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Research Article

Improvement of Bacterial Quality of Chicken Meat Patties During Frozen Storage by Using Oregano, Thyme, and Clove Oleoresins

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Abstract | Essential oils (EOs) are becoming more popular as natural preservatives alternatives to synthetic ones due to their powerful antibacterial action and increasing consumer concerns about synthetic preservatives. However, applications of EOs in food have been hampered by their pungent odor, which has a negative impact on customer acceptance. Therefore, this study was conducted to investigate the effect of using low concentration (0.002%) of thyme (T), oregano (O), clove (CL), and their combination (TO, TCL, OCL, and TOCL) on the bacterial quality of chicken meat patties during frozen storage at −18 °C for 12 weeks by enumeration of aerobic plate count (APC), psychrotrophic, *Enterobacteriaceae*, and lactic acid bacterial (LAB) counts. The results revealed that the sole effect of T, CL, and O oleoresins caused non-significant reductions in all investigated bacterial counts. Among the treated groups (T, CL, or O alone), it was found that O oleoresin achieved the highest reduction rate, followed by CL while the least reduction rate was recorded in samples treated with T. Therefore, addition of O into the mixture of T-O, O-CL, or T-O-CL improved their antibacterial activity. Furthermore, treatment of chicken patties with TCL, TO, OCL, as well as TOCL completely suppressed the growth of LAB (< 2 $\log_{10}$ CFU/g) at 0-time of examination and during the entire freezing storage. Additionally, the highest reduction rate in APC, psychrotrophic, and *Enterobacteriaceae* count was observed in samples treated with mixtures of three oleoresins (T-CL-O) as compared with other groups. In conclusion, the food industry could use a mixture of these oleoresins with a low concentration as a natural source of antibacterial during the processing of chicken meat products to improve their bacterial quality and extend their shelf life without causing odor problems.

Keywords | Clove, Oregano, Thyme, Chicken meat patties, Frozen storage

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INTRODUCTION

In recent years, consumption of chicken meat and its products has greatly increased globally. The majority of consumers prefer chicken meat due to its lower price in comparison to beef and pork meats. Chicken meat is characterized by high nutritional value due to its high protein content and low fat content, besides relatively high polyunsaturated fatty acids concentrations (Kralik et al., 2018). Moreover, chicken meat is suitable for processing different meat products than other types of meats due to its light color, good texture, and neutral flavor and consequently produce favorable flavor and texture profiles according to the consumer and market requirements (Barbut, 2012). Furthermore, fast meals such as chicken meat patties become widely consumed in the world as a result of the change in consumers' lifestyles. However, chicken meat and its products become highly perishable due to its richness in essential nutrients, along with high pH value and water activity, therefore it acts as a favorable media for the growth of pathogenic and spoilage bacteria that cause public health hazards to consumers (Abdel-Naeem et al., 2021). In this context, chicken meat and its products should be handled, prepared, and preserved in a way that keeps them safe until human consumption (Fratianni et al., 2010).

Consumers' awareness about using microbiologically safe food that is healthy, shelf-stable, minimally processed, and without synthetic chemical preservatives has been increased (Kamat and Balasubramaniam, 2020). Food processors are looking for discovering natural preservatives to address all consumers' needs (Petrou et al., 2012). Spices and herbs have been added in foods to improve their color, flavor, and aroma. They have powerful antimicrobial, antioxidant, and preservative actions (Embuscado, 2015). Hundreds of years ago, antimicrobial compounds derived from plants, have been known as inhibitory action against pathogenic and spoilage bacteria such as thyme, oregano, and their essential oils (EOs) (Burt and Reinders, 2003). Essential oils are natural and aromatic liquids extracted from different parts of plants such as seeds, flowers, roots, leaves, etc. (Gokoglu, 2019). They are non-phytotoxic oils and are considered generally recognized as safe (GRAS) by FDA (Lucera et al., 2012). Oleoresins exhibited excellent antibacterial, antiparasitic, antiviral, insecticidal, antimycotic, and antitoxigenic properties (Bishop, 1995; Karpouhtsis et al., 1998; Juglal et al., 2002; Mourey and Canillac, 2002; Pessoa et al., 2002; Mari et al., 2003). The antimicrobial activity of oleoresins is depending on the concentration of different phytochemicals that exist in different plant ingredients such as alkaloids, phenolic, saponins, terpenes, tannins, thymol, and carvacrol (Sharma et al., 2012). Although all aforementioned advantages of EOs, their addition in meat products should be with suitable concentrations due to their strong odor and flavor that could negatively affect the sensory characteristics of such products (Lambert et al., 2001; Proestos and Komaitis, 2008).

