Jurnal Teknologi, 49(F) Dis. 2008: 27–38

STABILITY OF BROMELAIN-POLYPHENOL COMPLEX IN PINEAPPLE JUICE

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Abstract. Pineapple posseses many beneficial properties for the human health due to high content of bromelain. The most natural ways to consume bromelain is by eating fresh fruit or by drinking fresh pineapple juice. However, processed pineapple juice is relatively easy and convenient to buy, transport and stored for later use. Canned or packed juices are usually processed under high temperature. This heat treatment for sterilisation purposes may contribute to enzyme and other heat sensitive components in the juice be denatured, especially bromelain. Polyphenol compounds was previously reported to stabilize bromelain. In this work, we reported the stability of bromelain in pineapple juice complexed with polyphenol compounds from fresh apple juice. The mixed pineapple-apple juice was prepared under various heating temperatures and sterilisation holding times. The bromelain concentration and activity in prepared juices were determined using HPLC using spesific column. Our study shows that the bromelain-polyphenol complex enhanced bromelain thermal stability and the shelf life of juice when stored at lower temperature.

Keywords: Bromelain; polyphenol; pineapple; apple; stability

Abstrak. Nanas mempunyai banyak kelebihan untuk kesihatan kerana mempunyai kandungan bromelain yang tinggi. Pengambilan bromelain secara semulajadi adalah dengan memakan buah atau meminum jus yang segar. Walau bagaimanapun, jus nanas yang telah diproses adalah lebih senang dan mudah untuk dibeli, dibawa serta disimpan. Proses mengetin atau membungkus jus kebiasaannya menggunakan suhu tinggi. Rawatan kaedah pemanasan ini adalah untuk pensterilan, yang menyebabkan enzim dan sebatian lain yang sensitif dalam jus akan ternyahasli terutama bromelain. Dalam kajian terdahulu, sebatian polifenol didapati dapat menstabilkan bromelain. Dalam penyelidikan ini, kami mendapati bromelain dalam jus nanas dapat distabilkan dengan sebatian polifenol dari epal yang segar. Campuran jus nenas-epal disediakan dengan menggunakan suhu dan masa pensterilan yang berlainan. Kepekatan dan aktiviti bromelain dalam jus yang dikaji ditentukan menggunakan HPLC (Kromatografi Cecair Berprestasi Tinggi) dengan menggunakan kolum yang khusus. Kajian kami menunjukkan kompleks bromelain-polifenol menambah kestabilan bromelain dan jangka hayat jus apabila disimpan pada suhu yang rendah.

Kata kunci: Bromelain; polifenol; nanas; epal; stabiliti

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1.0 INTRODUCTION

Bromelain or it scientific name sulphydryl proteolytic enzyme or cysteine proteinase is a mixture of sulphur-containing protein-digesting enzymes, called proteolytic enzymes or proteases. Bromelain is mainly comprised of cysteine proteases, with smaller amounts of acid phosphatase, peroxidase, amylase and cellulase. Bromelain is measured in MCUs (milk clotting units) or GDUs (gelatin digestion units). One GDU equals 1.5 MCU. The general benefits of bromelain may be summarized as follows [1]:

(i) Prevents edema formation and reduces existing edemas
(ii) Reduces the blood level of fibrinogen
(iii) Supports fibrinolysis
(iv) Activates plasmin
(v) Prolongs the prothrombin and partial thromboplastin time (after relatively high doses)
(vi) Prevents aggregation of blood platelets
(vii) Prevents adhesion of platelets to endothelial cells of blood vessels
(viii) Reduces the blood level of plasmakinins
(ix) Reduces the level of prostaglandine E2 and of thromboxane A2 in exsudates during acute inflammation
(x) Acts as an antiinflammatory agent
(xi) Induces the secretion of interleukin (Il)-1, Il-6, Il-8 and tumor necrosis factor (TNF)-a from blood monocytes and granulocytes
(xii) Supports the oxidative burst and the cytotoxicity of granulocytes against tumor cells
(xiii) Increases the tissue permeability of antibiotic drugs
(xiv) Prevents metastases in a mouse model
(xv) Supports skin debridement of burns

Pineapple fruit is rich with vitamin C which also known as ascorbic acid. It is useful for the prevention of recurrent ear infections, colds, and flu. Vitamin C is the body's primary water-soluble antioxidant, defending all aqueous areas of the body against free radicals that attack and damage normal cells. Pineapple is a good source of trace minerals. The fate of these components is usually compromised during thermal treatment of such juices for sterility purposes.

