FOOD

RESEARCH

Effect of high pressure processing on the microbiological, physicochemical and enzymatic properties of jackfruit (*Artocarpus heterophyllus* L.) bulb

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Abstract

The effect of high pressure processing (HPP) on the microbiological, physicochemical and enzymatic properties of jackfruit bulbs at different pressures and holding times was studied. The pressure and holding time used in this study were 300, 400, 500 and 600 MPa at 3, 5, 10 and 15 min, respectively. The aforementioned treatments significantly ($p<0.05$) reduced the microbial load to non-detectable level. However, the HP-treated samples exhibited no significant differences ($p>0.05$) in terms of the proximate composition and ΔE indicator of total colour difference. HPP significantly ($p<0.05$) increased the hardness and chewiness of the treated samples. In terms of enzymatic property, polygalacturonase (PG) and pectin esterase (PE) contents were significantly ($p<0.05$) reduced by HPP. These results suggested that HPP had successfully inactivated the vegetative microorganisms and at the same time, retained the physicochemical properties of the jackfruit bulbs.

1. Introduction

Jackfruit (*Artocarpus heterophyllus* L.), also known as nangka (Javan and Malay), jacquier (French), khnaor (Cambodia), langka (Philippine), or khanum (Thailand), is one of the local non-seasonal tropical fruits that is widely cultivated throughout the Southeast Asia region. Jackfruit is extensively planted for local and export markets. It is rich in carbohydrate, fibre, potassium and carotene. It has a fibrous, thin, soft and musky flesh, and emits a strong aroma when it ripens (Saxena *et al.*, 2011). However, fresh edible fruit bulbs have a relatively short shelf life once the protective outer layer is removed. For instance, fresh edible jackfruit bulbs stored at chilled temperature only have a shelf life of 7 days (Farheen *et al.*, 2014). The factors contributing to the short shelf life of fresh fruit include bacteria contamination, storage temperature, humidity, handling methods and air composition surrounding the fruit (Kusumaningrum *et al.*, 2015).

High pressure processing (HPP) is a non-thermal processing method that has the capability to meet the consumer's desire for high quality fresh food that has extended shelf life without any addition of preservatives and additives. HPP is increasingly utilized in food processing as it offers an alternative method to preserving food; via the inactivation of the vegetative cells of microorganisms and enzymes without negatively affecting the textural, nutritional and sensory attributes (Hite, 1899; Smelt, 1998; Oey, Van der Plancken, Van Loey *et al.*, 2008). Notably, the nutrient and flavour components of food remain intact as HPP does not induce any changes to the covalent bonding of food structure molecules (Simonin *et al.*, 2012).

Numerous studies on the effect of HPP along with the application of high temperature have been conducted on different types of fruit purees or vegetable juices, with exceptional results obtained in the context of extending the shelf life and retaining the physicochemical properties of the aforementioned products (Houška *et al.*, 2006; Paciulli *et al.*, 2016). Despite the fact that HPP treatment coupled with high thermal treatment could extend the shelf life of products, the application of heat will invariably induce damaging effects on the products, especially in term of their colour and texture. On the contrary, there were studies reporting that the treatment of HPP in combination with vacuum packaging successfully retained the appealing appearance of fresh-cut peaches, while at the same time extended the shelf life of the peaches to up to 21 days (Denoya *et al.*, 2015). Also, several studies on the inhibition of enzyme activity by HPP were conducted and the results showed that HPP could induce irreversible inactivation of several enzymes which were responsible for unfavourable colour changes in fruit products (Bermejo-Prada *et al.*, 2014; Rao *et al.*, 2014; Chakraborty *et al.*, 2015). Generally, the

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aforementioned studies using HPP, without thermal treatment, showed promising outcomes on the shelf life extension of fruit products. At the same time, the appearance of the fruit products is also retained. However, to the best of our knowledge, the use of HPP on fresh jackfruit bulbs has not been investigated. The importance of this research revolved around the preservation of fresh jackfruit bulbs using HPP without any thermal treatment in order to retain the appearance of fresh jackfruit bulbs. Thus, in this study, our primary objective was to examine the effect of HPP at different pressures (300, 400, 500 and 600 MPa) and holding times (3, 5, 10 and 15 mins) on the microbiological, physicochemical and enzymatic properties of jackfruit bulbs.

2. Materials and methods

2.1 Materials

All different types of agar such as plate count agar, MacConkey agar, eosin methylene blue agar and potato dextrose agar were obtained from Merck (Darmstadt, Germany). Other chemicals such as acids (H_2SO_4, HCl), alkaline (NaOH), salt buffer (NaCl, sodium acetate, borate buffer, potassium phosphate), indicator (2-cyanocetamide, bromothymol blue), solvent (hexane) that were used were of analytical grade (Merck, Darmstadt, Germany). Pectin and polygalaturonic acid were purchased from Sigma-Aldrich (St. Louis, USA).

2.2 Sample preparation

Jackfruits (*Artocarpus heterophyllus*) were provided by Duria Manufacturing Sdn. Bhd. (Kamunting, Perak). The jackfruit bulbs were manually removed from ripened jackfruits. Each product pouch contained approximately 350 g of these bulbs that was vacuum-sealed using nylon cast polypropylene and stored at chilled temperature (4°C).

2.3 High pressure processing method

Nylon-packaged samples were subjected to high pressure treatments of 300, 400, 500 and 600 MPa for 3, 5, 10 and 15 mins at room temperature using a Hiperbaric 120 processing unit (Hiperbaric España, Burgos, Spain). At the end of each treatment, the packaged samples were stored at 4°C prior to microbiological and physicochemical analyses.