Most of the previous studies have been used high concentrations of thyme, oregano, and clove in processing chicken meat patties and examined their antibacterial activities during chilling storage (Ntzimani et al., 2011; Petrou et al., 2012), which could negatively affect the sensory attributes of the products. Hence, due to the flavor considerations from using high concentrations of EOs, as well as the studies examining the antibacterial activity of using a low concentration of these EOs during frozen storage of chicken meat patties are scarce. Therefore, the objective of the present study was to explore the effect of using very low concentration (0.002%) of thyme, oregano, clove oleoresins, and their combination on the bacterial quality of chicken meat patties during frozen storage at −18 °C for 12 weeks by enumeration of aerobic plate count, psychrotrophic, *Enterobacteriaceae*, and lactic acid bacterial counts.

MATERIALS AND METHODS

Preparation of chicken meat patties ingredients

Deboned chicken meat (about twenty-four kg of a mixture of the thigh and breast meat) was obtained from a local poultry processing plant, trimmed from all visible fat and connective tissue, and kept at −18°C for the next day. Seasonings mix and sodium tripolyphosphate were obtained from Loba Chemie, Mumbai, India. Moreover, the starch and sodium chloride were acquired from a local market in Cairo, Egypt.

Products formulation

A simple traditional formulation was used to prepare a base batter as follows: 80% chicken breast and thigh (40% each), 7.5% sunflower oil, 1.8% sodium chloride, 0.3% sodium tripolyphosphate, 0.3% seasonings mix, 5% water and 5% starch. From the base batter, seven groups were prepared by addition of 0.002% from each EOs as follow: thyme (T), oregano (O), clove (CL), a mixture of thyme and oregano (TO), a mixture of thyme and clove (TCL), a mixture of oregano and clove (OCL), and finally a mixture of thyme, oregano and clove (TOCL) oleoresins.

Chicken meat patties processing and storage

Three independent replicates for each chicken meat patties formula were processed to investigate the effect of using thyme, oregano, clove oleoresins, and their combination on the bacterial quality of chicken meat patties during frozen storage at −18 °C for 12 weeks. For each replicate, the chicken breast and thigh meat were minced through a 4.5-mm plate grinder. The minced chicken meat was mixed with salt, polyphosphates, seasonings, water, oil, and starch. The mixture was divided into seven groups (T; O; CL; TO; TCL; OCL and TOCL). Afterward, the mixture of each formula was mixed by hand for 5 min and manually formed into discs of 75 grams and 1cm thickness using manual former. The chicken patties were placed in plastic packaging films, held at −40 °C for 30 min, then placed in plastic containers and stored at −18 °C for 12 weeks. For each replicate, samples were withdrawn from each formula for analysis on the first day (0-time) and every two weeks.

Bacteriological examination

Ten grams of chicken meat patties were taken under complete aseptic condition and homogenized in a sterile homogenizing bag with 90 mL of sterile Ringer's solution (Oxoid BR0052G, Hampshire, England) using a stomacher (Lab Blender Seward 80 BA 6020) for 3 min to get a dilution of 10^{-1}, from which tenfold serial dilutions were performed. Aerobic plate count (APC) was counted after spreading of 0.1 mL from the diluted tubes over the surface of double sets of Plate Count Agar (PCA, Oxoid CM0325B, Hampshire, England), then incubated at 35°C for 48 h (Ryser and Schuman, 2015). While, to enumerate the psychrotrophic bacteria, another set of plate count agar were inoculated and incubated at 7 °C for 7–10 days (Vasavada and Critzer, 2015). *Enterobacteriaceae* bacterial counts were enumerated after incubation of the inoculated Violet Red Bile Glucose agar plates (VRBG, Oxoid CM1082B, Hampshire, England) at 37 °C for 24 h (Kornacki et al., 2015). In addition, Lactic acid bacteria (LAB) were determined after incubation of the inoculated deMan Rogosa Sharpe agar plate at 30 °C for 48 h (MRS, Oxoid CM 0361, Hampshire, England) according to Njongmeta et al. (2015).

Statistical analysis

Statistical data analysis for the three independent replicates was carried out using SPSS statistics 17.0 for windows (SPSS Inc, Chicago, IL, USA). The difference between the means values (reduction rate) of APC, psychrotrophic, *Enterobacteriaceae*, and lactic acid bacteria among different treatments at every two weeks was determined using one-way analysis of variance (ANOVA), and multiple comparisons of means were done using Post Hoc (least square difference test, LSD) procedure. Differences were considered significant at the $P < 0.05$ level.

