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Research Article

Antibacterial efficacy of essential oil of *Thymus capitatus*, lactic acid and acetic acid against *Escherichia coli* in craw chicken meat.

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Abstract

Essential oils (EO) and their components are known by their antimicrobial activity and therefore might be used as ingredients in certain food products to preserve them against spoilage and prolong their shelf life. It was the purpose of this work to evaluate the antibacterial efficacy of EO of *Thymus capitatus,* alone and in combination with lactic acid and acetic acid against *Escherichia coli* ATCC 25922 in fresh chicken meat experimentally contaminated. EO and both organic acids (OAs) were tested at concentrations ranging from 0.1 to 1.0 % (v / v) and 0.25 to 1.0 % (v/v), respectively. The antibacterial effect was demonstrated by counting the colony forming units (CFU), after culturing of the ground meat in mixture with appropriate antibacterial agent, on McConkey agar medium. The results showed a reduction in microbial charge ranged between 0.32 and 2.22 log CFU/ml, at the concentrations of essential oil cited above. Acetic acid was ineffective at 0.25% to 1.0%, while 1% lactic acid led to a reduction of 2.03 log CFU/ ml. The combination effect of 0.5 % EO, 0.25 % acetic acid and 0.25 % lactic acid, was comparable to 1.0 % EO employed alone. In order to preserve the chicken meat, during refrigerated conservation, against deterioration possibly due to *E. coli*, EO of *T. capitatus* could be employed at lower concentration if combined with lactic acid and acetic acid.

Introduction

Poultry meat such as chicken meat is a very popular food commodity around the world due to its low cost of production (Barbut, 2002; Patsias et al., 2008). It has has many desirable nutritional characteristics such as a low lipid content and relatively high concentration of polyunsaturated fatty acids (Bourre, 2005). However, poultry meat belongs to a class of highly perishable foods (Dias et al., 2013). Spoilage of raw meat may occur in two ways during refrigeration: microbial growth and oxidative rancidity (Sebranek et al., 2005). If not properly handled and preserved, fresh meat and meat products, support growth of spoilage and pathogen bacteria, leading to loss of quality and potential public health problems (Vernozy et al., 2002). These bacteria, viz., *Pseudomonas*, *Acinetobacter*, *Brochothrix thermosphacta*, *Lactobacillus* spp., *Escherichia coli* etc., and yeast and mold cause quality defects such as off-flavor, off-odor etc (Jayasena et Jo, 2013). Deterioration is further accelerated by some intrinsic factors including pH and water activity of fresh meat (Jayasena et Jo, 2013). In general, most fresh meat has a water activity value higher than 0.85 and its pH value falls within the favorable pH range for spoilage bacteria of meat (Dave et Ghaly, 2011).

As a result, extending the shelf life of perishable chicken products is a major concern for the poultry industry (Wang et al., 2004). In this regard, many synthetic additives have been used over the years (Chen et al., 1992). Unfortunately, these additives have been accused for some carcinogenic and toxic properties (Jayasena et Jo, 2013). Owing to this, consumers increasingly demand use of natural products as alternative preservatives in foods, as the safety of synthetic additives has been questioned in last years (Imaida et al., 1983). Natural components from different sources including animals, bacteria, algae and mushrooms have been reported in literature. While, bio-preservatives are mainly derived from plant extracts (Vaithiyanathan et al., 2011). Essential oils – volatile, aromatic oily liquids obtained from plant material –

are regarded as prime candidates for use as natural antimicrobials in food (Hulankova et al., 2013). Their antimicrobial properties have been long recognized (Burt, 2004). Essential oils have been shown to possess antibacterial activities against several microorganisms associated with meat, including gram-negative and gram-positive bacteria (Karabagias et al., 2011). Major groups of compounds that are responsible for antimicrobial activity from plants include phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids, and alkaloids (Ciocan et Bara, 2007; Lai et Roy, 2004).

