Effect of ultrasound pre-treatment on the physical, microbiological, and antioxidant properties of *calçots*

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Abbreviations

ΔE^*: Colour difference; BI: Browning Index; DPPH$^{\cdot}$ 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power; h^o: Hue angle; TAC: Total Antioxidant Capacity; TPC: Total Phenolic Content; US: Ultrasound.

Abstract

The effect of ultrasound (US) treatment (40 kHz, 250 W) for 0, 10, 25 and 45 min on the physical and microbiological quality, total antioxidant capacity (TAC) and total phenolic content (TPC) of *calçots* (*Allium cepa* L.) was evaluated. Moreover, the effect of roasting (270 °C, 8 min) and *in vitro* simulated digestion on the antioxidant properties was studied. Overall, US treatment had no effect of the physical quality and antioxidant properties of *calçots* regardless the treatment time, while thermal processing produced an increase on the TAC and maintenance in TPC. Furthermore, the digestion process caused a remarkable decrease on the TAC and TPC, but that decrease was higher in roasted than in fresh samples. The microbial load of all US-treated fresh samples was below 6 log (cfu g^{-1}) and a decrease of 1-log reduction was observed after treating for 45 min. Those results indicated that US pre-treatment had no negative effects on the quality of *calçot* while produced a decrease on the microbial load at high processing times.

Keywords: *Allium cepa* L.; thermal processing; gastrointestinal digestion; antioxidant activity; novel technologies

1. Introduction

Calçots (*Allium cepa* L.) are the immature floral stems of second-year onion resprouts of the 'Ceba Blanca Tardana de Lleida' onion landrace. The singularity of the production of this product has helped to confer protected status from the European Union and 'Calçot de Valls' being awarded with the Protected Geographical Indication (EC No 905/2002) (Simó et al. 2013; Zudaire et al. 2017). An increased demand and interest for *calçots* has motivated researches to explore new postharvest techniques such as minimal processing or ultrasound (US) treatment, thus maintaining their physical, microbiological, and nutritional quality.

Thermal pasteurization and sterilization are two common techniques used for the inactivation of microorganisms in food products. However, the effectiveness of those methods is based on long exposure time and high temperatures, which generally results in a deterioration in functional properties, sensory characteristics, and nutritional value (Piyasena et al. 2003). In recent years, emerging non-thermal technologies, such as high pressure, pulsed electric fields, ultraviolet light, intense pulsed light, and US treatments, have been widely studied for application in food industry (de São José et al. 2014). High energy (high power, high-intensity) US are usually applied in the food industry with frequencies ranging between 20 and 100 kHz. This technology has become an attractive option for food processors because only consumed a fraction of the time and energy normally need for traditional processes, reduces processing cost, guarantees food safety, improves food quality, reduced chemical and physical risks, and is considered environmentally friendly (Awad et al. 2012; Chemat et al. 2011; Wang et al. 2015; Welti-Chanes et al. 2017).

Previous studies suggested US processing as a promising technology if it used as an auxiliary pre-treatment to sanitizers in reducing initial microbial populations of foods (Ding et al. 2015). However, the effect of US on the total antioxidant capacity (TAC) of food is a controversial issue. On the one hand, the generation of reactive oxygen species such as hydroxyl radicals could affect the quality of some foods by reducing the TAC (Kentish and Ashokkumar 2011). On the other hand, those species could impose oxidative stress to fresh products and hence, induce the TAC of fruits and vegetables (de São José et al. 2014). For example, the application of US (20 kHz, 400 W) for 10 min had no remarkable effect on the TAC and total phenolic content (TPC) of mushrooms (Lagnika et al. 2013). However, TPC of minimally processed pineapples increased after US treatment (37 kHz) at 25 or 29 W for 10-15 min (Yeoh and Ali 2017).

