

Article

Changes in Microstructure, Germination, Sprout Growth, Phytochemical and Microbial Quality of Ultrasonication Treated Adzuki Bean Seeds

Kai-Ying Chiu

Department of Post-Modern Agriculture, Mingdao University, Peetow, Changhua County 523, Taiwan; cky@mdu.edu.tw; Tel.: +886-0953-835-832

Abstract: Sprout is a nutritious ready-to-eat vegetable. In this study, we examined the effects of ultrasonication treatments (treated with 28, 40 or 80 kHz frequency for 10 min at 25 °C) on the changes in microstructure, seed hydration and germination, sprout growth, sprout quality, and microbial load of two adzuki bean (*Vigna angularis*) varieties (Kaohsiung 8 and Kaohsiung 10). The results indicated that the ultrasonication at 40 kHz or 80 kHz improved seed hydration and enhanced germination; this is linked to the ultrasound-induced microstructure changes in the hilum and seed coat surface of treated seeds. The ultrasonication-increased sprout growth and yield were possibly attributable to the increased α -amylase activity during germination. Ultrasonication also improved total polyphenols, total flavonoids, and total saponins contents and further enhanced the anti-oxidative activities (FRAP and DPPH) of produce sprouts. The results further indicated that the 40 kHz or 80 kHz treatment decreased microbial loads of treated sprouts to an acceptable level; therefore, these two treatments can be used for germination improvement and an effective microbial control treatment for adzuki bean sprout production.



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Keywords: adzuki bean; germination; microbial load; phytochemical; seed hydration; sprout; ultrasonication

1. Introduction

Adzuki bean (*Vigna angularis*), a member of the family fabaceae, has been cultivated for centuries in Asian countries such as China, Japan, Korea, and Taiwan [1]. The seeds of the adzuki bean have a sweet and nutty taste and plays an important role in the human diet owing to their richness in starch and protein in addition to high levels of dietary fiber, minerals, and vitamins [2]. It also contains large amounts of polyphenols, flavonoids, and saponins [1], which are known to have anti-inflammatory and anti-bacterial properties that exhibits many beneficial effects for human health. In Chinese folk medicine, adzuki bean seeds are used to treat diuretic functions and other diseases such as dropsy and beriberi [1].

Soybean (*Glycine max*), mung bean (*Vigna radiata*), alfalfa (*Medicago sativa*), and sprouts are the products obtained from the germination of seeds that are harvested before the development of true leaves and they are intended to be eaten whole. Sprout is a nutritious ready-to-eat vegetable worldwide [3]. Adzuki bean seeds are also consumed as sprouts because of their soft and crispy texture and attractive fragrance. However, the seed coat of the adzuki bean is hard when compared to other legumes [4]. This hard seed coat characteristic is linked to the polyphenols and suberin compounds accumulated in the palisade layer of seed coat, which reduces water permeability [5]. As a result, the germinations of some adzuki bean varieties are relatively low compared to other legumes such as soybean and mung bean [6] and consequently affects their sprouts production. Another concern is that the produced sprouts are vulnerable to microbial contamination, which can result from the microbial load on the harvested seeds and the microbial multiplication during sprouting [7]. Therefore, an effective seed treatment, which can improve seed

germination and the diminishing of microbial contaminants, is helpful for adzuki bean sprouts production.

Ultrasonication is a technique that involves the application of ultrasound (frequency ranges generally between 20 kHz and 100 kHz) to interact with the materials by using a liquid medium [8]. It is often used in the food industry for improving physical and chemical reactions of treated materials in various applications [9,10]. Ultrasonication is also known to stimulate germination in many legumes such as mung bean [11], pea (*Pisum sativum*) [12], chickpea (*Cicer arietinum*) [13], and soybean [14]. The ultrasonically increased germination is caused by the mechanical effects of ultrasonic acoustic cavitation. The oscillation and collapse of cavitation bubbles driven by ultrasonic frequencies would impose a mechanical pressure on the cell wall of treated seeds and increases the porosity of cell wall, which subsequently improves water uptake. The improved water uptake in germinating seeds would result in a germination improvement [15,16]. Ultrasonication is also reported to improve sprout production and reduce microbial load in pea sprouts [12]. Ultrasonication treatment is also able to reduce the level of microbial load on alfalfa and broccoli seeds but the germination of treated seeds is decreased to some extent [17]. Thus, the efficacy of ultrasonication on seed decontamination appears to be species-dependent.

