Quality Assurance and Safety of Crops & Foods, 2016; 8 (4): 575-582

Wageningen Academic Publishers

Application of plant hydrosols for decontamination of wheat, lentil and mung bean seeds prior to sprouting

N. Sahan and F. Tornuk*

Food Engineering Department, Chemical and Metallurgical Engineering Faculty, Yildiz Technical University, Davutpasa Campus, 34210 Esenler, Istanbul, Turkey; ftornuk@yildiz.edu.tr

Received: 21 January 2016 / Accepted: 15 March 2016

RESEARCH ARTICLE

Abstract

It is a requirement for both minimally processed and ready-to-eat fruit and vegetable industry and consumers to decrease use of harmful chemical antimicrobial agents such as chlorinated compounds in fresh product sanitation since they have a number of negative health and environmental effects. In this study, washing treatments with summer savoury, bayleaf and thyme hydrosols that are by-products essential oils were assessed for decontamination of wheat, mung bean and lentil seeds contaminated with *Salmonella* Typhimurium and *Staphylococcus aureus* prior to sprout production. Initial levels of *S. aureus* on wheat, lentil and mung bean were 4.86, 4.24 and 4.23 log colony forming units/g, respectively. Hydrosol treatments reduced *S. aureus* numbers significantly (P<0.05) depending on treatment time while the pathogen was not detected on mung bean soaked in the thyme hydrosol for 40 min. Sensitivity of *S.* Typhimurium was higher than *S. aureus* and thyme hydrosol achieved elimination of *Salmonella* on all of the seeds. Sanitising ability of the hydrosols were in order of thyme>summer savoury>bayleaf. Therefore, this study confirmed that plant hydrosols, especially thyme had potential to be used as a natural disinfectant for sanitation of seeds prior to germination in order to ensure their microbiological safety.

Keywords: seed sprout, germination, hydrosol, decontamination, thyme

1. Introduction

Nutrition habits have changed in recent years due to rapidly changing human lifestyles and consumer demands for healthier foods. Consumers now increasingly prefer foods that are ready to eat, minimally processed and/or can be prepared quickly and easily. Sprouting of seeds is considered as a simple and natural way of production of foods with high nutritional values. Sprouting has been known as a traditional method especially in Far East countries such as China and Japan since ancient times (Plaza *et al.*, 2003). Several plant seeds such as mung bean, soy bean, alfalfa and broccoli are used for sprout production in many parts of the World (Waje and Kwon, 2007) and it is well-known that sprouts are richer than the seeds in terms of the abundance of nutritional compounds such as minerals, vitamins, unsaturated fatty acids and phenolics (Alvarez-Jubete *et al.*, 2010; Calzuola *et al.*, 2004, 2006; Dhakal *et al.*, 2009; Gharachorloo *et al.*, 2012; Ozturk *et al.*, 2012a).

Besides the nutritional advantages of seed sprouts, they may possess a critical health concern since they may be vehicles of a high microbial load and also probably several foodborne pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes, Staphylococcus aureus* and *Salmonella*. As they are generally eaten raw, an effective decontamination procedure is required before consumption. In this respect, raw sprout consumption has caused numerous intensive foodborne illnesses due to the consumption of contaminated sprouts in all over the world (Breuer *et al.*, 2001; Mahon *et al.*, 1997; Painter *et al.*, 2013; Sivapalasingam *et al.*, 2004; Taormina *et al.*, 1999).

In order to ensure the microbiological safety of seed sprouts, a number of decontamination processes such as chlorine washing, ozone treatment, irradiation, heat treatment, high hydrostatic pressure, electrolysed water and their combinations have been tested (Bari *et al.*, 2003, 2004; Jaquette *et al.*, 1996; Lang *et al.*, 2000; Piernas and Guiraud,

1997; Sharma and Demirci, 2003; Tornuk *et al.*, 2011b; Wuytack *et al.*, 2003). However, no single treatment has been found to be able to ensure complete safety of seed sprouts microbiologically before consumption (Singh *et al.*, 2003; Taormina *et al.*, 1999). Moreover, in the case of insufficient sanitation treatment, pathogenic bacteria present in the seed can multiply easily and reach to very high counts during sprouting procedure with the help of mild environmental conditions (Goni *et al.*, 2013; Stewart *et al.*, 2001).

