Efficiency of Ultrasonic Treatment on Postharvest Quality and Bioactive Compounds of 'Kim Ju' Guava Fruit During Short-Term Storage at Room Temperature

Suriyan Supapvanich* and Chollawit Kijka

Department of Agricultural Education, Faculty of Industrial Education and Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

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Abstract

The purpose of this recent work was to investigate the efficiency of ultrasonic (US) treatment on the postharvest quality and bioactive compounds of 'Kim Ju' guava fruits during storage at room temperature (RT) (28±1°C) for 6 d. The fruit samples were sonicated at 40 kHz and 150 w for 10 min. Visual appearance, colour attributes, weight loss, total soluble solids (TSS), titratable acidity (TA), texture, pectin substances, antioxidant activity, total phenols and flavonoids contents of the fruits were monitored during storage. The fruits treated with US had better visual appearance than that of untreated fruits. US treatment could delay weight loss but it had no effect on all colour attributes, TSS and TA of fruits. Fruit softening was inhibited by US treatment due to delay in the formation of increased soluble pectin and decreased insoluble pectin contents. Moreover, US treatment could enhance antioxidant activity and the total phenols and flavonoids contents. Nevertheless, there was no change of ascorbic acid content in fruits during storage. These results suggest that US treatment is an effective postharvest approach, which could preserve postharvest quality and level of bioactive compounds of 'Kim Ju' guavas during short-term storage at RT.

Keywords: guava; ultrasound; bioactive compound; firmness; fruit
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1. Introduction

Guava (*Psidium guajava* L.) fruit is a commercial fruit in Southeast Asian countries including Thailand. Thailand has been widely known as a potential country producing tropical fruits such as durian, rambutan, papaya, pineapple, and guava [1]. Guava has been produced for domestic and international markets. Three commercial cultivars such as 'Klomsali', 'Salithong', and 'Kim Ju' have been commonly grown in Thailand [2]. They are white guavas and they are best consumed at full mature green stage when the flesh is still crisp. Besides their crispy texture and bright-green skin, white flesh and sweet-and-sour taste are other attractive characteristics of Thai guava. Among the commercial cultivars of guava, 'Kim Ju' is one of the most popular cultivars in the domestic and export market. In the domestic market, guava fruits are mostly kept at room temperature (RT) rather than storage in a cold room, a condition which limits its shelf-life and marketing period. Skin browning, rotting, softening, and weight loss are the main

*Corresponding author: Tel.: (+66) 900124901
E-mail: suriyan.su@kmitl.ac.th

factors affecting quality and marketability of guava fruits [3, 4]. Guava is classified as a climacteric fruit that exhibits peaks of respiratory rate and ethylene production during the ripening process [5, 6]. At the average atmospheric temperature in Thailand (28-32°C), guavas typically undergo rapid deterioration within 3-4 days [7]. Recently, many postharvest treatments such as heat treatment [8], calcium treatment [9], exogenous plant-growth regulators treatment [4, 7] have been applied to maintain postharvest quality and reduce the deterioration of guavas during storage.

The potential of ultrasonic (US) treatment has been recognized in food industry since the 1970s. It has been used in food processing and extraction due to its cavitation effect [10]. Recently, US application has been accepted as an effective physical treatment for controlling postharvest quality and inducing pathogenic resistance in fruits and vegetables such as tomatoes [11, 12], table grapes [13] and loquats [14]. Many previous works suggested that US could enhance defence mechanisms, antioxidant activities, secondary metabolites biosynthesis [12, 15, 16] as well as strengthen cell walls and membrane structures in plants [17, 18]. It has also been claimed that US treatment for an appropriate time could enhance product quality, reduce chemical hazards, lower energy consumption and be an environmentally friendly technique [10]. However, the investigation of US effects on the postharvest quality of Thai guava fruits has not been reported yet. Thus, the purpose of this study was to investigate the efficiency of US treatment on physiochemical quality and bioactive compounds of 'Kim Ju' guava during short-term storage at RT.

