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Antimicrobial activity of essential oils of three herbs against *Listeria monocytogenes* on chicken frankfurters

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ABSTRACT

Listeria monocytogenes is a foodborne pathogen responsible for listeriosis. The inhibitory effect of essential oils (1% v/w) of *Thymus daenensis* Celak (Lamiaceae), *Thymbra spicata* L. (Lamiaceae) and *Satureja bachtiarica* Bunge (Lamiaceae) applied to the surface of chicken frankfurters was determined on *L. monocytogenes* inoculated at low (10^3 CFU/g) and high (10^6 CFU/g) populations and stored for seven and 14 days. The results showed that *L. monocytogenes* populations increased during seven and 14 days of storage at 4 °C on control frankfurters without essential oil. The application of 1 % essential oil (v/w) of herbs to frankfurter surfaces can significantly reduce ($p<0.05$) the *L. monocytogenes* populations as compared to control in two inocula treatments after seven and 14 days of storage at 4 °C.

Key words: Iranian medicinal herbs, *Listeria monocytogenes,* chicken frankfurters, essential oil

IZVLEČEK

PROTIMIKROBNA AKTIVNOST ETERIČNIH OLJ TREH ZELIŠČ PROTI PATOGENU *Listeria monocytogenes* V PIŠČANČJIH HRENOVKAH

Listeria monocytogenes je povzročitelj listerioze, ki se pojavlja v živilih. Proučevan je bil inhibitorni učinek eteričnih olj (1% v/w) zelišč *Thymus daenensis* Celak (Lamiaceae), *Thymbra spicata* L. (Lamiaceae) in *Satureja bachtiarica* Bunge (Lamiaceae), nanešenih na površino piščančjih hrenovk, ki so bile inokulirane z nizko (10^3 CFU/g) oziroma visoko populacijo (10^6 CFU/g) listerije ter shranjene za 7 oziroma 14 dni. Rezultati so pokazali, da se populacije *L. monocytogenes* povečajo tekom 7 oziroma 14 dni shranjevanja pri 4 °C na primerjalnih hrenovkah. Uporaba 1 % (v/w) eteričnih olj zelišč pri hrenovkah lahko značilno ($p<0.05$) zmanjša populacijo *L. monocytogenes* v primerjavi s kontrolo.

Ključne besede: iranska zdravilna zelišča, *Listeria monocytogenes,* piščančje hrenovke, eterična olja

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1 INTRODUCTION

Foodborne illness resulting from consumption of food contaminated with pathogenic bacteria has been of vital concern to public health. *Listeria monocytogenes* is a Gram-positive bacterium responsible for the severe foodborne illness listeriosis. This disease is primarily transmitted through various foods, fish, dairy products, cured or processed meat, egg, poultry, seafood, salad, fruits and vegetables (Garcia et al., 2004). A severe infection, listeriosis has been associated with a mortality rate as high as 30-40% (Datta, 2003).

Ready-to-eat (RTE) meat products have been introduced for the convenience of consumers; however, many have few barriers to microbial growth. Frequently, refrigeration is the only barrier for these types of foods, and temperature abuse at any of the links of the supply chain from the processing plants to the consumer's refrigerator could accelerate the growth of *L. monocytogenes*. Most RTE foods receive little or no final heat treatment before being consumed because such foods are assumed to be and are often labelled as fully cooked (Hao et al., 1998). There have been reported illnesses resulting when supposedly RTE foods were not reheated before consumption (Pinner et al., 1992; Schuchat et al., 1991). There have also been a large number of recalls of RTE meat due to contamination by *L. monocytogenes* (Kathariou, 2000).
To reduce health hazards and economic losses due to foodborne bacteria, the use of natural products as antibacterial compounds seems to be an interesting way to control the presence of pathogenic bacteria and to extend the shelf life of processed food (Dorman and Deans, 2000). Essential (volatile) plant oils occur in edible, medicinal and herbal plants and have been widely used as flavouring agents in foods since the earliest recorded history. It is well-established that many essential oils have antimicrobial activity against a wide range of spoilage and pathogenic bacteria (Deans and Ritchie, 1987; Alzoreky and Nakahara, 2003; Kim *et al.*, 1995).

A lot of references on anti-*listeria* and antibacterial efficiency of essential oils are available in the literature (Chauhan et al., 2007; Rasooli et al., 2006; Stonsaovapak et al., 2000). For example, Ghasemi Pirbalouti et al., 2009 reported that the essential oil of *Thymus daenensis* and *Thymus* sp. flowers exhibited antibacterial activities against *L. monocytogenes in vitro*.

The Iranian medicinal plants, including *Thymbra spicata, Satureja bachtiarica* and *Thymus daenensis* have been utilised as spice and culinary plants by the indigenous people of west provinces, Iran (Ghasemi Pirbalouti, 2009).

However, no study investigating the antimicrobial effect of *Thymus daenensis, Thymbra spicata* and *Satureja bachtiarica* on *L. monocytogenes* on RTE chicken frankfurters could be found in the literature surveyed. The objective of this study was to determine the growth inhibition of *L. monocytogenes* (isolated from chicken meat) on chicken frankfurters and the efficacy of essential oils in inhibiting *L. monocytogenes* growth on chicken frankfurters.