Plant derived compounds, such as essential oils and hydrosols, have been well demonstrated to have strong antibacterial activity against a wide spectrum of foodborne pathogens (Burt, 2004; Sagdic and Ozcan, 2003). Hydrosols are obtained by steam distillation of aerial parts of aromatic plants as byproducts of essential oils and have numerous advantages such as readily availability, cheapness and ease of production (Tornuk *et al.*, 2011a). Although convenience of plant hydrosols have been proven for decontamination of fresh-cut fruit and vegetables in several studies (Ozturk *et al.*, 2012b; Sagdic *et al.*, 2013; Tornuk *et al.*, 2011a, 2014; Törnük and Dertli, 2015), they have not been tested for their efficiency for elimination of pathogens from plants that are used for sprout production. Therefore, this study was conducted to determine the ability of thyme (*Thymus vulgaris* L.), summer savoury (*Satureja hortensis* L.) and bayleaf (*Laurus nobilis* L.) hydrosols for sanitation of wheat, lentil and mung bean that were contaminated with *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *S. aureus.*

2. Materials and methods

Materials

Bread wheat (*Triticum aestivum*), lentil (*Lens culinaris*) and mung bean (*Vigna radiata*) seeds were purchased from a local market in Istanbul, Turkey. Dried leaves of thyme (*T. vulgaris* L.), summer savoury (*S. hortensis* L.) and bayleaf (*L. nobilis* L.) were provided from a spice warehouse in Kayseri, Turkey.

Bacterial cultures, namely *S. aureus* ATCC 25923 and *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028 were obtained from Erciyes University Food Engineering Department, Kayseri, Turkey. Frozen stock bacterial cultures were activated in Nutrient Broth (Merck, Darmstadt,, Germany) for 24 h at 37 °C twice before use.

Isolation of plant hydrosols

Hydrosols were obtained based on the method described by Törnük and Dertli (2015). 100 g of dried leaves of thyme, bayleaf and summer savoury was weighed and placed into the flask (5 l of volume) and incorporated with 1 l of distilled water. Hydrodistillation was performed using a Clavenger apparatus (Ildam, Ankara, Turkey) for 2 h. The hydrosol was separated from the corresponding essential oil by the cooling tunnel and stored in dark bottles (1 l of volume) at 4 °C until use.

Inoculation of wheat, lentil and mung bean seeds

Prior to experiments, possible impurities, such as stem, soil, weed, etc., were removed manually from wheat, lentil and mung bean. Then the seeds were washed with tap water by gently rinsing for a while in order to decrease the native microbial load and dried in a biosafety cabinet for 2 h at ambient temperature. Dip inoculation procedure was performed for contamination of the seeds with the bacterial strains. For this aim, 400 g of each surface-dried seed was dipped in the inoculum suspension (1:1, w:v) for 20 min with the targeted contamination level of ~10^4-10^5 colony forming units (cfu)/g. Then the seeds were drained and dried in the biosafety cabinet for 2 h.

Soaking of the seeds with plant hydrosols

In order to determine the efficiency of plant hydrosols for decontamination of lentil, wheat and mung bean seeds, the contaminated seeds were soaked in thyme, summer savoury and bayleaf hydrosols. Soaking procedure was carried out based on the method previously described by Sagdic *et al.* (2013), Törnük and Dertli (2015) and Tornuk *et al.* (2011a). Sterile tap water was used as the control soaking material. For soaking, 100 g of the each contaminated seeds was submerged in sterile beakers containing each plant hydrosol (400 ml) for 0, 20, 40 or 60 min. In the preliminary studies, the hydrosol treatments were confirmed for the absence of their negative effects on seed germination ability.

Microbiological analysis and determination of growth inhibition rate

Following the washing treatment for certain periods, 10 g of the each seed sample was aseptically weighed into sterile stomacher bags and combined with 90 ml of sterile Ringer solution (Merck). Then the mixture was homogenised using a Stomacher apparatus (Golden Motor Industry Group Co., Limited, Zhejiang, China P.R.) for 90 s and appropriate decimal dilutions were prepared. The dilutions were spread plated onto petri plates containing dried xylose lysine deoxycholate (Merck) and Baird Parker Agar (Merck) for enumeration of *S.* Typhimurium and *S. aureus*, respectively. Following the incubation 37 °C for 24 h, specific colonies were counted and the cfu were converted to logarithmic values.

Growth inhibition rates (GIRs) for *S.* Typhimurium and *S. aureus* provided by hydrosol washing was calculated by the following equation (Sağdıç, 2003):

$$GIR(\%) = \frac{(P_C - P_T)}{P_T} \times 100$$

where P_C and P_T were populations of control sample and the sample washed with the hydrosol for certain treatment times, respectively.

Statistical analysis

The analyses were performed in duplicate. The data obtained by the hydrosol treatments were subjected to two-way analysis of variance using statistical analysis software (SAS 8.2; SAS Institute, Cary, NC, USA). Significant differences between the data were determined by Duncan's multiple range test at a significance level of 95%.

3. Results

Table 1 shows the effects of washing with thyme, summer savoury and bayleaf hydrosols on *S. aureus* populations of wheat, lentil and mung bean seeds. Initial inoculation counts of the strain were 4.86, 4.24 and 4.92 log cfu/g for wheat, lentil and mung bean, respectively. In general, tap water washing showed limited efficiency for removal of *S. aureus* from the seeds. Thyme hydrosol provided the highest (P<0.05) reduction on the bacterial population for all seeds. In case of mung bean, soaking in the thyme hydrosol reduced *S. aureus* count under the detection levels within 40 min. In terms of the antibacterial efficiency, thyme hydrosol was followed by summer savoury and bayleaf, respectively. Among the seeds, hydrosol soaking treatments exhibited the maximum inhibition on *S. aureus* for mung bean independently of treatment time.