2.4 Microbiological analysis

Aerobic mesophilic microorganisms, total presumptive coliform, total presumptive *Escherichia coli* (*E. coli*), yeast and mold loads of jackfruit samples were analysed according to the FDA's Bacteriological Analytical Manual (BAM) standard methods (Tournas *et*

al., 1998; Feng *et al.*, 2002). In short, 25 g of sample was homogenized with 225 mL of 0.1% peptone water and further decimal dilutions were made with the same 0.1% peptone water. Each diluted sample (1 mL) was spread-plate onto a petri dish filled with plate count agar (PCA) and incubated at 37°C for 48 hrs. Total presumptive coliform, *E. coli*, yeast and mould loads were analysed in a similar way using MacConkey agar, eosin methylene blue (EMB) agar and potato dextrose agar (PDA), respectively. All microbial data were expressed as logarithms of number of colony forming units (log_{10} CFU g^{-1}).

2.5 Physicochemical analysis

2.5.1 Proximate analysis

The moisture, crude protein, ash, fiber, lipid and carbohydrate contents were determined according to the AOAC (1995) methods. The moisture content was determined according to AOAC no. 934.06 method. Crude protein content was determined based on Kjeldahl method with a conversion factor of 6.25 (AOAC no. 960.52). Ash content was determined by incineration in a muffle furnace at 550°C (AOAC no. 923.03). Total fiber content was estimated using acid/alkaline hydrolysis of insoluble residues (AOAC no. 962.09). Lipid content was determined by firstly extracting the lipid using an automated Soxtec^{TM} 8000 extraction unit, followed by the gravimetric determination of the total fat content (AOAC no. 960.39). Finally, the carbohydrate content was calculated by deducting all the aforementioned contents from a total of 100%.

2.5.2 Colour analysis

The colour of the samples was measured using a Minolta Chroma Meter CR-410 (Konica Minolta Instrument, Osaka, Japan). The colour was expressed in $L*$ value representing the lightness of the sample, $a*$ value representing the redness of sample and $b*$ value representing the yellowness of the sample. Hue angle, $h*$ represents the relative amounts of redness and yellowness, and was calculated using Equation (1):

$$h^* = tan^{-1}(b^*/a^*) \tag{1}$$

The total colour difference (ΔE) was determined using the following Equation (2):

$$\Delta E = \sqrt{(a^* - a_0^*)^2 + (b^* - b_0^*)^2 + (L^* - L_0^*)^2} \tag{2}$$

where L_0*, a_0*, b_0* are the control values for untreated jackfruit bulbs.

2.5.3 pH analysis

Three jackfruit bulbs from each treatment were homogenized using a blender, MX-896TM (National,

Selangor, Malaysia). The pH of the samples was then determined using a pH meter, PT15 (Sartorius, China).

2.5.4 Texture analysis

The texture of the samples was analyzed using a TA-XT2*i* texture analyzer unit (Stable Micro Systems, Surrey, UK). The parameters were set according to the method described by Caner *et al.* (2008), with slight modifications. Briefly, the following settings were used: pre-test speed 5.0 mm/s, test speed 1.0 mm/s and post-test speed 8.0 mm/s; penetration depth of 4.0 mm and a rest period of 5 s between two cycles; and trigger force 5.0 N. A cylinder probe (2 mm diameter) was used and always returned to the trigger point prior to the second cycle. Measurements were made on three jackfruit bulbs per pack of sample. The values for hardness and chewiness were then calculated using the Exponent Stable Micro Systems version 4.0.13.0 equipment software (Stable Micro Systems, Surrey, UK).

2.6 Enzyme activity

2.6.1 Preparation of jackfruit samples for enzyme assays

The extraction of polygalacturonase (PG) and pectin esterase (PE) were performed as described by Chakraborty *et al.* (2015), and Hagerman and Austin (1986), respectively. In brief, PG was extracted from 3 g of homogenized sample using 30 mL of 1.2 M sodium chloride solution (pH 3) for 30 mins and centrifuged at $16000 \times g$ (Thermo Fisher Scientific, Waltham, USA) for 30 mins at 4°C. Meanwhile, PE was extracted from the homogenized sample using 1.5 M sodium chloride solution for 1 h, and centrifuged at $16000 \times g$ for 30 mins at 4°C. Crude enzyme extract was then obtained from the supernatant, which was further filtered using a 0.8 μm cellulose acetate filter paper.

2.6.2 Polygalacturonase activity

Assay of PG was performed according to the method described by Gross (1982). Briefly, 100 μL of the crude enzyme extract was mixed with 300 μL of 0.3% (w/v) polygalacturonic acid solution prepared in 50 mM sodium acetate buffer at pH 5.5. The mixture was incubated at 35°C for 30 mins prior to the addition of 2 mL of 0.1 M borate buffer prepared at pH 9 and 400 μL of 1% 2-cyanocetamide. Then, the mixture was incubated at 100°C for 10 mins. After 10 mins the mixture was cooled using an ice bath. Then, an aliquot of the mixture was measured for its absorbance at 276 nm using a spectrophotometer (Cary 60 UV-VIS, Agilent Technologies, Santa Clara, USA), against a blank buffer (aforementioned mixture without the addition of crude enzyme extract). A standard curve to calculate the

equivalent reducing sugar produced from crude enzyme was prepared using monogalacturonic acid. PG activity was expressed as the amount of reducing sugar produced per gram of protein (Gross, 1982).

2.6.3 Pectin esterase activity

PE activity was assayed according to the method of Hagerman and Austin (1986). In short, 0.1 mL of crude enzyme extract was adjusted to pH 7.5 and mixed with 2 mL of 0.5% citrus pectin and 0.2 mL of 0.01% bromothymol blue; both prepared with 0.7 mL of 0.0003 M potassium phosphate buffer at pH 7.5. The absorbance of the reaction was measured at 620 nm at 25°C. The PE activity was estimated from the slope of linear portion of the curve of reaction time against absorbance. PE activity unit is defined as the change in absorbance per minute per gram of fresh weight of sample (Hagerman and Austin, 1986).

2.7 Statistical analysis

All experiments were duplicated and all data were subjected to one-way analysis of variance (ANOVA) using Minitab Statistical Software Release 16.1 (Minitab Inc., PA, USA). Statistical significance at $p < 0.05$ was established using Tukey's test to evaluate the differences between mean values.

