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Effects of high pressure in association with pH and salt on the allergenicity, proteolytic and fibrinolytic activities of pineapple juice

J. Liang, J. Pan*, Z.Y. Wu, M. Ge and J.F. Xu

Engineering Research Centre of Bio-Process, Ministry of Education of China, Hefei University of Technology, 193 Tun Xi Road, Hefei, Anhui 230009, China P.R.; hfut20022013@sina.com

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RESEARCH ARTICLE

Abstract

The objective of this study was to investigate the influence of high pressure in association with pH and NaCl concentration on the allergenicity and proteolytic and fibrinolytic activities of pineapple juice. Pineapple juice was treated with various pressures (0-500 MPa) and adjusted to various pH values (2.7-5.0) and NaCl concentrations (0-0.8%), and its allergenicity and proteolytic and fibrinolytic activities were measured. Pressure influenced proteolytic activity, fibrinolytic activity and allergenicity in a similar trend but at different strengths. Although 400 MPa was taken as a good condition for retaining the activities and decreasing the allergenicity of pineapple juice, desensitisation was insufficient with high hydrostatic pressure alone. High pressure in combination with adjustment of pH reduced proteolytic activity and allergenicity, and the optimum pH range was expanded and became more alkaline. However, this combination increased fibrinolytic activity and its optimum pH was barely changed. High pressure in combination with adjustment of NaCl concentration changed these three activities in a similar trend, but they were variably decreased at NaCl concentrations of >0.4%. Allergenicity decreased fastest, following by proteolytic activity and fibrinolytic activity. At a pressure of 400 MPa, a pH of 3.2 and a NaCl concentration of 0.6%, allergenicity and proteolytic and fibrinolytic activities were 41.92%, 64.35% and 93.86%, respectively, indicating this processing is suitable for pineapple juice.

Keywords: allergenicity, fibrinolytic activity, high hydrostatic pressure, pineapple juice, proteolytic activity

1. Introduction

Pineapple (*Ananas comosus* L. Merr.), which belongs to the Bromeliaceae family, is an important fruit crop in many tropical and subtropical countries. It is commonly consumed as fresh and processed products such as pineapple juice, which has filled market shelves for years, principally because of its unique pleasant aroma and characteristic flavour (Laorko *et al.*, 2013; Rattanathanalerk *et al.*, 2006).

The beneficial components of pineapple play a primary role in reducing the risk of chronic diseases. Bromelain is a major proteolytic enzyme complex found in pineapple (Hale *et al.*, 2005) and exhibits various activities both *in vitro* and *in vivo* such as fibrinolytic activity, antithrombotic activity, tumour growth-modulating activity and anti-inflammatory activity (Chobotova *et al.*, 2010; Gregory and Kelly, 1996; Hale, 2004). Tumour formation, shrinkage and apoptosis were observed when an induced papilloma was treated with bromelain in mice (Beez *et al.*, 2007). Bromelain increases fibrinolytic activity in a dose-dependent manner in inflammatory animal models (Pirotta and De Giuli-Morghen, 1978). Platelet counts decreased dramatically when humans with ADP-induced platelet aggregation or thrombocytosis received bromelain *per os* (Heinicke *et al.*, 1972). Interestingly, when bromelain is inactivated, all these effects vanish, which suggests they are dose dependent and related with the proteolytic activity of this enzyme (Morita *et al.*, 1979).

Traditional thermal treatments of foods, such as pasteurisation and sterilisation, are also suitable for juice stabilisation. However, these processes often have undesirable effects on the flavour, nutritional contents, physicochemical properties and proteolytic activity of juices (Bhattacharya *et al.*, 2009; Mosqueda-Melgar *et al.*, 2008). Consumers demand high-quality, convenient and minimally processed natural fruit products with a fresh appearance, texture and flavour, and they should be free from preservatives while retaining their bioactive functions (Jang *et al.*, 2002). High hydrostatic pressure (HHP) processing is deemed to be a suitable non-thermal food treatment method and has been successfully applied in commercial liquid food production such as that of juices (Lee *et al.*, 2015). HHP could be potentially used instead of thermal pasteurisation in the juice industry (Butz *et al.*, 2003; Hendrickx and Knorr, 2002).