Many vegetables including *calçots*, onion, or carrots can be either eaten raw or after cooking. *Calçot* are usually eaten after roasting process. Culinary processes produce significant changes such as degradation of thermolabile compounds and formation others due to heat-induced chemical reactions. Roasting could affect phenolic compounds and, consequently the TAC of foods (Juániz et al. 2016). Furthermore, the TPC and TAC of fruit and vegetables could also be affected by the human digestion process. During gastrointestinal digestion, polyphenols could suffer changes due to their interaction with other food components, degradation or metabolization. These structural changes could affect both their uptake and bioactivity and hence, the TAC (Bouayed et al. 2012).

The objective of this study was to evaluate the effects of US processing for either 10, 25, or 45 min on the physical and microbiological quality, TPC, and TAC of raw and roasted *calçots*. Moreover, an *in vitro* simulated gastrointestinal digestion of both raw and roasted samples was carried out to evaluate the resistance of the TAC and TPC to gastrointestinal digestion.

2. Material and Methods

2.1 Plant Material

Calçots were provided by the 'Cooperativa Agricola Valls' (Tarragona, Spain) at commercial size. The *calçots* had the European quality label PGI 'Calçot de Valls' establishing that their diameter and size are within the legal ranges (D.A.R.P. 2009). Samples cultivated in northeast Spain (41º13'47''N, 01º13'12''E) during the crop growing seasons of 2016 and 2017. Pre-conditioning was conducted according to the study of Aguiló-Aguayo et al. (2015) which consisted of cutting roots and external leaves from the edible part as well as removing the outer peel. Fresh *calçots* were immersed in a 10 L bath which contained 100 mg L^{-1} of sodium hypochlorite at room temperature under continuous agitation for 60 s. Samples were further rinsed with tap water for 1 min, dried at room temperature, and labelled as Control.

2.2 Sonication

Eight *calçots* for each time and repetition were directly immersed in a sonicator bath (Frequency 40 kHz, Power 250 W, JP SELECTA S.A., Barcelona, Spain) and the treatment time (0, 10, 25, 45 min) was varied for each batch. The surface of water (tap water) in the bath was kept at the same level during each experiment but without temperature controller (initial temperature 17 ± 1 °C). All samples were weighed before and after US treatment. All samples were dried at room temperature. On each treatment time and repetition half of fresh-cut *calçots* were taken to firmness, colour and total aerobic count measurements. The rest were roasted as Zudaire et al. (2017) described. Briefly, calçots were roasted at 270 °C for 8 min using a Self Cooking Center (Mod SCC WE 101, Rational AG, Landsberg am Lech, Germany) and then, cooled into a blast chiller (Infrico, Cordoba, Spain) until they reached 3 °C. After conducting those assays, both fresh and roasted samples were crushed, powered and frozen with liquid nitrogen and stored at −80 °C for nutritional analysis and gastrointestinal digestion.

2.3 Colour

The colour of the white shaft was measured with a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan). Colour was measured using CIE L*, a*, b* coordinates with illuminant D65 which approximates to daylight and 10° observer angle. L* defines the lightness, and a* and b* define the red-greenness and blue-yellowness, respectively. These values were used to calculate the browning index (BI) and hue angle (h^o) as previously described by Liu et al. (2016) and Colás-Medà et al., (2016), respectively. Furthermore, difference from the control (ΔE^*) was calculated following the methodology described by Wibowo et al. (2015).

2.4 Firmness

To assess changes on texture, firmness (N) was measured at 5 cm from the roots set in transversal position using the TA.TX2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, England) attached with a Warner-Blatzler blade (HDP/BSK: Blade set with knife). The sample was placed into the press holder, and then the blade was moved downwards at different rates: pre-test rate: 5 mm s^{-1}; test rate: 1 mm s^{-1}; post-test rate: 10 mm s^{-1} to 60 mm below the bottom of the holder. Data acquisition rate was 200 pulses per sec.