2. Materials and Methods

2.1 Plant materials preparation

Guava (*Psidium guajava* L.) cv. 'Kim Ju' fruits at commercial maturity (100 days after anthesis) were harvested from an orchard in Rachaburi province, Thailand. Then, the fruits were delivered to Laboratory at Department of Agricultural Education, King Mongkut's Institute of Technology Ladkrabang within 3 h. The fruit samples were then screened on the basis of uniform size (180-200 g per fruit) and quality (being without damages and diseases). Afterwards, the selected guava fruits were cleaned by rinsing with chlorinated water and dried at RT for 10 min.

2.2 Treatments

In our preliminary experiment, the effect of ultrasonic (US) treatment at 40 kHz and 150 w, for 5, 10, 15 or 20 min compared with the control treatment (without US) on visual appearance of the fruit during storage at RT (28 ± 2 °C) for 5 d was investigated. The result indicated that the visual appearance of fruit treated with US for 5 min was not different from untreated fruit. Moreover, browning skin was obviously found in the fruit samples treated with US for 20 min. US treatment for 10 or 15 min maintained the visual appearance of the fruit being storage in a superior way than other treatments (data not shown). Therefore, US treatment for 10 min was selected to study its effect on physicochemical quality of 'guavas during short-term storage at RT. In this study, the US treatment was operated using an ultrasonicator at 40 kHz and 150 w (GT-1860QTS, China). The guava fruits were treated for 10 min and the fruit samples dipped in water for 10 min were used as negative control samples. After treatment, each individual guava fruit was wrapped in a LLDPE film (commercial plastic film for guava) and then stored at RT for 6 d. The physicochemical quality parameters such as visual appearance, superficial colour attributes, total soluble solids (TSS), total acidity (TA), texture, pectin substances, antioxidant activity, phenolic compounds, flavonoids and ascorbic acid contents of both US treated and untreated guava fruits were monitored during storage.

2.3 Visual appearance and superficial colour measurement

The visual appearance of guavas during storage was evaluated by taking photographs. Photographs of the fruit samples were at day 0, 2, 4 and 6. Colour attributes such as *L**, *a**, *b**, hue and chroma values were determined using a Minolta colorimeter CR-300 (Minolta Camera Co., Japan).

2.4 Total soluble solids (TSS) and titratable acidity (TA) measurement

TSS content of guava fruit was determined using a refractometer (ATAGO, Japan). The data were expressed as percentage of TSS (%). TA content was assayed using the titration method of Association of Official Analytical Chemists [19]. The guava juice was titrated with 0.1 N NaOH using phenolphthalein as an indicator. The volume of titrated NaOH was used for calculation. The data were shown as the percentage of citric acid (% citric acid).

2.5 Texture measurement

The texture of guava fruits was measured using a TA Plus Texture Analyzer (Lloyds, England). A cylindrical probe (3 mm diameter) was used to measure the hardness of fruits at the operation rate of 1 mm sec^{-1}. The maximum force (Newton, N) was recorded as hardness.

2.6 Soluble- and insoluble-pectin substances determinations

Acetone insoluble solid (AIS) of guava was prepared according to the method of Supapvanich *et al*. [4]. All gained AIS was then used to extract pectin substances. The soluble pectin in AIS was extracted with 50 mM ethylenediaminetetraacetic acid (EDTA) consisting of 50 mM sodium acetate (pH 7) for 6 h at RT. The filtrate was collected and soluble pectin was precipitated using absolute ethanol. The cake was again extracted with 50 mM sodium carbonate (Na_2CO_3) consisting of 20 mM sodium borohydride ($NaBH_4$) for 24 h at 4±1°C followed by at ambient temperature for 2 h. The insoluble pectin was precipitated using absolute ethanol. Both the soluble and insoluble pectins were assayed using the method described by Ahmed and Labavitch [20]. The data were expressed as gram of galacturonic acid per kilogram fresh weight of sample (g kg^{-1}).

2.7 Antioxidant activity determination

Guava pulp (5g) was extracted with 60 % (v/v) ethanol. The extract was used to determine ferric reducing antioxidant potential (FRAP), and the concentrations of total phenolic compounds and flavonoids. FRAP was assayed using the method of Benzie and Strain [21]. The sample was mixed with FRAP reagent, consisting of acetate buffer (pH 3), 10 mM 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) and 20 mM ferric chloride hexahydrate in the ratio of 10:1:1. The mixture was incubated at RT for 30 min and the absorbance at 630 nm wavelength was then recorded. FRAP was computed and expressed as mmole Trolox equivalents per kg fresh weight of sample (mmol kg^{-1}).

