Efficiency of a commercial liquid spice extracts mix for the decontamination of *Listeria monocytogenes* and *Escherichia coli* O157:H7 from meat surface

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Abstract— ***Listeria monocytogenes* and *Escherichia coli* O157:H7 are of primary importance due to their high prevalence in raw meat. In this study, efficiency of a recently released commercial liquid spice extract mix (Asatim®, Kayseri, Turkey) obtained from dried parts of sorrel (*Rumex acetosella* L.), milfoil (*Achillea millefolium* L.) and ribwort plantain (*Plantago lanceolata* L.) was used to decontaminate *L. monocytogenes* and *E. coli* O157:H7 inoculated previously onto the meat surface at room temperature. Initial inoculation levels of *L. monocytogenes* and *E. coli* O157:H7 were 5.60 and 4.70 log cfu/cm^2. The meat blocks were washed with the above extracts for 0, 0.5, 1 or 2 min, and the remaining cells were recounted. In the results, it was observed that the washing treatment for 0.5 min lowered the number of *L. monocytogenes* and *E. coli* O157:H7 by 1.25 and 0.35 log cfu/cm^2 respectively as compared to the no wash control while no statistical difference was observed on *E. coli* O157:H7 counts between the extract and sterile distilled water washes in 0.5 min treatment. However, extending of washing time caused additional reductions on both *L. monocytogenes* and *E. coli* O157:H7, nearly around to 3 log cfu/cm^2. It can be concluded that the liquid spice extract mix might be a novel sanitizing agent for the meat industry to reduce the number of common food-borne pathogens, *L. monocytogenes* and *E. coli* O157:H7 from meat surfaces effectively.***

Keywords— **Meat surface, *L. monocytogenes*, *E. coli* O157:H7, spice extract.**

I. INTRODUCTION

Meat is a nutritious media that provides a high opportunity for the growth of spoilage microorganisms and common foodborne pathogens [1]. Contamination of meat begins with the slaughter of the animal and continues in the sequential stages such as cut up and storage depending of the hygienic conditions. Contamination of meat carcasses with undesired microorganisms has been a wide problem in the worldwide when the meats are exposed to warm conditions [2]. Various carcass decontamination methods including immersion, flooding, deluging, cascading water, rinsing or spray washing have been introduced in meat industry [3]. These methods comprise physical, chemical and biological treatments [4], and they aim to reduce or eliminate the bacteria that may be pathogenic to humans or lead to meat spoilage [5]. Organic acids, especially lactic and acetic acid are most widely used antimicrobials in the chemical decontamination treatments [6]. Especially, acid washing has the advantage to provide immediate decontamination (via the physical dislocation) of meat surfaces [7]. However, bacteriocins and chlorine based chemicals are other antimicrobial compounds used in post-harvest meat decontamination [1].

Antibacterial activity of spices and their derivatives have also been reported against a number of foodborne pathogens including *E. coli, L. monocytogenes, Salmonella* Typhimurium and *Staphylococcus aureus* [8, 9]. Various extracts of herbs and spices have been used in meat products as flavoring materials for long

times [10]. A number of studies have been carried out about the antibacterial activities of plant-derived antimicrobials in meat products [10, 11, 12]. However, possibility of use of plant derived materials in carcass decontamination is still in need of investigation. The aim of this study was to investigate the antibacterial efficiency of a recently released commercial liquid spice extract mix against *E. coli* O157:H7 and *L. monocytogenes* inoculated to meat surface, thereby to determine its possibility to use as a decontamination agent on the carcasses.

II. MATERIALS AND METHODS

A. Materials

The commercial liquid spice extract mix was obtained from the manufacturer (Asatim®, Kayseri, Turkey) and stored at 4 ^{o}C until use. *E. coli* O157:H7 ATCC 33150 and *L. monocytogenes* ATCC 7644 were provided from Kayseri Agriculture Control Protection Management, Turkey. Bacterial cultures were maintained on Nutrient Agar (Merck, Darmstadt, Germany) at 4 ^{o}C and activated in Nutrient Broth (Merck, Darmstadt, Germany) for 24 h at 37 ^{o}C.

B. Preparation of Meat Samples

Fresh lean beef plate cuts were purchased from a local market in Kayseri, Turkey and cut into 5x5 cm square cubes by hand.

C. Inoculation of Meat Samples with Bacterial Cultures

Dip inoculation method was used for inoculation of the meat samples with *L. monocytogenes* and *E. coli* O157:H7. Inoculum solutions were prepared by transferring 5mL of 24h Nutrient Broth culture into 500mL of Ringer Solution. Meat cubes (n=10) were immersed into the inoculum solution and shaken for 1 min to distribute the inoculum homogenously and then kept in the safety cabinet for 20 min at 22±2 ^{o}C. Targeted final inoculum levels were approximately 10^5-10^6 cfu/cm^2 on contacted meat surfaces.

D. Decontamination Study and Enumeration of Bacteria

Decontamination process was made according to the method described by Carpenter et al. [6] with some modifications. Meat cubes were decontaminated by dipping in sterile holders containing 100 mL of the liquid spice mix extract for 0, 0.5, 1 and 2 min. Control samples were washed in sterile distilled water. Following the decontamination, samples were allowed to air for 20 min at room temperature.