3. Results and discussion

3.1 Effect of HPP on microbial populations of jackfruit bulbs.

The total plate count, coliform, *E. coli* and yeast and mould counts in untreated jackfruit bulbs were 4.38, 4.91, 3.43 and 4 $\log_{10} CFU$ g^{-1}, respectively. However, for HP-treated samples, the microbial counts were all below the detection limit. After HPP at or over 300 MPa for 3 mins, the counts of microbial populations were significantly ($p < 0.05$) reduced by 4 log cycles. These results are in accordance with the results of Leyva-Daniel *et al.* (2017) who reported that HPP at 600 MPa for 15 min at ambient temperature reduced the microbial load of honey to non-detectable levels. Vega-Gálvez *et al.* (2016) reported non-detectable levels of microbial load after gooseberry pulp subjected to designed experimental high-pressure parameters. Also, Gao *et al.* (2016) reported similar results where HP-treated strawberry samples showed non-detectable total aerobic bacteria and yeasts and moulds. All the aforementioned results of microbial inactivation as reported by a various group of researchers demonstrated the lethality of HPP on microorganisms. According to Linton and Patterson (2000), HPP induced changes in the cell morphology and biochemical reactions inhibited genetic mechanisms and caused protein denaturation, which eventually led to

microbial inactivation. However, some researches revealed that the inactivation of microorganisms via HPP might differ for different species and stages of bacteria growth. As revealed by Daher *et al.* (2017), the bacteria under stationary and dormant phases of growth are pressure labile as compared to those in the exponential growth phase. However, in general, cocci bacteria and spores are more stable towards pressure. Also, as reported by Lima Tribst *et al.* (2009), yeast and mould and vegetative bacteria in fruits are susceptible to HPP inactivation due to the low pH of fruits. However, Basak *et al.* (2002) demonstrated that *Saccharomyces cerevisiae* in orange juice was not inactivated even though the juice was subjected to 400 MPa of high pressure. The author further concluded that the resistance of this microorganism towards pressure increased due to the high concentration of sugar in the product. Thus, it is important to investigate and understand the matrix of different fruits in order to achieve the desired level of microorganisms inactivation. From our study, we believe that a minimum level of pressure and holding time of 300 MPa and 3 min were sufficient to prevent spoilage induced by pathogenic microorganisms, thus securing the shelf life and quality of jackfruit bulbs.

3.2 Physicochemical analysis

3.2.1 Effect of HPP on proximate analysis

In general, there were no significant differences (p>0.05) among untreated and HP-treated jackfruit bulbs in terms of their moisture, ash, fat, crude fibre and carbohydrate contents. These results are similar to the

proximate contents reported by Goswami *et al.* (2011) and Swami *et al.* (2012). As shown in Table 1, the moisture, ash, fat, fibre and carbohydrate contents for untreated samples were 78.54±1.59%, 1.11±0.39%, 0.15±0.08%, 7.16±2.59% and 11.79±4.24% respectively. HP-treated samples exhibited no significant differences (p>0.05) in terms of the aforementioned contents when compared with untreated samples as the covalent bonding of molecule structures in the food system remained intact (Simonin *et al.*, 2012). However, there was a significant decrease in the protein content as the pressure was increased. The protein content for untreated samples was 1.25±0.18%, whereas, for HP-treated samples, the protein content ranged from 0.43±0.15% to 1.05±0.30% (Table 2). The decrease in protein content indicates the denaturation of protein that occurred as the pressure was increased (Kunugi and Tanaka, 2002). In this case, HPP induced conformation changes to protein molecules to a certain degree without breaking the covalent bonding of food structures molecules (Simonin *et al.*, 2012). Thus, only the protein content was affected while the overall quality of HP-treated jackfruit bulbs in terms moisture, fibre, fat and carbohydrate contents were preserved and remained similar to the quality of fresh jackfruit bulbs.

3.2.2 Effect of HPP on colour and pH

The colour for untreated jackfruit bulbs in terms of *L**, *a** and *b** values were 68.13±2.64, 8.14±0.51 and 56.68±2.27, respectively. Hue angles for untreated and HP-treated jackfruit bulbs shown in Table 2 indicate that all samples fell within the yellowish colour region. There

Table 1. Effects of HPP at different pressures and holding times on the proximate content of jackfruit bulbs.

Treatments (MPa/min)	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fibre (%)	Carbohydrate (%)
Control	78.54±1.59[a]	1.11±0.39[a]	1.25±0.18[a]	0.15±0.08[a]	7.16±2.59[a]	11.79±4.24[a]
300/3	80.74±1.65[a]	1.03±0.16[a]	0.77±0.16[ab]	0.10±0.02[a]	9.49±0.92[a]	8.87±3.35[a]
300/5	78.26±1.39[a]	0.98±0.05[a]	0.48±0.08[b]	0.11±0.01[a]	8.84±4.35[a]	11.32±3.32[a]
300/10	79.73±0.20[a]	0.97±0.19[a]	0.72±0.24[ab]	0.11±0.02[a]	8.10±4.78[a]	10.38±4.54[a]
300/15	80.08±0.49[a]	0.93±0.15[a]	0.76±0.30[ab]	0.11±0.01[a]	6.29±1.37[a]	11.84±1.71[a]
400/3	79.35±0.26[a]	0.90±0.16[a]	0.91±0.23[ab]	0.11±0.01[a]	6.79±4.15[a]	11.94±4.03[a]
400/5	79.04±1.60[a]	0.82±0.19[a]	0.53±0.30[b]	0.13±0.02[a]	8.67±3.85[a]	8.83±3.24[a]
400/10	80.09±0.26[a]	1.04±0.10[a]	0.43±0.01[b]	0.10±0.01[a]	5.96±1.45[a]	11.39±1.61[a]
400/15	80.22±0.09[a]	0.83±0.10[a]	0.72±0.15[ab]	0.11±0.01[a]	6.88±1.01[a]	11.25±1.16[a]
500/3	78.73±1.83[a]	0.87±0.09[a]	0.67±0.22[ab]	0.13±0.02[a]	7.92±4.04[a]	11.62±3.37[a]
500/5	78.37±0.82[a]	1.07±0.27[a]	0.68±0.30[ab]	0.10±0.02[a]	5.36±1.05[a]	11.42±0.27[a]
500/10	80.81±0.24[a]	0.84±0.24[a]	0.81±0.16[ab]	0.11±0.01[a]	6.66±1.56[a]	11.77±1.16[a]
500/15	78.61±1.07[a]	1.04±0.24[a]	0.43±0.15[b]	0.12±0.03[a]	9.76±5.10[a]	11.11±1.07[a]
600/3	79.31±0.90[a]	0.86±0.16[a]	1.05±0.30[ab]	0.11±0.01[a]	8.77±4.44[a]	9.13±5.11[a]
600/5	80.78±1.02[a]	0.98±0.08[a]	0.58±0.14[b]	0.12±0.03[a]	9.56±3.76[a]	9.12±3.53[a]
600/10	80.18±0.88[a]	0.94±0.23[a]	0.71±0.13[ab]	0.09±0.02[a]	9.67±3.04[a]	8.40±2.27[a]
600/15	80.85±0.64[a]	0.96±0.06[a]	0.67±0.30[ab]	0.13±0.01[a]	8.87±4.37[a]	8.52±4.37[a]