2.8 Total phenols and flavonoids contents determinations

The concentrations of total phenols and flavonoids were assayed using the methods described by Slinkard and Singleton [22] and Jia *et al*. [23], respectively. Total phenol determination was begun when 1 ml of the extract was reacted with 1 ml of 50 % (v/v) Folin-Ciocalteu reagent and 2 ml of saturated Na_2CO_3 solution. The absorbance at 750 nm wavelength was measured. The data was calculated and expressed as g gallic acid per kg fresh weight (g kg^{-1}). Flavonoids determination was initiated when 0.25 ml of the extract was mixed with 1.25 ml of distilled

water and 0.075 ml of 0.5 % $NaNO_2$, and the mixture was then incubated for 6 min. After incubation, 0.15 ml of 10 % $AlCl_3$-$6H_2O$ was added into the mixture and left for 5 min before 0.5 ml of 1.0 M NaOH was added. Absorbance at 510 nm wavelength was measured and the data were computed and shown as mg catechin equivalents per kilogram fresh weight of sample (mg kg^{-1}).

2.9 Ascorbic acid determination

A 5 g sample of guava was extracted with cold 5% metaphosphoric acid. Ascorbic acid concentration was determined using the method described by Hashimoto and Yamafuji [24]. The 0.8 ml of extract was well-mixed with 0.4 ml of 2 % 2,6-dichlorophenolindophenol and then 0.4 ml of 2 % thiourea and 0.4 ml of 1 % dinitrophenol hydrazine were added. The reaction was incubated at 37 °C for 3 h and 2 ml of 85 % H_2SO_4 was then added. Absorbance at 540 nm wavelength was measured and the data were expressed as g ascorbic acid per kilogram fresh weight of sample (g kg^{-1}).

2.10 Statistical analysis

The presented data were the mean of 4 fruit samples (n = 4) with standard deviation (SD). T-test was used for statistical analysis. Differences at $P < 0.05$ were considered as significant.

3. Results and Discussions

3.1 Visual appearance and superficial colour attributes

Figure 1 shows the visual appearance of 'Kim Ju' guava fruits treated without and with US during storage at RT for 6 d. The US treatment inhibited fruits rotten and retained desirable visual appearance during storage. The rotten fruits were detected in the control at day 4 of storage, whilst no rot was found on the US treated fruits over the storage. Brown flecks occurred evidently on the control fruits' skin after storage for 4 days but were not found on the skin of US treated fruits over the storage period. The overall colour appearance of both control and US treated fruits did not change markedly during the storage. Table 1 shows that no significant differences in colour attributes between control and US treated fruits were found throughout the storage. L^* value tended to decrease, while a^* and hue values remained constant; b^* and chroma values increased slightly in the both treatments during storage. The negative values of a^* and hue exhibited the green colour of guava fruit which were concomitant with the visual colour appearance shown in Figure 1. We also found that no significant differences in colour attributes existed between control and US treated fruits over the storage ($P > 0.05$). This s suggested that US treatment did not affect colour of the fruit skin; however, it reduced fruit decay and brown flecks on fruit skin during storage at RT for 6 day. Cao *et al*. [25] suggested that US destroys or removes contaminated microorganisms by cavitation effects. The cavitation provides free radicals in aqueous medium which attack the cell wall structures of microorganisms leading the weakening of cell wall and cell injury. Previous works reported that US treatment markedly reduced the numbers of bacteria, yeasts and mould in postharvest commodities and in turn alleviated deterioration incidence and prolonged commercial shelf-life [10, 26]. Moreover, da Silva and Dobránszki [27] suggested that US also induces antioxidative defence systems in plants. It is commonly recognised that skin browning of fruits and vegetables is caused by the oxidative reaction of polyphenol oxidase (PPO) and phenolic compounds. Lo'ay and Taher [28] suggested that the reaction of PPO and phenolic compounds caused the skin browning of guava fruit. The antioxidants induced by US prevented the oxidative reaction between PPO and phenolic compounds resulting in the inhibition of browning incidence [10]. Furthermore, Nadar