Bromelain is a significant functional and allergenic ingredient of pineapple. Daily administration of bromelain *per os* for 18 weeks can induce the dose-dependent production of an anti-bromelain antibody in mice (Hale *et al.*, 2004). Immunogenic responses such as immunoglobulin E (IgE)-mediated respiratory and gastrointestinal allergic reactions occur when bromelain is given orally (Baur, 1999; Gailhofer *et al.*, 1988), meaning pineapple juice is not suitable for people who are allergic to pineapple. Although high pressure processing preserves the bio-functions of pineapple juice, partial allergenicity persists. It is important to determine how to produce pineapple juice that has good bio-functions and low allergenicity. The aim of this study was to determine the effects of high pressure on three activities (allergenicity and proteolytic and fibrinolytic activities) of pineapple juice and the relationship between them, and to generate useful data for producing HHP-processed pineapple juice with higher bioactivity and lower allergenicity.

2. Materials and methods

Materials

Fresh pineapples (*A. comosus*) were purchased at a local market. Thrombin, urokinase and fibrinogen from bovine plasma were purchased from Sigma-Aldrich (St. Louis, MO, USA). Casein was purchased from the National Institutes for Food and Drug Control (Beijing, China P.R.). Human sera were provided by the First Affiliated Hospital of Anhui Medical University (Hefei, China P.R.). They were collected from five patients with a clinical history of allergic reactions to pineapple, each of whom tested positive to pineapple. Serum IgE specific to pineapple was determined using ImmunoCap™ (Phadia, Uppsala, Sweden). Written informed consent was obtained from all study participants. Allergic sera were pooled to screen the IgE-binding pattern of the major pineapple allergens. Goat anti-human IgE-linked horseradish peroxidase (HRP) was purchased from Abcam (Cambridge, UK). Chemicals and solvents of analytical and molecular biology grades were obtained from Sigma-Aldrich (Bornem, Belgium), Tedia (Fairfield, USA) and Bio-Rad (Nazareth, Belgium). Enzyme-linked immunosorbent assay (ELISA) plates were purchased from Costar (Los Angeles, CA, USA) and ultrafiltration centrifuge tubes were purchased from Millipore (Billerica, MA, USA). Other products were purchased from Sangon BioTech (Shanghai, China P.R.).

Sample treatment with HHP and adjustment of pH and NaCl concentration

Pineapples were peeled and squeezed with a small manual juicer (G008; SPL Jiangmen, China P.R.). The juice was filtered through six layers of cheese cloth and centrifuged at 4,000×*g* at 4 °C for 30 min (3K15; Sigma Laborzentrifugen, Osterode am Harz, Germany). The supernatant was retained and portioned.

For the proteolytic and fibrinolytic activity assays, the centrifuged pineapple juice was adjusted to different pH values (2.7, 3.2, 3.7, 4.2, 4.7 and 5.0) and NaCl concentrations (0.1, 0.2, 0.4, 0.6 and 0.8%). Of note, 0.8% was selected as the maximum NaCl concentration mainly because it was the maximum concentration that people found acceptable in pineapple juice in our taste test. The samples were dispensed in sterile PET bottles and put into sealed plastic bags filled with water, and then the air was squeezed out. Samples were placed under different pressures (0, 100, 200, 300, 400 and 500 MPa) to determine the effects on proteolysis, fibrinolysis and allergenicity. Samples were placed under 400 MPa to evaluate the effects of different pH values and NaCl concentrations on these three parameters. The traditional high pressure equipment was composed of a 1 l high pressure vessel (Kefa, Bao Tou, China P.R.) and a high pressure intensifier pump (Dalong, Shanghai, China P.R.). The vessel was capable of producing a maximum pressure of 600 MPa. The temperature (0-60 °C) was thermally regulated with a heating/cooling water jacket. The pressure-conducting fluid was dioctyl sebacate. The compression and decompression rate was 40 MPa/s and variation around the set point of the HHP unit was 5%.