In Table 2, *Salmonella* populations of wheat, lentil and mung bean seeds soaked in summer savoury, bayleaf and thyme hydrosols at different treatment times were indicated. Inoculation levels of the untreated wheat, lentil and mung bean were 4.25, 4.85 and 4.55 log cfu/g, respectively. Soaking treatments in the hydrosols enabled significant (P<0.05) reductions on *S.* Typhimurium populations within the first 20 min while water washing was ineffective at this time, as expected. Summer savoury and thyme hydrosols totally inhibited the bacteria that were inoculated to mung bean seeds in 20 min of the treatment. Extending of the treatment time to 60 min with thyme hydrosol also provided complete elimination of the population in the case of lentil and wheat. Although bayleaf soaking reduced significantly (P<0.05) *Salmonella* counts as compared to the control, the reduction level was lower (P<0.05) than those were provided by summer savoury and thyme for all the seed samples tested. In general, thyme hydrosol was the most

Table 1. Counts (log 10 cfu/g) of *Staphylococcus aureus* after soaking of wheat, lentil and mung bean with the plant hydrosols.[1,2]

Hydrosol type	Treatment time (min)			
Wheat	**0**	**20**	**40**	**60**
Control	4.86±0.35^{Aa}	4.52±0.19^{Aa}	4.47±0.31^{Aa}	4.48±0.40^{Aa}
Bayleaf	4.86±0.35^{Aa}	4.55±0.12^{BAa}	4.41±0.36^{Ba}	4.31±0.26^{Ba}
Summer savoury	4.86±0.35^{Aa}	4.33±0.25^{Ba}	4.25±0.33^{Ba}	3.68±0.20^{Cb}
Thyme	4.86±0.35^{Aa}	3.79±0.13^{Bb}	3.44±0.06^{Bb}	2.94±0.28^{Cc}
Lentil	**0**	**20**	**40**	**60**
Control	4.24±0.18^{Ba}	4.60±0.17^{Aa}	4.64±0.24^{Aa}	4.51±0.20^{BAa}
Bayleaf	4.24±0.18^{Aa}	3.85±0.17^{BAb}	3.60±0.35^{Bb}	3.09±0.10^{Cb}
Summer savoury	4.24±0.18^{Aa}	2.85±0.17^{Bd}	3.09±0.10^{Bc}	2.85±0.17^{Bc}
Thyme	4.24±0.18^{Aa}	3.09±0.12^{Bc}	2.94±0.34^{CBc}	2.70±0.00^{Cc}
Mung bean	**0**	**20**	**40**	**60**
Control	4.92±0.36^{Aa}	4.56±0.29^{BAa}	4.58±0.11^{BAa}	4.26±0.06^{Ba}
Bayleaf	4.92±0.36^{Aa}	4.55±0.26^{BAa}	4.23±0.05^{BCb}	3.96±0.13^{Cb}
Summer savoury	4.92±0.36^{Aa}	3.86±0.36^{Bb}	3.09±0.10^{Cc}	3.00±0.00^{Cc}
Thyme	4.92±0.36^{Aa}	2.70±0.00^{Bc}	<1.00^{Cd}	<1.00^{Cd}

[1] Results are mean ± standard deviation. cfu = colony forming units.

[2] Different superscript letters in the same column indicate significant difference (P<0.05) between the results; Different superscript capital letters in the same line indicate significant difference (P<0.05) between the results.

Table 2. Counts (log 10 cfu/g) of *Salmonella* Typhimurium after soaking of wheat, lentil and mung bean with the plant hydrosols.[1,2]

Hydrosol type	Treatment time (min)			
Wheat	**0**	**20**	**40**	**60**
Control	4.25±0.32^{Aa}	4.38±0.02^{Aa}	4.60±0.38^{Aa}	4.23±0.09^{Aa}
Bayleaf	4.25±0.32^{Aa}	3.94±0.31^{Aa}	4.06±0.35^{Ab}	3.85±0.00^{Ab}
Summer savoury	4.25±0.32^{Aa}	4.02±0.02^{Aa}	3.50±0.23^{Bc}	3.24±0.28^{Bc}
Thyme	4.25±0.32^{Aa}	2.85±0.17^{Bb}	2.70±0.00^{Bd}	<1.00^{Cd}
Lentil	**0**	**20**	**40**	**60**
Control	4.85±0.36^{Aa}	4.79±0.22^{Aa}	4.43±0.23^{Aa}	4.51±0.37^{Aa}
Bayleaf	4.85±0.36^{Aa}	4.48±0.27^{Aa}	4.48±0.37^{Aa}	4.42±0.27^{Aa}
Summer savoury	4.85±0.36^{Aa}	3.83±0.16^{Bb}	3.62±0.35^{Bb}	3.00±0.00^{Cb}
Thyme	4.85±0.36^{Aa}	3.87±0.15^{Bb}	3.24±0.07^{Cb}	<1.00^{Dc}
Mung bean	**0**	**20**	**40**	**60**
Control	4.55±0.17^{Aa}	4.39±0.39^{Ab}	4.29±0.36^{Aa}	4.54±0.34^{Aa}
Bayleaf	4.55±0.17^{Aa}	4.80±0.02^{Aa}	3.83±0.15^{Bb}	3.27±0.31^{Cb}
Summer savoury	4.55±0.17^{Aa}	<1.00^{Bc}	<1.00^{Bc}	<1.00^{Bc}
Thyme	4.55±0.17^{Aa}	<1.00^{Bc}	<1.00^{Bc}	<1.00^{Bc}