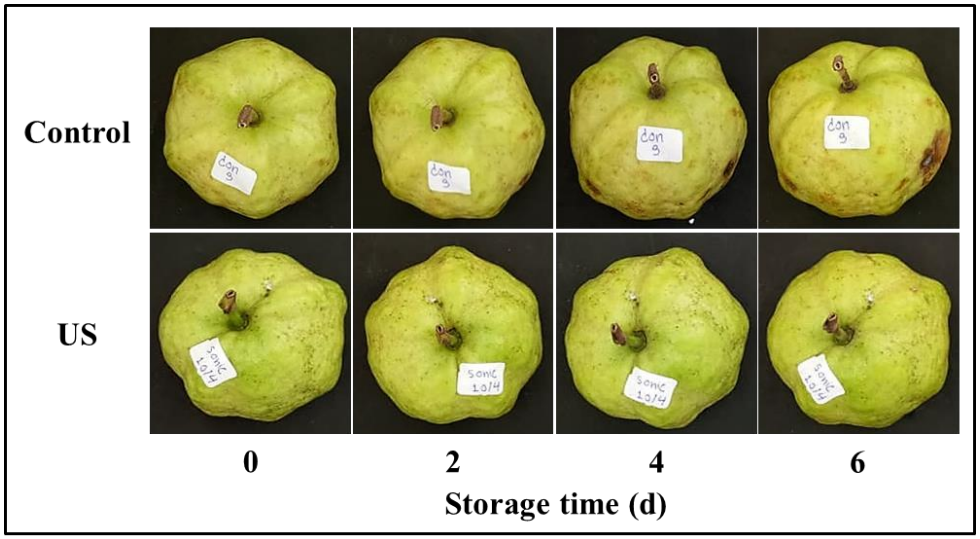

Figure 1. Visual appearance of 'Kim Ju' guava fruits treated with and without US during storage at RT (28±1ºC) for 6 days

Table 1. *L**, *a**, *b**, hue and chroma values of guava fruits treated with and without US during storage at RT (28±1ºC) for 6 days

Storage time (day)	Treatments	Colour attributes				
		L*	***a****	***b****	**hue**	**chroma**
0	Control	63.90±1.30	-7.31±1.44	37.32±0.94	101.28±1.87	37.51±0.84
	US	63.75±1.65	-7.68±0.86	37.25±1.25	101.65±1.25	38.05±1.26
2	Control	63.87±0.33	-7.90±1.24	37.94±1.92	101.21±1.66	39.41±1.37
	US	63.23±1.91	-7.65±0.56	37.53±0.95	101.53±0.98	38.31±0.89
4	Control	62.06±1.48	-7.35±1.76	38.11±1.32	100.94±2.31	38.22±1.33
	US	61.29±0.91	-7.56±0.70	37.80±0.85	101.31±1.04	38.59±0.84
6	Control	61.74±2.00	-7.02±1.96	38.36±1.52	100.41±2.45	39.04±1.75
	US	61.18±1.71	-7.71±0.85	38.26±1.12	101.42±1.42	39.13±1.00

Data are shown as mean ± SD (n = 4).

and Rathod [29] concluded that hydrodynamic cavitation from US treatment causes the damage of enzyme structure. Therefore, the skin browning of 'Kim Ju' guava fruit was inhibited by US treatment due to increased antioxidant system against oxidative browning reaction and hydrodynamic cavitation effect on enzyme structure.

3.2 Weight loss, TSS and TA

The fresh weight loss and taste-related parameters such as TSS and TA of guava fruit are shown in Figure 2. The loss of fresh weight was delayed by US which was significantly lower than that of control fruits over storage time ($P < 0.05$). The loss of fresh weight of control fruits reached