For assessment of allergenicity, the centrifuged pineapple juice was extracted using solid ammonium sulphate, which was slowly added with continuous stirring until saturation was reached at 40%. The obtained mixtures were continuously stirred for more than 2 h and then dispensed into polyethylene tubes and centrifuged at 6,000×*g* for 20 min. The supernatant was discarded and the pellet was dissolved in ddH_2O. The solution was desalted using ultrafiltration centrifugal filters. All the steps were performed at 0-4 °C. The high pressure processing was the same for the proteolytic and fibrinolytic activity assays.

All the samples were generated from the same batch of treated pineapple juice and stored at -20 °C until tested. Control samples were kept at atmospheric pressure (0.1

MPa), a pH of 3.7 and 20 °C. All samples were kept at high pressure for 20 min.

Allergenicity

Allergenicity was tested by an indirect ELISA. A 96-well ELISA plate was coated with 0.1 ml of samples prepared for allergenicity testing, which had been dissolved in carbonate buffer (0.05 M; pH=9.6) and incubated at 4 °C overnight. After incubation, the plate was washed with phosphate-buffered saline (PBS) containing 0.05% Tween (PBST) three times (5 min per time). All reagents used to make PBST were purchased from Sangon BioTech. The plate was blocked with PBST containing 5% skimmed milk (0.2 ml/well) at 37 °C for 2 h. Thereafter, the blocking buffer was removed and sera of allergic patients (0.1 ml of a 1:200 dilution) was added to each coated well. The plate was incubated at 37 °C for 1 h and washed three times with PBST (5 min per time), followed by the addition of human IgE-linked HRP (0.1 ml/well, 1:2,000 dilution) and incubation at 37 °C for 1 h. Finally, 100 μl of substrate (3,3',5,5'-tetramethylbenzidine) was added to each well. The plate was incubated at room temperature for 10-30 min. The reaction was terminated by adding 100 μl of H_2SO_4 (2 M) to each well. Absorbance at 450 nm was measured using a microplate reader (Epoch; Bio-Tek, Winooski, VT, USA). The antigenic response of pressure-treated samples was tested with a standard curve of bromelain, which was correlated with a Napierian logarithm. All analyses were conducted three times and the average values were converted to their equivalent concentrations in mg/l (bromelain/pineapple juice). The original allergen content of control samples was 0.955±0.043 mg/ml, which was defined as 100% allergenicity. The definition of relative allergenicity was as follows:

Relative allergenicity =

$$\left(\frac{\text{allergenicity of treated samples}}{\text{allergenicity of control samples}}\right) \times 100\% \qquad (1)$$

Proteolytic activity

Proteolytic activity was detected with the Folin-phenol method (Zhang *et al.*, 2010). Tyrosine and casein were used as a standard and a substrate, respectively. The sample (1.0 ml) was mixed with casein solution (1.0 ml). The reaction was carried out at 40 °C for 10 min and stopped by the addition of 2 ml of trichloroacetic acid (0.4 mol/l). The reaction mixture was then centrifuged at 8,000×*g* for 10 min. Thereafter, 1 ml of the obtained supernatant was transferred to another tube and mixed with 5 ml of Na_2CO_3 (0.4 mol/l) and 1 ml of Folin-phenol solution. The mixture was kept at 40 °C for 20 min and absorbance was measured at 660 nm. One unit of protease activity was defined as the amount of product released by enzymes that equalled 1 μg of tyrosine/min/ml under the standard assay conditions. All experiments were performed three times and mean values were calculated. The standard curve that should pass through the origin was generated, whose x-axis was optical density at 280 nm and y-axis was tyrosine concentration. One unit of proteolytic activity was defined as the amount of casein hydrolysed in 1 ml of pineapple juice to produce 1 μg of tyrosine. The proteolytic activity of control samples was 74.67±2.66 U/ml, which was defined as 100% proteolytic activity. The definition of relative bromelain activity was as follows:

Relative proteolytic activity =

$$\left(\frac{\text{proteolytic activity of treated samples}}{\text{proteolytic activity of control samples}}\right) \times 100\% \qquad (2)$$