Regarding the meat and meat products, EOs from oregano, rosemary, thyme, clove, balm, ginger, basilica, coriander, marjoram, and basil have shown a greater potential to be used as an antimicrobial agent (Barbosa et al., 2009; Chouliara et Kontominas, 2006; Dzudie et al., 2004; Fratianni et al., 2010; Govaris et al., 2010; Menon et Garg, 2001; Skandamis et Nychas, 2001; Skandamis et al., 2002; Solomakos et al., 2008; Tsigarida et al., 2000). The most important compounds of thyme EO are the phenols thymol (44–60%) and carvacrol (2.2–4.2%), which constitute the major and more active constituents (Di Pasqua et al., 2005), as well as the monoterpene hydrocarbons p-cymene (18.5–23.5%) and c-terpinene (16.1–18.9%), (Baranauskiene et al., 2003; Daferera et al., 2000). Furthermore, organic acids are natural constituents of plant and animal tissues; they exhibit antibacterial activity while many of them have been classified as: generally recognized as safe [GRAS] (Friedly et al., 2009). The antimicrobial activity of organic acids is attributed to pH reduction, depression of internal pH of microbial cells by ionization of acid molecules, and disruption of substrate transport by altering cell membrane permeability (Jay et al., 2005).

Thyme essential oils are highly aromatic natural components. In fact, higher concentrations of these compounds are usually needed in food (including meat) to obtain the same antimicrobial effect as in vitro (Barbosa et al., 2009). The aim of this study on the combined effect of EO of *T. captatus* and organic acids was to evaluate the possibility of lowering the individual concentrations of this EO to a sensory acceptable level while maintaining the overall antimicrobial effect.

Materials and Methods

Bacterial strain

Regarding the meat and meat products, several pathogenic microorganisms including *E. coli* can result in foodborne illnesses to consumers. In the current study commercial strain of *E. coli* (ATCC-25922) was used in different essays. Inocula used in different essay were set to 0.5 McFarland or an optical density from 0.08 to 0.13 at 625 nm wavelength, which corresponds to 10^8 CFU/mL (CLSI, 2006).

Antimicrobial agents

Essential oil

Essential oil used in this study was obtained from *T. capitatus*, collected in the area of Tlemcen, located in the North-west of Algeria. Extraction was carried out by hydro-distillation for 2 hours using a standard Clevenger-type apparatus as recommended in the European pharmacopoeia. Oil extracts were dehydrated with anhydrous sodium sulphate (Na_2SO_4) and stored in glass tubes at 4°C protected from the light until testing. Essential oil was tested over a concentration range of 0.1 to 1% (v / v).

Organic acids

Both acetic acid and citric acid were purchased from Sigma-Aldrich and tested over a concentration range of 0.25 to 1 % (v/v).

Experimental design

The protocol applied in the current study has been inspired of the works of Solomakos et al. (2008**)** with some modifications. Chicken meat conserved in sterile food plastic bags was purchased from a local butcher and transported, as soon as possible, under refrigerated conditions, to the laboratory. The outer surface of each slices of meat was sterilized by immersion in 70% (v/v) ethanol and then burning the residual. Ground meat (10g) was diluted in saline solution (89mL). Saline solution contains appropriate concentration of antimicrobial indicated above. Then 1mL of bacterial suspension was added. In each essay 1% (v/v) tween 80 was used to enhance dissolution of essential oil. To evaluate the combined effect of EO/OA, the same previous steps were achieved using different combined fractions of antimicrobials (data not shown in the current study). Samples of ground meat in saline without any antimicrobials served as control. Prior to microbial analysis, all samples, were serially diluted (1:10, 1:100 and 1:1000) in sterile peptone saline solution. The dilutions were inoculated on duplicate plates of McConkey agar (Sigma-Aldrich). After incubation at 37 °C for 24 h, populations of *E. coli* were enumerated on the plates. The experiment was repeated five times separately for each essay.

Results

The antibacterial effect of EO at 0.1%, 0.25%, 0.50 and 1% on *E. coli* was realized five times separately, on 5 samples of fresh chicken meat. Values obtained (Table 1) at different concentrations of EO represent the mean counts (log CFU/mL) and their standard deviation (SD) of *E. coli* population on McConkey agar medium after incubation 24 h at 37°C in aerobic conditions.