Values are means ± standard deviation (n = 6). Means with different letter superscript in each column are significantly different (p<0.05).

Table 2. Effects of HPP at different pressures and holding times on the colour (L*, a*, b*, h* and Δ E values), and pH of jackfruit bulbs.

Treatments (MPa/min)	Colour			Hue angle, h* (°)	Δ E	pH
	L* value	a* value	b* value			
Control	68.13±2.64[ab]	8.14±0.51[bcdef]	56.68±2.27[ab]	81.83±0.32[abc]	0.00±0.00	5.27±0.10[a]
300/3	67.03±3.03[ab]	9.59±0.79[abc]	59.68±3.40[ab]	80.86±0.78[bcde]	4.92±2.80[a]	5.10±0.05[b]
300/5	65.38±4.46[b]	9.02±0.50[abcde]	54.85±2.98[b]	80.65±0.53[bcde]	4.26±1.54[a]	5.10±0.03[b]
300/10	68.39±3.03[ab]	7.69±2.04[cdef]	56.07±5.42[ab]	82.23±1.73[ab]	5.55±2.89[a]	5.08±0.02[bc]
300/15	70.18±3.30[ab]	6.89±1.44[ef]	53.35±4.86[ab]	82.61±1.56[ab]	6.13±3.60[a]	5.08±0.02[bc]
400/3	72.09±3.25[a]	6.64±1.02[f]	59.58±3.78[ab]	83.46±0.63[a]	4.58±2.07[a]	5.09±0.02[bc]
400/5	70.69±3.55[ab]	8.00±1.93[bcdef]	59.13±5.25[ab]	82.35±1.49[ab]	6.45±3.26[a]	5.07±0.02[bcd]
400/10	69.22±5.74[ab]	7.35±0.85[def]	55.82±3.47[ab]	82.48±0.89[ab]	5.90±2.97[a]	5.07±0.02[bc]
400/15	72.50±4.53[a]	8.53±0.73[abcdef]	57.54±5.03[ab]	81.55±0.62[abcd]	6.14±2.40[a]	5.04±0.02[bcd]
500/3	67.90±2.55[ab]	8.14±2.13[bcdef]	56.19±3.98[ab]	81.80±1.91[abc]	4.64±2.40[a]	5.06±0.04[bcd]
500/5	69.53±3.12[ab]	10.12±1.50[ab]	57.26±2.12[ab]	79.57±1.39[cde]	4.22±1.80[a]	5.05±0.03[bcd]
500/10	70.54±2.47[ab]	9.51±0.64[abcd]	59.13±3.36[ab]	80.84±0.82[bcde]	5.16±1.74[a]	5.06±0.03[bcd]
500/15	70.75±4.14[ab]	8.89±1.09[abcde]	60.54±3.57[a]	81.60±1.28[abc]	6.39±3.16[a]	5.03±0.02[bcd]
600/3	69.85±2.09[ab]	9.74±1.28[abc]	60.83±4.16[a]	80.93±0.81[bcde]	6.18±2.36[a]	5.03±0.03[bcd]
600/5	65.79±2.99[b]	10.44±0.85[a]	56.93±4.43[ab]	79.57±0.97[de]	5.16±1.56[a]	5.03±0.03[bcd]
600/10	66.66±2.23[ab]	9.30±1.22[abcd]	56.23±4.52[ab]	80.60±1.06[bcde]	4.89±1.98[a]	5.01±0.03[cd]
600/15	65.77±1.71[b]	10.45±0.73[a]	56.41±3.29[ab]	79.46±1.18[e]	4.44±1.69[a]	4.98±0.03[d]

Values are means ± standard deviation (n = 8). Means with different letter superscript in each column are significantly different (p<0.05).
L* value represents the lightness of sample; a* value represents the redness of sample; b* value represents the yellowness of sample; h* represents the relative amounts of redness and yellowness; Δ E represents the total colour difference of sample

were no distinct colour differences observed immediately after the HPP of jackfruit bulbs. This visual observation is in line with the results reported by Oey, Lille, Van Loey et al. (2008), Daoudi et al. (2002) and Ahmed et al. (2005), whereby no visual colour differences were observed immediately after the HPP of fruit-based food products. The visual observation from this study was also coherent with the ΔE values obtained, which were not significantly (p<0.05) different between untreated and HP-treated jackfruit bulbs. The yellow appearance of jackfruits bulbs is due to the presence of many carotenoid pigments within the cell wall (De Faria et al., 2009). As revealed by Oey, Lille, Van Loey et al. (2008), carotenoids are pressure-stable pigments in most plant matrix, thus they remained unchanged when subjected to HP treatment. However, some research work disclosed that carotenoid globules diffused easily through plant tissues after pressure treatment due to the modifications to the cell wall membrane (Vázquez-Gutiérrez et al., 2011; Serment-Moreno et al., 2017). Based on the insignificant ΔE values obtained in this study, we believe that the carotenoid pigments remained intact within the cell wall of jackfruit bulbs after HP treatment. As a result, the colour of HP-treated jackfruit bulbs remained unchanged.

