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Ultrasonication-enhanced microbial safety of sprouts produced from selected crop species

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Summary

Seed sprouts are susceptible to microbial contamination; therefore, use of an effective sanitization treatment on these products is of great importance. This study investigated the effects of ultrasonication, heating and chemical washing on sanitization of nine crop seeds and the microbial safety of sprouts produced from decontaminated seeds. Most of the sanitization treatments improved seed germination (except dry heating), reduced microbial loads on produced sprouts, and increased sprouts yield. However, only ultrasonication treatment reduced both the total aerobic counts and total coliforms counts to < 3 log $_{10}$ CFU g^{-1} level on produced alfalfa, mung bean pea and radish sprouts. The sprouts produced from ultrasonication-treated alfalfa, mung bean, pea and radish seeds also outyielded their respective non-treated sprouts by 88, 25, 73 and 56 %. Therefore, ultrasonication can be used as a seed sanitization treatment for producing alfalfa, mung bean, pea and radish sprouts.

Introduction

There has been an increasing interest in consuming various seed sprouts worldwide because of their high nutrient value and widespread availability. However, consumption of fresh and uncooked sprouts might impose a safety risk, because of their potential for microbial growth during the sprouting process. Among the sprouts available in the market, alfalfa and mung bean sprouts are most often implicated in outbreaks of food-borne diseases (CERNA-CORTES et al., 2013; TABAN et al., 2013). The source of microbial contamination on sprouts is mainly from the micro-biological load of the seeds. The sprouting process also provides suitable conditions for propagation of microorganisms if they are present in the seeds. Therefore, sanitization procedures for reducing the possible contamination of pathogenic microbes are generally performed on the selected seeds prior to sprouting (PEÑAS et al., 2008).

Many chemicals, such as aqueous ClO_2, ozone and organic acids, have been studied for their efficacies of reducing microbial loads in seed sprouts (KIM and SONG, 2009; SCOUTEN and BEUCHAT, 2002; SIKIN et al., 2013). However, the application of chemical disinfectants in food processing is always concomitant with health concerns. Heating treatment is also applied extensively to inactivate microbes (RAJKOVIC et al., 2010; SIKIN et al., 2013). But the efficacy of heating treatment depends on the treatment temperature and duration, and it often leads to the loss of nutrients, development of undesirable colors and deterioration of organoleptic properties of the treated food (OMS-OLIU et al., 2012). The germination of heat-treated seeds is also occasionally decreased to an unacceptable level (BARI et al., 2009; CHIU and SUNG, 2014).

Ultrasonication has long been used in many industrial sectors as an effective tool for surface cleaning (FARMER et al., 2000). It has also been used as an alternative to heat processing in the food industry (AWAD et al., 2012; DE SÃO JOSÉ et al., 2014). However, only limited research data are currently available on the efficacy of using ultrasonication to decontaminate the seeds (YALDAGARD et al., 2008). The ultrasonication treatment was reported to reduce the level of microbial load on alfalfa and broccoli seeds to some extent, but the germination percentages of treated seeds were decreased to below 85 % (KIM et al., 2006). On the other hand, ultrasonication treatment was reported to have satisfactory results for reducing the microbial loads of pea seeds and improving their germination percentage and sprout growth (CHIU and SUNG, 2014). It appears that the efficacy of ultrasonication on seed sanitization is species-dependent.

The objective of this study was to investigate the effects of ultrasonication on the sanitization and germination of seeds of alfalfa (*Medicago sativa*), mung bean (*Vigna radiate* (L.) R. Wilczek), adzuki bean (*Vigna angularis*), pea (*Pisum sativum* L.), soybean (*Glycine max* (L.) Merr.), peanut (*Arachis hypogaea* L.), radish (*Raphanus sativus* L.), purple cabbage (*Brassica oleracea* capitata f. rubra) and cauliflower (*Brassica oleracea* botyritis) (*Medicago sativa*). Several other sanitization treatments, including heating and chemical sanitizer, were also tested and compared with ultrasonication. The effects of these seed treatments for inactivating microbes on the subsequently produced sprouts were also examined. Knowledge of these variations would help identify the potential value of ultrasonication on safety control of produced sprouts.

Materials and methods

Seed materials

The commercially-produced seeds of alfalfa, mung bean, adzuki bean, pea, soybean, peanut, radish, purple cabbage and cauliflower were purchased from a local market and stored at 4 °C until treatments were applied.

Seed sanitization treatments

For aqueous chemical treatments, four chemical solutions were prepared and evaluated for their effectiveness in inactivating microbes on the tested crop seeds: NaOCl (1 g L^{-1}), vinegar (350 mL L^{-1}), ethanol (350 mL L^{-1}), NaCl (10 g L^{-1}). The experiments were performed by dispersing 20 g of seed samples in 200 mL of respective chemical solutions for 15 min at 25 °C. For heating treatments, the seed samples of each variety were soaked in a 55 °C hot water bath (wet heating) for 15 min or in a 70 °C oven (dry heating) for 72 h. The ultrasonication treatment was performed by dispersing 20 g of seed samples in 200 mL of double-distilled water in an ultrasonic bath (4HT-1014-6, Crest Ultrasonics, Trenton, NJ, USA) without mechanical agitation while applying a power input of 40 kHz for 1 min at 25 °C. The water in the ultrasonic tank was degassed for 40 min prior to the treatment. A sub-sample of non-treated seeds was prepared by soaking the seeds in double-distilled water at 25 °C for 15 min, and was used as a control.

Seed germination and sprout production

Fifty seeds (three replicates) subjected to various decontamination treatment were germinated. The treated seeds were placed on two pieces of moistened filter paper in a sterilized plastic tray. The plastic trays were sprinkled with distilled water every 6 h. Seeds were

germinated in a growth chamber (ES4-1S, Saint Tien Co. LTD, Kaoshiung city, Taiwan) in the dark at 20 °C for 6 days. Germination was counted daily for 6 days; with seeds recorded as germinated when radicles were protruding and visible (2 mm in length), and mean germination time (MGT) was calculated (CHIU et al., 2006). A sub-sample of the treated seeds (10 g) from each sanitization treatment was also germinated under the same conditions for 6 days (the growth period generally used for commercial-scale production of seed sprouts in Taiwan), and the growing sprouts were collected for hypocotyl and radicle lengths and sprout fresh weight measurements.