[1] Results are mean ± standard deviation. cfu = colony forming units.
[2] Different superscript letters in the same column indicate significant difference (P<0.05) between the results; Different superscript capital letters in the same line indicate significant difference (P<0.05) between the results.

efficient for *S.* Typhimurium inhibition, followed by summer savoury and bayleaf, respectively.

GIR(%) is the percent ratio between inhibition obtained by decontamination treatment and control treatment and gives clear idea about the decontamination efficiency of an antimicrobial agent. In this study, GIRs of *S. aureus* and *S.* Typhimurium that were inoculated to mung bean, wheat and lentil were calculated as affected by the hydrosol soaking treatments and they were exhibited in Figure 1. In general, it was obvious that thyme hydrosol caused the highest GIRs on both bacterial strains for all seeds, followed by summer savoury and bayleaf and GIRs increased depending on the longer treatment time with thyme hydrosol. Thyme hydrosol treatment for 40 and 20 min provided 100% inhibition for both *S. aureus* and *S.* Typhimurium on mung bean seeds, respectively (Figures 1C and 1F). 100% GIR caused by summer savoury was only observed at mung bean that was contaminated with *S.* Typhimurium.

4. Discussion

Plant hydrosols obtained from summer savoury, bayleaf and thyme were assessed for their sanitation efficacy on several seeds including lentil, wheat and mung bean that are commonly used as raw materials for sprout production in different regions of the world. Minimally processed ready-to-eat fresh fruits and vegetables such as seed sprouts are among the main vehicles for transmission of common foodborne pathogens. In several cases, *Salmonella* spp., *L. monocytogenes, S. aureus, Bacillus cereus* and *Aeromonas hydrophila* have been isolated from seed sprouts (Beuchat, 1996). At the same time, a number of outbreaks of foodborne illnesses associated with sprout consumption has been documented up to date (Buchholz *et al.*, 2011; King *et al.*, 2012; Mahon *et al.*, 1997; Michino *et al.*, 1999; Mohle-Boetani *et al.*, 2009; Slayton *et al.*, 2013).

Results have shown that in spite of the promotive conditions of sprouting process for microbial growth, seeds appeared to be the main source of microbial contamination in the outbreaks of infection resulted from the sprout consumption. Therefore, it is recommended that seeds should be sanitised just prior to sprouting with one or combined treatments that is effective to reduce/eliminate pathogens from the seeds (NACMCF, 1999). NACMCF (1999) also comments that minimum 5-log reduction in the levels of pathogens such as *Salmonella* and *E. coli* is required as a result of sanitation treatment. For this purpose, a number of sanitation treatments have been assessed for