Fibrinolytic activity

Fibrinolytic activity was tested with the modified fibrin plate method (Astrup and Mullertz, 1952). Agar solution (10 g/l) was prepared using PBS (0.05 mol/l). Thereafter, 0.5 ml of fibrinogen solution (10 g/l) and 20 μl of thrombin solution (100 U/ml) were added to 15 ml of heat-melted agar solution, which was cooled to 50 °C. The mixture was homogenised, poured into Petri dishes and left to stand for 1 h to form fibrin clots. The urokinase standard was diluted to 20-250 U/ml, and a calibration curve was generated. The mixture was injected into holes with a diameter of 3 mm that had been punched in fibrin plates. The fibrin plates were kept at ambient temperature for 10 min, incubated at 37 °C for 18 h, stained with Coomassie brilliant blue R-250 solution (0.1% R-250, 12% methanol and 10% acetic acid) for 15 min and finally destained until the lysed circles were clear. The two orthogonal diameters of the lysed circles were measured, and the area of the lysed circles was calculated. The standard curve was plotted with the logarithms (log C) of urokinase activity C (U/ml) as the x-axis and the logarithms (log A) of the area of lysed circle A (mm^2) as the y-axis. All experiments had three replications, the average values of which are reported. The fibrinolytic activity of control samples was 247.88±13.21 U/ml, which was defined as 100% fibrinolytic activity. The definition of relative fibrinolytic activity was as follows:

Relative fibrinolytic activity =

$$\left(\frac{\text{fibrinolytic activity of treated samples}}{\text{fibrinolytic activity of control samples)}}\right) \times 100\% \qquad (3)$$

Statistical analysis

Linear fit and regression analysis and variance analysis were carried out using Origin 8.0 (OriginLab Corporation, Northampton, MA, USA) and SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The significant level was established as P<0.05.

3. Results and discussion

Effects of HHP on allergenicity and proteolytic and fibrinolytic activities

The influence of pressure on the relative allergenicity and proteolytic and fibrinolytic activities of pineapple juice are shown in Figure 1. All three parameters exhibited a similar trend with increasing pressure and were all significantly influenced by high pressure ($P<0.05$); however, the extent of the effect differed among the three parameters. The proteolytic activity, fibrinolytic activity and allergenicity of pineapple juice positively correlated with a pressure of 100-300 MPa, and reached a maximum of 174.49, 121.99 and 111.26%, respectively, when the pressure was 300 MPa. As the pressure increased further, these parameters decreased to varying degrees. At 400 MPa, proteolytic activity, fibrinolytic activity and allergenicity were 93.51, 118.48 and 85.68%, respectively, which suggests that there were major differences among these parameters, with allergenicity falling below the value in control samples. When the pressure reached 500 MPa, the differences among the parameters were less evident. Thus, 400 MPa is a good condition for retaining the activities of pineapple juice and desensitisation. However, desensitisation was insufficient with HHP alone; therefore, the effectiveness of a pressure of 400 MPa with various pH values and NaCl concentrations was assessed.

The most important protein related to the allergenicity, proteolytic activity and fibrinolytic activity of pineapple juice is bromelain (Hale, 2004; Pirotta and De Giuli-Morghen, 1978). Bromelain mainly comprises multiple glycosylated enzyme species of the papain superfamily, which have different proteolytic activities, molecular masses of 20-31 kDa and isoelectric points of 4.8-10 (Harrach *et al.*, 1995). HHP influences the bromelain conformation, further affecting its function. The obvious increase in fibrinolytic activity in comparison with proteolytic activity is probably because bromelain acts in different manners on different substrates. Proteolytic activity is aimed at casein, while fibrinolytic activity simultaneously degrades fibrous protein directly and transforms plasminogen into fibrinolysin to degrade fibrin (Taussig and Batkin, 1988). The action mechanism of the fibrinolytic activity of bromelain was seldom discussed in previous research, and some investigators suggested that the fibrinolytic function was connected with the proteolytic activity (Morita *et al.*, 1979). In this report, we studied high pressure-treated pineapple juice, a complex matrix rather than pure bromelain solution; therefore, the activities may be affected by constituents of the matrix.

