Journal of Food Science and Agricultural Technology (2019) 5 (Spcl. Iss.): 220-222

Journal of Food Science and Agricultural Technology

International peer-reviewed scientific online journal

Published online: http://rs.mfu.ac.th/ojs/index.php/jfat

Original Research Article

Efficacy of organic acids and hot water treatment to inactivate *E. coli* inoculated on sunflower sprouts

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ARTICLE INFO

Article history:

Received 31 July 2018

Received in revised form 31 December 2018

Accepted 08 January 2019

Keywords:

Sunflower sprouts

Organic acids

E. coli

Hot water

ABSTRACT

In Thailand, the consumption of sunflower sprouts has increased due to their nutritional value. However, there have been the outbreaks of foodborne illness caused by sprouts contaminated with enteric pathogens. The objective of this research was to evaluate the efficiency of lactic acid (1%, 2.5% and 5%(v/v)), acetic acid (1%, 2.5% and 5%(v/v)) and hot water (60°C, 70°C for 20s, 180s) on reduction of E. coli which is artificially inoculated on sunflower sprouts. The results showed that more than 2.4 log CFU/g reduction of *E. coli* was achieved when sprouts were treated with 5% lactic acid, followed by the hot water treatment at 70°C for 180s and 5% acetic acid. However, the hot water treatments also caused detrimental impact on the color and firmness of sunflower sprouts. This study suggests the method to decontaminate the pathogen on sunflower sprouts.

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Published by School of Agro-Industry, Mae Fah Luang University

ISSN: 2408-1736

INTRODUCTION

Nowadays, sunflower sprouts are popular oriental foods and their consumption has been growing in Thailand. They are a good and inexpensive source of healthy fats, fiber, protein and iron. Normally, sunflower sprouts are often consumed raw or lightly cooked so they are at risk for being a vehicles of transmission in outbreaks of foodborne illness. Pathogen microorganisms such as *Salmonella* Thyphimurium, *E. coli* O157:H7 and *Listeria monocytogenes* have been reported to contaminate in seed sprouts (Kim et al., 2009; Neo et al., 2013; Lee et al., 2018). Several methods have been evaluated for decontamination of sprouts such as chemical disinfectants (hydrogen peroxide, hypochorite, chlorine dioxide, calcium hydrogen peroxide, ethanol and ozone) and physical treatments (heat and irradiation) (Zhang et al., 2011). Currently, chlorine is widely used in food processing plants to remove dirt and contamination, but this sanitation procedure can only achieve <2 log reduction in the population of *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes* (Kim et al., 2009). Moreover, consumers are concerned about the safety of artificial chemical that are used to control these foodborne pathogen. Therefore, the objective of this research was to compare the efficacy of organic acid and hot water treatment for reducing the *E. coli* on sunflower sprouts.

MATERIALS AND METHODS

Bacterial cultures and preparation of inocula

E. coli obtaining from the department of food technology and nutrition, faculty of natural resources and agro-industry, Kasetsart university was cultured in 10ml tryptic soy broth (TSB) with 0.6% yeast extract at 37°C for 24h, harvested by centrifugation at 3,000 x g for 10 min and washed three times with 0.1% peptone water. Bacterial counts were obtained by plating 0.1 ml of a 10-fold serial dilution on tryptic soy agar (TSA) with 0.6% yeast extract at 37°C for 24h. The population of *E. coli* was approximately 7-8 log CFU/ml.

Inoculation of E. coli on sunflower sprouts

Sunflower sprouts were purchased from a local market and unwashed sprouts were stored at 4°C during experiments within 2 days. Prior to inoculation, the sprouts were removed from the refrigerator to room temperature for 45 min. Approximately 400 g of sprouts was submerged in 2L of *E. coli* (ca. 6 log CFU/ml) suspension for 45 min with gentle stirred of magnetic stirrer. The inoculated sprouts were dried on sterile plate in a laminar flow for 4 h.