Ultrasonication treatments have been reported to improve germination in different varieties of bean seeds. However, there are limited reports about its effect on adzuki bean seed germination (40 kHz of ultrasound treatment for 1 min at 25 °C) [7]. This research aimed to find the best ultrasonication frequency to produce enhanced germination response for the tested two adzuki bean varieties. Special attention was given to the contents of several phytochemicals including total polyphenols, total flavonoids, total saponins, and the antioxidant activities in the ultrasonication treated seeds were also examined. The effects of ultrasonication treatments to reduce microbial contaminations on the produced sprouts were also examined.

2. Materials and Methods

2.1. Seed Materials

Two commercially produced adzuki bean (*Vigna angularis*) varieties Kaohsiung 8 (KH 8, 100 seeds, and 16.8 g in weight) and Kaohsiung 10 (KH 10, 100 seeds, and 15.4 g in weight) were used in all the experiments. The seeds with about 19% seed moisture content on dry weight basis were stored at 4 °C before they were used for testing.

2.2. Ultrasonication Treatment

The ultrasonication treatments were conducted by soaking 100 g of seeds in 2 L of deionized water in an ultrasonic bath with a power of 300 W (RingTech, MU 301-30L, Taichung, Taiwan) [12]. The applied frequencies were 28 kHz, 40 kHz, or 80 kHz, respectively, for 10 min at 25 °C. A subsample of non-treated control was prepared by soaking the seeds in deionized water for 10 min at 25 °C.

2.3. Seed Hydration and Germination Determinations

Fifty seeds sampled from each ultrasonication treatment were germinated in a growth chamber (ES4-1S, Saint Tien Co. Ltd., Kaoshiung city, Taiwan) in the dark at 20 °C for 6 days. The germination percentage and mean germination time (MGT) were calculated following the procedures detailed by Chiu and Sung [12]. For water uptake determination, thirty seeds were weighed to obtain the initial weight and then the weights were measured at the following periods: 1, 2, 3, 4, 5, and 6 days. The moisture uptake was determined as a function of seed fresh weight increase and seed weight difference at each sampling time.

A subsample of the seeds of 10 g from each treatment was germinated under the similar conditions for 6 days, and the growing sprouts were collected for hypocotyl length, radicle lengths, and sprout fresh weight measurements.

2.4. α -Amylase Activity Determinations

For α -amylase activity determination, the adzuki bean seeds were ground and sifted through a 0.75 mm sieve. Approximately 1 g of ground sample was added to a centrifuge tube containing 5 mL of 50 mM sodium phosphate buffer (pH 8). Extraction was performed for 30 min at 30 °C and then the centrifuge tube was centrifuged for 10 min at 3000 g. The supernatant was used as α -amylase extract. Alpha-amylase activity was determined by measuring the reducing sugars produced by its action on soluble starch by using a 3,5-dinitrosalicylic acid method [18].

2.5. Scanning Electron Microscopy Examinations

The adzuki bean seed was cut with a razor blade and was attached to stubs using a double-sided adhesive tape. The prepared sample was coated with 30 nm of gold using a Technics Hummer V sputter coater. The coated sample was scanned and examined using a Hitachi S-3400N microscope operating at 30 kV.

2.6. Phytochemicals and Anti-Oxidative Activities Determinations

The adzuki bean sprouts were dried and ground and sifted through a 0.75 mm sieve. Five grams of the ground adzuki bean sample was added to 100 mL of acidified ethanol (60% ethanol with 2% HCl, pH 1.5) and extracted following the procedures detailed by Zhao et al. [19]. The produced extract was used for phytochemical contents and anti-oxidative activities determinations. Total polyphenols were determined using the Folin–Ciocalteu reagent method [20]. The content of total polyphenols was calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of sprout sample fresh weight. Total flavonoids were estimated by using an AlCl₃ method [21]. Total flavonoids content was calculated and expressed as milligrams of catechin equivalents per gram of sprout sample fresh weight. Total saponins determination was conducted using an anisaldehyde method [22]. The content of saponins was calculated and expressed as mg g⁻¹ bean sprout sample fresh weight.

Free radical scavenging ability of the bean sprout sample extract was carried out tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay method [22]. The results were expressed as percentage inhibition at 100 μ g mL⁻¹. The Ferric Reducing Antioxidant Power (FRAP) ability of the sprout sample was determined using the method of Benzie and Strain [23]. The results were expressed as μ mol trolox equivalent per g⁻¹ sprout sample FW.

2.7. Data Analysis

A complete randomized block design with three replicates was used to evaluate the effects of ultrasonication treatments on the two tested adzuki bean seeds. The data were presented by using mean \pm standard deviation (SD) and statistical significances were determined according to Fisher's least significant difference (LSD) test.