Microbial analyses

Microbial analyses were performed on the seeds that have received sanitization treatments and on the sprouts produced from the treated seeds. In each test, the treated seeds or their sprouts (25 g) were added to 225 mL of 8.5 g L^{-1} NaCl solution, then the solution was homogenized and diluted (a six-fold dilution) with distilled water. Resultant solution was surface-deposited on a Sanita-kun cultural medium sheet (Chisso Corporation, Tokyo, Japan) containing a transparent cover film, an adhesive sheet, a layer of nonwoven fabric and a water-soluble compound film designed for detection of aerobic microorganisms (MORITA et al., 2003). The sheets were kept in a temperature-controlled incubator according to the manufacturer's instructions for counting of total aerobes (the compound film containing a red color chromogen generated from 2,3,5-triphenyltetrazolium chloride by microbial metabolism), total coliform (the compound film containing an indigo blue color chromogen generated from β-glucuronidase of *E. coli*) or total mould (the compound film containing a blue or indigo blue chromogen color generated from esterase of mould). All the sheets were incubated at 35 °C for 48 h, 35 °C for 24 h and 25 °C for 5 days for total aerobes, *E. coli* and mold counting, respectively. Microbial counts were expressed in colony-forming units per g fresh weight ((log $_{10}$ CFU g^{-1} fresh weight).

Experimental design

A randomized complete block design with three replicates was used to evaluate the effects decontamination on the tested seeds. The results were analyzed using the Statistical Analysis System (SAS Institute, Raleigh, N.C., U.S.A.) for two-way analyses of variance (ANOVA) to determine differences in the germination, sprout growth and microbial parameters among the tested crop seeds that have been subjected to each treatment. Differences among treatments were studied with Duncan's multiple range test and differences at $p < 0.05$ were considered to be significant.

Results and discussion

Effects of seed sanitization treatments on microbial loads of tested crop seeds

Seeds usually contain high microbial loads, ranging from 3 to 6 log $_{10}$ CFU g^{-1} (PEÑAS et al., 2009). In this study, the initial total aerobic counts determined from the non-treated control seeds of tested crop species, prior to sprouting, ranged from 1.82 (adzuki bean seeds) to 4.71 (radish seeds) log $_{10}$ CFU g^{-1} fresh weight (Tab. 1). The seeds of radish, cauliflower and purple cabbage of Brassicaceae family had higher total aerobic counts than alfalfa, adzuki bean, mung bean, pea, peanut and soybean seeds of family Fabaceae. It appears that the pod wall tissues of fruit pod presented in Fabaceae species may act as a physical barrier, which can affect the infection and propagation of microorganisms during seed development. The total aerobic count values obtained from the examined alfalfa, mung bean, soybean and radish seeds are lower than the values of the alfalfa seeds (4.01 log $_{10}$ CFU g^{-1}) reported by SOYLEMEZ et al. (2001), the mung bean seeds (4.4 log $_{10}$ CFU g^{-1}) reported by GABRIEL (2005), the soybean seeds (4.6 log $_{10}$ CFU g^{-1}) reported by MOLINOS et al. (2009) and the radish seeds (6.3 log $_{10}$ CFU g^{-1}) reported by BANG et al. (2011). The non-treated control seeds also exhibited various levels of total coliforms counts (1.61 - 3.76 log $_{10}$ CFU g^{-1} fresh weight) and total mould counts (1.69 - 3.58 log $_{10}$ CFU g^{-1} fresh weight). The observed microbial contaminations on the tested crop seeds were expected because the selected seeds were obtained from the plants grown on open fields without special measures.

As shown in Tab. 1, most of the sanitization treatments were able to substantially reduce the microbial loads on the selected crop seeds. From the decontaminated seeds the measured total aerobic bacterial counts, total coliforms counts and total mould counts ranged between 0.53 and 3.82, between 0.17 and 3.32, and between 0.36 and 3.18 log $_{10}$ CFU g^{-1} fresh weights, respectively. The lowest average of total aerobic count (0.89 log $_{10}$ CFU g^{-1} fresh weight) across all the tested seeds was obtained from dry heating-treated seeds, followed by ethanol-treated (1.25 log $_{10}$ CFU g^{-1} fresh weight) and ultrasonication-treated (1.93 log $_{10}$ CFU g^{-1} fresh weight) seeds (Tab. 1). The dry heating also effectively reduced the total coliforms counts and total mould counts to the lowest levels (averages of 0.58 and 0.65 log $_{10}$ CFU g^{-1} fresh weights across all the examined species, respectively) comparing to other sanitization treatments. These results suggest that the dry heating is the most effective seed sanitization approach comparing to other treatments. However, the ultrasonication treatment also reduced the total aerobic counts, total coliforms counts and total mould counts to 1.93, 1.55 and 1.51 log $_{10}$ CFU g^{-1} (average of all the tested crop species) on the treated seeds. These values were significantly lower than the data obtained from the non-treated control seeds (Tab. 1).

Effects of seed sanitization treatments on germination of tested crop seeds

The effects of seed sanitization treatments on the germination responses of tested crop seeds are shown in Tab. 2. Among the non-treated control seeds, the germination percentages ranged from 30.80 to 100 %, with an average of 75.47 % across all the tested species. The seeds of alfalfa, mung bean, pea, peanut, soybean, radish and cauliflower generally had an acceptable germination percentage. However, extremely lower germination percentages were obtained from the purple cabbage (30.80 %) and adzuki bean seeds (34.47 %) (Tab. 1). These results suggest that some germination enhancement treatments for these two crop seeds should be taken prior to producing their sprouts (CHIU et al., 2006).

The germination percentages of de-contaminated seeds ranged between 6.57 and 100 % (Tab. 2). Most of the sanitization treatments (except dry heating) improved seed germination, among which the ultrasonication treatment raised the germination percentage of tested seeds to 93.56 % (the average of all the tested crop species) (Tab. 2). Both NaOCl and ethanol treatments also elevated the germination percentage of tested crop species to 89.97 and 87.82 %, respectively. It appears that the sanitization treatments (i.e., ultrasonication, chemical washings and wet heating) may suppress the incidence of the seed-borne microorganisms (Tab. 1) that could damage the growing embryos during seed germination (NWACHUKWU and UMECHURUBA, 2001). On the other hand, the lowest germination percentage was obtained from dry heating treated-seeds, with average of 61.1 % germination across all the tested crop species, with the poorest germination percentage of 6.5 % obtained from adzuki bean seeds (Tab. 2). This result reconfirms that the dry heating is in some cases (except the radish and cauliflower seeds) harmful to the viability of treated seeds (BARI et al., 2009), even though it is an effective seed sanitization treatment (Tab. 1).