RESULTS AND DISCUSSION

The effect of thyme, clove, oregano EOs and their combination on the reduction rate of APC of chicken meat patties

Aerobic plate count (APC) is an index of meat quality, which gives a general idea about the hygienic measures during the processing of further processed chicken meat products (Aberle et al., 2001). It estimates the level of live aerobic bacteria in meat products, which helps in assessing their shelf life, keeping quality, and post-processing contamination (Maturin and Peeler, 1998). Although Levine (1987) reported that APC is not a definite indication of food safety for consumption, nevertheless it is of great importance in judging the hygienic condition under which food has been prepared, handled, and stored.

The reduction rate in aerobic plate count ($\log_{10}$ CFU/g) of chicken patties treated with different oleoresins during frozen storage at −18 °C for 12 weeks is presented in Table 1. APC counts reduction rate of T, CL or O treated samples were ranged from 0.32–0.99, 0.34–1.00, and 0.45–1.19 $\log_{10}$ CFU/g, at 0-time of the examination till the end of frozen storage (12 weeks), respectively. The individual effect of T, CL, and O oleoresins caused non-significant ($P > 0.05$) reductions in APC counts while their combination achieved a significant effect. Among the treated groups (T, CL, or O alone), it was found that O oleoresin achieved the highest reduction rate, followed by CL while the least reduction rate was recorded in samples treated with T. In this regard, mixing of CL or O with T improved its antibacterial activity since, T-CL or T-O induced a significant ($P < 0.05$) reduction in APC of treated samples especially at 2, 4, 6 and 8 weeks of freezing storage as compared with samples treated with T only. Furthermore, a mixture of O-CL or O-CL-T resulted in a significant ($P < 0.05$) reduction of APC of treated samples (at 0 time of examination till the end of storage period) as compared with samples treated with T, CL, or O. Interestingly, treatment of chicken patties with the mixtures of three oleoresins (T-CL-O) exhibited significant ($P < 0.05$) higher reduction rate (1.96-2.98 $\log_{10}$ CFU/g) in APC among all treated groups.

Our results are in agreement with those reported by Tsigarida et al. (2000), who found that treatment of vacuum-packed beef muscle with oregano EO (0.8%) resulted in a significant reduction in the APC by 2-3 $\log_{10}$ CFU/g as compared with control samples. Similarly, Skandamis and Nychas (2001) observed that treatment of minced beef with oregano EO (1%) induced immediate suppression in APC by 1 $\log_{10}$ CFU/g. It is noteworthy that, these authors obtained a higher reduction rate in APC in their studies than our finding due to using a higher concentration of such EOs than the concentration used in the present study (0.002%). Furthermore, Radha Krishnan et al. (2014) noticed that using a mixture of oregano and clove EOs (0.5% each) in the treatment of chicken meat samples cause a significant reduction in the APC as compared with the control group (5.81 vs 7.15 $\log_{10}$ CFU/g). Accordingly, Al-Hijazeen (2014) indicated that oregano EO (0.01- 0.03%) could be used as a good preservative in ground chicken meat.

Treatment of chicken meat with 1% clove extract significantly reduced the APC as compared with control samples (5.88 vs 7.22 $\log_{10}$ CFU/g) during chilling storage at 4 °C (Zhang et al., 2016). Moreover, Zengin and Baysal (2015) observed that the addition of thyme and clove EOs (2 minimum inhibitory concentrations) during the formulation of ground beef samples induced an immediate and significant reduction in APC when compared with control samples. Likewise, Giatrakou et al. (2010) obtained a 5-day shelf-life extension of 0.2 % thymol treated ready to cook poultry product as compared to the control sample, which was microbiologically rejected on day 4 of storage. In contrast to our findings, a higher bacterial reduction rate in thymol oil-treated chicken sausage than clove oil (0.125%) treated one was obtained by Sharma et al. (2017).

The effect of thyme, clove, oregano EOs and their combination on the reduction rate of the psychrotrophic bacterial count of chicken meat patties

Psychrotrophic bacteria can grow at cold storage conditions and result in deterioration, spoilage as well as off-flavor even at low temperatures which shorten the shelf life of poultry products (Carrizosa et al., 2017). The growth of psychrotrophic pathogens in refrigerated poultry products is a food safety concern where raw poultry products are refrigerated or frozen before cooking (Ryser, 1999). In this regard, the determination of psychrotrophic bacterial count can be considered as an overall indicator for the shelf life of poultry products (Capita et al., 2001).