3. Results and Discussion

3.1. Ultrasonication Treatment on Seed Hydration

The hydration process in seeds is the first step toward germination. This process is essential to change the status of seed development from a state of quiescence to active growth. The rate of water uptake is initially controlled by the seed coat. Bewley et al. [24] reported that depending on the seed coat permeability, seeds can hydrate following two different behaviors: downward concave shape and sigmoidal shape. In this study, both varieties Kaohsiung 8 (KH 8) and Kaohsiung 10 (KH 10) exhibited sigmoidal behavior on seed hydration (Figure 1), which was characterized by rapid water uptake at the early phase followed by slow water uptake and finally a much faster rate of water uptake. Significant hydration difference existed between the non-treated control seeds of two varieties; the KH 8 seed exhibited faster and higher water uptake than the KH 10 seed. This difference in hydration process might be linked to the different testa microstructures observed between

the two tested varieties. As is shown in Figure 2, only one macrosclereids layer was found in the seed coat of KH 8, while the seed coat of KH 10 had two macrosclereids layers. The two macrosclereids layer microstructure may affect the entrance of water and subsequently slow down the hydration of the KH 10 seed [24].

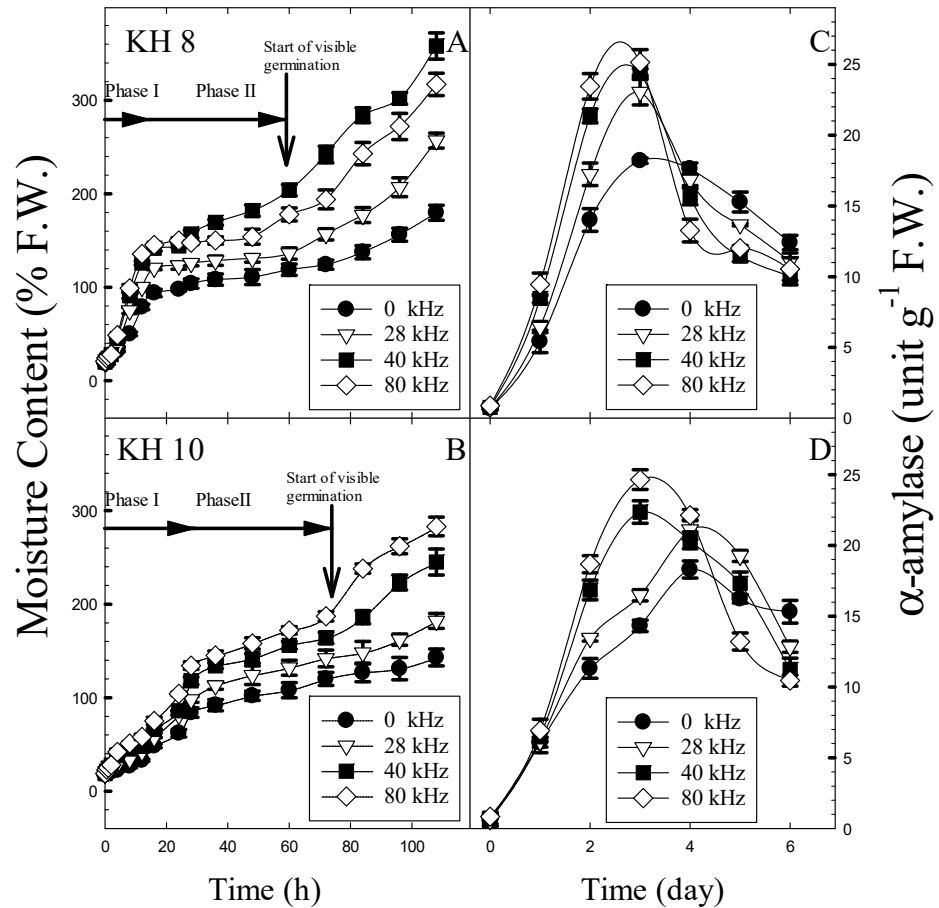


Figure 1. Water absorption and α -amylase activity characteristics of two adzuki bean varieties. Kaohsiung 8 (KH 8) and Kaohsiung 10 (KH 10) seeds were subjected to different ultrasonication treatments (28 kHz, 40 kHz and 80 kHz frequencies for 10 min under 25 °C) during different germination periods.