2.5 Dry matter determination

Due to differences in water content between fresh and roasted samples, total antioxidant capacity and total phenolic content calculations were made on a dry weight (dw) basis. For determination of DM content, 4-5 g of fresh or roasted sample (as triplicate) were dried in a convection oven at 105 °C for at least 40 h until reaching a constant weight.

2.6 Determination of Total Antioxidant Capacity

TAC was determined using two different methods: 2,2-diphenyl-1-picrylhydrazyl ($DPPH^\bullet$) radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. The extraction and assays were carried out according to the methods described by Plaza et al. (2016). Results were expressed on a dry weight (dw) basis as mol of ascorbic acid equivalents per kg.

2.7 Determination of TPC

The extraction and determination of TPC were determined by the Folin-Ciocalteu method (Singleton et al. 1999), following the modifications described by Altisent et al. (2014). Results were expressed on a dry weight (dw) basis as g of gallic acid equivalent per kg.

2.8 Microbial quality

The total aerobic count of *calçots* was analysed in triplicate as described by Alegre et al. (2011). Briefly, the edible part of two *calçots* per treatment were cut and 10 g of were diluted in 90 mL of buffered peptone water (Oxoid LTD, Basingstoke, Hampshire, England) in a sterile bag and homogenized in a masticator paddle blender (IUL Masticator Basic 400 ml, IUL Instruments, Barcelona, Spain) at 250 impact s^{-1} for 90 s in triplicate. Further ten-fold dilutions were made with saline peptone (SP; 8.5 g L^{-1} NaCl and 1 g L^{-1} peptone). Aliquots of serial dilutions were spread in duplicate onto plates with Plate Count Agar (Biokar Diagnostics, Beauvais, France) and were incubated at 30 ± 1 °C for 3 d. The results were represented as log colony forming units (cfu) per gram basis on fresh weight. Microbiological analyses were performed in triplicate.

2.9 *In vitro* gastrointestinal digestion

In vitro gastrointestinal digestion was performed according to the method described by Minekus et al. (2014) with minors modifications (Zudaire et al. 2017). The simulated digestion was performed in triplicate for each treatment for raw and roasted samples. A blank was prepared using only distilled water instead of sample following the same procedure. Results were compared with non-digested samples. Determinations of TAC using both the FRAP and $DPPH^{\cdot}$ methods and TPC were performed after digestion.

2.10 Statistical analysis

All data were firstly evaluated for normal distribution (Shapiro-Wilk W Test) and homogeneity of variance (Levene's Test) of residues. Significant differences between results were calculated by using one-way analysis of variance (ANOVA). In case of non-normality or unequal variances the non-parametric equivalents (Wilcoxon/Kruskal-Wallis Tests) were used. Differences were were significant at $p<0.05$ (95 % confidence level). In case of significant differences, multiple comparison of means was established with the Post Hoc Tukey-Kramer HSD or Student's test. All statistical analyses were performed with JMP 8 software (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1 Effect of US processing on physicochemical and antioxidant parameters

The colour of a food is an important freshness-related attribute for consumers and colour changes in a food product may affect their overall acceptability (Pingret et al. 2013). Previous studies suggested that US processing could affect the colour attributes of fruit and vegetables (Alexandre et al. 2012; Fava et al. 2011). However, in the current study, no significant differences were observed in colour parameters of *calçot* samples after sonication (Table 1). Birmpa, Sfika, & Vantarakis (2013) reported significant colour changes in lettuce leaves after US processing (37 kHz, 30 W L^{-1}) for 30, 45, or 60 min. The authors of that study suggested that a significant non-enzymatic browning could be responsible for the observed colour changes. The ΔE^* combines the change in L^*, a^*, and b^* values to quantify the colour deviation from a standard reference sample. Those samples with $\Delta E^* > 3$ display a visible colour deviation (Wibowo et al. 2015). As expected, and shown in Table 1, US-treated *calçots* showed a $\Delta E^* < 3$. Moreover, BI values of all samples were similar and there were no significant differences ($p<0.05$) among them. Similar results were obtained previously after US processing (40 kHz, 500 W) of strawberries (do Rosário et al. 2017).