The HPP had significant (p<0.05) effect on the pH of HP-treated jackfruit bulbs. The pH of fresh untreated jackfruit bulbs was 5.27±0.04, whereas the pH for treated jackfruit bulbs varied from 4.98 to 5.10. Previous work by other researchers showed that the pH of HP-

treated fruit beverages decreased along with the increase in pressure due to HPP intensifying the ionic dissociation of water, consequently resulting in the release of H^+ ions (Jayachandran et al., 2015). A similar result was reported by Kaushik et al. (2014), whereby the pH of mango pulp decreased by 0.26 units when subjected to pressure treatment. Notably, the decrease in pH is critical towards the survival and growth of microorganisms. Specifically, a low pH inhibits the growth of neutrophilic microorganisms. As mentioned earlier, the pH of HP-treated jackfruit bulbs in our study showed a decrease in value. We believe that HPP induced disruption on the ionic bonding which subsequently led to an increase in the amount of proton ion released from acid molecules present in the fruit. Eventually, this led to a decrease in the pH value of our jackfruit bulbs.

3.2.3 Effect of HPP on texture

Generally, HPP had significant (p<0.05) effects on the hardness and chewiness of treated jackfruit bulbs (Table 3). The hardness of untreated jackfruit bulbs was 116.18±32.84 g. Meanwhile, the hardness of HP-treated jackfruit bulbs increased by almost three-fold. A similar trend was observed for the chewiness of treated jackfruit bulbs. This was possibly due to the de-esterification chemical reaction induced by HPP on pectin esterase (PE) present in jackfruit bulbs. As disclosed by Oey, Lille, Van Loey et al. (2008) and Tangwongchai et al. (2000), the PE released during HPP induced the demethylation process once it comes into contact with

highly methylated pectin substrate. A gel network with divalent ions was then formed between the de-esterified pectin (low-methoxy-pectin), resulting in an increase in hardness. Thus, in our case, an increase in the treatment pressure eventually led to a tendency of forming a harder and firmer texture in jackfruit bulbs.

Table 3. Effects of HPP at different pressures and holding times on the texture of jackfruit bulbs.

Treatments (MPa/min)	Hardness (g)	Chewiness
Control	116.18±32.84[e]	76.19±34.40[d]
300/3	244.70±56.00[cde]	89.34±47.62[cd]
300/5	268.46±45.11[abcd]	171.55±36.92[ab]
300/10	229.06±90.91[abcde]	131.04±42.37[abcd]
300/15	275.13±98.72[abcd]	162.44±40.65[abcd]
400/3	218.40±38.51[bcde]	102.57±36.09[bcd]
400/5	232.43±82.01[abcde]	134.71± 54.71[abcd]
400/10	304.74±64.23[abc]	159.47± 46.86[abc]
400/15	208.43±42.82[bcde]	107.55±26.57[bcd]
500/3	315.89±33.62[ab]	153.07±41.38[abcd]
500/5	214.98±67.15[abcde]	107.10±43.94[bcd]
500/10	269.83±76.74[abcd]	138.31±28.78[abcd]
500/15	271.95±45.65[abcd]	136.10±24.87[abcd]
600/3	282.97±75.06[abc]	139.88±45.58[abcd]
600/5	337.05±98.24[a]	192.19±57.49[a]
600/10	246.51±41.11[abcd]	163.11±43.66[abc]
600/15	239.50±85.00[abcde]	130.17±42.27[abcd]

Values are means ± standard deviation (n = 6). Means with different letter superscript in each column are significantly different ($p<0.05$).

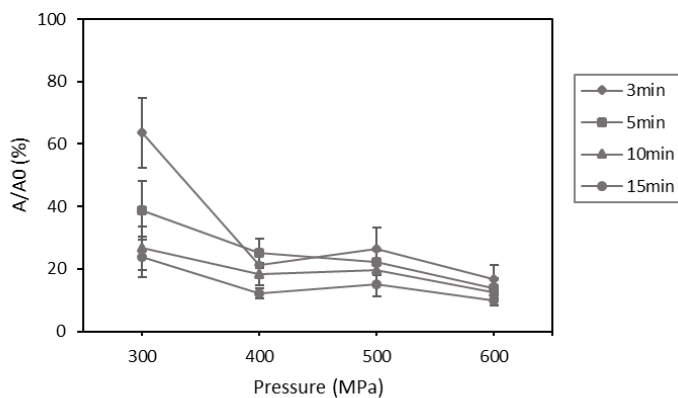## 3.3 Enzyme activity

3.3.1 Effect of HPP on PG activity

Figure 1. Residual PG enzyme activity of HP-treated jackfruit bulbs at: ◆ 3min; ■ 5min; ▲ 10min; ● 15min.

The data shows that HPP significantly ($p<0.05$) reduced the PG enzyme residual activity to 10% when the treatment of 600 MPa for 15 mins was applied (Figure 1). The results are in agreement with those reported by Bermejo-Prada et al. (2014), Fachin et al. (2002), Tangwongchai et al. (2000) reported that the PG present in cherry tomatoes was almost completely inactivated when the cherry tomatoes were subjected to a pressure of 500 MPa at 20°C. In addition, Shook et al.