The results of reduction rate in psychrotrophic bacterial count (log_{10} CFU/g) of chicken patties treated with different oleoresins during frozen storage at –18°C for 12 weeks are summarized in Table 2. Our results revealed that the effect of oleoresins (T, CL, or O) on the psychrotrophic bacterial count of treated samples followed the same pattern of the results of APC, where chicken patties treated with O had the highest reduction rate (0.70 – 1.10 log_{10} CFU/g) followed by samples treated with CL (0.47 – 1.00 log_{10} CFU/g) while, the lowest reduction rate was observed in samples treated with T (0.20 – 0.92 log_{10} CFU/g). Furthermore, the addition of CL with T during the formulation of chicken patties significantly ($P < 0.05$) improve its antibacterial activity against psychrotrophic bacterial count as compared with samples treated with T only nonetheless such mixture showed the same effect of CL or O. Moreover, the use of O into the mixture of T-O, O-CL, or T-O-CL significantly reduced the psychrotrophic bacterial count during the entire storage periods (12 weeks) in comparison to other groups (T, CL, O or T-CL). Interestingly, the highest reduction rate in psychrotrophic bacterial count (2.00 – 2.57 log_{10} CFU/g) was achieved in samples treated with mixtures of three oleoresins (T-CL-O) as compared with other treated samples.

The results obtained are in consistent with those reported by Jaworska et al. (2021), who showed that treatment of minced poultry meat with oregano extract (0.003%) completely reduced the psychrotrophic bacteria throughout the storage period at 4 °C for 15 days. In another study, Hać-Szymańczuk et al. (2019) noticed that the addition of oregano EO (0.1%) to mechanically separated chickens meat decreased the psychrotrophic bacterial count as compared to their counterpart control samples at the end of frozen storage at –18 °C for 9 months. In contrast to our findings in this study, Zengin and Baysal (2015) observed that ground beef samples treated with thyme or clove EOs induced a non-significant reduction in psychrotrophic bacterial count as compared with control samples (7.05 log_{10} CFU/g). Nonetheless, the combination between thyme (0.125%) and clove (0.25%) EOs produced a higher reduction rate against psychrotrophic bacteria in chicken sausage than using 0.125% of holy basil oil (Sharma et al., 2017). Furthermore, Giatrakou et al. (2010) observed that treatment of ready to cook chicken products with thyme (0.2%) cause a significant reduction in the psychrotrophic count at 4 °C for 12 days. In another regard, Jaworska et al. (2021) found that treatment of minced poultry meat with thyme EO (0.003%) resulted in a non-significant reduction in psychrotrophic bacteria (5.14 log_{10} CFU/g) when compared with control (4.97 log_{10} CFU/g) during the chilling storage at 4 °C for 15 days.

The effect of thyme, clove, oregano EOs and their combination on the reduction rate of *Enterobacteriaceae* bacterial count of chicken meat patties

Enterobacteriaceae count is used to evaluate the general hygienic condition of food and their presence in cooked food indicates insufficient cooking or contamination after processing (CFS, 2014). They play a crucial role in meat spoilage because of their capability to convert amino acids into malodorous volatile chemicals like foul-smelling diamines and sulfuric compounds (Baylis, 2006). Furthermore, this group of microorganisms is considered as a safety indicator due to their presence in food with high numbers is refers to the presence of food-borne pathogens (Jay et al., 2005).

The reduction rate in *Enterobacteriaceae* count (log_{10} CFU/g) of chicken patties treated with different oleoresins during frozen storage at –18 °C for 12 weeks is illustrated in Table 3. There was a non-significant ($P > 0.05$) reduction in *Enterobacteriaceae* count (0.98 – 1.20, 1.14 –1.33 and 1.27 – 1.45 log_{10} CFU/g, respectively) between samples treated with T, CL, or O. However, treatment of chicken patties with TCL or TO resulted in significant ($P < 0.05$) reduction in this bacterial count compared to samples treated with T or CL. Furthermore, treatment of chicken patties with OCL caused a significant ($P < 0.05$) reduction in *Enterobacteriaceae* count compared to samples treated with one of the three oleoresins (T, CL, or O). It is important to emphasize that the highest reduction rate in *Enterobacteriaceae* count (2.43 – 2.90 log_{10} CFU/g) was obtained in samples treated with TCLO as compared with other treatments.

Our findings in this study are in agreement with those of Chouliara et al. (2007), who noticed that *Enterobacteriaceae* counts are not detected (< 1 log_{10} CFU/g) in 0.1% oregano treated fresh chicken breast meat on day 15 up to 25 of chilling storage. In the same regard, oregano EO (0.1%) could limit the growth of *Enterobacteriaceae* in mechanically deboned chicken meat and extend its shelf life to 9 months under frozen storage conditions at – 18 °C (Hać-Szymańczuk et al., 2019). Furthermore, Koplay and Sezer (2013) found that the addition of clove EO (7%) to red beef meat decreased the *Enterobacteriaceae* count during chilling storage at 4 °C for 14 days. Moreover, Radha Krishnan et al. (2014) found that treatment of raw chicken meat with oregano (1%) or clove (1%) EOs reduced the *Enterobacteriaceae* count (4.26 vs 4.41 $\log_{10}$ CFU/g) when compared with the control group (4.68 $\log_{10}$ CFU/g) during chilling storage at 4 °C for 15 days, however, their combination (0.5% each) reduced the count to 4.11 $\log_{10}$ CFU/g. A lower *Enterobacteriaceae* count in thyme EO (0.1%) treated lamb meat (2.8 $\log_{10}$ CFU/g) in comparison to the control group (6 $\log_{10}$ CFU/g) was obtained by Karabagias et al. (2011).