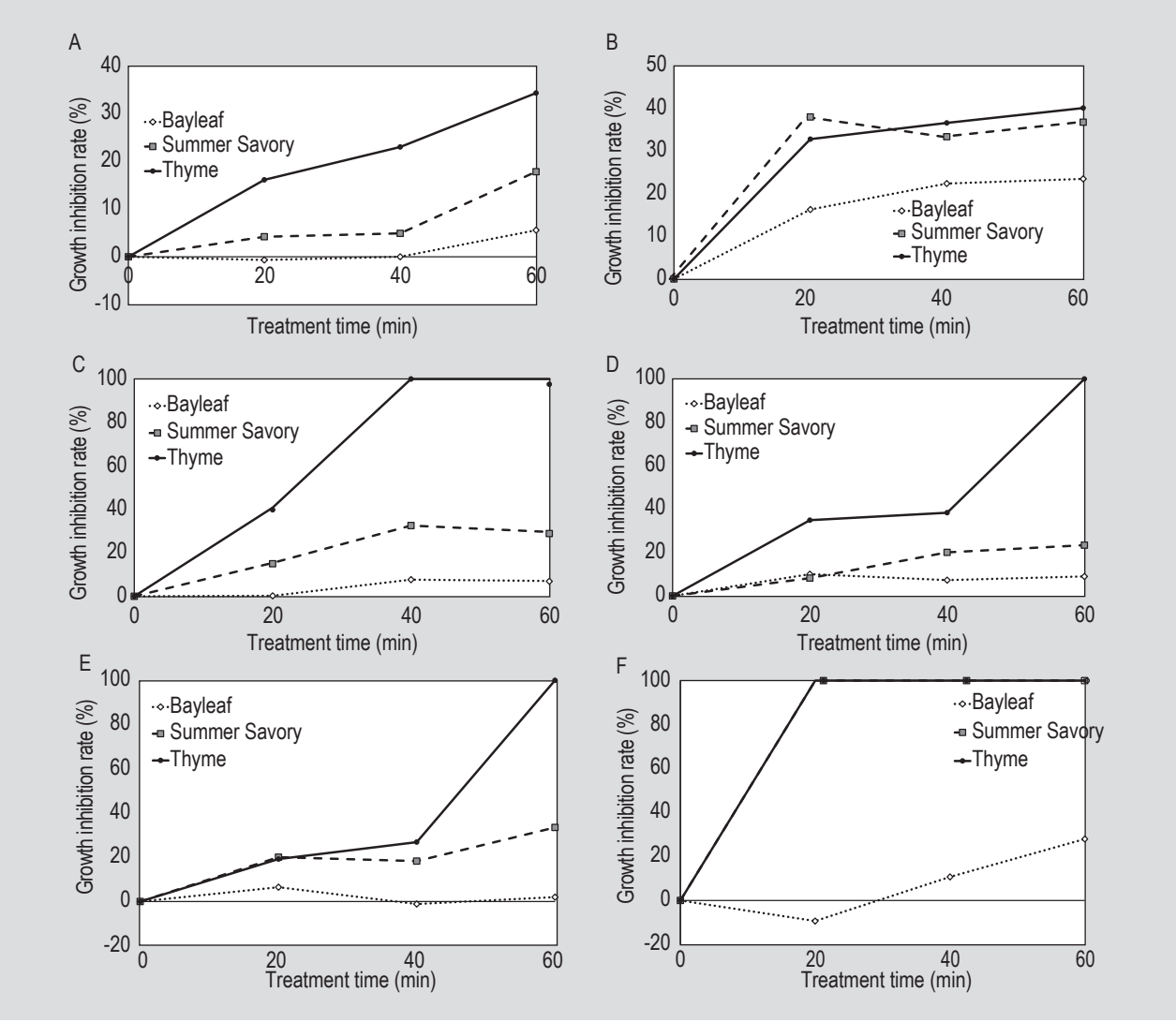

Figure 1. Growth inhibition rates (GIRS, %) of *Staphylococcus aureus* and *Salmonella* Typhimurium as affected by hydrosol soaking treatments. (A) GIR of *S. aureus* inoculated to wheat seed; (B) GIR of *S. aureus* inoculated to lentil seed; (C) GIR of *S. aureus* inoculated to mung bean seed; (D) GIR of *S.* Typhimurium inoculated to wheat seed; (E) GIR of *S.* Typhimurium inoculated to lentil seed; (E) GIR of *S.* Typhimurium inoculated to mung bean seed.

decontamination of seeds-sprouts and complete elimination or efficient reduction of pathogens has been achieved in several studies. Lang *et al.* (2000) investigated two-step organic acid-hypochlorite treatments for elimination of *E. coli* O157:H7 from artificially contaminated alfalfa seeds. Lactic acid (5% v/v) and acetic acid (5% v/v) treatments at 42 °C for 10 min reduced the population about 6 log, as analysed by using Sorbitol MacConkey (SMAC) Agar as medium while lower reduction levels were observed when plating with Brain Heart Infusion Agar, indicating the presence of injured cells that were unable to grow on SMAC Agar. Neetoo *et al.* (2009) reported that 600 MPa high pressure treatment at 40 °C for 2 min was adequate in eliminating a 5-log-unit *E. coli* O157:H7 population on the seeds with no adverse effect on seed viability. In that study, the process of 550 MPa for 2 min at 40 °C was found to be the most desirable, achieving favourable final germination percentages and sprout sizes. Choi *et al.* (2016) investigated the efficiency of ClO_2 (200 µg/ml, 5 min) or dry-heat (80 °C/ 23% relative humidity, 24 h) on germination ability of alfalfa, broccoli, kohlrabi, kyona, mustard, pak choi, red kohlrabi, red radish, red young radish, tatsoi, and violet radish seeds and on reduction of *E. coli* O157:H7 or *S. enterica* population inoculated to pak choki seeds. In the results, pak choi, red radish, and tatsoi seeds showed higher tolerance to ClO_2 and dry-heat treatments while sequential treatments of pak choi seeds with ClO_2, drying, and dry-heat are effective in reducing large numbers of *E. coli* O157:H7 and *S. enterica* without loss of seed sprouting ability. Electrolysed water has been suggested as an alternative method for decontamination of seeds and sprouts (Issa-Zacharia *et al.*, 2011; Kim *et*