Table 1. Antibacterial effect of EO at different concentrations, against *E. coli* in craw chicken meat. values are expressed in log CFU/mL.

Essays	**Control**	**EO**			
	0 **%**	0.1%	0.25%	0.5%	1%
1	6.50	6.00	7.00	6.00	5.20
2	7.00	6.20	5.70	4.20	4.00
3	6.20	8.00	6.00	6.00	3.90
4	5.30	5.30	5.00	3.70	4.50
5	6.95	4.85	4.90	6.70	3.25
Mean ± SD	6.39±0.69	6.07±1.21	5.72±0.85	5.32±1.29	4.17±0.73

These results were converted into histogram as it can be shown in Figure 1. Bars which were made by Excel computer software represent the mean values of *E. coli* (log UFC/mL) and their standard deviations in the five samples.

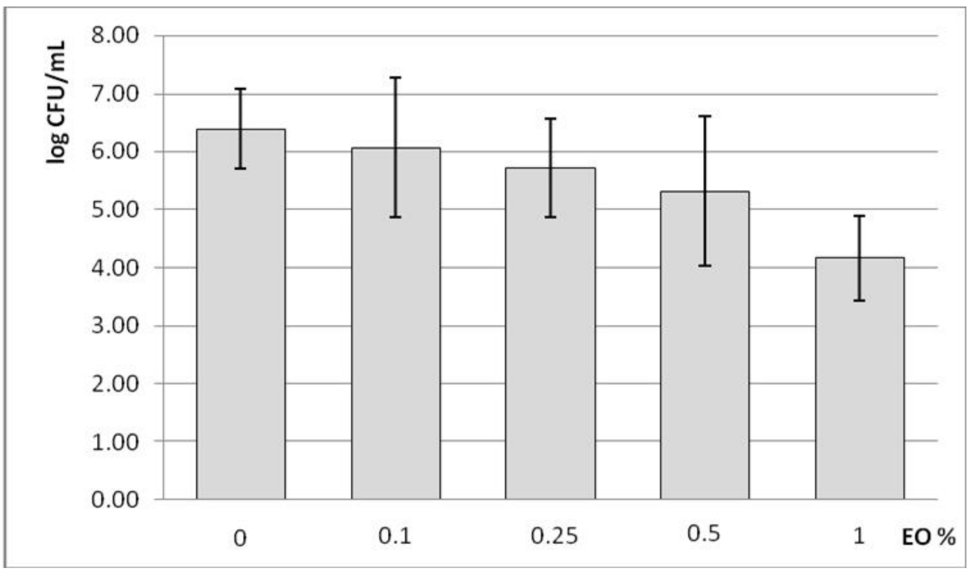

Figure 1. Antibacterial effect of different concentrations of *T. capitatus* EO, against *E. coli* in craw chicken meat.

Results showed that the *T. capitatus* EO has a low in vivo activity against the tested bacteria in the ground chicken meat in comparison with control,. The average efficacy of EO was in the following order: 1% EO, 0.5% EO, 0.25% EO and 0.1% EO. Therefore, reduction in microbial charge was ranged between 0.32 and 2.22 log CFU/ml.

The antibacterial efficacy against *E. coli* in craw chicken meat was also tested with two organic acids, acetic acid and lactic acid. The mean counts (log CFU/mL) and their standard deviation (SD) of *E. coli* population, at different concentrations of each organic acid, are presented in Table 2.

Table 2. Antibacterial effect of organic acids at different concentrations, against *E. coli* in craw chicken meat. values are expressed in log CFU/mL.