Table 1: Reduction rate in aerobic plate count ($\log_{10}$ CFU/g) of chicken patties treated with different oleoresins during frozen storage at –18 °C for 12 weeks.

Treatments	Storage period (weeks)						
	0	2	4	6	8	10	12
T	$0.32^{c,t} \pm 0.02$	$0.42^{d,t} \pm 0.00$	$0.42^{d,t.} \pm 0.03$	$0.43^{d,tu} \pm 0.06$	$0.53^{d,tu} \pm 0.05$	$0.76^{d,u} \pm 0.06$	$0.99^{d,u} \pm 0.03$
CL	$0.34^{c,t} \pm 0.04$	$0.66^{cd,tu} \pm 0.02$	$0.67^{cd,tu} \pm 0.01$	$0.67^{cd,tu} \pm 0.06$	$0.70^{cd,u} \pm 0.01$	$0.80^{d,u} \pm 0.04$	$1.00^{d,u} \pm 0.02$
O	$0.45^{c,t} \pm 0.05$	$0.73^{cd,tu} \pm 0.02$	$0.73^{cd,tu} \pm 0.10$	$0.73^{cd,tu} \pm 0.11$	$0.81^{cd,u} \pm 0.05$	$0.91^{cd,uv} \pm 0.02$	$1.19^{cd,v} \pm 0.01$
TCL	$0.62^{bc,t} \pm 0.04$	$0.93^{bc,tu} \pm 0.01$	$0.93^{bc,tu} \pm 0.02$	$0.94^{bc,tu} \pm 0.30$	$0.95^{bc,tu} \pm 0.08$	$0.98^{cd,tu} \pm 0.05$	$1.27^{cd,u} \pm 0.04$
TO	$0.66^{bc,t} \pm 0.01$	$1.04^{bc,tu} \pm 0.02$	$1.05^{bc,u} \pm 0.04$	$1.06^{bc,u} \pm 0.21$	$1.07^{bc,u} \pm 0.06$	$1.22^{bc,uv} \pm 0.06$	$1.49^{bc,v} \pm 0.01$
OCL	$0.94^{b,t} \pm 0.05$	$1.12^{b,tu} \pm 0.04$	$1.13^{b,tu} \pm 0.07$	$1.15^{b,tu} \pm 0.09$	$1.22^{b,tu} \pm 0.01$	$1.39^{b,uv} \pm 0.00$	$1.62^{b,v} \pm 0.06$
TOCL	$1.96^{a,t} \pm 0.05$	$2.21^{a,t} \pm 0.02$	$2.21^{a,t} \pm 0.03$	$2.26^{a,tu} \pm 0.06$	$2.27^{a,tu} \pm 0.02$	$2.61^{a,uv} \pm 0.02$	$2.98^{a,v} \pm 0.00$

T: Thyme; CL: Clove; O: Oregano; TO: Thyme+Oregano; TCL: Thyme+Clove; OCL: Oregano+Clove; TOCL: Thyme+Oregano+Clove. $^{a–d}$Means with different superscripts within the same column are significantly ($P < 0.05$) different. $^{t–v}$Means with different superscripts within the same row are significantly($P < 0.05$) different. Values represent the mean of 3 independent replicates ± *SE*.

Table 2: Reduction rate in psychrotrophic bacterial count ($\log_{10}$ CFU/g) of chicken patties treated with different oleoresins during frozen storage at –18 °C for 12 weeks.