Chemical sanitizers and hot water treatments

The sunflower sprouts were weighed and divided into sets of 100g. A 100g of sunflower sprouts was transferred into 1L of lactic acid and acetic acid (1, 2.5 and 5% (v/v)) and hot water (60°C, 70°C for 20, 180s) at room temperature with gentle stirring for 5 min. The sprouts without sanitizer were served as control.

Microbiological analysis

After treatments with sanitizers and hot water, a 10g of sunflower sprouts transferred into sterile stomacher bags and added with 90 ml of 0.1% peptone water. The sunflower sprouts were homogenate using a stomacher for 1 min. The homogenate was pour plate with tryptic soy agar (TSA) containing 0.6% yeast extract. The plates were incubated at 37°C for 24h. Microbial counts are expressed as the log CFU/g.

Physical quality during storage

Quality assessment was performed on the sunflower sprouts subjected to hot water treatment. The quality assessment was performed by measuring the color and the texture for 4 days at 4°C and room temperature of storage. Sunflower sprouts without treatment were set as a control. The color of the sunflower sprouts was determined by L* values with Hunter Lab (Mini scan XE plus, USA) (Phua et al., 2014). The firmness was measured using a texture analyzer (Stable Micro System, USA) with the force required for the probe (25 mm in diameter) of the texture analyzer to compress the samples by a distance of 1 mm. The sample of sprouts (8g) was pressed under a flat surface metal cylinder until it reached a flat slab and compressed twice at the constant compression rate of 60 mm/ min for 7 min (D'ambrosio et al., 2017).

Statistical analysis

All experiments were conducted in replicate at least 3 times. The data were expressed as the mean values and standard deviations. Duncan 's multiple range tests were performed to analyze the data using the SPSS program.

RESULTS AND DISCUSSION

Effect of organic acid and hot water treatment on the survival of *E. coli* inoculated on sunflower sprouts

The Initial population of *E. coli* on sunflower sprouts was approximately 6 log CFU/g. The sunflower sprouts were treated with organic acid and hot water to inactivate *E. coli* (Table 1). It was found that 5% acetic acid gave the highest reduction of 2.4 log CFU/g followed by the hot water at 70°C for 180s and 60°C for 180s, respectively. However, there was no significant difference (P>0.05). Acetic acid (1%) treatment was the least effective method with a reduction of only 0.31 log CFU/g. In general, texture of sprouts may be concerned after heat treatment. Thus, hot water treatment was study for the treatment influences physical of sunflower sprouts during storage. Mode of action of organic acids are dependent upon their pH and Pka value. Only in its undissociated form an organic acid crosses the cell wall of bacteria and perform its antibacterial activity. The low pH suppresses the enzymatic reaction in the cell, which consequently decreases the intracellular pH, and ATP-dependent H+ ion pump or other pH control system. These are effect to interfere the nutrient transport, membrane leakage and distruption of membrane permeability (Huang and Chen, 2011; Inatsu et al., 2017).

Table 1. Effect of organic acid and hot water treatment on the survival of *E. coli* inoculated on sunflower sprouts.

Treatment	Survival cells (log CFU/g)	Reduction (log CFU/g)
Control	6.9 ± 0.07^{a}	0.04 ± 0.07^{a}
Hot water		
60°C, 20s	5.07 ± 0.03^{g}	1.87 ± 0.03^{g}
60°C, 180s	4.96 ± 0.04^{gh}	1.98 ± 0.04^{gh}
70°C, 20s	4.78 ± 0.02^{hi}	2.16 ± 0.02^{hi}
70°C, 180s	4.74 ± 0.06^{i}	2.20 ± 0.06^{i}
Lactic acid		
1%	6.40 ± 0.02^{c}	0.54 ± 0.02^{c}
2.5%	6.06 ± 0.12^{d}	0.88 ± 0.12^{d}
5%	4.54 ± 0.22^{i}	2.40 ± 0.22^{i}
Acetic acid		
1%	6.63 ± 0.14^{b}	0.31 ± 0.14^{b}
2.5%	5.81 ± 0.08^{e}	1.13 ± 0.08^{e}
5%	5.58 ± 0.05^{f}	1.36 ± 0.05^{f}

Values not followed by the same letter are significantly different (p<0.05, n=3).