Tab. 1: Effects of seed decontamination treatments on the microbial loads of various tested crop seeds.

	Alfalfa	Mung bean	Adzuki Bean	Pea	Soybean	Peanut	Radish	Purple cabbage	Cauliflower	Mean
	Total aerobic bacterial count (log $_{10}$ CFU g^{-1} fresh weight)									
Control	3.91 ± 0.21^{a}	2.77 ± 0.17^{a}	1.82 ± 0.12^{a}	2.67 ± 0.17^{a}	3.28 ± 0.21^{a}	3.32 ± 0.21^{a}	4.71 ± 0.29^{a}	4.58 ± 0.29^{a}	4.43 ± 0.28^{a}	3.56 ± 0.97^{a}
NaOCl (1 g/L)	2.91 ± 0.24^{b}	2.69 ± 0.15^{a}	1.73 ± 0.11^{a}	1.72 ± 0.11^{b}	1.98 ± 0.12^{c}	2.15 ± 0.13^{c}	3.32 ± 0.21^{bc}	3.65 ± 0.23^{b}	3.82 ± 0.24^{b}	2.70 ± 0.80^{b}
Wet heating (55 °C)	2.57 ± 0.21^{c}	2.05 ± 0.08^{c}	1.30 ± 0.04^{b}	1.33 ± 0.05^{c}	2.65 ± 0.11^{b}	2.57 ± 0.11^{b}	3.07 ± 0.13^{c}	2.68 ± 0.11^{c}	2.32 ± 0.11^{d}	2.26 ± 0.57^{c}
Dry heating (70 °C)	0.87 ± 0.08^{e}	0.66 ± 0.03^{e}	0.81 ± 0.06^{d}	0.84 ± 0.04^{d}	1.08 ± 0.05^{e}	0.96 ± 0.04^{f}	0.98 ± 0.04^{f}	0.83 ± 0.04^{f}	0.92 ± 0.05^{f}	0.89 ± 0.12^{e}
Vinegar (350 mL/L)	1.59 ± 0.13^{d}	1.26 ± 0.07^{d}	0.76 ± 0.04^{d}	1.22 ± 0.06^{c}	1.62 ± 0.09^{d}	1.62 ± 0.09^{d}	1.92 ± 0.10^{e}	1.78 ± 0.09^{e}	1.62 ± 0.09^{e}	1.48 ± 0.34^{d}
Ethanol (350 mL/L)	1.32 ± 0.11^{d}	0.91 ± 0.05^{e}	0.53 ± 0.03^{e}	1.21 ± 0.06^{c}	1.19 ± 0.06^{e}	1.32 ± 0.07^{e}	1.78 ± 0.09^{e}	1.62 ± 0.09^{e}	1.44 ± 0.08^{e}	1.25 ± 0.36^{d}
NaCl (10 g/L)	3.62 ± 0.18^{a}	2.44 ± 0.12^{b}	1.11 ± 0.07^{c}	1.71 ± 0.11^{b}	2.65 ± 0.17^{b}	2.32 ± 0.15^{bc}	3.65 ± 0.24^{b}	3.82 ± 0.25^{b}	3.21 ± 0.21^{c}	2.76 ± 0.92^{b}
Ultrasonication	2.54 ± 0.22^{c}	1.81 ± 0.11^{c}	1.22 ± 0.06^{bc}	1.32 ± 0.07^{c}	2.07 ± 0.11^{c}	1.88 ± 0.10^{d}	2.34 ± 0.13^{d}	2.28 ± 0.12^{d}	2.12 ± 0.13^{d}	1.93 ± 0.44^{c}
	Total coliforms count (log $_{10}$ CFU g^{-1} fresh weight)									
Control	3.13 ± 0.16^{a}	1.66 ± 0.10^{b}	1.61 ± 0.10^{a}	2.19 ± 0.14^{a}	3.76 ± 0.13^{a}	2.71 ± 0.17^{a}	2.79 ± 0.15^{a}	2.33 ± 0.14^{a}	2.36 ± 0.13^{a}	2.55 ± 0.68^{a}
NaOCl (1 g/L)	2.66 ± 0.22^{b}	1.31 ± 0.08^{c}	1.48 ± 0.09^{b}	1.67 ± 0.10^{b}	3.32 ± 0.27^{b}	2.03 ± 0.13^{bc}	1.66 ± 0.10^{bc}	1.86 ± 0.12^{c}	2.01 ± 0.14^{b}	2.14 ± 0.89^{b}
Wet heating (55 °C)	2.48 ± 0.20^{c}	1.87 ± 0.08^{a}	1.04 ± 0.04^{c}	0.83 ± 0.03^{d}	1.33 ± 0.05^{d}	1.92 ± 0.08^{c}	1.91 ± 0.08^{b}	2.12 ± 0.09^{b}	2.12 ± 0.09^{b}	1.72 ± 0.51^{c}
Dry heating (70 °C)	0.69 ± 0.06^{d}	0.49 ± 0.02^{g}	0.21 ± 0.01^{g}	0.17 ± 0.02^{f}	0.85 ± 0.04^{e}	0.41 ± 0.02^{e}	0.46 ± 0.01^{d}	0.54 ± 0.02^{f}	0.87 ± 0.04^{e}	0.58 ± 0.21^{e}
Vinegar (350 mL/L)	0.95 ± 0.08^{d}	0.71 ± 0.04^{e}	0.61 ± 0.03^{e}	0.84 ± 0.04^{d}	1.41 ± 0.07^{d}	1.34 ± 0.07^{d}	1.64 ± 0.09^{c}	1.41 ± 0.07^{d}	1.41 ± 0.07^{d}	1.14 ± 0.36^{d}
Ethanol (350 mL/L)	0.61 ± 0.04^{d}	0.55 ± 0.03^{fg}	0.48 ± 0.03^{f}	0.61 ± 0.03^{e}	1.17 ± 0.06^{e}	0.44 ± 0.02^{e}	0.46 ± 0.01^{d}	1.13 ± 0.06^{e}	1.25 ± 0.06^{d}	0.74 ± 0.32^{e}
NaCl (10 g/L)	2.86 ± 0.19^{ab}	1.11 ± 0.07^{d}	1.04 ± 0.07^{c}	1.64 ± 0.11^{b}	2.81 ± 0.19^{c}	2.18 ± 0.14^{b}	2.89 ± 0.09^{a}	2.06 ± 0.14^{bc}	1.81 ± 0.09^{c}	2.07 ± 0.70^{b}
Ultrasonication	2.61 ± 0.22^{bc}	0.67 ± 0.04^{ef}	0.85 ± 0.05^{d}	1.04 ± 0.06^{c}	3.12 ± 0.17^{c}	1.29 ± 0.07^{d}	1.63 ± 0.08^{c}	1.51 ± 0.07^{d}	1.37 ± 0.06^{d}	1.55 ± 0.76^{c}
	Total mould count (log $_{10}$ CFU g^{-1} fresh weight)									
Control	3.43 ± 0.18^{a}	2.08 ± 0.13^{a}	1.69 ± 0.11^{a}	2.37 ± 0.15^{a}	3.58 ± 0.22^{a}	2.94 ± 0.18^{a}	3.52 ± 0.22^{a}	3.19 ± 0.20^{a}	3.15 ± 0.20^{a}	2.94 ± 0.68^{a}
NaOCl (1 g/L)	1.87 ± 0.15^{d}	1.64 ± 0.10^{c}	1.42 ± 0.09^{b}	1.08 ± 0.07^{c}	2.07 ± 0.13^{c}	1.38 ± 0.09^{c}	1.54 ± 0.10^{d}	1.48 ± 0.09^{d}	1.28 ± 0.08^{c}	1.55 ± 0.30^{d}
Wet heating (55 °C)	2.51 ± 0.21^{c}	1.94 ± 0.08^{b}	1.15 ± 0.05^{c}	1.02 ± 0.04^{c}	1.83 ± 0.08^{d}	2.17 ± 0.09^{b}	2.35 ± 0.10^{c}	2.33 ± 0.10^{c}	2.20 ± 0.09^{b}	1.92 ± 0.50^{c}
Dry heating (70 °C)	0.76 ± 0.07^{e}	0.55 ± 0.03^{e}	0.43 ± 0.02^{f}	0.36 ± 0.02^{e}	0.94 ± 0.04^{f}	0.62 ± 0.03^{d}	0.66 ± 0.03^{e}	0.65 ± 0.03^{e}	0.89 ± 0.04^{d}	0.65 ± 0.19^{f}
Vinegar (350 mL/L)	0.82 ± 0.07^{e}	0.92 ± 0.05^{d}	0.59 ± 0.03^{e}	0.69 ± 0.04^{d}	1.04 ± 0.06^{ef}	1.45 ± 0.08^{c}	1.75 ± 0.09^{d}	1.55 ± 0.08^{d}	1.49 ± 0.08^{c}	1.14 ± 0.40^{e}
Ethanol (350 mL/L)	0.88 ± 0.06^{e}	0.69 ± 0.04^{e}	0.50 ± 0.03^{ef}	0.83 ± 0.04^{d}	1.27 ± 0.07^{e}	0.58 ± 0.03^{d}	0.87 ± 0.05^{e}	0.87 ± 0.05^{e}	0.98 ± 0.05^{d}	0.83 ± 0.22^{f}
NaCl (10 g/L)	3.15 ± 0.21^{b}	1.62 ± 0.11^{c}	1.06 ± 0.07^{c}	1.66 ± 0.11^{b}	2.75 ± 0.18^{b}	2.23 ± 0.15^{b}	3.18 ± 0.21^{b}	2.73 ± 0.18^{b}	2.34 ± 0.15^{b}	2.33 ± 0.72^{b}
Ultrasonication	2.04 ± 0.08^{d}	1.01 ± 0.05^{d}	0.87 ± 0.05^{d}	1.02 ± 0.05^{c}	2.38 ± 0.13^{c}	1.42 ± 0.08^{c}	1.74 ± 0.09^{d}	1.68 ± 0.09^{d}	1.54 ± 0.08^{c}	1.51 ± 0.48^{d}