Treatments	Storage period (weeks)						
	0	2	4	6	8	10	12
T	$0.20^{e,t} \pm 0.02$	$0.30^{e,t} \pm 0.00$	$0.34^{d,t} \pm 0.04$	$0.38^{d,t} \pm 0.03$	$0.53^{d,t} \pm 0.02$	$0.53^{d,t} \pm 0.09$	$0.92^{d,u} \pm 0.07$
CL	$0.47^{de,t} \pm 0.09$	$0.50^{de,t} \pm 0.02$	$0.54^{cd,t} \pm 0.02$	$0.56^{cd,t} \pm 0.01$	$0.65^{cd,tu} \pm 0.03$	$0.69^{cd,tu} \pm 0.01$	$1.00^{cd,u} \pm 0.02$
O	$0.70^{cd,t} \pm 0.01$	$0.75^{cd,tu} \pm 0.04$	$0.75^{c,tu} \pm 0.02$	$0.76^{c,tu} \pm 0.03$	$0.77^{cd,tu} \pm 0.01$	$0.89^{cd,tu} \pm 0.04$	$1.10^{cd,u} \pm 0.04$
TCL	$0.72^{c,t} \pm 0.03$	$0.75^{cd,t} \pm 0.02$	$0.75^{c,t} \pm 0.10$	$0.77^{c,t} \pm 0.04$	$0.95^{c,tu} \pm 0.07$	$0.97^{c,tu} \pm 0.01$	$1.27^{c,u} \pm 0.03$
TO	$1.01^{bc,t} \pm 0.01$	$1.03^{bc,t} \pm 0.02$	$1.13^{b,tu} \pm 0.03$	$1.29^{b,tu} \pm 0.05$	$1.33^{b,tu} \pm 0.04$	$1.33^{b,tu} \pm 0.02$	$1.39^{b,u} \pm 0.02$
OCL	$1.34^{b,t} \pm 0.04$	$1.34^{b,t} \pm 0.03$	$1.43^{b,t} \pm 0.07$	$1.45^{b,t} \pm 0.00$	$1.45^{b,t} \pm 0.03$	$1.57^{b,t} \pm 0.07$	$1.63^{b,t} \pm 0.06$
TOCL	$2.00^{a,t} \pm 0.04$	$2.00^{a,t} \pm 0.06$	$2.23^{a,tu} \pm 0.05$	$2.24^{a,tu} \pm 0.03$	$2.26^{a,tu} \pm 0.02$	$2.53^{a,u} \pm 0.06$	$2.57^{a,u} \pm 0.07$

T: Thyme; CL: Clove; O: Oregano; TO: Thyme+Oregano; TCL: Thyme+Clove; OCL: Oregano+Clove; TOCL: Thyme+Oregano+Clove. $^{a–e}$Means with different superscripts within the same column are significantly ($P < 0.05$) different. $^{t–u}$Means with different superscripts within the same row are significantly($P < 0.05$) different. Values represent the mean of 3 independent replicates ± *SE*.

Table 3: Reduction rate in *Enterobacteriaceae* count ($\log_{10}$ CFU/g) of chicken patties treated with different oleoresins during frozen storage at –18 °C for 12 weeks

Treatments	Storage period (weeks)						
	0	2	4	6	8	10	12
T	$0.98^{d,t} \pm 0.02$	$1.00^{d,t} \pm 0.07$	$1.10^{d,t} \pm 0.02$	$1.16^{d,t} \pm 0.09$	$1.18^{d,t} \pm 0.02$	$1.18^{d,t} \pm 0.04$	$1.20^{e,t} \pm 0.03$
CL	$1.14^{d,t} \pm 0.04$	$1.14^{d,t} \pm 0.03$	$1.15^{d,t} \pm 0.07$	$1.15^{d,t} \pm 0.02$	$1.20^{d,t} \pm 0.04$	$1.29^{d,t} \pm 0.02$	$1.33^{e,t} \pm 0.05$
O	$1.27^{cd,t} \pm 0.04$	$1.34^{cd,t} \pm 0.03$	$1.34^{cd,t} \pm 0.01$	$1.38^{cd,t} \pm 0.00$	$1.40^{cd,t} \pm 0.06$	$1.45^{cd,t} \pm 0.05$	$1.45^{de,t} \pm 0.02$
TCL	$1.54^{bc,t} \pm 0.03$	$1.59^{bc,t} \pm 0.05$	$1.60^{c,t} \pm 0.04$	$1.63^{c,t} \pm 0.01$	$1.69^{c,t} \pm 0.08$	$1.70^{c,t} \pm 0.05$	$1.73^{cd,t} \pm 0.04$
TO	$1.57^{bc,t} \pm 0.01$	$1.62^{bc,tu} \pm 0.02$	$1.67^{bc,tu} \pm 0.11$	$1.69^{c,tu} \pm 0.05$	$1.70^{c,tu} \pm 0.02$	$1.72^{c,tu} \pm 0.01$	$1.96^{bc,u} \pm 0.00$
OCL	$1.90^{b,t} \pm 0.06$	$1.92^{b,t} \pm 0.01$	$2.00^{b,tu} \pm 0.02$	$2.14^{b,tu} \pm 0.02$	$2.15^{b,tu} \pm 0.05$	$2.20^{b,tu} \pm 0.03$	$2.29^{b,u} \pm 0.02$
TOCL	$2.43^{a,t} \pm 0.01$	$2.55^{a,tu} \pm 0.04$	$2.58^{a,tu} \pm 0.04$	$2.62^{a,tu} \pm 0.02$	$2.67^{a,tu} \pm 0.05$	$2.76^{a,tu} \pm 0.04$	$2.90^{a,u} \pm 0.08$

T: Thyme; CL: Clove; O: Oregano; TO: Thyme+Oregano; TCL: Thyme+Clove; OCL: Oregano+Clove; TOCL: Thyme+Oregano+Clove. $^{a–e}$ Means with different superscripts within the same column are significantly ($P < 0.05$) different. $^{t–u}$ Means with different superscripts within the same row are significantly($P < 0.05$) different. Values represent the mean of 3 independent replicates ± *SE*.