Essays	**Control**	**Acetic acid**			**Lactic acid**		
	0 **%**	0.25%	0.5%	1%	0.25%	0.5%	1%
1	6.00	6.00	6.04	6.30	6.80	6.50	5.20
2	6.17	6.11	5.95	5.14	5.80	5.00	4.00
3	5.90	6.47	5.39	5.76	6.00	6.14	4.10
4	6.36	6.32	6.60	6.32	5.40	5.50	4.00
5	6.07	5.30	6.17	5.04	6.00	6.70	3.05
Mean ± SD	6.1±0.18	6.04±0.45	6.03±0.44	5.71±0.61	6.00±0.51	5.96±0.71	4.07±0.76

Application of acetic acid alone didn't lead to any significant inhibition of *E. coli* in comparison with control, whereas the application of lactic acid alone at 1% inhibited relatively the growth of this microorganism. Indeed, a difference of about 2logCFU/mL was observed between 1% EO and the control essay.

Data of combined antibacterial effect of different fractions of organic acids with EO are not shown in this work. In fact, combination of 0.5% EO, 0.25% acetic acid and 0.25% lactic acid has shown a significant antibacterial on E. coli, in comparison with control. This result looks like to the one obtained with 1% EO employed alone.

Discussion

Essential oils and organic acids have long been known to possess great potentials as antimicrobial compounds. *T. capitatus*, is one of famous herbals used in this regard. Its EO contains more than 60 ingredients, most of which posses important antioxidant and antimicrobial properties (Baranauskiene et al., 2003). From Table 1 and Figure 1, the mean count values of E. coli obtained in different concentrations of EOs revealed no significantly important efficacy, compared to control; this may be due to the EO concentrations used in the current study. Although insufficient, at 1% EO, the reduction of 31.31 % (2.22 logCFU/mL) of the initial bacterial charge is contestable and could be explored.

Some of the organic acids (acetic, lactic, benzoic, sorbic) have a long history of use in the food industry as food preservatives (Skrivanova et al., 2006). Thus, it seems that the antimicrobial properties of organic acids are of pivotal importance for their beneficial effects (Roth and Kirchgessner, 1998). Occurrence of colour changes in meat to brighter or brownish due to application of organic acids with regard to decontamination of carcasses and meat cuts has been studied and discussed previously by many authors, e.g. in a review by Smulders and Greer (1998). Furthermore, in a study effect of organic acids was more pronounced in cultures of *Clostridium perfringens*, which is a Gram positive bacterium, than in *E. coli* and *Salmonella* sp., which are Gram negative bacteria belonging to Enterobacteriaceae (Skrivanova et al., 2006). In accordance with results shown in Table 2, it can be noted that lactic acid was more efficacious than acetic acid, since microbial charge of E. coli was diminished in 1% lactic acid by 2.03logCFU/mL, compared to control.

Since higher concentrations of EOs are generally required to ensure their antimicrobial activity for food preservation as compared to in vitro system, their application may be limited due to changes in organoleptic and textural quality of food or interactions of EOs with food components (Gutierrez et al., 2008). That why currently, several research focused on the combined antibacterial evaluation of EO with other antimicrobials. This led, in certain cases, to enhancement of antibacterial efficacy of EO and diminution of its concentrations compared to those usually used. Furthermore, many reports about the combined effect of thyme EO with other biologically active natural compounds have been cited in the literature (Solomakos et al., 2008; Solomakos et al., 2008; Turgis et al., 2012). The combined use of EOs and organic acids appears to be a promising natural alternative. In fact, combined effects of EO with acetic and lactic acids, were little studied to date. In a study, combined treatment of lactic acid and clove oil synergistically reduced microbial counts without affecting sensory qualities such as color and odor compared to controls (Jayasena et Jo, 2013).

In the current study, combined effect of 0.5% EO with 0.25% of each one of the two organic acids used, was comparable to 1% EO applied alone. This effect was not sufficient but convincing that EO efficacy could be enhanced at lower concentration when used in combination with organic acids and hence the suitable sensory level can be respected.

Conclusion

In conclusion, treatment of ground chicken meat with 0.5 % essential oil, 0.25 % acetic acid and 0.25 % lactic acid, showed an antibacterial activity against *E. coli* ATCC 25922 relatively equivalent to antibacterial effect obtained with 1.0 % EO, when used alone. Although, these results are interesting, since at 0.5 % of EO sensory level was acceptable, the overall antimicrobial effect was not yet sufficient.

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