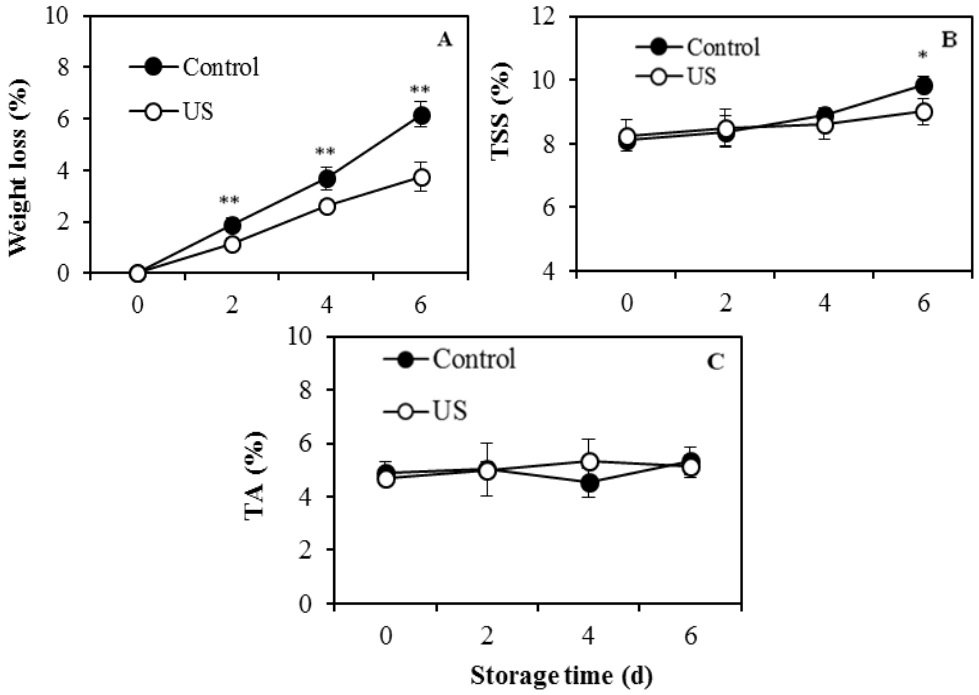

Figure 2. Weight loss (A), TSS (B), and TA (C) of guava fruits treated without and with US during storage at RT (28±1ºC) for 6 days. Vertical bars represent SD of means. Asterisks indicate the differences between treatments [** ($P < 0.01$), * ($P < 0.05$)].

6.16% whereas that of US treated fruits reached 3.74% at the end of storage. The TSS of both control and US treated fruits was not significantly different although a slight increase in TSS was found in control fruits during storage for 4 days. On day 6 of storage, an increase in TSS of both treatments was found and TSS of control fruits was significantly higher than that of US treated fruits ($P < 0.05$). However, US treatment did not significantly affect TA of the guava fruit over the storage. The increased weight loss of guavas was positively concomitant with skin browning during storage, as described by Supapvanich *et al.* [4]. The recent study showed that the lower weight loss of guava fruits was related to the lower level of skin browning. The reduction of weight loss by US might be related to the strengthening of cell walls and cell membranes. Chen *et al*. [16] reported that US reduced lipid peroxidation and dysfunction of plant membrane occurred due to the inducements of antioxidant enzymes and bioactive compounds. Zhi *et al*. [18] reported that US treatment strengthened cell wall structure and reduced membrane peroxidation of jujube fruit during storage period. Lagnika *et al.* [30] also reported that US treatment could delay the increased weight loss of white mushroom during postharvest storage. The increase in TSS of control fruits might be related to the ripening process. It is commonly recognised that guavas are a climacteric fruit for which an increase in TSS has been used as an indicator of the ripening process [1]. Xu *et al.* [10] suggested that hydrodynamic cavitation from US treatment could delay the ripening process by altering ethylene biosynthesis and ethylene signalling pathways.

3.3 Hardness and pectin substances

Fruit softening is an important factor limiting the success of Thai guava fruit industry because the fruit is typically consumed when its texture is still firm and crisp [7]. Figure 3 shows the effect of US treatment on texture (hardness) and pectin substances of 'Kim Ju' guava fruit during storage at RT. The hardness of guava fruits treated with US slightly decreased during