Table 4: Reduction rate in lactic acid bacterial count ($\log_{10}$ CFU/g) of chicken patties treated with different oleoresins during frozen storage at −18 °C for 12 weeks.

Treatments	Storage period (weeks)						
	0	2	4	6	8	10	12
T	$1.83^{b,t} \pm 0.10$	$2.03^{b,t} \pm 0.27$	$2.37^{b,tu} \pm 0.10$	$2.45^{b,u} \pm 0.07$	$2.56^{b,u} \pm 0.05$	$2.60^{c,u} \pm 0.19$	$2.65^{c,u} \pm 0.53$
CL	$1.95^{b,t} \pm 0.04$	$2.10^{b,t} \pm 0.04$	$2.52^{b,u} \pm 0.07$	$2.55^{b,u} \pm 0.08$	$2.64^{b,u} \pm 0.06$	$2.70^{c,u} \pm 0.10$	$2.72^{c,u} \pm 0.73$
O	$2.14^{b,t} \pm 0.02$	$2.35^{b,t} \pm 0.05$	$2.61^{b,u} \pm 0.08$	$2.65^{b,u} \pm 0.10$	$2.74^{b,u} \pm 0.12$	$3.08^{b,v} \pm 0.09$	$3.17^{b,v} \pm 0.06$
TCL	$>4.30^{a,t} \pm 0.00$	$>4.55^{a,t} \pm 0.00$	$>4.92^{a,t} \pm 0.00$	$>4.99^{a,t} \pm 0.00$	$>5.13^{a,t} \pm 0.00$	$>5.50^{a,t} \pm 0.00$	$>5.60^{a,t} \pm 0.00$
TO	$>4.30^{a,t} \pm 0.00$	$>4.55^{a,t} \pm 0.00$	$>4.92^{a,t} \pm 0.00$	$>4.99^{a,t} \pm 0.00$	$>5.13^{a,t} \pm 0.00$	$>5.50^{a,t} \pm 0.00$	$>5.60^{a,t} \pm 0.00$
OCL	$>4.30^{a,t} \pm 0.00$	$>4.55^{a,t} \pm 0.00$	$>4.92^{a,t} \pm 0.00$	$>4.99^{a,t} \pm 0.00$	$>5.13^{a,t} \pm 0.00$	$>5.50^{a,t} \pm 0.00$	$>5.60^{a,t} \pm 0.00$
TOCL	$>4.30^{a,t} \pm 0.00$	$>4.55^{a,t} \pm 0.00$	$>4.92^{a,t} \pm 0.00$	$>4.99^{a,t} \pm 0.00$	$>5.13^{a,t} \pm 0.00$	$>5.50^{a,t} \pm 0.00$	$>5.60^{a,t} \pm 0.00$

T: Thyme; CL: Clove; O: Oregano; TO: Thyme+Oregano; TCL: Thyme+Clove; OCL: Oregano+Clove; TOCL: Thyme+Oregano+Clove. $^{a-c}$ Means with different superscripts within the same column are significantly ($P < 0.05$) different. $^{t-v}$ Means with different superscripts within the same row are significantly($P < 0.05$) different. Values represent the mean of 3 independent replicates ± *SE*.

The effect of thyme, clove, oregano EOs and their combination on the reduction rate of LAB of chicken meat patties

LAB is mainly accompanied by the spoilage of processed meat products more than fresh meat due to the addition of carbohydrates during the processing of such products leading to slime, gas, greening, and sour off-flavors. The spoilage ability of different LAB strains is mainly differing according to water activity and pH of the meat, carbon dioxide, and oxygen levels as well as storage and cooking temperatures. The reduction rate of the lactic acid bacterial count of chicken patties treated with T, CL, or O was 1.83 − 2.65, 1.95 − 2.72, and 2.14 − 3.17 $\log_{10}$ CFU/g, respectively (Table 4). There was a significant ($P < 0.05$) reduction in LAB count in samples treated with O at 10 and 12 weeks of frozen storage as compared with samples treated with T or CL. Surprisingly, LAB counts of samples treated with TCL, TO, OCL as well as TOCL were under the detectable levels (< 2 $\log_{10}$ CFU/g) at 0-time of examination and during the entire freezing storage. In this context, such treatments induced more than 4.30-5.60 $\log_{10}$ CFU/g reduction rate in LAB at 0-time of the examination till the end of frozen storage (Table 4).