Results are means of three determinations ± SD. Means in the same column followed by the same letter are not significantly different at LSD test $P<0.05$.

Tab. 2: Effects of seed decontamination treatments on the germination percentage, mean germination time of tested seeds and the fresh weights of produced crop sprouts.

	Alfalfa	Mung bean	Adzuki Bean	Pea	Soybean	Peanut	Radish	Purple cabbage	Cauliflower	Mean
	Germination percentage (%)									
Control	93.52 ± 2.32^{bc}	97.14 ± 2.41^{a}	34.47 ± 2.11^{d}	81.67 ± 2.33^{d}	78.08 ± 2.59^{c}	84.04 ± 2.11^{c}	99.33 ± 0.94^{a}	30.80 ± 0.50^{d}	80.20 ± 1.31^{d}	75.47 ± 24.08^{b}
NaOCl (1 g/L)	98.01 ± 2.01^{ab}	99.33 ± 0.94^{a}	94.91 ± 3.68^{a}	89.49 ± 2.22^{bc}	97.82 ± 1.96^{a}	99.33 ± 0.94^{a}	99.33 ± 0.94^{a}	32.99 ± 0.41^{c}	98.47 ± 1.23^{a}	89.97 ± 20.45^{a}
Wet heating (55 °C)	91.01 ± 3.36^{c}	97.68 ± 2.60^{a}	89.90 ± 3.72^{a}	88.90 ± 2.54^{bc}	80.40 ± 2.63^{bc}	83.46 ± 2.48^{c}	99.67 ± 0.47^{a}	32.08 ± 0.95^{cd}	94.17 ± 2.36^{b}	84.14 ± 19.47^{ab}
Dry heating (70 °C)	70.86 ± 3.20^{d}	77.74 ± 0.97^{b}	6.57 ± 0.30^{e}	76.34 ± 2.20^{e}	42.36 ± 1.56^{d}	57.42 ± 1.98^{d}	99.33 ± 0.94^{a}	24.34 ± 0.61^{e}	91.68 ± 1.57^{bc}	60.74 ± 29.28^{c}
Vinegar (350 mL/L)	93.61 ± 1.92^{bc}	99.33 ± 0.94^{a}	79.22 ± 3.23^{b}	88.59 ± 2.53^{c}	78.36 ± 2.24^{c}	82.57 ± 1.69^{c}	99.33 ± 0.94^{a}	36.80 ± 0.60^{b}	98.74 ± 1.20^{a}	84.06 ± 18.61^{ab}
Ethanol (350 mL/L)	99.33 ± 0.94^{a}	99.14 ± 1.02^{a}	92.52 ± 3.43^{a}	93.52 ± 2.32^{ab}	81.00 ± 2.36^{bc}	88.90 ± 1.45^{b}	99.67 ± 0.47^{a}	35.70 ± 0.58^{b}	99.67 ± 0.47^{a}	87.82 ± 19.40^{a}
NaCl (10 g/L)	94.61 ± 2.70^{abc}	98.54 ± 1.60^{a}	66.14 ± 3.77^{c}	88.69 ± 2.53^{bc}	84.50 ± 2.07^{b}	91.68 ± 2.30^{b}	99.67 ± 0.47^{a}	32.10 ± 0.52^{cd}	89.50 ± 1.53^{c}	82.83 ± 20.34^{ab}
Ultrasonication	99.33 ± 0.94^{a}	96.98 ± 1.21^{a}	93.43 ± 2.72^{a}	96.94 ± 1.64^{a}	99.67 ± 0.47^{a}	99.33 ± 0.94^{a}	99.33 ± 0.94^{a}	58.60 ± 0.96^{a}	98.40 ± 0.80^{a}	93.56 ± 12.57^{a}
	Mean germination time (days)									
Control	1.43 ± 0.08^{bc}	3.13 ± 0.20^{a}	5.26 ± 0.33^{bc}	3.96 ± 0.25^{ab}	2.82 ± 0.18^{bc}	4.62 ± 0.29^{a}	2.06 ± 0.13^{a}	6.24 ± 0.39^{a}	3.48 ± 0.22^{a}	3.67 ± 1.46^{ab}
NaOCl (1 g/L)	1.39 ± 0.11^{cd}	2.15 ± 0.13^{c}	5.63 ± 0.35^{b}	3.83 ± 0.24^{abc}	1.98 ± 0.12^{f}	3.58 ± 0.22^{c}	1.68 ± 0.10^{bc}	6.06 ± 0.38^{ab}	2.88 ± 0.18^{bc}	3.26 ± 1.62^{bc}
Wet heating (55 °C)	1.70 ± 0.14^{ab}	2.73 ± 0.11^{b}	4.76 ± 0.20^{cd}	3.54 ± 0.15^{cd}	2.51 ± 0.10^{de}	3.87 ± 0.16^{bc}	2.01 ± 0.08^{a}	6.09 ± 0.25^{ab}	3.23 ± 0.13^{ab}	3.36 ± 1.30^{abc}
Dry heating (70 °C)	1.75 ± 0.15^{a}	3.14 ± 0.14^{a}	6.34 ± 0.29^{a}	4.18 ± 0.19^{a}	4.41 ± 0.20^{a}	5.01 ± 0.23^{a}	2.03 ± 0.09^{a}	6.32 ± 0.29^{a}	3.23 ± 0.15^{ab}	4.03 ± 1.58^{a}
Vinegar (350 mL/L)	1.25 ± 0.10^{cd}	2.34 ± 0.12^{c}	4.74 ± 0.25^{d}	3.86 ± 0.21^{abc}	3.07 ± 0.16^{b}	3.88 ± 0.21^{bc}	1.89 ± 0.10^{ab}	5.88 ± 0.31^{ab}	2.98 ± 0.16^{bc}	3.33 ± 1.37^{abc}
Ethanol (350 mL/L)	1.34 ± 0.10^{cd}	2.43 ± 0.13^{bc}	4.78 ± 0.25^{cd}	2.71 ± 0.14^{e}	2.73 ± 0.15^{cd}	3.48 ± 0.18^{c}	1.78 ± 0.09^{b}	5.62 ± 0.30^{bc}	2.74 ± 0.15^{c}	3.08 ± 1.30^{bc}
NaCl (10 g/L)	1.27 ± 0.08^{cd}	2.11 ± 0.14^{c}	4.55 ± 0.30^{d}	3.73 ± 0.25^{bc}	2.36 ± 0.16^{e}	4.12 ± 0.27^{b}	2.02 ± 0.13^{a}	5.98 ± 0.39^{ab}	3.09 ± 0.20^{b}	3.25 ± 1.41^{bc}
Ultrasonication	1.09 ± 0.09^{e}	1.86 ± 0.10^{d}	3.98 ± 0.21^{e}	3.16 ± 0.17^{de}	1.86 ± 0.10^{f}	3.28 ± 0.18^{c}	1.54 ± 0.08^{c}	5.19 ± 0.28^{c}	2.69 ± 0.14^{c}	2.75 ± 1.24^{c}
	Sprout fresh weight (g 10 g^{-1} seeds)									
Control	78.4 ± 4.1^{cd}	102.1 ± 6.4^{bcd}	167.6 ± 9.5^{e}	203.5 ± 9.8^{c}	60.5 ± 3.8^{d}	32.8 ± 2.1^{e}	72.1 ± 4.5^{d}	21.2 ± 1.3^{bc}	40.3 ± 2.5^{d}	76.51 ± 51.17^{d}
NaOCl (1 g/L)	94.4 ± 7.7^{b}	105.6 ± 6.6^{bcd}	221.1 ± 9.9^{ab}	292.7 ±8.3^{b}	82.5 ± 5.1^{bc}	74.2 ± 4.6^{c}	82.1 ± 5.1^{bc}	22.4 ± 1.4^{bc}	66.4 ± 4.1^{b}	116.28 ± 80.83^{abcd}
Wet heating (55 °C)	72.3 ± 5.9^{d}	96.7 ± 4.0^{cd}	168.3 ± 6.9^{e}	226.9 ± 9.3^{c}	76.8 ± 3.2^{c}	58.4 ± 2.4^{d}	76.2 ± 3.1^{cd}	21.8 ± 0.9^{bc}	54.6 ± 2.2^{c}	94.50 ± 60.31^{bcd}
Dry heating (70 °C)	72.9 ± 6.4^{d}	93.8 ± 4.3^{d}	122.5 ± 5.6^{f}	211.6 ± 9.7^{c}	42.5 ± 1.9^{e}	28.4 ± 1.3^{e}	70.6 ± 3.2^{d}	19.8 ± 0.8^{c}	42.8 ± 1.8^{d}	78.30 ± 56.38^{cd}
Vinegar (350 mL/L)	48.2 ± 3.8^{e}	97.3 ± 5.2^{cd}	212.2 ± 8.9^{bc}	314.6 ± 16.7^{b}	78.6 ± 4.2^{c}	78.9 ± 4.2^{c}	89.4 ± 4.7^{b}	22.9 ± 1.2^{bc}	57.9 ± 3.1^{c}	111.44 ± 88.27^{abcd}
Ethanol (350 mL/L)	95.9 ± 7.0^{b}	114.3 ± 6.1^{b}	193.7 ± 9.8^{cd}	327.5 ± 17.4^{ab}	92.1 ± 4.9^{b}	70.6 ± 3.8^{c}	88.6 ± 4.5^{b}	23.1 ± 1.1^{b}	59.8 ± 3.2^{c}	118.77 ± 86.77^{abc}
NaCl (10 g/L)	86.3 ± 5.7^{bc}	104.4 ± 6.9^{bcd}	222.3 ± 8.9^{ab}	322.7 ± 10.8^{ab}	107.3 ± 7.1^{a}	87.2 ± 5.8^{b}	75.2 ± 4.9^{cd}	21.4 ± 1.4^{bc}	55.9 ± 2.7^{c}	121.52 ± 89.24^{ab}
Ultrasonication	147.7 ± 6.0^{a}	127.8 ± 6.8^{a}	243.9 ± 9.7^{a}	352.7 ± 8.9^{a}	115.6 ± 5.1^{a}	107.8 ± 5.4^{a}	112.8 ± 5.8^{a}	42.8 ± 2.2^{a}	78.2 ± 4.2^{a}	146.28 ± 91.02^{a}