al., 2003; Sharma and Demirci, 2003). In these studies, although significant reductions in native population and the counts of artificially contaminated pathogens were achieved, complete elimination did not occur. In addition, germination of the treated seeds were negatively influenced. Gaseous antimicrobial agents has been tested as alternatives for seed disinfection. Delaquis *et al.* (1999) reported that *S.* Typhimurium and *E. coli* O157:H7 that were inoculated to mung bean seeds were not detected by enrichment of seeds treated with 242 µl of acetic acid per litre of air for 12 h at 45 °C while *L. monocytogenes* was recovered by enrichment from two of 10-25 g seed samples treated in this manner. Application of 0.33 mg of ozone/g wheat/min was found to be effective for significant inactivation of fungal spores of wheat. However, 20, 30 or 45 min of ozonation reduced the wheat germination capability to 85.4, 80.0 and 61.3%, respectively (Wu *et al.*, 2006).

Considering the reports and literature up to date, a number of methods have been suggested for providing microbiological safety of seed sprouts. Decontamination of seeds before germination seems to be better than sprout treatment for safer sprouts. Sprouting conditions are also taken into consideration in order to minimise the microbial growth. Increasing humidity and temperature have been shown to encourage microbial growth during seed germination (Choi *et al.*, 2016; Tornuk *et al.*, 2011b). As seen in the literature, elimination of the pathogens has been successful by the treatments of seeds. In our study, we also achieved complete elimination of both *S. aureus* and *S.* Typhimurium by the treatment of seeds with thyme hydrosol with different treatment times. Hydrosols have a number of advantages that give them superiority as compared to other sanitation methods. For example: (1) they are not toxic and/or carcinogen like chlorine based compounds; (2) possibility of lack of germination of the seeds treated with hydrosols is low; and (3) they are cheap and easy to be produced.

5. Conclusions

In this study, hydrosols obtained from bayleaf, summer savoury and thyme were used for decontamination of mung bean, wheat and lentil seeds prior to germination in order to ensure the microbiological safety of the seed sprouts for human consumption. The seeds that were artificially inoculated with *S. aureus* and *S.* Typhimurium strains separately were soaked with the hydrosols up to 60 min. Thyme hydrosol treatment achieved complete elimination of *S. aureus* from mung bean and *Salmonella* from all the seeds. Summer savoury and bayleaf hydrosols also reduced the pathogen numbers significantly during the treatment. The antibacterial efficiency was in the order of thyme > summer savoury > bayleaf. In conclusion, this study confirmed that the plant hydrosols, especially thyme hydrosol could be used as a safe disinfectant for seeds to be used for sprout production before germination process.

References

Alvarez-Jubete, L., Wijngaard, H., Arendt, E.K. and Gallagher, E., 2010. Polyphenol composition and *in vitro* antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. Food Chemistry 119: 770-778.

Bari, M.L., Al-Haq, M.I., Kawasaki, T., Nakauma, M., Todoriki, S., Kawamoto, S. and Isshiki, K., 2004. Irradiation to kill *Escherichia coli* O157:H7 and *Salmonella* on ready-to-eat radish and mung bean sprouts. Journal of Food Protection 67: 2263-2268.

Bari, M.L., Nazuka, E., Sabina, Y., Todoriki, S. and Isshiki, K., 2003. Chemical and irradiation treatments for killing *Escherichia coli* O157:H7 on alfalfa, radish, and mung bean seeds. Journal of Food Protection 66: 767-774.

Beuchat, L.R., 1996. Pathogenic microorganisms associated with fresh produce. Journal of Food Protection 59: 204-216.

Breuer, T., Benkel, D.H., Shapiro, R.L., Hall, W.N., Winnett, M.M., Linn, M.J., Neimann, J., Barrett, T.J., Dietrich, S., Downes, F.P., Toney, D.M., Pearson, J.L., Rolka, H., Slutsker, L., Griffin, P.M. and Investigation Team, 2001. A multistate outbreak of *Escherichia coli* O157:H7 infections linked to alfalfa sprouts grown from contaminated seeds. Emerging Infectious Diseases 7: 977-982.

Buchholz, U., Bernard, H., Werber, D., Böhmer, M.M., Remschmidt, C., Wilking, H., Deleré, Y., Van der Heiden, M., Adlhoch, C., Dreesman, J., Ehlers, J., Ethelberg, S., Faber, M., Frank, C., Fricke, G., Greiner, M., Höhle, M., Ivarsson, S., Jark, U., Kirchner, M., Koch, J., Krause, G., Luber, P., Rosner, B., Stark, K. and Kühne, M., 2011. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. New England Journal of Medicine 365: 1763-1770.

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology 94: 223-253.

Calzuola, I., Luigi Gianfranceschi, G. and Marsili, V., 2006. Comparative activity of antioxidants from wheat sprouts, *Morinda citrifolia*, fermented papaya and white tea. International Journal of Food Sciences and Nutrition 57: 168-177.