In addition, appearance and texture changes are two key characteristics determining the acceptability of fresh-cut fruit and vegetables (Toivonen and Brummell 2008). The texture of a food treated by US can be determined by the structure changes of proteins and enzymes during sonication (de São José et al. 2014). In the current study, as shown in Table 1, no significant differences were observed between the firmness and weight of the control and US-treated samples ($p<0.05$). Results were comparable to those previously reported by Ding et al. (2015), who observed that the firmness of strawberries after US (40 kHz, 240 W) treatment for 10 min did not change significantly. In addition, Alexandre et al. (2012) observed a higher firmness retention (16 %) in US-treated (2 min, 15 ± 2 °C, 35 kHz, 120 W) strawberries when compared to water-washed strawberries.

Besides physical attributes of foods such as colour or firmness, US treatment could affect minor components associated with TAC and phytochemical content. In the current study, two methods, DPPH and FRAP, were used to investigate the changes in total TAC of *calçots* after US treatment. Antioxidant capacity of *calçots* before and after processing are shown in Figure 1. Although higher treatment times resulted in a significant decrease in the TAC of the samples (data not shown), US processing for either 10, 25, or 45 min had no effect on the TAC of *calçots* (p<0.05). Results obtained using the FRAP were in line with those obtained using the $DPPH^{\cdot}$ method. Results obtained herein were in agreement with those reported by Wang et al. (2015) who showed that US treatment (8 min, 25 °C, 20 kHz, 106.19 W L^{-1}) had no effect on the TAC of cherry tomatoes. Similar results were also reported after processing of eggplant (Colucci et al. 2018). However, Muzaffar et al. (2016) and Gani et al. (2016) recently reported an increase of TAC in US-treated (25 °C, 33 kHz, 60 W) at different times (0, 10, 20, 30, 40 and 60 min) cherries and strawberries when compared to the untreated samples.

The TPC of the control and US-treated *calçots* is shown in Figure 1. In the current study, treating for either 10, 25, or 45 min did not affect the TPC of the samples when compared to the untreated control (p<0.05). Results were in agreement with those obtained by Santos et al. (2015) who reported that both TAC and TPC of fresh-cut mango were maintained after US processing (25 °C, 25 kHz, 55 W L^{-1}) for 30 min. Previous authors observed a decrease in the TPC of US-treated fruit and vegetables caused by a oxidation due to hydroxyl radicals formed by cavitation (de São José et al. 2014; Rawson et al. 2011). However, Yeoh & Ali (2017) showed that the TPC of fresh-cut pineapple was increased after processing at 25 and 29 W for 10-15 min. The calculated TPC of the untreated and US-treated *calçots* correlates well with the observed TAC before and after processing.

3.2 Effect of thermal processing on the nutritional quality of *calçots*

Calçots are generally eaten cooked after roasting. However, vitamins, phenolic compounds, and other health-promoting compounds have been shown to be heavily lost during thermal processing

(Kapusta-Duch et al. 2016; Soares et al. 2017). The effects of thermal processing on the TAC and TPC of *calçots* are shown in Figure 1. Overall, TAC of all samples increased after roasting ($p<0.05$). In the same way, Juániz et al. (2016) reported that TAC of chopped onions increased after cooking (150 °C for 10 min + 110 °C for 5 min). In summary, the increase of TAC after roasting (270 °C, 8 min) could be due to: (1) liberation of high amount of antioxidant compounds due to thermal destruction of cell walls and sub cellular compartments; (2) production of antioxidant compounds with high radical scavenging activity; (3) suppression of oxidation capacity of antioxidant compounds due to the thermal inactivation of oxidative enzymes; (4) production of new no-nutrient antioxidants or the formation of new compounds such as Maillard reactions' compounds which could have antioxidant activity (Jiménez-Monreal et al. 2009; Morales and Babbel 2002).