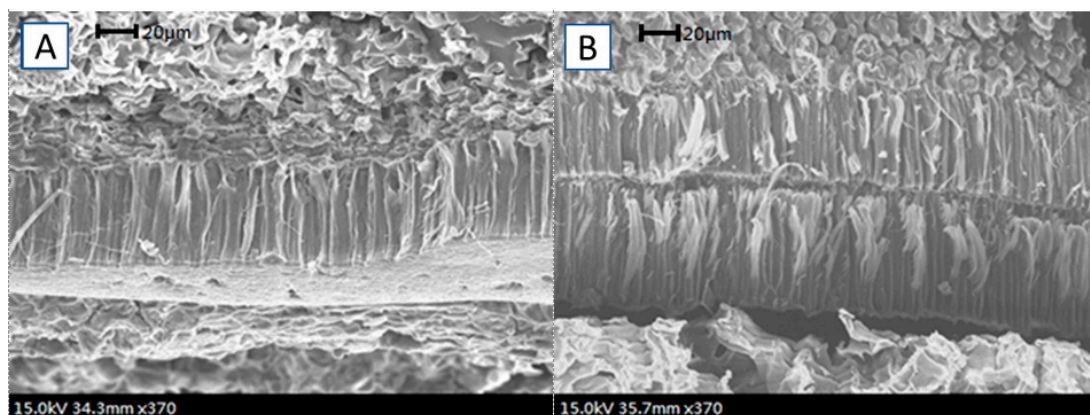


Figure 2. The microstructure of macrosclereids layers of adzuki bean seeds of two varieties: (A) Kaohsiung 8 and (B) Kaohsiung 10 grain microstructure (SEM, 15 kV, 370 X).

Hilum is known to be the main water intake route during legume seed hydration. As is shown in Figure 3A, the hilum of the tested adzuki bean seed had some visible intercellular spaces which were porous. These porous spaces were involved in the exchange of gases and water between the seed and the external environment [15]. Moreover, the seed coat surfaces of non-treated adzuki bean seeds were almost not porous (Figure 3C) in contrast to the hila which were relatively porous (Figure 3A).