Our results are similar to those reported by Petrou et al. (2012), who found that the addition of oregano EO (0.25%) in chicken fillets resulted in suppression of LAB in chicken breast stored at 4 °C for 14 days. Additionally, Chouliara et al. (2007) noticed that using oregano EO (0.1%) in fresh chicken breast meat produced 1 $\log_{10}$ CFU/g reduction in LAB count while at concentration 1% completely inhibits its growth throughout the storage period at 4 °C for 12 days. Likewise, Koplay and Sezer (2013) recorded a reduction rate of 1 $\log_{10}$ CFU/g (on days 7 and 9), 2.5 $\log_{10}$ CFU/g (on day 11 and 13), and 2 $\log_{10}$ CFU/g at the end of chilling storage (14 days) in LAB count of 7% clove EO treated red meat as compared to control samples. Furthermore, Fratianni et al. (2010) observed that 0.5% thyme EO treated chicken breast meat showed significant inhibition of LAB growth until the end of chilling storage (3 weeks). In another study, Giatrakou et al. (2010) explored that 0.2% thyme oil-treated ready to cook poultry product had a lower LAB count than the control sample. On the other hand, Emiroğlu et al. (2010) found that the application of antimicrobial films with thyme and oregano EOs (5% each) in ground beef patties produced no significant effect on LAB. In the same regard, Roller (1995) declared that LAB is the most resistant bacteria to the activity of oleoresins.

The antimicrobial activity of oleoresins is mainly related to their phenolic compounds such as carvacrol in oregano, thymol in thyme, and eugenol in clove (Shan et al., 2005). These phenolic compounds make the destruction of the bacterial cell wall, disturbance in the cell membrane, increase the cell membrane permeability, and release of bacterial cell constituents. Furthermore, they make alterations in the structure of phospholipid and fatty acid and coagulation of cytoplasmic protein beside they interfere with DNA and RNA synthesis and consequently cause bacterial cell death (Tiwari et al., 2009). Moreover, Akagawa et al. (2003) attributed the antimicrobial activity of oleoresins to the action of polyphenols which produce hydroperoxides.

The antimicrobial activity of EOs was higher against the Gram-positive bacteria than the Gram-negative bacteria. This was explained by Nazzaro et al. (2013), who reported that Gram-negative bacteria have a more complex cell wall with a thicker peptidoglycan layer than Gram-positive bacteria which hinders the diffusion of hydrophobic compounds of different oleoresins. In the same regard, Tiwari et al. (2009) found that the Gram-positive bacteria are highly sensitive to the phenolic compounds of the

EOs, which interfere with enzymes that are responsible for energy production at low concentrations, however, at high concentrations, they make proteins denaturation. Nonetheless, it was reported that clove, thyme, and oregano EOs are active against both Gram-positive bacteria such as *L. monocytogenes*, *S. aureus*, Enterococcus, and *Bacillus cereus,* as well as Gram-negative bacteria such as *E. coli*, Salmonella species, *Y. enterocolitica*, and *P. aeruginosa* (Lopez et al., 2005; Sakkas and Papadopoulou, 2017).

CONCLUSIONS AND RECOMMENDATIONS

It was concluded that using low concentration (0.002%) of thyme, clove, and oregano EOs induced non-significant reductions in APC, psychrotrophic *Enterobacteriaceae*, and LAB counts of chicken meat patties. However; their combination achieved a significant effect especially in the mixture containing oregano EO. Moreover, treatment of chicken patties with TCL, TO, OCL, as well as TOCL completely suppressed the growth of LAB (< 2 $\log_{10}$ CFU/g) at 0-time of examination and during the entire freezing storage. A synergetic powerful antimicrobial activity against the APC, psychrotrophic bacteria, Enterobacteriaceae were recorded in samples treated with mixtures of three oleoresins (T-CL-O). Accordingly, such a mixture can prevent the microbial spoilage of chicken products and extend their shelf life during frozen storage.

NOVELTY STATEMENT

The addition of essential oils (EOs) plays an important role in improving the flavor, aroma as well as bacterial quality of chicken meat products. However, most previous studies used high concentrations of meat products which resulted in a negative impact on the flavor of such products. Moreover, most researchers studied their effect under chilling storage conditions. Therefore, the novelty of the present study was to explore the effect of using a very low concentration (0.002%) of thyme, oregano, clove oleoresins, and their combination on the bacterial quality of chicken meat patties during frozen storage at –18 °C for 12 weeks.

AUTHOR'S CONTRIBUTION

All authors shared the same effort during performing this study

Conflict of interest

The authors have declared no conflict of interest.

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