Moreover, there were no significant differences ($p>0.05$) between TPC of fresh and roasted *calçots* (270 °C, 8 min) at each processing time. However, Sharma et al. (2015) reported that heating at 80 °C, 100 °C, and 120 °C for 30 min increased and at 150 °C for 30 min decreased the total phenolic content for all studied onion varieties. Furthermore, Guillén et al. (2017) showed that cooking (90-100 °C) reduced the initial phenolic content in broccoli, green beans, artichokes and carrots. Notwithstanding, Rawson et al. (2013) reported that the decrease observed in total phenolic content was higher in boiled (30 min) than in roasted (160 °C, 15 min) fennel slices.

3.3 Effect of US processing on the microbiological quality of *calçots*

There are indications that suggest that US can be used in the food industry, alone or associated with chemical sanitizers, to remove dirt and food residues as well as to inactivate microorganisms from the surfaces of fruit and vegetables (de São José et al. 2014). Microbial inactivation occurs because of cavitation. In the current study, processing for 10 min did not significantly reduce the total aerobic count in the US-treated *calçots* when compared to the untreated samples (Figure 2). However, US processing for 45 min significantly reduced the microbial load (around 1.0-log) of the samples ($p<0.05$). In all cases, the microbial load was not higher than 6 log (cfu g^{-1}). Bilek &

Turantaş (2013) recently suggested that US processing for 10 min, alone or in combination with other strategy, is generally enough to decontaminate fruit and vegetables. Indeed, Ding et al. (2015) reported that US treatment (40 kHz, 240 W) for 10 min removed 0.71 log cfu g^{-1} for total aerobic bacteria on cherry tomatoes. In the same way, Cao et al. (2010) observed that numbers of aerobic microorganism of strawberries decreased from 2.15 ± 0.02 to 1.49 ± 0.01 log_{10} cfu g^{-1} after US treatment (20 °C, 40 kHz, 350 W) for 10 min.

3.4 Resistance of TAC and TPC to a simulated gastrointestinal digestion

In reference to evaluation the biological activity of *calçots* is much more relevant to know TAC and TPC potentially available for further intestinal absorption and/or protection than the quantification in the food matrix (Carbonell-Capella et al. 2014). Results obtained herein suggested that the TAC and TPC were statistically lower after gastrointestinal digestion when compared to the control (p<0.05; Figure 3). Similar results were reported by Ramírez-Moreno et al. (2018), where TAC and TPC of blackberry juice treated with US (20 kHz, 1500 W) at different times (0, 15 and 25 min) and amplitudes (60 and 80 %) decreased drastically after *in vitro* digestion. Recent studies have evaluated the effect of US treatment on the bioaccessibility of other compounds such as lycopene. For example, Anese et al. (2013, 2015) studied the effect of US treatment on the bioaccessibility of lycopene of tomato pulp. Despite the high decrease observed in TAC values, control (0 min) and US-treated samples (10 and 25 min) presented lower decrease (around 60 %) than roasted samples (70-90 %). The same tendency was observed in TPC values and *calçots* (raw or roasted) treated for 10 min presented the lowest values (around 70 %). The observed differences could be due to the sensitivity and instability to the pH changes and enzymatic activity during *in vitro* digestion of antioxidant compounds formed in the thermal processing. In the recent study carried out by de Lima et al. (2017), the effect of three different cooking methods (boiling, steaming and microwave) on the bioaccessibility of TAC and TPC of cassava. In that study a drastic decrease of TAC and TPC after *in vitro* digestion was observed and the bioaccessibility was similar in all studied samples. Recent studies have evaluated the effect of different cooking treatment on the bioaccessibility of other compounds. For example, (Palmero et al. 2014) studied the effect of thermal treatment on the bioaccessibility of β-carotene of orange carrots and lycopene of red carrots and tomatoes. The vast majority of research on roasting and subsequent digestion has been carried out with cereals or coffee/cacao beans (Ribas-Agustí et al. 2017).