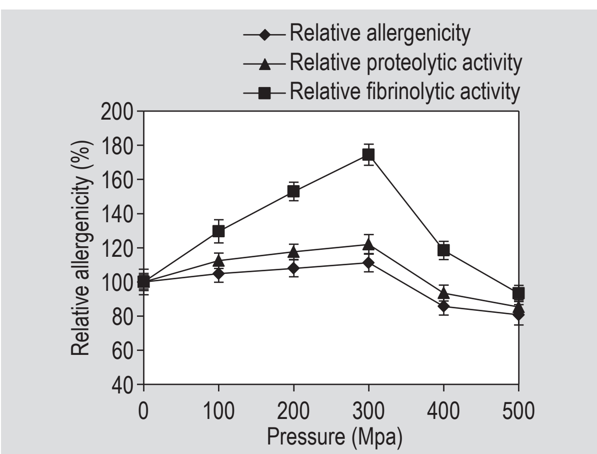

Figure 1. Changes in the relative allergenicity and proteolytic and fibrinolytic activities of pineapple juice treated with various pressures. Control samples were kept at atmospheric pressure (0.1 MPa), and the relative values in these samples were defined as 100%. All samples were processed at 20 °C in native pH with a hold time of 20 min in three parallel experiments.

The relative allergenicity and proteolytic and fibrinolytic activities peaked at 300 MPa and decreased as the pressure increased further. There are two possible reasons for this. Firstly, enzymes and their substrates are often isolated by membranes in integral plant tissues, which are destroyed by high pressure (e.g. less than 300 MPa), thereby increasing enzymatic reactions. Secondly, HHP treatment exposes thiol groups of proteins (Rawdkuen *et al.*, 2009). Given that bromelain is a cysteine sulfhydryl protease and that sulfhydryl is an essential group related to catalytic activity (Chen and Yan, 1990), an increased sulfhydryl content would increase enzyme activity.

Effects of HHP and pH on allergenicity and proteolytic and fibrinolytic activities

Although high pressure alone could preserve the activities of pineapple juice, the decrease in allergenicity was not satisfactory (Figure 1). To further reduce the allergenicity of pineapple juice, treatment with high pressure (400 MPa) in combination with adjustment of the pH to various values (2.7-5.0) was carried out.

Proteolytic activity was highest in the natural pH of pineapple juice (pH=3.7) at atmospheric pressure, and decreased when the pH was increased or decreased (Figure 2). When the pressure was 400 MPa, proteolytic activity was highest at pH 4.2 and only changed minimally at pH 3.2-4.2, which implies that the optimum pH became more alkaline at 400 MPa and that the pH range was expanded. Proteolytic activity was much lower at 400 MPa than at atmospheric pressure when comparing corresponding pH values (Figure 2).

Figure 3 shows the effects of high pressure in combination with various pH values on fibrinolytic activity. At atmospheric pressure, the optimum pH ranged from 3.7

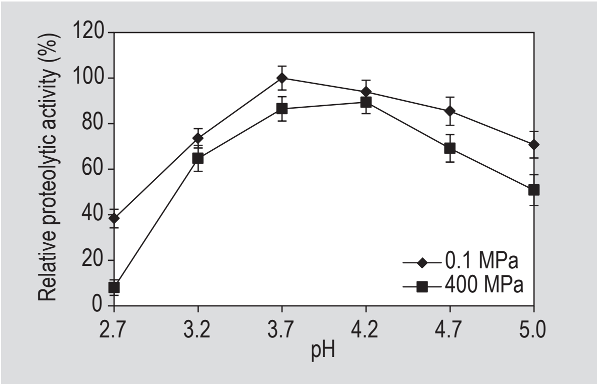

Figure 2. The effects of high pressure combined with different pH values on the relative proteolytic activity of pineapple juice. Control samples were kept at atmospheric pressure (0.1 MPa), native pH and 20 °C, and the relative values in these samples were defined as 100%. Each sample was used for three replicates.