Calzuola, I., Marsili, V. and Gianfranceschi, G.L., 2004. Synthesis of antioxidants in wheat sprouts. Journal of Agricultural and Food Chemistry 52: 5201-5206.

Choi, S., Beuchat, L.R., Kim, H. and Ryu, J.-H., 2016. Viability of sprout seeds as affected by treatment with aqueous chlorine dioxide and dry heat, and reduction of *Escherichia coli* O157:H7 and *Salmonella enterica* on pak choi seeds by sequential treatment with chlorine dioxide, drying, and dry heat. Food Microbiology 54: 127-132.

Delaquis, P.J., Sholberg, P.L. and Stanich, K., 1999. Disinfection of mung bean seed with gaseous acetic acid. Journal of Food Protection 62: 953-957.

Dhakal, K., Jeong, Y.-S., Lee, J.-D., Baek, I.-Y., Ha, T.-J. and Hwang, Y.-H., 2009. Fatty acid composition in each structural part of soybean seed and sprout. Journal of Crop Science and Biotechnology 12: 97-101.

Gharachorloo, M., Tarzi, B.G., Baharinia, M. and Hemaci, A.H., 2012. Antioxidant activity and phenolic content of germinated lentil (*Lens culinaris*). Journal of Medicinal Plants Research 6: 4562-4566.

Goni, M.G., Moreira, M.R., Viacava, G.E. and Roura, S.I., 2013. Optimization of chitosan treatments for managing microflora in lettuce seeds without affecting germination. Carbohydrate Polymers 92: 817-823.

Issa-Zacharia, A., Kamitani, Y., Miwa, N., Muhimbula, H. and Iwasaki, K., 2011. Application of slightly acidic electrolyzed water as a potential non-thermal food sanitizer for decontamination of fresh ready-to-eat vegetables and sprouts. Food Control 22: 601-607.

Jaquette, C.B., Beuchat, L.R. and Mahon, B.E., 1996. Efficacy of chlorine and heat treatment in killing *Salmonella stanley* inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage. Applied and Environmental Microbiology 62: 2212-2215.

Kim, C., Hung, Y.-C., Brackett, R.E. and Lin, C.-S., 2003. Efficacy of electrolyzed oxidizing water in inactivating *Salmonella* on alfalfa seeds and sprouts. Journal of Food Protection 66: 208-214.

King, L.A., Nogareda, F., Weill, F.-X., Mariani-Kurkdjian, P., Loukiadis, E., Gault, G., Jourdan-DaSilva, N., Bingen, E., Macé, M., Thevenot, D., Ong, N., Castor, C., Noël, H., Van Cauteren, D., Charron, M., Vaillant, V., Aldabe, B., Goulet, V., Delmas, G., Couturier, E., Le Strat, Y., Combe, C., Delmas, Y., Terrier, F., Vendrely, B., Rolland, P. and De Valk, H., 2012. Outbreak of Shiga toxin-producing *Escherichia coli* O104:H4 associated with organic fenugreek sprouts, France, June 2011. Clinical Infectious Diseases 54: 1588-1594.

Lang, M.M., Ingham, B.H. and Ingham, S.C., 2000. Efficacy of novel organic acid and hypochlorite treatments for eliminating *Escherichia coli* O157:H7 from alfalfa seeds prior to sprouting. International Journal of Food Microbiology 58: 73-82.

Mahon, B.E., Pönkä, A., Hall, W.N., Komatsu, K., Dietrich, S.E., Siitonen, A., Cage, G., Hayes, P.S., Lambert-Fair, M.A., Bean, N.H., Griffin, P.M. and Slutsker, L., 1997. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. Journal of Infectious Diseases 175: 876-882.

Michino, H., Araki, K., Minami, S., Takaya, S., Sakai, N., Miyazaki, M., Ono, A. and Yanagawa, H., 1999. Massive outbreak of *Escherichia coli* O157: H7 infection in schoolchildren in Sakai city, Japan, associated with consumption of white radish sprouts. American Journal of Epidemiology 150: 787-796.

Mohle-Boetani, J.C., Farrar, J., Bradley, P., Barak, J.D., Miller, M., Mandrell, R., Mead, P., Keene, W.E., Cummings, K., Abbott, S. and Werner, S.B., 2009. *Salmonella* infections associated with mung bean sprouts: Epidemiological and environmental investigations. Epidemiology and Infection 137: 357-366.

National Advisory Committee on Microbiological Criteria for Foods (NACMCF), 1999. Microbiological safety evaluations and recommendations on sprouted seeds. International Journal of Food Microbiology 52: 123-153.

Neetoo, H., Pizzolato, T. and Chen, H., 2009. Elimination of *Escherichia coli* O157:H7 from alfalfa seeds through a combination of high hydrostatic pressure and mild heat. Applied and Environmental Microbiology 75: 1901-1907.