(2001) concluded that a pressure treatment of 600 MPa at temperatures of 25 and 45°C induced total inactivation of PG present in tomatoes. The inactivation of enzymes via HPP was further explained in the early works of Knorr (1993), in which it was reported that high pressure induced changes in the structural conformation of the enzyme. Ludikhuyze et al. (2001) revealed that the mechanism of enzyme inactivation via HPP is similar to the mechanism of protein denaturation since protein denaturation was a result of conformation changes. The disruption in structural conformation, even to a small degree and particularly at the active site of the enzyme, would eventually lead to the enzyme losing its capability to synthesize biological activity (Tsou, 1986). Thus, from this study, we believe that the enzyme activity of PG from jackfruit can easily be altered by pressure as up to 90% inactivation of PG was achieved within the studied pressures and holding times.
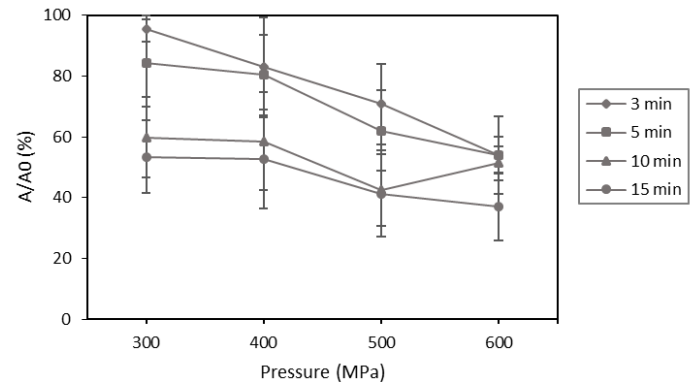
3.3.2 Effect of HPP on PE activity

Figure 2. Residual PE enzyme activity of HP-treated jackfruit bulbs at: ◆ 3min; ■ 5min; ▲ 10min; ● 15min.

Figure 2 shows that HPP significantly ($p<0.05$) reduced the PE enzyme residual activity to 37% at 600 MPa for 15 min. The results are in accordance with those reported by Houben et al. (2012), whereby high pressure treatment of 800 MPa for 10 min at ambient temperature induced up to 30% PE inactivation in tomato puree. Meanwhile, 50% PE inactivation was obtained by Rao et al. (2014) when they subjected their samples (peach juice) to a treatment of 600 MPa for 25 mins at ambient temperature. These results indicated that jackfruit PE appeared to be very pressure-stable even when subjected to 600 MPa high pressure treatment. There were studies reporting that PE from some fruit source is pressure-stable due to the protection of food components present in the fruit composition (Balogh et al., 2004; Katsaros et al., 2017). In other words, PE is more resistant to pressure when embedded in an intact tissue as compared to when it is in its purified form. However, Knorr et al. (2006) revealed that enzymes' resistance to inactivation is very likely attributed to the presence of different isomers of a particular enzyme. Also, from the study of

orange PE which was carried out at a molecular level, it was revealed that HPP alone is insufficient to induce a tertiary structural conformation change in PE from its secondary structure molecule phase (Alexandrakis *et al.*, 2014). This results in PE being able to maintain its substrate-enzyme binding site. Thus, in this context, we believe that the PE from jackfruit is also pressure-stable, whereby the HP-treated jackfruit PE maintained its secondary structure similar to untreated protein molecules. Only partial inactivation of PE enzyme could be achieved within the studied pressures and holding times.

4. Conclusion

HPP successfully inactivated the vegetative pathogenic microorganisms and had no significant effects on the proximate content of jackfruit bulbs. In addition, HPP had no significant (p<0.05) effect on ΔE indicator of total colour difference but significantly (p<0.05) increased the hardness and chewiness of jackfruit bulbs. Also, HPP significantly (p<0.05) reduced the activity of PG and PE, albeit to a varying degree of success.

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References

Ahmed, J., Ramaswamy, H.S. and Hiremath, N. (2005). The effect of high pressure treatment on rheological characteristics and colour of mango pulp. *International Journal of Food Science and Technology,* 40(8), 885-895. https://doi.org/10.1111/j.1365-2621.2005.01026.x

Alexandrakis, Z., Katsaros, G., Stavros, P., Katapodis, P., Nounesis, G. and Taoukis, P. (2014). Comparative Structural Changes and Inactivation Kinetics of Pectin Methylesterases from Different Orange Cultivars Processed by High Pressure. *Food and Bioprocess Technology,* 7(3), 853-867. https://doi.org/10.1007/s11947-013-1087-7

AOAC. (1995). Official methods of analysis of AOAC International. Maryland, USA: AOAC

Balogh, T., Smout, C., Nguyen, B.L., Van Loey, A.M. and Hendrickx, M. E. (2004). Thermal and high-pressure inactivation kinetics of carrot pectin methylesterase: from model system to real foods. *Innovative Food Science and Emerging Technologies,* 5(4), 429-436. https://doi.org/10.1016/j.ifset.2004.06.002

Basak, S., Ramaswamy, H. and Piette, J. (2002). High pressure destruction kinetics of Leuconostoc mesenteroides and Saccharomyces cerevisiae in single strength and concentrated orange juice. *Innovative Food Science and Emerging Technologies,* 3(3), 223-231. https://doi.org/10.1016/S1466-8564(02)00008-5

Bermejo-Prada, A., Van Buggenhout, S., Otero, L., Houben, K., Van Loey, A. and Hendrickx, M. E. (2014). Kinetics of thermal and high-pressure inactivation of avocado polygalacturonase. *Innovative Food Science and Emerging Technologies,* 26, 51-58. https://doi.org/10.1016/j.ifset.2014.05.005

Caner, C., Aday, M.S. and Demir, M. (2008). Extending the quality of fresh strawberries by equilibrium modified atmosphere packaging. *European Food Research and Technology,* 227(6), 1575-1583. https://doi.org/10.1007/s00217-008-0881-3

Chakraborty, S., Baier, D., Knorr, D. and Mishra, H. N. (2015). High pressure inactivation of polygalacturonase, pectin methylesterase and polyphenoloxidase in strawberry puree mixed with sugar. *Food and Bioproducts Processing,* 95, 281-291. https://doi.org/10.1016/j.fbp.2014.10.016

Daher, D., Le Gourrierec, S. and Pérez-Lamela, C. (2017). Effect of high pressure processing on the microbial inactivation in fruit preparations and other vegetable based beverages. *Agriculture,* 7(9), 72. https://doi.org/10.3390/agriculture7090072

Daoudi, L., Quevedo, J., Trujillo, A.-J., Capdevila, F., Bartra, E., Mínguez, S. and Guamis, B. (2002). Effects of High-Pressure Treatment on the Sensory Quality of White Grape Juice. *High Pressure Research,* 22(3-4), 705-709.