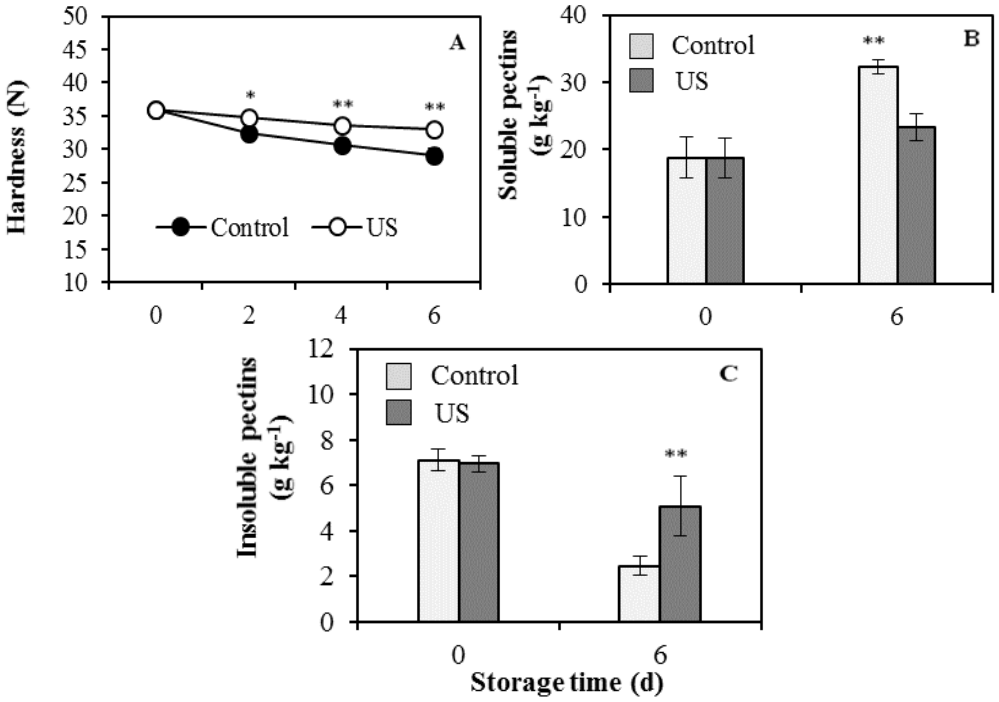

Figure 3. Hardness (A) and the concentrations of soluble pectin (B), and insoluble pectin (C) of guava fruits treated without and with US during storage at RT (28±1 ºC) for 6 days. Vertical bars represent SD of means. Asterisks indicate the differences between treatments [** ($P < 0.01$), * ($P < 0.05$)].

storage, whereas that of control was markedly decreased ($P < 0.01$). The change of texture was related to the changes of soluble and insoluble pectin substances during storage. It is commonly acknowledged that the modification of pectin substances, especially the depolymerisation of pectin polymers, causes fruit softening [7, 9]. Our previous studies found that the softening of 'Kim Ju' guava fruit was accompanied by increased soluble pectin and decreased insoluble pectin during storage [4, 7]. The recent result showed that US treatment obviously delayed the increase of soluble pectin and the reduction of insoluble pectin during storage. The soluble pectin and insoluble pectin of US treated guava fruit were significantly lower and higher, respectively, than those of control fruits ($P < 0.05$). Zhi *et al*. [18] suggested that US induced cellular calcium distribution, resulted in the creation of calcium pectate and inhibition of the generation of water- and CDTA-soluble pectin fractions in jujube fruit. Moreover, the increase of intercellular Ca^{2+} by US treatment was reported by Wang *et al*. [17].

3.4 Antioxidant activity and bioactive compounds

Figure 4 showed the effect of US on antioxidant activity (FRAP) and bioactive compound contents of 'Kim Ju' guavas during storage. It was found that US enhanced FRAP and the total contents of phenols and flavonoids of guavas compared to control fruits. However, there was no effect on ascorbic acid content of fruits during storage. This was in the agreement with the findings of Ding *et al*. [11], who observed that the combined US and slightly acidified electrolytic water treatment had no effect on ascorbic acid content of cherry tomatoes. Both FRAP and total phenols content of US treated guavas were significantly higher than those of control fruit throughout storage time ($P < 0.05$). The flavonoids content of US treated fruits was