Results are means of three determinations ± SD. Means in the same column followed by the same letter are not significantly different at LSD test P<0.05.

The mean germination times (MGT) of tested crop seeds are also shown in Tab. 2. Among the non-treated control seeds, the calculated MGTs ranged between 1.43 and 6.24 days, with an average of 3.67 days across all the tested species. Among the tested species, alfalfa seeds had the shortest MGT, followed by radish seeds, soybean seeds and mung bean seeds, while the longest MGT was obtained from purple cabbage seeds (Tab. 2).

Treating the seeds with sanitization treatments (except dry heating) improved their MGT, among which the ultrasonication treatment showed the greatest improvements in MGT (average of 2.74 days across all the crop species) (Tab. 2). Similar improvements in MGT were also observed in the ultrasonication-treated chick peas, wheat, melon and broccoli seeds (GOUSSOUS et al., 2010; KIM et al., 2006). On the contrary, the dry heating treatment resulted in an increase in MGT (an average of 4.05 days across all species), suggesting that the sprouting processing of treated seeds were slowed down to some extent (Tab. 2). YILDDIRIM et al., (2011) reported that the ultrasonication enhanced germination speed was attributable to the large and rapid oscillations in bubble size that lead to disruption of plant cell walls, thereby increasing water diffusion rate by the seed. Recent study of DA SILVA and DOBRÁNSZKI (2014) further indicated that the ultrasonication increased the organogenesis, possibly resultant from the enhanced activities of antioxidant enzymes such as superoxide dismutase and catalase that protected cell membranes against stress caused by sound irradiation. These enhanced antioxidant enzymes were reported as one of the main reasons for priming-improved germination speed (CHIU et al., 2006). Thus, the ultrasonication may be beneficiary to germinating seeds by activating the antioxidant enzymes that protecting the seeds from possible soaking injury during imbibition. In this regard, a detailed physiological and biochemical examinations should be conducted on ultrasonication-treated seeds in the future.

Effects of seed sanitization treatments on microbial loads of pea sprouts

Tab. 3 presents the results of microbial loads on the sprouts produced from the control and decontaminated seeds. While the microbial loads of control seeds were kept at relatively low levels prior to sprouting (Tab. 1), microbial populations increased substantially during sprouting. The sprouts produced from non-treated control seeds exhibited high levels of total aerobic bacterial counts, ranged from 5.74 (radish seeds) to 12.75 log $_{10}$ CFU g^{-1} fresh weights (purple cabbage seeds) (Tab. 3). They also exhibited high levels of total coliforms counts (ranged between 3.42 and 7.35 log $_{10}$ CFU g^{-1} fresh weight) and total mould counts (ranged between 3.78 and 8.12 log $_{10}$ CFU g^{-1} fresh weight) (Tab. 3). The increased microbial populations are due to the microbes present on the seeds and the favorable environmental conditions (e.g., water activity, temperature) in which they were grown GHANDI and MATTHEWS, 2003). The extremely higher total aerobic bacterial counts obtained from non-treated control purple cabbage (12.75 log $_{10}$ CFU g^{-1} fresh weights and adzuki bean (11.81 log $_{10}$ CFU g^{-1} fresh weights) sprouts were not anticipated (Tab. 3). These results are believed to be associated with the poor germinations (30.80 and 34.47 %, respectively) and longer MGT (6.24 and 5.26 days, respectively) of these two crop species (Tab. 2). The organic residues left within the seeds will be further hydrolyzed and utilized by the rapidly propagated microorganisms if their fail to germinate. As a result, an extremely higher microbial population was observed on the purple cabbage or adzuki bean sprouts.

As shown in Tab. 3, most of the seed sanitization treatments were able to reduce the microbial loads of produced sprouts, but each with different magnitude of reduction. From the decontaminated-seeds produced sprouts the measured total aerobic bacterial counts, total coliforms counts and total mould counts ranged from 2.01 to 11.99, from 1.21 to 9.23, and from 1.64 to 9.94 log $_{10}$ CFU g^{-1} fresh weights, respectively. Microbial sanitization using ultrasonication has been investigated for application to a range of liquid foods (CHANDRAPALA et al., 2012). It is also a safe and clean technology with high potential for quality and safety improvements of seeds and sprouts (CHEMAT et al., 2011; CHIU and SUNG, 2014). In this study, the ultrasonication appeared to be the most effective approach for reducing the total aerobic counts, total coliforms counts and total mould counts because their counts were reduced to lowest levels (averages of 3.53, 2.41 and 2.55 log $_{10}$ CFU g^{-1} fresh weights respectively, across all the examined species) comparing to other sanitization treatments (Tab. 3).

It is generally accepted that the total coliforms populations should be kept at the level below 3 log CFU g^{-1} on the raw and prepared vegetables (including salad vegetables) (STANNARD, 1997). Same standard is also applied to the salad vegetable in Taiwan. Based on this scale, only the soybean sprouts produced from ultrasonication-treated seeds are not suitable for consumption of raw-seed sprouts, and the other crop sprouts are acceptable (Tab. 3). However, if a standard of < 3 log CFU g^{-1} on total aerobic bacterial counts is also considered, only ultrasonication-treated alfalfa, mung bean, pea and radish sprouts are acceptable (Tab. 3). Some of other seed sanitization treatments are also capable of reducing both total aerobic bacterial counts and coliforms counts to < 3 log CFU g^{-1}, but their applications are limited to pea (NaOCl, wet heating), and radish (vinegar, ethanol) sprouts (Tab. 3). These results indicate that a further sanitization procedure for the sprouts produced from decontaminated alfalfa, mung bean, adzuki bean, soybean, peanut, purple cabbage and cauliflower seeds should be performed if the produced sprouts are consumed raw in salads and sandwiches.