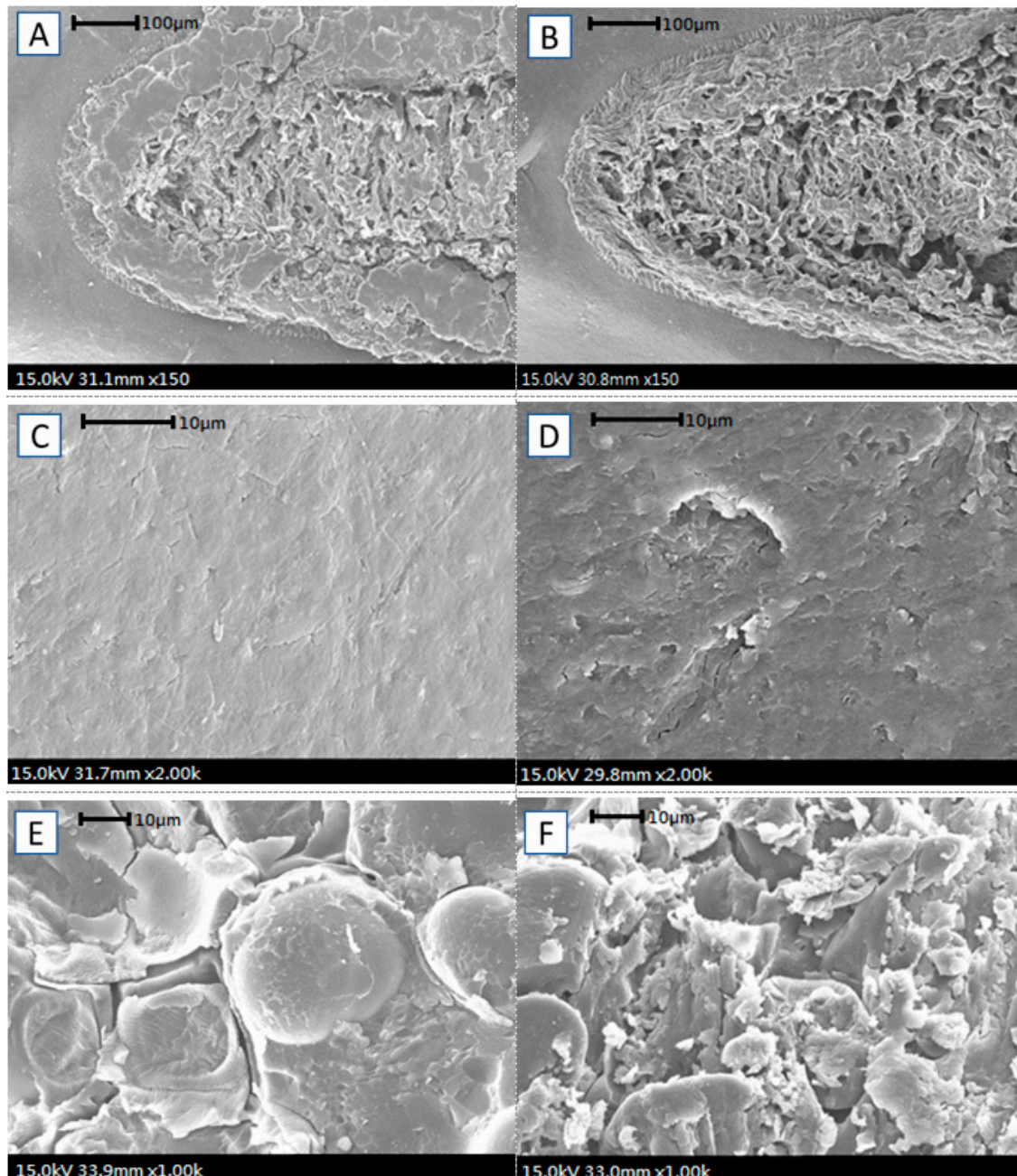


Figure 3. The microstructure of the hila, seed coat surfaces, and starch granules of adzuki bean seeds of two varieties: (A) hilum of Kaohsiung 8, non-treated control seed (1.5 kV, 150 X); (B) hilum of Kaohsiung 8, 80 kHz ultrasonication treated seed (1.5 kV, 150 X); (C) seed coat surface of Kaohsiung 8, non-treated seed (1.5 kV, 2.0 kX); (D) seed coat surface of Kaohsiung 8, 80 kHz ultrasonication treated seed (1.5 kV, 2.0 kX); (E) starch granules of Kaohsiung 8, non-treated control seed (1.5 kV, 1.0 kX); (F) starch granules of Kaohsiung 8, 80 kHz ultrasonication treated seed (1.5 kV, 1.0 kX).

Ultrasound is defined as sound waves possessing a frequency that exceeds the hearing limit of the human ear (~20 kHz) [25]. The application of ultrasonication has demonstrated positive results in accelerating the hydration of mung bean seed [15]. As was shown in Figure 1, the water absorption of adzuki bean seeds that were subjected to ultrasonication treatment (28–80 kHz) increased substantially during seed hydration. The highest water absorption for KH 8 was obtained from the seeds subjected to 40 kHz frequency ultrasonication treatment and were followed by 80 kHz and 28 kHz treatments at the end of 50 h of seed hydration (Figure 1A). On the other hand, the water uptake of KH 10 followed the descending order of 80 kHz > 40 kHz > 28 kHz (Figure 1B). The increased water absorptions observed in the treated seeds were believed to be linked to the ultrasonication-induced microstructure changes observed on the hila and seed coat surfaces (Figure 3). The hilum of adzuki bean seeds subjected to 40 kHz (Figures are not shown) and 80 kHz ultrasonication had considerably more intercellular space than their respective controls (Figure 3B). Moreover, the seed coat surface of 80 kHz treated seeds appeared to have some visible cracks (Figure 3D) in contrast to the seed coat of the control seeds (Figure 3C).

3.2. Ultrasonication Treatment on Seed Germination and α -Amylase Activity

Non-treated control seeds of varieties KH 8 and KH 10 had 66.1% and 34.5% of germination percentage and 4.43 days and 5.65 days of mean germination time (MGT), respectively (Table 1). The germination percentage of variety KH 10 was considerably lower than KH 8; this is possibly caused by its slower water absorption and the different testa microstructure (Figures 1 and 2). All the ultrasonication treated seeds of KH 8 and KH 10 showed improved germination percentage and accelerated germination speed in comparison to their respective controls (Table 1). However, 80 kHz treated seeds performed better than the 28 kHz and 40 kHz treated seeds (Table 1). The best MGT was 2.43 days and 2.78 days for KH 8 and KH 10, respectively (40 kHz treated seed) (Table 1).

Table 1. Effects of different ultrasonication treatments (28 kHz, 40 kHz and 80 kHz) on the germination percentage, mean germination time, hypocotyl length, radicle length, and sprout yield of 6-days-old sprouts of two adzuki bean varieties.

Variety	Ultrasonication Treatment (kHz)	Germination (%)	Mean Germination Time (days)	Hypocotyl Length (cm)	Radicle Length (cm)	Sprout Fresh Weight (g 10 g ⁻¹ seeds)
KH8	0	66.1 ± 4.1	4.43 ± 0.1	5.43 ± 0.0	6.72 ± 0.1	212.4 ± 4.4
	28	87.2 ± 2.3	3.48 ± 0.2	5.92 ± 0.0	7.62 ± 0.0	238.6 ± 9.8
	40	97.2 ± 2.4	2.43 ± 0.1	7.25 ± 0.0	8.46 ± 0.1	331.5 ± 6.8
	80	92.3 ± 2.2	2.63 ± 0.2	6.73 ± 0.1	7.64 ± 0.1	283.4 ± 5.8
KH10	0	34.5 ± 3.4	5.65 ± 0.7	2.42 ± 0.0	1.24 ± 0.0	167.6 ± 3.4
	28	78.5 ± 2.2	4.63 ± 0.2	3.84 ± 0.0	2.83 ± 0.0	211.4 ± 4.3
	40	94.9 ± 1.5	3.12 ± 0.1	5.56 ± 0.0	6.25 ± 0.0	253.9 ± 5.2
	80	94.6 ± 1.6	2.78 ± 0.1	6.84 ± 0.1	7.47 ± 0.1	274.8 ± 6.7
LSD _{0.05}		3.16	0.14	0.30	0.10	13.13