Effect of hot water treatment on physical properties of sunflower sprouts during storage

Changes in color of untreated and hot water treated sunflower sprouts was measured using L* values for 4 days at 4°C and room temperature. Since sunflower sprouts are white in color, their brightness may change if they are physically injured by the treatment (Usall et al., 2016).

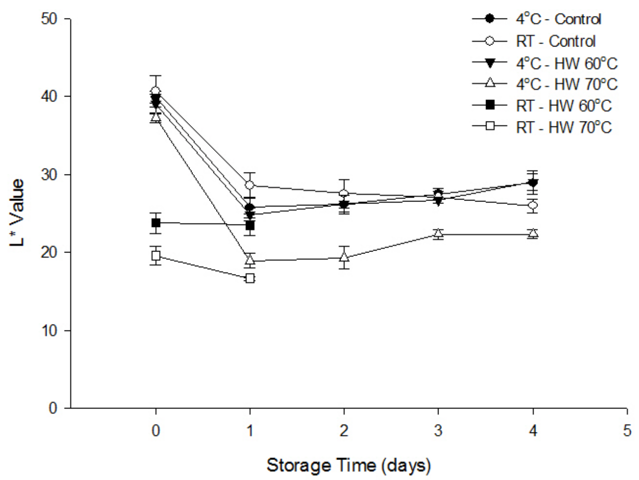

Figure 1. Changes in the L* value of hot water treated sunflower sprouts during storage for 4 days at 4°C and room temperature.

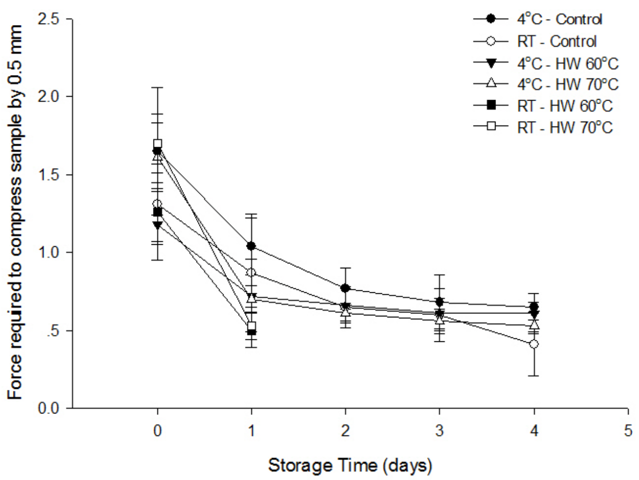

Figure 2. Changes in the firmness of hot water treated sunflower sprouts during storage for 4 days at 4°C and room temperature.

At room temperature, L* values of the treated sprouts dropped significantly on day 0 and continued to decrease at day 2. While the L* values of untreated control continued to decrease for the subsequent 4 days (Figure 1). In the case of firmness, the firmness of treated sunflower sprouts during storage at 25°C dramatically decreased on day 1 and could not analysis because the sprouts were fragile (Figure 2). In this study, hot water (70°C, 20s) was found to be the most effective method for the inactivation of the inoculated *E. coli*. Hot water treatment did have significant undesirable impact on the color and firmness of the sunflower sprouts after treatment or during storage that exhibited the treated sunflower sprouts had a shorter shelf life than untreated control. It may also suffer physical damage during the hot water treatment. The sprouts are fragile and delicate in nature. Also, the research indicates that 70°C of hot water was too high to retain physical quality of sunflower sprouts.

CONCLUSIONS

Organic acid and hot water treatments have the effective to reduce *E. coli* on sunflower sprouts. However, lactic acid and hot water treatments were most effective decontamination method to reduce *E. coli*. Hot water, on the other hand, had significant impacts on the color and firmness of the sunflower sprouts, affecting to the marketing and consumer acceptable of the product.

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