De Faria, De Rosso, V. and Mercadante, A. (2009). Carotenoid composition of jackfruit (Artocarpus heterophyllus), determined by HPLC-PDA-MS/MS. *Plant Foods for Human Nutrition,* 64(2), 108-115. https://doi.org/10.1007/s11130-009-0111-6

Denoya, G.I., Vaudagna, S.R. and Polenta, G. (2015). Effect of high pressure processing and vacuum packaging on the preservation of fresh-cut peaches. *LWT - Food Science and Technology,* 62(1), 801-806. https://doi.org/10.1016/j.lwt.2014.09.036

Fachin, D., Loey, A.V., VanLoeyIndrawati, A., Ludikhuyze, L. and Hendrickx, M. (2002). Thermal and High-Pressure Inactivation of Tomato Polygalacturonase: A Kinetic Study. *Journal of Food Science,* 67(5), 1610-1615. https://doi.org/10.1111/j.1365-2621.2002.tb08692.x

Farheen, T., Ranganna, B. and Munishamanna, K.B. (2014). Vacuum packaging of minimally processed jackfruit bulbs for long distance transportation. *Mysore Journal of Agricultural Sciences,* 48(3), 351-357.

Feng, P., Weagant, S.D., Grant, M.A., Burkhardt, W., Shellfish, M. and Water, B. (2002). BAM: Enumeration of *Escherichia coli* and the Coliform Bacteria. Bacteriological Analytical Manual. Retrieved from FDA BAM website: https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm064948.htm

Gao, G., Ren, P., Cao, X., Yan, B., Liao, X., Sun, Z. and Wang, Y. (2016). Comparing quality changes of cupped strawberry treated by high hydrostatic pressure and thermal processing during storage. *Food and Bioproducts Processing,* 100, 221-229. https://doi.org/10.1016/j.fbp.2016.06.017

Goswami, C., Hossain, M., Kader, H. and Islam, R. (2011). Assessment of physicochemical properties of jackfruits'(*Artocarpus heterophyllus* Lam) pulps. *Journal of Horticulture, Forestry and Biotechnology,* 15(3), 26-31.

Gross, K.C. (1982). A rapid and sensitive spectrophotometric method for assaying polygalacturonase using 2-cyanoacetamide. *HortScience,* 17(6), 933-934.

Hagerman, A.E. and Austin, P.J. (1986). Continuous spectrophotometric assay for plant pectin methyl esterase. *Journal of Agricultural and Food Chemistry,* 34(3), 440-444. https://doi.org/10.1021/jf00069a015

Hite, B.H. (1899). The effect of pressure in the preservation of milk : a preliminary report. West Virginia, USA: West Virginia Agricultural Experiment Station.

Houben, K., Jamsazzadeh Kermani, Z., Van Buggenhout, S., Jolie, R.P., Van Loey, A.M. and Hendrickx, M. E. (2012). Thermal and High-Pressure Stability of Pectin methylesterase, Polygalacturonase, β-Galactosidase and α-Arabinofuranosidase in a Tomato Matrix: Towards the Creation of Specific Endogenous Enzyme Populations Through Processing. *Food and Bioprocess Technology,* 6(12), 3368-3380. https://doi.org/10.1007/s11947-012-0984-5

Houška, M., Strohalm, J., Kocurová, K., Totušek, J., Lefnerová, D., Tříska, J. and Paulíčková, I. (2006). High pressure and foods—fruit/vegetable juices. *Journal of Food Engineering,* 77(3), 386-398. https://doi.org/10.1016/j.jfoodeng.2005.07.003

Jayachandran, L.E., Chakraborty, S. and Rao, P.S.

(2015). Effect of high pressure processing on physicochemical properties and bioactive compounds in litchi based mixed fruit beverage. *Innovative Food Science and Emerging Technologies,* 28, 1-9. https://doi.org/10.1016/j.ifset.2015.01.002

Katsaros, G., Alexandrakis, Z. and Taoukis, P. (2017). Kinetic Assessment of High Pressure Inactivation of Different Plant Origin Pectinmethylesterase Enzymes. *Food Engineering Reviews*, 3, 170-189. https://doi.org/10.1007/s12393-016-9153-3

Kaushik, N., Kaur, B.P., Rao, P.S. and Mishra, H.N. (2014). Effect of high pressure processing on color, biochemical and microbiological characteristics of mango pulp (*Mangifera indica* cv. Amrapali). *Innovative Food Science and Emerging Technologies,* 22(Suppl. C), 40-50. https://doi.org/10.1016/j.ifset.2013.12.011

Knorr, D. (1993). Effects of high-hydrostatic-pressure processes on food safety and quality. *Food Technology,* 47, 156-161.