Air dried meat samples were placed into Stomacher bags with sterile stainless steel spatula. Sterile Ringer solution (100mL) was added to the each Stomacher bag and the contents were homogenized with a stomacher (IUL, Nr 1147/470, Spain). Serial dilutions of the homogenates were prepared with sterile Ringer solutions and drop (0.1mL) plated in duplicate onto Oxford Listeria Selective Agar (Merck, Germany) and Sorbitol MacConkey Agar (Merck, Germany) for the enumeration of *L. monocytogenes* and *E. coli* O157:H7, respectively. The plates were incubated at 37 ^{o}C for 48h and colonies were counted.

III. RESULTS AND DISCUSSION

Table 1. *L. monocytogenes* counts after the decontamination process with different treatment times.

Time (min)	Microbial counts (log cfu/cm^2)	
	Water wash	Extract wash
0	5.60±0.20^{Aa}	5.60±0.20^{Aa}
0.5	5.09±0.10^{Ac}	4.35±0.05^{Bb}
1	5.39±0.10^{Ab}	3.00±0.00^{Bc}
2	5.30±0.00^{Ab}	2.89±0.24^{Bc}

A-B: Differences in uppercase letters indicate statistical difference (P<0.05) in a row, a-c: Differences in lowercase letters indicate difference (P<0.05) in a column.

Changes in *L. monocytogenes* counts by the decontamination processes with different times are shown in Table 1. Initial *L. monocytogenes* population of meat cubes were 5.60 cfu/cm^2. Water and extract washes for 0.5 min provided significant (P<0.05) reductions on *L. monocytogenes* numbers. Extending of water wash treatment time did not cause additional

reductions; however, a significant (P<0.05) increase occurred from treatment time for 0.5 min to 1 min. Time was significantly (P<0.05) effective on the reduction of extract wash of the meat samples. Extract wash for 2 min reduced listerial counts around to 2.89 log cfu/cm^2 from 5.60 log cfu/g.

Table 2. *E. coli* O157:H7 counts after the decontamination process with different treatment times.

Time (min)	Microbial counts (log cfu/cm^2)	
	Water wash	Extract wash
0	4.70±0.00^{Aa}	4.70±0.00^{Aa}
0.5	4.37±0.10^{Ab}	4.35±0.00^{Ab}
1	4.33±0.10^{Ab}	3.24±0.07^{Bc}
2	4.30±0.00^{Ab}	3.09±0.10^{Bcd}

A-B: Differences in uppercase letters indicate statistical difference (P<0.05) in a row, a-d: Differences in lowercase letters indicate difference (P<0.05) in a column.

Table 2 shows the changes in *E. coli* O157:H7 counts resulting from the wash treatments. 0.5 min treatment of meat cubes with the liquid extract caused a reduction level of 0.35 log cfu/cm^2. A better inhibition occurred with the extract wash treatment for 1 min. In this time, the reduction level on *E. coli* O157:H7 number was 1.46 log cfu/cm^2. However, water wash did not result in additional significant (P>0.05) reductions while extending the treatment time.

Overall, it can be seen from Table 1 and Table 2 that, *L. monocytogenes* was more susceptible to the liquid extract than that of *E. coli* O157:H7, and the reduction level on *L. monocytogenes* count was higher.

Efficacy of various antimicrobials to decontaminate meat or poultry carcasses from *E. coli* O157:H7 and *L. monocytogenes* as well as other pathogens and natural microflora has been well investigated. For example, Carpenter et al. [6] determined 0.68, 1.06 and 0.88 log cfu/m^2 reductions in *E. coli* O157:H7 numbers of beef plates by %2 acetic acid, lactic acid and levulinic acid washes for 20s, respectively. Hardin et al. [13] reported that washing the beef carcasses with water following 2% lactic or acetic acid spray performed better inhibition than washing with water alone on *E. coli* O157:H7 or *S.* Typhimurium counts while lactic acid was more effective than acetic acid. Castillo et al. [14] tested the efficiency of citric acid-acidified sodium chlorite spray wash against *E. coli* O157:H7 on beef carcasses, a reduction of 4.5 log cycles was observed. Acuff et al. [15] demonstrated that water spray wash of beef carcasses at 95^{o}C at 24 psi for 5s caused significant reductions on total coliform, thermotolerant coliform, *S.* Typhimurium and *E. coli* O157:H7 counts. Again, Latha et al. [16] examined the antibacterial efficiency of spray wash treatments of pork carcasses with potassium sorbate (5%) and a combination of sodium chlorite, sodium acetate, sodium citrate, sodium lactate and potassium sorbate. In the results, *Staphylococcus aureus*, *E. coli*, *S.* Typhimurium, *Yersinia enterocolitica, L. monocytogenes, Serratia marcescens, Pseudomonas aeruginosa and Proteus vulgaris* were found to be more sensitive to salt combination treatment as compared to potassium sorbate alone.

IV. CONCLUSIONS

The liquid spice extract mix used to decontaminate the meat surface is a novel product that has been recently launched to the market as an antimicrobial additive for the meat products such as sausage and sucuk (traditional fermented Turkish sausage). Furthermore, it was aimed in this study to investigate the efficiency of the extract as an antimicrobial wash agent for the meat carcasses. As a conclusion, it might be said that the liquid extract was effective against *L. monocytogenes* and *E. coli* O157:H7 on meat surface, and it might be a convenient agent to use for decontamination of meat carcasses.

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