2 MATERIALS AND METHODS

2.1. Plant material

The *Thymus daenensis* Celak (Lamiaceae), *Thymbra spicata* L (Lamiaceae) and *Satureja bachtiarica* Bunge (Lamiaceae) were collected from mountain areas of Zagross, West-South Iran, during May–Nov, 2008. Their identity was confirmed by Ghahraman (1987–1989), Mozaffarian (2007), and Rechinger (1963-1998), and voucher specimens were deposited at the Researches Centre of Medicinal Plants, Islamic Azad University-Shahrekord Branch, Iran.

2.2. Preparation of Extracts

The leaves and flowers of plants were powdered (200 g) and subjected to hydro-distillation (2000 ml distilled water) for 4 h using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (British Pharmacopoeia, 1988). The extracts were filtered using Whatman No. 1 filter paper and then were stored in universal bottles and refrigerated at 4 °C prior to use.

2.3. Food sample preparation

Chicken frankfurters were purchased at a local supermarket and brought immediately to the laboratory. The frankfurters were cut into 10 g portions, and the surface of each sample was exposed to UV light (λ = 260 nm) for 10 min to kill surface contaminants.

2.4. Preparation of Listeria monocytogenes

The strain of *L. monocytogenes* was isolated from chicken meat and kindly provided by the Department of Microbiology, Faculty of Veterinary Medicine Faculty, Islamic Azad University- Shahrekord, Iran. Bacterial cultures were maintained frozen in broth at -20 °C until use. Prior to use, the

cultures were activated in at 37 °C for 24 h. The contents of the tube were transferred to a 100-ml flask containing sterile BHI broth (Merk, Germany) at log phase (OD_{600}=0.4-0.5) to scale up the bacterial suspension volume. An initial bacterial suspension containing 1x10^6 CFU/ml was made from the flask broth culture.

2.5. Treatments of food samples

The inhibitory effect of essential oils (1% v/w) of *Thymus daenensis*, *Thymbra spicata* and *Satureja bachtiarica* applied to the surface of chicken frankfurters (10g of each) was determined after *L. monocytogenes* was inoculated (100 µl with 10^3 CFU/g and 100 µl with 10^6 CFU/g) and stored for seven and 14 days at 4°C. The treated samples were contaminated with *L. monocytogenes* on the surface using a pipette, and then the bacterium was spread with a sterile bent glass rod. A control consisted of chicken frankfurters inoculated with *L. monocytogenes* with no essential oil. Inoculated samples in Petri-dishes were left undisturbed for 30 min to allow residual moisture to be absorbed. The number of *L. monocytogenes* on the frankfurters was determined with plate count method at seven and 14 (at 4 °C) days of storage. Three replications of the treatments were performed.

2.6. Microbial analysis

Three samples from each inoculation level of *L. monocytogenes* at seven and 14 days of storage were assayed (at 4 °C). Corresponding control samples were also assayed. Samples were serially diluted (1:10), and 100 µl was spread plated onto BHI agar (Merk, Germany). The plates were incubated at 37 °C for 24 h to determine the population of *Listeria*. Selected presumptive colonies of *L. monocytogenes* were confirmed by Henry illumination and biochemical tests (Mytle et al., 2006).

2.7. Analysis of data

The numbers of *L. monocytogenes* was statistically analysed by one ways ANOVA, and statistical significance between treated and control groups was analysed by means of Student's *t*-test (*P-values* < *0.05*) by the program "SAS version 6.12 full".

3 RESULTS AND DISCUSSION

During seven and 14 days of storage of frankfurters, the control samples had higher levels of bacteria than the treated samples (5.3 x 10^4 CFU/g to 7.7 x 10^5 CFU/g) (Table 1). The amount of bacteria on the controls was not "increased," but rather, the essential oils prevented bacterial growth on the treated frankfurters. The treatment group had inhibited bacterial growth relative to the growth observed in the control group.

The results indicated that after seven days of storage at 4 °C, essential oil treatments were able to significantly reduced ($p<0.05$) to 3.2 x 10 to 6.6 x 10^2 CFU/g, as compared to control (5.3 x10^4 CFU/g) in the frankfurters with low inoculum (Table 1.). The essential oil of *Satureja bachtiarica* was least efficient in decreasing the *L. monocytogenes* count with low inoculum after 7 and 14 days at 4 °C, whereas, nitrite and essential oils of *Thymus daenensis* and *Thymbra spicata* were highly efficient in decreasing the *L. monocytogenes* count with low inoculum after 7 and 14 days at 4 °C.