4. Conclusions

The physical and microbiological quality and antioxidant capacity of fresh-cut *calçots* after ultrasound treatment was measured and those samples were also roasted (270 °C, 8 min) and digested. Minimally processed *calçots* pre-treated with ultrasounds (40 kHz, 250 W) for 10, 25 or 45 min retained colour, firmness and weight after processing. Ultrasound pre-treatment had no effect on the antioxidant properties of fresh-cut *calçots*, but both the thermal process (270 ° C, 8 min) and the *in vitro* digestion produced a considerable reduction. Although microbial load of all samples was lower than 6 log (cfu g^{-1}), only a decrease could be observed in those samples treated for 45 min. Therefore, pre-treatment with ultrasound showed potential to be used as a complementary treatment in the food industry. It is necessary to emphasize that this study was a first step to optimize the treatment conditions. Additional studies into the effect of ultrasound on the enzymatic activity in this type of fresh-cut vegetables should be undertaken in future works.

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Figure Captions

Fig. 1. Effect of US and thermal processing on the TAC measured using the DPPH$^{\cdot}$ (A) and FRAP (B) methods and on the TPC (C) of US- and thermally-treated *calçots*.

Lower case letters indicate significant differences between fresh samples (black bars) and capital letters indicate significant differences between roasted (270 °C, 8 min) samples (grey bars). * indicates significant differences between fresh and roasted samples. The criterion for statistical significance was $p<0.05$. The error bars represent the standard errors of the mean of three independent measurements.

Fig. 2. Effect of US processing on the total aerobic count of fresh-cut *calçots*.

Lower case letters indicate significant differences between samples. The criterion for statistical significance was $p<0.05$. The error bars represent standard errors of the mean of independent measurements.

Fig. 3. Resistance of TAC assessed using the DPPH (A) and FRAP (B) method and TPC (C) of US- and thermally-treated *calçots* to a simulated gastrointestinal digestion.

Lower case letters indicate significant differences between samples (grey bars) after *in vitro* simulated digestion. * indicates significant differences between undigested (black bars) and digested samples (grey bars). The criterion for statistical significance was $p<0.05$. The error bars represent the standard errors of the mean of three independent measurements.

Table 1. Colour parameters, firmness, and weight of untreated and US-treated *calçots* (fresh). Values represent the means of independent experiments ± standard deviation. Different letters in the same column indicate significant differences between samples (p<0.05).

Sample	*h*°	BI	Δ*E**	Firmness (N)	Weight (g)
0 min (control)	104.54 ± 3.31^{a}	7.50 ± 1.87^{a}	-	138.00 ± 36.94^{a}	52.89 ± 14.04^{a}
10 min	103.46 ± 2.28^{a}	7.41 ± 2.20^{a}	4.70 ± 3.08^{a}	123.08 ± 41.80^{a}	54.73 ± 13.91^{a}
25 min	105.79 ± 3.19^{a}	6.41 ± 1.65^{a}	5.93 ± 4.86^{a}	102.08 ± 33.73^{a}	51.23 ± 16.56^{a}
45 min	105.00 ± 2.78^{a}	7.28 ± 1.77^{a}	4.04 ± 2.48^{a}	121.95 ± 30.93^{a}	56.27 ± 15.17^{a}