Growth and yield of sprouts

The effects of seed sanitization treatments on the sprouts yields were also evaluated based on the produced sprout fresh weight (Tab. 3). The yields of sprouts produced from the non-treated control seeds were in the range of 40.4 to 203.5 g 10 g seeds^{-1}, with an average of 83.2 g 10 g seeds^{-1} across all the species. The averages of sprout yields (across all species) produced from the seeds that have received sanitization treatments ranged between 78.3 and 146.3 g 10 g^{-1} seeds (Tab. 2). The sprout yield differences were noticeable among the applied treatments. The highest average of yield increase was obtained from the ultrasonication-treated seeds, with 78 % more sprout yield than the average of non-treated control seeds. The lowest average of yield increase was obtained from the wet heating-treated seeds, with 14 % more sprout yield than the average of non-treated control seeds. These results re-confirm that the higher microbial loads on germinating seeds may have a negative impact on subsequent growth of sprouts (NWACHUKWU and UMECHURUBA, 2001).

Conclusion

The present study demonstrated that most of sanitization treatments (except dry heating) were able to improve or at least maintain the germination responses of tested crop seeds, decrease the seeds and sprouts microbial loads and enhanced sprouts growth. A single ultrasonication seed treatment can reduce the total coliforms counts to < 3 log $_{10}$ CFU g^{-1} fresh weight on nearly all the tested sprouts (except soybean). However, if both total aerobic bacterial counts and total coliforms were required to exhibit < 3 log $_{10}$ CFU g^{-1} fresh weight level, only the sprouts produced from ultrasonication-decontaminated seeds of alfalfa, mung bean, pea and radish seeds were acceptable. Both NaOCl and wet heating treatments were also capable of reducing the total aerobic bacterial counts and total coliforms

Tab. 3: Effects of seed decontamination treatments on the microbial loads of various tested crop sprouts.