Knorr, D., Heinz, V. and Buckow, R. (2006). High pressure application for food biopolymers. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics,* 1764(3), 619-631. https://doi.org/10.1016/j.bbapap.2006.01.017

Kunugi, S. and Tanaka, N. (2002). Cold denaturation of proteins under high pressure. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology,* 1595(1), 329-344. https://doi.org/10.1016/S0167-4838(01)00354-5

Kusumaningrum, D., Lee, S.-H., Lee, W.-H., Mo, C. and Cho, B.-K. (2015). A Review of Technologies to Prolong the Shelf Life of Fresh Tropical Fruits in Southeast Asia. *Journal of Biosystems Engineering*, 40(4), 345-358. https://doi.org/10.5307/JBE.2015.40.4.345

Leyva-Daniel, D.E., Escobedo-Avellaneda, Z., Villalobos-Castillejos, F., Alamilla-Beltrán, L. and Welti-Chanes, J. (2017). Effect of high hydrostatic pressure applied to a Mexican honey to increase its microbiological and functional quality. *Food and Bioproducts Processing,* 102, 299-306. https://doi.org/10.1016/j.fbp.2017.01.001

Lima Tribst, A.A., de Souza Sant'Ana, A. and de Massaguer, P.R. (2009). Microbiological quality and safety of fruit juices—past, present and future perspectives. *Critical Reviews in Microbiology,* 35(4), 310-339. https://doi.org/10.3109/10408410903241428

Linton, M. and Patterson, M.F. (2000). High pressure processing of foods for microbiological safety and

quality (a short review). *Acta Microbiologica et Immunologica Hungaria,* 47(2-3), 175-182.

Ludikhuyze, L., Van Loey, A., Denys, I.S. and Hendrickx, M.E. (2001). Effects of high pressure on enzymes related to food quality Ultra high pressure treatments of foods, p. 115-166. USA: Springer. https://doi.org/10.1007/978-1-4615-0723-9_5

Oey, I., Lille, M., Van Loey, A. and Hendrickx, M. (2008). Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. *Trends in Food Science and Technology,* 19(6), 320-328. https://doi.org/10.1016/j.tifs.2008.04.001

Oey, I., Van der Plancken, I., Van Loey, A. and Hendrickx, M. (2008). Does high pressure processing influence nutritional aspects of plant based food systems? *Trends in Food Science and Technology,* 19(6), 300-308. https://doi.org/10.1016/j.tifs.2007.09.002

Paciulli, M., Medina-Meza, I.G., Chiavaro, E. and Barbosa-Cánovas, G.V. (2016). Impact of thermal and high pressure processing on quality parameters of beetroot (Beta vulgaris L.). *LWT - Food Science and Technology,* 68(Suppl. C), 98-104. https://doi.org/10.1016/j.lwt.2015.12.029

Rao, L., Guo, X., Pang, X., Tan, X., Liao, X. and Wu, J. (2014). Enzyme activity and nutritional quality of peach (*Prunus persica*) juice: effect of high hydrostatic pressure. *International Journal of Food Properties,* 17(6), 1406-1417. https://doi.org/10.1080/10942912.2012.716474

Saxena, A., Bawa, A.S. and Raju, P.S. (2011). Jackfruit (*Artocarpus heterophyllus* Lam.) In Yahia, E.M. (Ed.). Postharvest Biology and Technology of Tropical and Subtropical Fruits. Vol. 2: Acai to citrus, p. 275-299. USA: Woodhead Publishing. https://doi.org/10.1533/9780857092885.275

Serment-Moreno, V., Jacobo-Velázquez, D.A., Torres, J.A. and Welti-Chanes, J. (2017). Microstructural and Physiological Changes in Plant Cell Induced by Pressure: Their Role on the Availability and Pressure-Temperature Stability of Phytochemicals. *Food Engineering Reviews*, 9(4), 314-334. https://doi.org/10.1007/s12393-017-9158-6

Shook, C.M., Shellhammer, T.H. and Schwartz, S.J. (2001). Polygalacturonase, pectinesterase, and lipoxygenase activities in high-pressure-processed diced tomatoes. *Journal of Agricultural and Food Chemistry*, 49(2), 664-668.

Simonin, H., Duranton, F. and de Lamballerie, M. (2012). New Insights into the High-Pressure Processing of Meat and Meat Products. *Comprehensive Reviews in Food Science and Food Safety,* 11(3), 285-306. https://doi.org/10.1111/j.1541-4337.2012.00184.x

Smelt, J. P. (1998). Recent advances in the microbiology of high pressure processing. *Trends in Food Science and Technology,* 9(4), 152-158. https://doi.org/10.1016/S0924-2244(98)00030-2

Swami, S.B., Thakor, N., Haldankar, P. and Kalse, S. (2012). Jackfruit and its many functional components as related to human health: a review. *Comprehensive Reviews in Food Science and Food Safety,* 11(6), 565-576. https://doi.org/10.1111/j.1541-4337.2012.00210.x

Tangwongchai, R., Ledward, D.A. and Ames, J.M. (2000). Effect of high-pressure treatment on the texture of cherry tomato. *Journal of Agricultural and Food Chemistry,* 48(5), 1434-1441. https://doi.org/10.1021/jf990796p

Tournas, V., Stack, M., Mislivec, P., Koch, H. and Bandler, R. (1998). Yeasts, molds and mycotoxins. Bacteriological Analytical Manual. Retrieved from website: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071435.htm

Tsou, C.-L. (1986). Location of the active sites of some enzymes in limited and flexible molecular regions. *Trends in Biochemical Sciences,* 11(10), 427-429. https://doi.org/10.1016/0968-0004(86)90178-7

Vázquez-Gutiérrez, J., Quiles, A., Hernando, I. and Pérez-Munuera, I. (2011). Changes in the microstructure and location of some bioactive compounds in persimmons treated by high hydrostatic pressure. *Postharvest Biology and Technology,* 61(2), 137-144. https://doi.org/10.1016/j.postharvbio.2011.03.008

Vega-Gálvez, A., Díaz, R., López, J., Galotto, M.J., Reyes, J.E., Perez-Won, M. and Di Scala, K. (2016). Assessment of quality parameters and microbial characteristics of Cape gooseberry pulp (*Physalis peruviana* L.) subjected to high hydrostatic pressure treatment. *Food and Bioproducts Processing,* 97, 30-40. https://doi.org/10.1016/j.fbp.2015.09.008

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