Chen et al. [26] reported that the germination percentage and the germination speed were possibly enhanced because ultrasound vibration caused changes in the cellular ultra-structure that could improve the release of enzymes from the cell wall and biochemical metabolism. Alpha-amylase is an enzyme found in the germinating seeds. The imbibition process causes the release of plant growth hormones (i.e., gibberellin) which stimulates the synthesis of amylase. In this study, we examined the activities of α -amylase in control and ultrasonication-treated adzuki seeds and the results were shown in Figure 1. During the different germination period, the maximum α -amylase activity was found at three days in KH 8 variety (18 unit/g FW), while the maximum α -amylase activity of KH 10 was found at four days.

Ultrasonication was shown to affect the water absorption pattern of treated adzuki bean seeds. Therefore, it was not surprising to find that the α -amylase activities were changed during seed hydration. As was shown in Figure 1, the α -amylase activities of the treated seeds of adzuki bean varieties were higher than that of the control seeds in the earlier germination phases. The maximum activities of 80 kHz treated seeds were increased by 53% and 39% for KH 8 and KH 10 compared to their respective controls. The maximum activities of 28 kHz treated seeds were also increased by 29% and 24% for KH 8 and KH 10 compared to their respective controls. The ultrasonication-stimulated α -amylase activities during the early phase of seed hydration were possibly caused by the ultrasound-induced starch granule damages (Figure 3F). The starch granules of non-treated adzuki seeds were characterized by a compact and homogeneous structure embedded in a matrix. However, this smooth starch granule structure was partially damaged by 80 kHz ultrasonication treatment (Figures for 28 kHz and 40 kHz treatments are not shown), especially for the KH 8 seed. The damaged starch granules would favor the starch hydrolysis through hydration-activated α -amylase activity and then enhance seed germination.

3.3. Effects of Ultrasonication Treatments on Sprouts Growths and Yields

Significant differences in sprout growth existed between the two tested varieties (Table 1). The sprouts of variety KH 8 exhibited longer hypocotyl and radicle lengths than the sprouts of variety KH 10. Variety KH 8 was also out yielded in sprouts than variety KH 10. The yields of sprouts produced from non-treated KH 8 and KH 10 were 212.4 g and 167.6 g per 10 g seeds, respectively.

Significant differences in hypocotyls and radicle lengths also existed among ultrasonication treatments. All the ultrasonication treatments increased hypocotyl and radicle growths. Among the treatments, KH 8 and KH 10 sprouts produced from 40 kHz and 80 kHz ultrasonication-treated seeds exhibited the highest sprout growth improvements (Table 1). Significant differences in sprout yield were also found among ultrasonication treatments. The greatest yield increases were obtained from the 40 kHz ultrasonication treated KH8 (331.5 g 10 g⁻¹ seeds) and 80 kHz ultrasonication treated KH 10 (274.8 g 10 g⁻¹ seeds) seeds.

3.4. Ultrasonication Treatment on Sprout Phytochemicals

Polyphenols and flavonoids are commonly known as the phytochemical molecules with antioxidative properties from plants [27]. Results of total polyphenols, total flavonoid, and total saponins content in the tested adzuki bean sprouts are shown in Table 2. The non-treated sprouts (control) of KH 8 contained more total polyphenols and total saponins but less total flavonoids than the non-treated sprouts of KH 10. The levels of total polyphenols found in our study were substantially higher than that of the levels reported by Świeca et al. [28]. They reported that the level of total polyphenols in the tested 5-days-old adzuki bean sprouts was around 0.11 mg per gram dry weight. However, in the present study, the levels of total polyphenols in the tested sprouts were around 4.05 mg/g DW (average of two varieties) (Table 2). The total flavonoid content of tested adzuki bean sprouts (1.31 mg/g DW, average of two varieties) was also considerably higher than the total flavonoid content of black bean (0.22 mg/g DW) reported by Borges-Martínez et al. [29].

Saponins are reported to have a plasma cholesterol lowering effect in humans and are important in reducing the risk of many chronic diseases [30]. In this study, total saponins were detectable in both KH 8 (12.43 mg per g DW) and KH 10 (10.33 mg per g DW) sprouts (Table 2) and these total saponins values were higher than the saponins level (2.06 mg/g) found in black bean sprout [31].