Figure 1

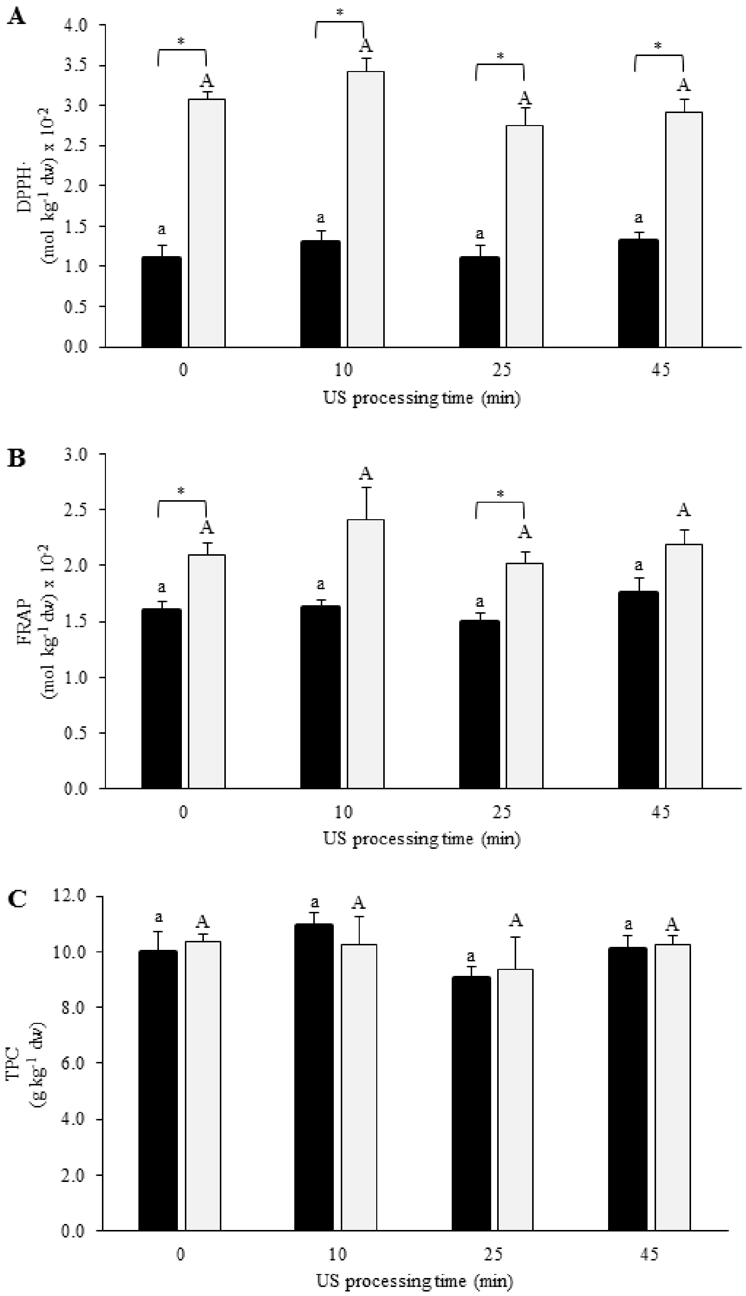

A
B
C
DPPH·
(mol kg-1 dw) x 10-2
FRAP
(mol kg-1 dw) x 10-2
TPC
(g kg-1 dw)
US processing time (min)
0
10
25
45
a
A
*