Table 1. Comparison of *L. monocytogenes* populations in frankfurters treated with different essential oils/plant extracts

Treatments	N (cfu/g)			
	Low inoculum		High inoculum	
	7 days	14 days	7 days	14 days
Control	5.3 × 10 ^{4}a	7.7 × 10^5	5.3 × 10 ^{7}	5.7 × 10^8
Thymus daenensis	3.2 × 10 *	4.7 × 10 *	3.7 × 10^3 *	3.8 × 10^2 *
Thymbra spicata	6.5 × 10 *	3.1 × 10 *	6.5 × 10^3 *	5.4 × 10^3 *
Satureja bachtiarica	6.6 × 10^2 *	4.8 × 10 *	4.9 × 10^4 *	6.1 × 10^4 *

a: Statistically significant by Student's *t*-test, *N=3* samples.
*: *P≤0.05* levels of significance.

During 7 and 14 days of storage of control frankfurters (without essential oil) with high inoculum at 4 °C, *L. monocytogenes* reached to 5.3 x 10^7 to 5.7 x 10^8 CFU/g (Table 1.).

The results showed that essential oil treatments were able to significantly reduced ($p<0.05$) to 3.7 x 10^3 to 4.9 x 10^4 CFU/g, as compared to control (5.3 x 10^7 CFU/g) with high inoculum after 7 days of storage at 4 °C. After 7 and 14 days at 4 °C, the essential oil of *Satureja bachtiarica* was least efficient at decreasing the *L. monocytogenes* count on frankfurters that received the high inoculum treatment, whereas the essential oil of *Thymus daenensis* was highly efficient at decreasing the

L. monocytogenes count on frankfurters with high inoculum.

The most active constituents (essential oils) of many spices have a wide spectrum of antimicrobial activity including aromatic phenolic compounds, such as thymol and carvacrol in oregano and thyme, eugenol in clove and cinnamon, and cinnamic aldehyde in cinnamon (Beuchat and Golden, 1989). These bioactive principles in the related dietary spices and medicinal herbs were also identified in other studies (Chauhan et al., 2007; Zampini et al., 2005). Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (Hara-Kudo et al., 2004).

Shan et al. (2007) showed that many herb and spice extracts contained high levels of phenolics and exhibited antibacterial activity against foodborne pathogens. Gram-positive bacteria such as *L. monocytogenes* were generally more sensitive to the tested extracts than Gram-negative ones. Also, Shan et al. (2007) reported a highly positive relationship (R^2=0.73) between antibacterial activity and phenolic content of the tested extracts against *L. monocytogenes*. According to a report of Rasooli et al. (2006), various concentrations of essential oils from *Thymus eriocalyx* and *Thymus x-porlock* tested on agar plates and in broth tubes showed very strong anti-*listeria* properties. Also, they reported that *Thymus x-porlock* was a stronger bactericidal agent than *Thymus eriocalyx* oil. Rožman and Jeršek (2009) confirmed that antimicrobial activity of rosemary (*Rosmarinus officinalis* L.) extracts was depended on selected rosemary extract, concentration of extracts, different species of *Listeria* and different strains of *Listeria monocytogenes*.

The essential oil and extract of some aromatic plants (for example, the mint family, *Lamiaceae*) with a higher percentage of carvacrol and thymol have a higher efficacy against bacterial strains (Rasooli et al., 2006). The essential oils of *Thymus daenensis* (Nickavar *et al.*, 2005), *Thymbra spicata* (Hanci et al., 2003) and *Satureja bachtiarica* (Sefidkon et al., 2007) contained high levels of phenolics monoterpenes (thymol and carvacrol) and exhibited antibacterial activity. They could be a potential source of inhibitory substances against some foodborne pathogens as well as a source of antioxidant agents.

Hao et al. (1998) applied extracts of various plants, including clove oil, to cooked chicken to determine their antimicrobial activity against the Scott A strain of *L. monocytogenes*. They reported no growth of the test strain of *Listeria* at 5 °C in untreated samples when a high population of 10^5 CFU/g inoculum was applied. In our study, the essential oil treatments significantly reduced the final *L. monocytogenes* populations after 7 and 14 days of storage at 4 °C as compared to the control.

Yuste and Fung, 2002 determined that 0.1% cinnamon reduced 10^4 CFU/g of *L. monocytogenes* Scott A in pasteurised apple juice (pH= 5.0) to undetectable cell numbers within 1 h of storage at 5 °C and 20 °C. In this study, the inhibitory effect of species of thyme on *L. monocytogenes* varied during storage for the same treatment. Mytle et al. (2006) reported that the application of 1% clove oil (v/w) to frankfurter surfaces or the inclusion of cloves or clove oil in the frankfurters, coupled with low temperature storage, can reduce the potential of *L. monocytogenes* contamination and growth without significantly changing flavour.

4 CONCLUSIONS

In conclusion, the *L. monocytogenes* populations in frankfurters treated with essential oils were significantly lower than in control samples throughout the storage period. The application of 1% thyme oil (v/w) applied to the surface of chicken frankfurters coupled with low temperature storage can reduce the potential of *L. monocytogenes* contamination.
In this study, the essential oils with high anti-*listeria* activity may be candidates for future studies of synergism, compatibility, and activity in foods or food-processing systems and mechanisms of activity against specific pathogens. The tested plant extracts may contain antimicrobial constituents, and further phytochemical and pharmacological studies will be necessary to isolate the active constituents and evaluate the antimicrobial activity against a wide range of microbial populations.

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