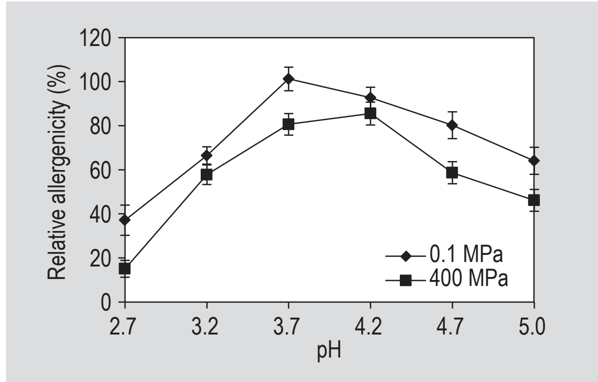

Figure 4. The effects of high pressure in combination with various pH values on the relative allergenicity of pineapple juice. Control samples were kept at atmospheric pressure (0.1 MPa), native pH and 20 °C, and the relative values in these samples were defined as 100%. Each sample was used for three replicates.

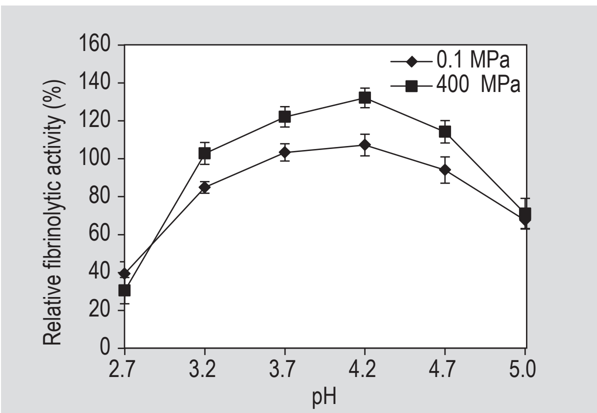

Figure 3. The effects of high pressure in combination with various pH values on the relative fibrinolytic activity of pineapple juice. Control samples were kept at atmospheric pressure (0.1 MPa), native pH and 20 °C, and the relative values in these samples were defined as 100%. Each sample was used for three replicates.

to 4.2. Fibrinolytic activity was highest within this range and lower outside it. Increased pressure enhanced fibrinolytic activity, especially at a pH of 3.2-4.7. However, in contrast to the influence of pressure and pH on proteolytic activity, the optimum pH for fibrinolytic activity was barely changed under high pressure.

Allergenicity was highest at a pH of 3.7 (Figure 4). The combination of pressure and pH facilitated desensitisation, and the pH at which allergenicity was highest became more alkaline and desensitisation was subtle. There were similarities in the effects of a combination of high pressure and pH adjustment on allergenicity and proteolytic activity (Figures 2 and 4). Bromelain is a pineapple thiol protease that has a single asparagine-linked heterooligosaccharide unit per molecule (Vanhoof and Cooreman, 1997; Yasuda *et al.*, 1970). Hale (2006) found that the proteolytic activity of bromelain and other cysteine proteases is critical for inducing immunogenicity. In addition, the use of inactive allergens (e.g. bromelain) may assist desensitisation, which is consistent with our finding that the allergenicity and proteolytic activity curves were similar (Figure 2). However, there are few studies of the epitopes of bromelain.

The three parameters decreased faster at pH values of <3.7 than at pH values of >3.7 (Figures 2-4). At a pressure of 400 MPa and a pH of 2.7, proteolytic activity, fibrinolytic activity and allergenicity decreased to 8.03, 30.46 and 15.09%, respectively, which was caused by bromelain unfolding at a low pH (Khan *et al.*, 2003). Ahmad and Khan (2006) selected a pH of 0.8-3.0 to study the acid-induced unfolding of bromelain. They showed that bromelain is maximally unfolded at a pH of 2.0, characterised by significant loss of secondary structure (80%) and almost complete loss of tertiary contacts. This could explain our finding that allergenicity, proteolytic activity and fibrinolytic activity were all significantly decreased at a pH of 2.7.