Ozturk, I., Sagdic, O., Hayta, M. and Yetim, H., 2012a. Alteration in α-tocopherol, some minerals, and fatty acid contents of wheat through sprouting. Chemistry of Natural Compounds 47: 876-879.

Ozturk, I., Tornuk, F., Sagdic, O. and Kisi, O., 2012b. Application of non-linear models to predict inhibition effects of various plant hydrosols on *Listeria monocytogenes* inoculated on fresh-cut apples. Foodborne Pathogens and Disease 9: 607-616.

Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J. and Griffin, P.M., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. Emerging Infectious Diseases 19: 407-415.

Piernas, V. and Guiraud, J.P., 1997. Disinfection of rice seeds prior to sprouting. Journal of Food Science 62: 611-615.

Plaza, L., De Ancos, B. and Cano, P.M., 2003. Nutritional and health-related compounds in sprouts and seeds of soybean (*Glycine max*), wheat (*Triticum aestivum*.L) and alfalfa (*Medicago sativa*) treated by a new drying method. European Food Research and Technology 216: 138-144.

Sagdic, O. and Ozcan, M., 2003. Antibacterial activity of Turkish spice hydrosols. Food Control 14: 141-143.

Sagdic, O., Ozturk, I. and Tornuk, F., 2013. Inactivation of non-toxigenic and toxigenic *Escherichia coli* O157:H7 inoculated on minimally processed tomatoes and cucumbers: Utilization of hydrosols of *Lamiaceae* spices as natural food sanitizers. Food Control 30: 7-14.

Sağdıç, O., 2003. Sensitivity of four pathogenic bacteria to Turkish thyme and oregano hydrosols. LWT – Food Science and Technology 36: 467-473.

Sharma, R.R. and Demirci, A., 2003. Treatment of *Escherichia coli* O157:H7 inoculated alfalfa seeds and sprouts with electrolyzed oxidizing water. International Journal of Food Microbiology 86: 231-237.

Singh, N., Singh, R.K. and Bhunia, A.K., 2003. Sequential disinfection of *Escherichia coli* O157:H7 inoculated alfalfa seeds before and during sprouting using aqueous chlorine dioxide, ozonated water, and thyme essential oil. LWT – Food Science and Technology 36: 235-243.

Sivapalasingam, S., Friedman, C.R., Cohen, L. and Tauxe, R.V., 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. Journal of Food Protection 67: 2342-2353.

Slayton, R.B., Turabelidze, G., Bennett, S.D., Schwensohn, C.A., Yaffee, A.Q., Khan, F., Butler, C., Trees, E., Ayers, T.L., Davis, M.L., Laufer, A.S., Gladbach, S., Williams, I. and Gieraltowski, L.B., 2013. Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 associated with romaine lettuce consumption, 2011. PLoS ONE 8: e55300.

Stewart, D., Reineke, K., Ulaszek, J., Fu, T. and Tortorello, M., 2001. Growth of *Escherichia coli* O157:H7 during sprouting of alfalfa seeds. Letters in Applied Microbiology 33: 95-99.

Taormina, P.J., Beuchat, L.R. and Slutsker, L., 1999. Infections associated with eating seed sprouts: an international concern. Emerging Infectious Diseases 5: 626-634.

Törnük, F. and Dertli, E., 2015. Decontamination of *Escherichia coli* O157:H7 and *Staphylococcus aureus* from fresh-cut parsley with natural plant hydrosols. Journal of Food Processing and Preservation 39: 1587-1594.

Tornuk, F., Cankurt, H., Ozturk, I., Sagdic, O., Bayram, O. and Yetim, H., 2011a. Efficacy of various plant hydrosols as natural food sanitizers in reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on fresh cut carrots and apples. International Journal of Food Microbiology 148: 30-35.

Tornuk, F., Ozturk, I., Sagdic, O. and Yetim, H., 2011b. Determination and improvement of microbial safety of wheat sprouts with chemical sanitizers. Foodborne Pathogens and Disease 8: 503-508.

Tornuk, F., Ozturk, I., Sagdic, O., Yilmaz, A. and Erkmen, O., 2014. Application of predictive inactivation models to evaluate survival of *Staphylococcus aureus* in fresh-cut apples treated with different plant hydrosols. International Journal of Food Properties 17: 587-598.

Waje, C. and Kwon, J.-H., 2007. Improving the food safety of seed sprouts through irradiation treatments. Food Science and Biotechnology 16: 171-176.

Wu, J., Doan, H. and Cuenca, M.A., 2006. Investigation of gaseous ozone as an anti-fungal fumigant for stored wheat. Journal of Chemical Technology and Biotechnology 81: 1288-1293.

Wuytack, E.Y., Diels, A.M.J., Meersseman, K. and Michiels, C.W., 2003. Decontamination of seeds for seed sprout production by high hydrostatic pressure. Journal of Food Protection 66: 918-923.