Table 2. Effects of different ultrasonication treatments (28 kHz, 40 kHz and 80 kHz) on the total polyphenols, total flavonoids, total saponins, and antioxidative abilities (FRAP and DPPH) of 6-days-old sprouts of two adzuki bean varieties.

Variety	Ultrasonication Treatment (kHz)	Total Polyphenols (mg gallic acid g ⁻¹ F.W.)	Total Flavonoids (mg CAE g ⁻¹ F.W.)	Total Saponins (mg g ⁻¹ F.W.)	FRAP (mmol Trolox 100 g ⁻¹ F.W.)	DPPH (% inhibition at 100 µg mL ⁻¹)	Total Polyphenols (mg gallic acid g ⁻¹ F.W.)
KH8	0	4.34 ± 0.04	1.13 ± 0.01	12.43 ± 0.10	8.22 ± 0.07	60.01 ± 1.2	4.34 ± 0.04
	28	6.62 ± 0.04	1.65 ± 0.01	15.10 ± 0.09	11.53 ± 0.09	62.38 ± 2.6	6.62 ± 0.04
	40	8.44 ± 0.06	1.60 ± 0.01	17.22 ± 0.11	13.93 ± 0.10	65.68 ± 1.2	8.44 ± 0.06
	80	5.89 ± 0.05	1.44 ± 0.02	15.49 ± 0.13	12.29 ± 0.06	61.01 ± 1.3	5.89 ± 0.05
KH10	0	3.76 ± 0.03	1.49 ± 0.01	10.33 ± 0.08	7.06 ± 0.05	58.37 ± 1.2	3.76 ± 0.03
	28	7.04 ± 0.06	2.64 ± 0.02	15.53 ± 0.13	12.46 ± 0.10	62.66 ± 1.3	7.04 ± 0.06
	40	8.89 ± 0.06	2.48 ± 0.02	18.45 ± 0.12	14.66 ± 0.09	64.50 ± 1.1	8.89 ± 0.06
	80	8.86 ± 0.07	2.04 ± 0.03	19.40 ± 0.16	15.70 ± 0.13	68.79 ± 1.7	8.86 ± 0.07
LSD _{0.05}		0.28	0.13	1.01	0.41	3.45	0.28

Ultrasonication treatment also affected the levels of total polyphenols, the total flavonoids contents and saponin in the sprouts of both adzuki bean varieties (Table 2). The highest levels of total polyphenols were found on the 40 kHz treated adzuki bean sprouts followed by 80 kHz and 28 kHz treated adzuki bean sprouts (Table 2). On the other hand, the highest levels of total flavonoids were found on the 28 kHz treated sprouts followed by 40 kHz and 80 kHz treated sprouts (Table 2). The contents of saponins for ultrasonication-treated KH8 sprouts were decreased in a descending order of 40 kHz > 80 kHz > 28 kHz, while the contents of saponins for ultrasonication-treated KH 10 sprouts were decreased in a descending order of 80 kHz > 40 kHz > 28 kHz.

3.5. Ultrasonication Treatment on Antioxidative Potentials

In this study two *in vitro* assays, ferric reducing antioxidant potential (FRAP) assay and DPPH radical scavenging capacity assay, were used to evaluate the antioxidant activities of two adzuki bean seeds (Table 2). The results of the FRAP assay showed that the sprout of variety KH 10 showed higher FRAP antioxidant activity (2.51 µmol Trolox per 100 g DW) than the sprout of KH 8 (2.14 µmol Trolox per 100 g DW). The FRAP of KH 8 and KH 10 sprouts were found lower than the adzuki bean sprout study of Bai et al. [32] (15.27 µmol Trolox per 100 g). The results of the DPPH assay showed that the sprout of variety KH 10 showed higher DPPH activity (88.6% inhibition at 100 µg/mL) than the sprout of KH 8 (82.4% inhibition at 100 µg/mL). The DPPH activities of KH 8 and KH 10 sprouts were found substantially higher than the soybean sprout study of Soedarjo et al. [33] (about 29% inhibition at 100 µg/mL). The results of the DPPH assay showed that the sprouts of variety KH 10 showed higher DPPH activity (88.6% inhibition at 100 µg/mL) than the sprouts of KH 8 (82.4% inhibition at 100 µg/mL).

A large variation in FRAP and DPPH ranging from 1.92 to 2.42 mg trolox g⁻¹ g dry weight and from 83.2% to 88.2%, respectively, was also observed for the ultrasonication-treated adzuki bean sprouts (Table 2). In general, the sprouts subjected to ultrasonication treatment tended to have relatively lower FRAP and DPPH activities as comparing to the control sprouts (Table 2). These results were different from the FRAP and DPPH activities of mung bean sprouts reported by Singh et al. [34].