According to the results presented in Figures 2-4, HHP combined with adjustment of the pH could significantly influence the allergenicity and proteolytic and fibrinolytic activities of pineapple juice ($P<0.05$), and there were obvious synergistic effects of HHP and pH on these three parameters ($P<0.05$). A pH of 2.7 or 5.0 combined with a pressure of 400 MPa remarkably reduced allergenicity, and proteolytic and fibrinolytic activities were also significantly decreased. Not only must the allergenicity of pineapple juice be reduced but its proteolytic and fibrinolytic activities should be retained, as much as possible. Therefore, a pH of 3.2 and a pressure of 400 MPa, at which the residual allergenicity and proteolytic and fibrinolytic activities were 57.82, 64.73 and 102.81%, respectively, seemed to be suitable for preserving the activities and decreasing the allergenicity of pineapple juice.

Effects of HHP and NaCl concentration on allergenicity and proteolytic and fibrinolytic activities

To further preserve the activities and decrease the allergenicity of pineapple juice, the effects of a pH of 3.2 and a pressure of 400 MPa in combination with various NaCl concentrations were studied.

Under a pressure of 0.1 or 400 MPa, proteolytic activity slightly positively correlated with the NaCl concentration when it was lower than 0.4% (Figure 5). This corroborates a report (Xue, 2011) that bromelain activity increases slightly when 0-0.2% NaCl is added to the enzyme solution. When the NaCl concentration was higher than 0.4%, it negatively correlated with proteolytic activity in an almost linear manner under a pressure of 0.1 and 400 MPa. The influence of the combination of high pressure and NaCl concentration on proteolytic activity was significant ($P<0.05$), although their synergistic effects were not.

The addition of NaCl affected proteolytic and fibrinolytic activities in a similar manner under a pressure of 0.1 MPa (Figure 6). When the NaCl concentration was higher than 0.4%, fibrinolytic activity decreased more slowly under HHP than under 0.1 MPa. The combination of high pressure and NaCl addition changed fibrinolytic activity significantly ($P<0.05$) and the synergistic effects were obvious ($P<0.05$).

There were similarities between the effects of NaCl addition on allergenicity and proteolytic activities (Figures 5 and 7). However, when the NaCl concentration was 0.4% or higher, allergenicity decreased faster than proteolytic activity under HHP. The combination of high pressure and NaCl addition changed allergenicity significantly ($P<0.05$) and the synergistic effects were obvious ($P<0.05$).

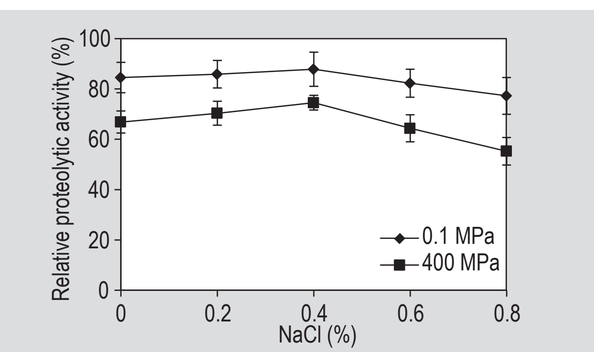

Figure 5. The effects of HHP in combination with various NaCl concentrations on proteolytic activity. Control samples were kept at atmospheric pressure (0.1 MPa), native pH and 20 °C, and the relative values in these samples were defined as 100%. Each sample was used for three replicates.

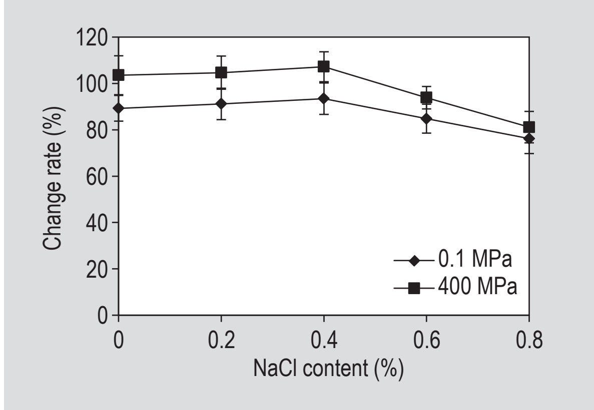

Figure 6. The effects of HHP in combination with various NaCl concentrations on fibrinolytic activity. Control samples were kept at atmospheric pressure (0.1 MPa), native pH and 20 °C, and the relative values in these samples were defined as 100%. Each sample was used for three replicates.