	Alfalfa	Mung bean	Adzuki Bean	Pea	Soybean	Peanut	Radish	Purple cabbage	Cauliflower	Mean
	Total aerobic bacterial count (log $_{10}$ CFU g^{-1} fresh weight)									
Control	6.31 ± 0.33^{a}	8.03 ± 0.49^{b}	11.81 ± 0.74^{a}	8.42 ± 0.53^{a}	6.12 ± 0.38^{b}	7.89 ± 0.49^{b}	5.74 ± 0.36^{a}	12.75 ± 0.80^{a}	8.17 ± 0.51^{a}	8.38 ± 2.36^{a}
NaOCl (1 g/L)	4.04 ± 0.33^{c}	3.13 ± 0.20^{e}	5.98 ± 0.37^{b}	2.62 ± 0.16^{d}	4.68 ± 0.29^{cd}	6.02 ± 0.38^{de}	3.75 ± 0.23^{b}	7.89 ± 0.49^{d}	6.16 ± 0.38^{b}	4.93 ± 1.62^{c}
Wet heating (55 °C)	3.26 ± 0.27^{de}	5.11 ± 0.21^{d}	6.27 ± 0.26^{b}	2.44 ± 0.10^{de}	4.98 ± 0.20^{cd}	6.89 ± 0.28^{cd}	3.71 ± 0.15^{b}	9.43 ± 0.39^{c}	5.27 ± 0.22^{c}	5.23 ± 1.97^{bc}
Dry heating (70 °C)	5.89 ± 0.51^{b}	9.78 ± 0.44^{a}	11.99 ± 0.55^{a}	6.17 ± 0.28^{b}	8.12 ± 0.37^{a}	9.12 ± 0.41^{a}	3.74 ± 0.17^{b}	10.38 ± 0.47^{b}	6.01 ± 0.27^{b}	7.89 ± 2.52^{a}
Vinegar (350 mL/L)	4.25 ± 0.34^{c}	7.96 ± 0.42^{bc}	6.65 ± 0.35^{b}	6.74 ± 0.36^{b}	6.04 ± 0.32^{b}	5.98 ± 0.32^{e}	2.68 ± 0.14^{c}	7.97 ± 0.42^{d}	6.07 ± 0.32^{b}	6.05 ± 1.60^{b}
Ethanol (350 mL/L)	3.87 ± 0.28^{cd}	4.69 ± 0.25^{d}	6.12 ± 0.33^{b}	3.01 ± 0.16^{cd}	4.39 ± 0.23^{d}	4.87 ± 0.26^{f}	2.57 ± 0.14^{c}	6.69 ± 0.36^{e}	4.32 ± 0.23^{d}	4.52 ± 1.26^{cd}
NaCl (10 g/L)	5.14 ± 0.34^{b}	7.25 ± 0.48^{c}	6.06 ± 0.40^{b}	3.4 ± 0.22^{c}	5.03 ± 0.33^{c}	7.04 ± 0.46^{c}	3.82 ± 0.25^{b}	9.67 ± 0.64^{bc}	6.47 ± 0.43^{b}	5.99 ± 1.82^{b}
Ultrasonication	2.78 ± 0.11^{e}	2.02 ± 0.11^{f}	4.71 ± 0.25^{c}	2.01 ± 0.12^{e}	3.48 ± 0.19^{e}	4.59 ± 0.25^{f}	2.42 ± 0.13^{c}	5.86 ± 0.31^{e}	3.87 ± 0.21^{d}	3.53 ± 1.27^{d}
	Total coliforms count (log 10 CFU g^{-1} fresh weight)									
Control	3.43 ± 0.18^{bc}	5.11 ± 0.32^{a}	7.35 ± 0.46^{a}	5.35 ± 0.33^{a}	6.87 ± 0.43^{a}	4.87 ± 0.30^{a}	3.42 ± 0.21^{a}	5.79 ± 0.36^{b}	4.58 ± 0.29^{a}	5.21 ± 1.30^{a}
NaOCl (1 g/L)	3.42 ± 0.28^{bc}	2.79 ± 0.17^{bc}	3.99 ± 0.25^{c}	1.89 ± 0.12^{e}	5.04 ± 0.31^{c}	3.77 ± 0.24^{c}	1.97 ± 0.12^{cd}	4.01 ± 0.25^{d}	3.19 ± 0.20^{c}	3.35 ± 0.96^{bc}
Wet heating (55 °C)	3.18 ± 0.26^{cd}	2.64 ± 0.11^{cd}	3.97 ± 0.16^{c}	1.21 ± 0.05^{f}	3.27 ± 0.13^{e}	4.08 ± 0.17^{bc}	2.09 ± 0.09^{c}	4.65 ± 0.19^{c}	3.25 ± 0.13^{c}	3.13 ± 0.99^{c}
Dry heating (70 °C)	2.22 ± 0.19^{e}	4.84 ± 0.22^{a}	7.07 ± 0.32^{a}	4.12 ± 0.19^{b}	5.74 ± 0.26^{b}	5.28 ± 0.24^{a}	1.65 ± 0.08^{ef}	9.23 ± 0.22^{a}	4.25 ± 0.19^{ab}	5.03 ± 2.42^{a}
Vinegar (350 mL/L)	2.17 ± 0.17^{e}	4.88 ± 0.26^{a}	3.18 ± 0.17^{de}	2.31 ± 0.12^{d}	4.02 ± 0.21^{d}	4.09 ± 0.22^{bc}	1.98 ± 0.11^{cd}	4.14 ± 0.22^{cd}	3.92 ± 0.21^{b}	3.41 ± 0.98^{bc}
Ethanol (350 mL/L)	1.63 ± 0.12^{f}	2.35 ± 0.12^{de}	3.57 ± 0.19^{cd}	1.47 ± 0.08^{f}	3.56 ± 0.19^{de}	3.67 ± 0.19^{c}	1.45 ± 0.08^{f}	4.08 ± 0.22^{d}	3.08 ± 0.16^{c}	2.77 ± 1.00^{cd}