Figure 2

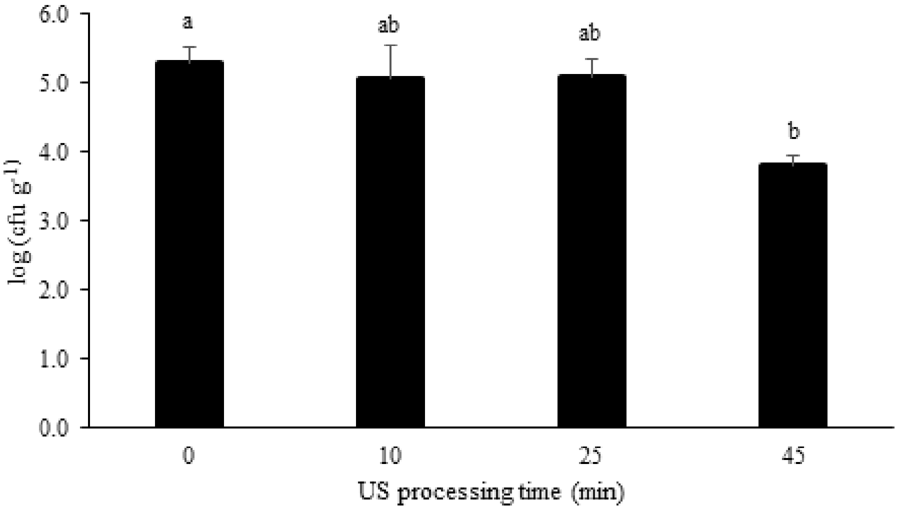
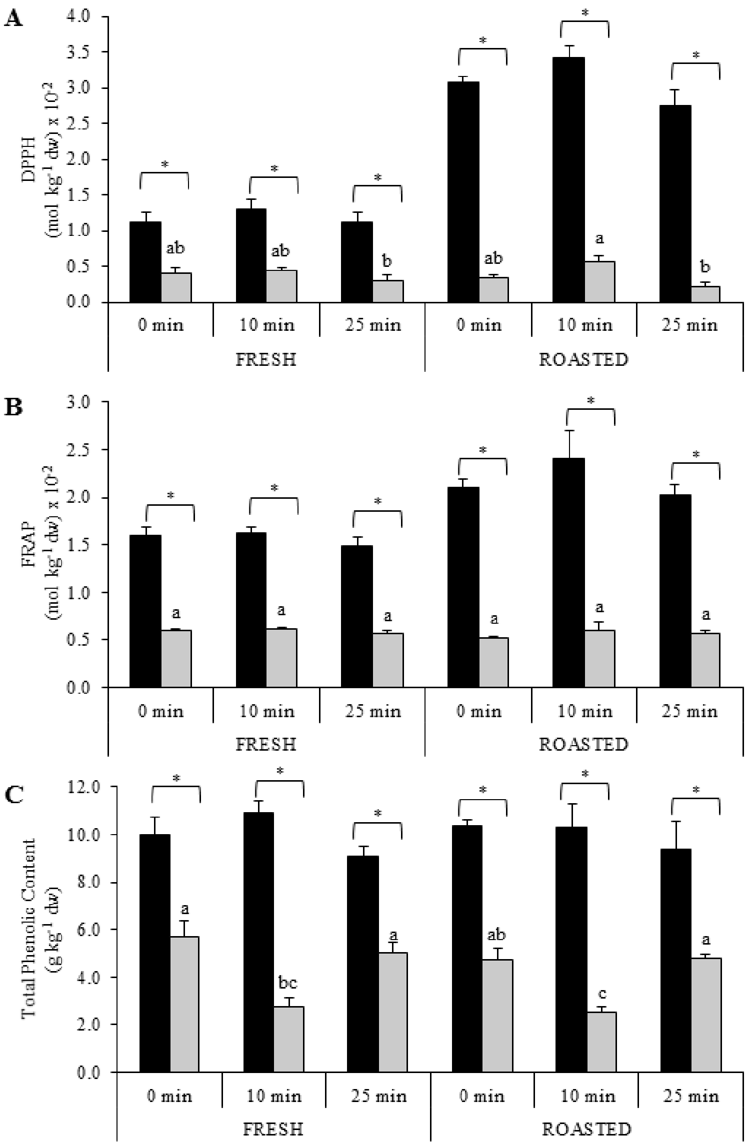

6.0
5.0
4.0
3.0
2.0
1.0
0.0
log (cfu g-1)
a
ab
ab
b
0
10
25
45
US processing time (min)

Figure 3



A
DPPH (mol kg-1 dw) x 10-2
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
ab
ab
b
a
0 min
10 min
25 min
FRESH
ROASTED
B
FRAP (mol kg-1 dw) x 10-2
C
Total Phenolic Content (g kg-1 dw)
12.0
10.0
8.0
6.0
4.0
2.0
bc
c