3.6. Effects of Ultrasonication Treatments on Microbial Loads

The sprouts produced from control sprouts of KH 8 and KH 10 exhibited total aerobic bacterial counts of 9.22 and 11.81 log₁₀ CFU g⁻¹ fresh weights, respectively (Table 3). They further exhibited total coliform counts of 6.29 and 7.35 log₁₀ CFU g⁻¹ fresh weight and total mould counts of 7.46 and 8.12 log₁₀ CFU g⁻¹ fresh weight, respectively (Table 3). These values were considerably higher than the microbial load in adzuki sprouts (11.81, 7.35 and 8.12 log₁₀ CFU g⁻¹ fresh weight for total aerobic, total coliform, and total mould counts, respectively) previously reported by Chiu [7]. The increased microbial populations on the

produced adzuki bean sprouts can be attributable to the microbes present on the seeds and the favorable environmental conditions (e.g., soaking water and sprouting temperature) in which they are grown [35].

Table 3. Effects of different ultrasonication treatments (28 kHz, 40 kHz and 80 kHz) on the microbial loads of 6-days-old sprouts of two adzuki bean varieties.

Variety	Ultrasonication Treatment (kHz)	Total Aerobic Bacterial Count	Total Coliforms Count	Total Mould Count
		(log ₁₀ CFU g ⁻¹ fresh weight)		
KH8	0	9.22 ± 1.74	6.29 ± 0.87	7.46 ± 0.43
	28	4.78 ± 0.63	2.38 ± 0.14	3.61 ± 0.23
	40	3.79 ± 0.18	1.48 ± 0.08	2.76 ± 0.03
	80	3.28 ± 0.07	0.10 ± 0.14	2.15 ± 0.11
KH10	0	11.81 ± 1.83	7.35 ± 0.48	8.12 ± 0.46
	28	5.88 ± 0.74	3.69 ± 0.09	4.91 ± 0.34
	40	4.13 ± 0.23	2.59 ± 0.24	2.69 ± 0.13
	80	3.93 ± 0.17	2.37 ± 0.33	2.33 ± 0.24
LSD _{0.05}		0.22	0.15	0.18

There are many physical, chemical, and biological methods available to decrease the microbial load of produced sprout [36]. Ultrasonication treatments have been reported to improve germination in different varieties of bean seeds. However, there was only limited report about its effect on adzuki bean seed germination (40 kHz of ultrasound treatment for 1 min at 25 °C) [7]. In this study, the efficacies of three ultrasonication treatments were compared. The de-contamination efficacy on produced sprouts for total aerobic, total coliforms, and total mould controls were significantly different among the tested ultrasonication treatments, with the descending order of 80 kHz > 40 kHz > 28 kHz (Table 3). To reiterate, significant differences existed between two varieties, with ultrasonication treated KH 8 sprouts generally having less microbial loads than KH 10 sprouts (Table 3).

4. Conclusions

This study provides information on seed germination, sprout yield, phytochemicals contents, antioxidative ability, and microbial status of two adzuki bean varieties. The results indicated that all the ultrasonication treatments were able to increase seed hydration, improve germinations, and increase α -amylase activities of two tested adzuki bean varieties and subsequently increase their sprout productions. The ultrasonication-improved seed hydration was linked to the ultrasound-induced microstructural changes on the hilum and the surface of seed coats in the treated adzuki bean seeds. Moreover, all the ultrasonication increased the contents of polyphenols, total flavonoids, and total saponins in the treated bean sprouts. Additionally, both FRAP and DPPH activities were enhanced in the ultrasonication-treated sprouts. As for food safety concerns, 80 kHz and 40 kHz ultrasonication treatments were capable of decreasing total aerobic counts of sprouts by 6.5 and 6.3 log unit (averages of two varieties), respectively, while the 28 kHz ultrasonication treatments were unable to decrease total aerobic counts adequately enough (≥ 5 log unit) to protect microbial safety for variety KH 8. Moreover, both 40 kHz and 80 kHz ultrasonication were capable of reducing the total coliforms and total mould counts to < 3 log₁₀ CFU g⁻¹ fresh weights on tested adzuki bean sprouts. Therefore, both the 40 kHz and 80 kHz ultrasonications are an appropriate choice as an effective pretreatment for adzuki bean sprout production. However, the 40 kHz treated KH 8 seeds produced highest sprout yield while the 80 kHz treated KH 10 seeds produced highest sprout yield. Thus, the efficacy of ultrasonication frequency on adzuki bean sprout production appears to be variety-dependent.

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Conflicts of Interest: The author declares no conflict of interest.

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