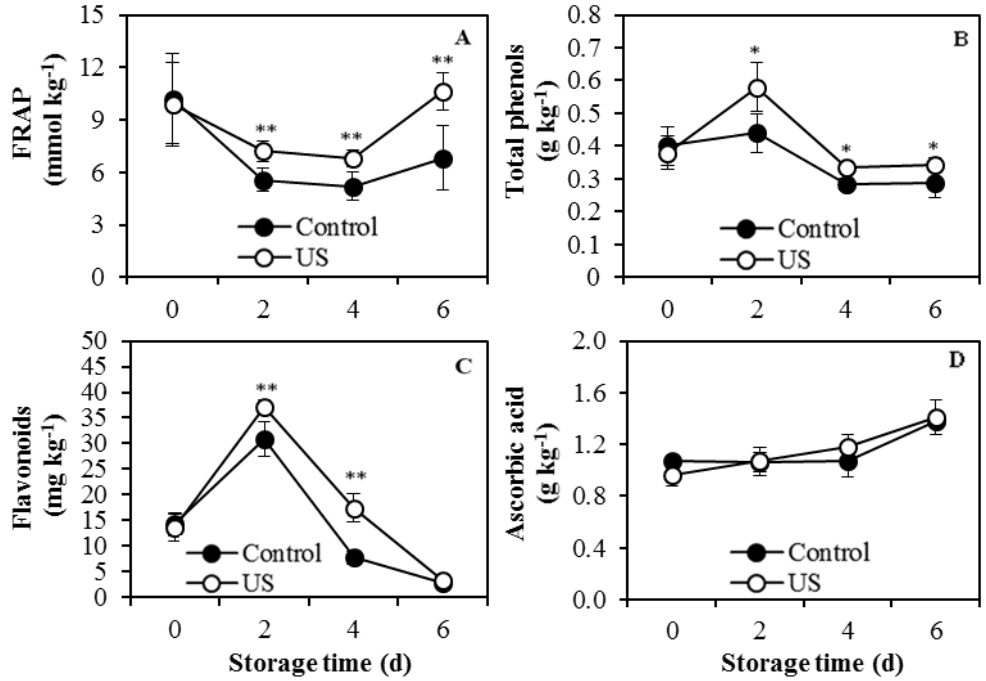

Figure 4. Antioxidant activity (FRAP) (A) and the contents of total phenols (B), flavonoids (C) and ascorbic acid (D) of guava fruit treated without and with US during storage at RT (28±1ºC) for 6 days. Vertical bars represent SD of means. Asterisks indicate the differences between treatments [** ($P < 0.01$), * ($P < 0.05$)].

significantly higher than that of control fruits during storage for the first 4 days of storage ($P < 0.01$) and it then declined and reached to the same level as the control fruits on day 6 of storage. The results indicated that US treatment enhanced antioxidant system and biosynthesis of secondary metabolites such as phenolic compounds and flavonoids contents of guava fruit during storage. It has been recognised that US is claimed as a physical elicitor of plant defence mechanisms [10]. The hydrodynamic cavitation from US creates abiotic stress in plant tissues which stimulates defence-related genes expression and the biosynthesis of secondary metabolites in order to protect against stressors [16]. Wu and Lin [15] and Chen *et al*. [16] proved that US treatment induces defence mechanism by stimulating phenylpropanoid pathway. It is widely recognised that phenylalanine ammonia lyase (PAL) is the key enzyme inducing the pathway. The effect of US treatment at hermetic dosage increases PAL activity and bioactive compounds including phenolic compounds and flavonoids, and this has been reported for tomatoes [11], fresh-cut pineapples [31], table grapes [13], and sweet potato slices [32].

4. Conclusions

The US treatment at the frequency of 40 kHz and the power of 150 w for 10 min could maintain desirable visual appearance, inhibit decay incidence and reduce increased weight loss of 'Kim Ju' guava fruits during storage at RT (28 ± 1 ºC). The US treatment could retard the increment of soluble pectin and the reduction insoluble pectin leading to the retention of fruit texture. Moreover, FRAP and the contents of total phenolic compounds and flavonoids were enhanced by US treatment. However, US treatment had no effect on colour attributes, TSS, TA and ascorbic acid content of the guava fruits. Thus, the US treatment is a potential postharvest

approach maintaining quality and enhancing nutritional values of 'Kim Ju' guava fruits during short term storage at RT.

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