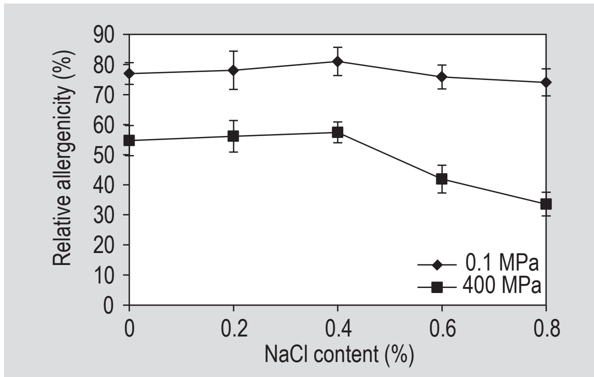

Figure 7. The effects of HHP in combination with various NaCl concentrations on allergenicity. Control samples were kept at atmospheric pressure (0.1 MPa), native pH and 20 °C, and the relative values in these samples were defined as 100%. Each sample was used for three replicates.

Figures 5-7 show that allergenicity, proteolytic activity and fibrinolytic activity were reduced to 33.56, 55.23 and 81.15%, respectively, at a pressure of 400 MPa, a pH of 3.2 and a NaCl concentration of 0.8%. Thus, a high fibrinolytic activity was retained and allergenicity and proteolytic activity were decreased. However, at a pressure of 400 MPa, a pH of 3.2 and a NaCl concentration of 0.6%, proteolytic and fibrinolytic activities were higher (64.35 and 93.86%, respectively) while allergenicity was remarkably decreased (41.92%). According to our taste test, a NaCl concentration of 0.6% did not make pineapple juice taste salty or influence its flavour, while a NaCl concentration of 0.8% made it taste slightly salty. Therefore, a pressure of 400 MPa, a pH of 3.2 and a NaCl concentration of 0.6% were most suitable for high pressure processing of pineapple juice.

4. Conclusions

In conclusion, the novelty of this work lies in its investigation of the influence of high pressure in combination with different pH values and NaCl concentrations on the allergenicity and proteolytic and fibrinolytic activities of pineapple juice. Pressure influenced the proteolytic activity, fibrinolytic activity and allergenicity in a similar trend but at different strengths. All the parameters were highest at a pressure of 300 MPa. At a pressure of 400 MPa, the activities varied, and allergenicity was lower than its initial value. At a pressure of 500 MPa, the differences among the parameters were less evident. Although 400 MPa was taken as a good condition for retaining the activities of pineapple juice and decreasing its allergenicity, desensitisation was insufficient with HHP alone. High pressure in combination with adjustment of pH also affected proteolytic activity, fibrinolytic activity and allergenicity, which were highest at a pH of 3.7, 4.2 and 3.7, respectively, at atmospheric pressure. High pressure and adjustment of pH reduced proteolytic activity and allergenicity, and its optimum pH range was expanded and became more alkaline. However, this combination increased fibrinolytic activity and its optimum pH was barely changed. High pressure in combination with adjustment of the NaCl concentration changed these three parameters in a similar trend, namely, they were almost not changed or slightly increased at NaCl concentrations of <0.4% but were variably decreased at NaCl concentrations of >0.4%. Allergenicity decreased fastest, followed by proteolytic activity and fibrinolytic activity. At a pressure of 400 MPa, a pH of 3.2 and a NaCl concentration of 0.6%, allergenicity and proteolytic and fibrinolytic activities were 41.92, 64.35 and 93.86%, respectively, indicating that this processing is suitable for pineapple juice.

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