NaCl (10 g/L)	4.02 ± 0.27^{a}	3.18 ± 0.21^{b}	4.55 ± 0.31^{b}	2.98 ± 0.20^{c}	3.07 ± 0.20^{e}	4.35 ± 0.29^{b}	2.97 ± 0.20^{b}	5.55 ± 0.37^{b}	4.06 ± 0.27^{b}	3.86 ± 0.84^{b}
Ultrasonication	2.58 ± 0.11^{d}	1.98 ± 0.11^{e}	2.87 ± 0.15^{e}	1.31 ± 0.07^{f}	3.35 ± 0.18^{e}	2.86 ± 0.15^{d}	1.79 ± 0.10^{de}	2.79 ± 0.15^{e}	2.13 ± 0.11^{d}	2.41 ± 0.64^{d}
	Total mould count (log $_{10}$ CFU g^{-1} fresh weight)									
Control	4.48 ± 0.24^{a}	5.58 ± 0.35^{b}	8.12 ± 0.51^{b}	5.65 ± 0.35^{a}	6.42 ± 0.41^{b}	7.23 ± 0.45^{a}	3.78 ± 0.24^{a}	6.78 ± 0.42^{b}	5.42 ± 0.34^{a}	5.95 ± 1.31^{a}
NaOCl (1 g/L)	2.68 ± 0.22^{e}	3.18 ± 0.20^{d}	4.78 ± 0.30^{e}	2.14 ± 0.13^{de}	3.51 ± 0.22^{de}	3.61 ± 0.22^{d}	1.89 ± 0.12^{d}	4.69 ± 0.29^{d}	3.02 ± 0.19^{e}	3.29 ± 0.97^{de}
Wet heating (55 °C)	3.12 ± 0.26^{c}	4.11 ± 0.17^{c}	4.95 ± 0.20^{e}	2.75 ± 0.11^{c}	5.71 ± 0.23^{c}	5.36 ± 0.22^{c}	2.79 ± 0.11^{b}	6.62 ± 0.27^{b}	4.17 ± 0.17^{c}	4.37 ± 1.28^{bc}
Dry heating (70 °C)	4.12 ± 0.36^{b}	5.31 ± 0.24^{b}	8.98 ± 0.41^{a}	2.33 ± 0.11^{de}	9.94 ± 0.47^{a}	7.84 ± 0.36^{a}	2.61 ± 0.12^{b}	9.23 ± 0.20^{a}	5.03 ± 0.23^{ab}	6.25 ± 2.90^{a}
Vinegar (350 mL/L)	2.21 ± 0.18^{e}	6.91 ± 0.37^{a}	5.13 ± 0.27^{de}	3.44 ± 0.18^{b}	6.99 ± 0.37^{b}	5.07 ± 0.27^{c}	2.27 ± 0.12^{c}	5.69 ± 0.30^{c}	3.64 ± 0.19^{d}	4.60 ± 1.71^{bc}
Ethanol (350 mL/L)	3.14 ± 0.23^{c}	4.27 ± 0.23^{c}	5.78 ± 0.31^{d}	1.99 ± 0.12^{ef}	4.22 ± 0.22^{d}	5.45 ± 0.29^{c}	2.29 ± 0.13^{c}	4.75 ± 0.25^{d}	4.27 ± 0.23^{c}	4.03 ± 1.25^{cd}
NaCl (10 g/L)	3.78 ± 0.25^{b}	5.05 ± 0.33^{b}	6.87 ± 0.41^{c}	2.42 ± 0.16^{cd}	5.37 ± 0.35^{c}	6.45 ± 0.43^{b}	3.62 ± 0.24^{a}	6.87 ± 0.45^{b}	4.81 ± 0.32^{b}	5.03 ± 1.47^{b}
Ultrasonication	2.55 ± 0.10^{de}	2.14 ± 0.11^{e}	2.86 ± 0.15^{f}	1.64 ± 0.09^{f}	3.01 ± 0.16^{e}	2.93 ± 0.16^{e}	1.88 ± 0.10^{d}	2.94 ± 0.16^{e}	2.96 ± 0.16^{e}	2.55 ± 0.50^{e}

Results are means of three determinations ± SD. Means in the same column followed by the same letter are not significantly different at LSD test P<0.05. 2.

counts to < 3 log $_{10}$ CFU g^{-1} fresh weights on pea sprouts, and the sprouts produced from ethanol-treated radish seeds also were acceptable at < 3 log $_{10}$ CFU g^{-1} fresh weights level. However, the ultrasonication treated seeds produced greater sprouts yield than other sanitizations-treated seeds, which gave an average of 78 % sprouts yield increase (across of all the tested crop species) comparing to non-treated seeds. Therefore, ultrasonication treatment can be used as a microbial control treatment for alfalfa, mung bean, pea and radish sprouts production.

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