Freshly juice is highly susceptible to spoilage because unheated juice is subject to rapid microbial, enzymatic, chemical and physical deterioration. Therefore, thermal treatment is important to minimize these undesirable reactions and at the same time, maintaining the quality of the juice. Most juices permits pasteurization as thermal treatment.

Pasteurization is a relatively mild form of heat treatment, generally at a temperature lower than the boiling point of water. Pasteurization uses of temperature near

100 °C to effect destruction of spoilage organisms. A potentially effective process for fresh juice involves heat cleaning, well-sorted whole fruit for up to 1 minute at 80 °C. This greatly reduces surface contamination without influencing the quality of fresh juice. A great deal of research is underway combining surface heat with other decontamination practices. Whether these treatments will qualify as producing "fresh" juice for labeling purposes remains to be seen.

Pasteurization extends the storage life of bottled fruits and juices by several months. Pasteurization is often combined with another form of preservation such as concentration, acidification and chemical preservation. There are other newer methods in juice processing. Some methods are still under extensive research and expensive to commercialise. Table 1 summarised some newer Juice Processing Methods.

The extraction and purification of phytochemical from natural sources are often used in the preparation of dietary supplements, food additives, pharmaceuticals, cosmetic products and functional food ingredients. In this study, apple was chosen as a source of polyphenol to be complex with bromelain enzyme in pineapple juice. Apples (*Rosaceae malus sp.*) have been one of the human diets since ancient times and are one of the most commonly consumed fruits in worldwide. They are eaten both raw and in processed products such as juice, cider, brandy, jam and vinegar. Polyphenols compound can extent length of heating time without denature the bromelain [2]. Apple polyphenols have been shown to be safe and posse no toxic at average dietary level [3]. Despite in previous study reported that phenolic from apple waste beneficially influences key stages of carcinogenesis in colon cells in vitro [4]. Therefore, the compound might play a role in human health.

Apple fruits are rich in quercetin glycosides, epicatechin, chlorogenic acid (the major polyphenol compounds), procyanidins and dihydrochalones (such as phloridzin), which have been shown to exert strong antioxidant activity [3, 5]. Apple

Table 1 Some newer juice processing methods

Process	Description	Status
Aseptic	High temperature short time	Widespread commercialization
Hyperbaric Pressure	High MPa pressure	Approaching commercialization
Hyperbaric + CO^2	Combines pressure and low pH	Scale-up evaluations
Pulsed electric field	High KV/cm, Microsec field	Actively being researched
Ultrasonic	High intensity ultrasound	Potential synergistic hurdle
Ohmic heating	Resistance-generated heat	Actively being researched
Membrane	Physical removal of microbes	Effective for clear juices
Pulsed light	High intensity UV to visible	Effective for clear juices
Magnetic field	Low and High frequency/ intensity	Highly experimental
Irradiation	Electrons, gamma or x-rays	Commercially feasible
Nonthermal plasma	Electric discharge into liquids	Highly experimental
Preservatives	"Natural" herbs, spices, etc.	Actively being researched
Hydrodynamic shock	Instant high pressure	Highly experimental

juice which is high in antioxidants [6] was chosen as polyphenol complex due to its common application as fruit juice, having a good taste when blended with pineapple juice and with high antioxidant properties. Antioxidants play importance role in preventing the progression of oxidative pathologies and may provide protection to foods and drinks.

This experimentation was focus on gathering data for designing a suitable blend of pineapple juice and polyphenol complex using apple juice in order to achieve high bromelain concentration and its activity in the processed pineapple fruit juice under minimal thermal treatment. The first part of the experiment was to determine the suitable heating time of samples in order to achieve high bromelain content. The second part of the experiment was to determine the storage condition of the samples by determining the spoilage time.

2.0 METHODOLOGY

2.1 Material

Pineapple fruits were collected from Pineapple Cannery of Malaysia Sdn Bhd at Pekan Nenas, Johor Malaysia and apples (Washington Apple, Inc) were obtained from supermarket. Standard bromelain, sodium phosphate and sodium chloride were purchased from Sigma Aldrich.

The experiment was conducted following the process flow presented in Figure 1.

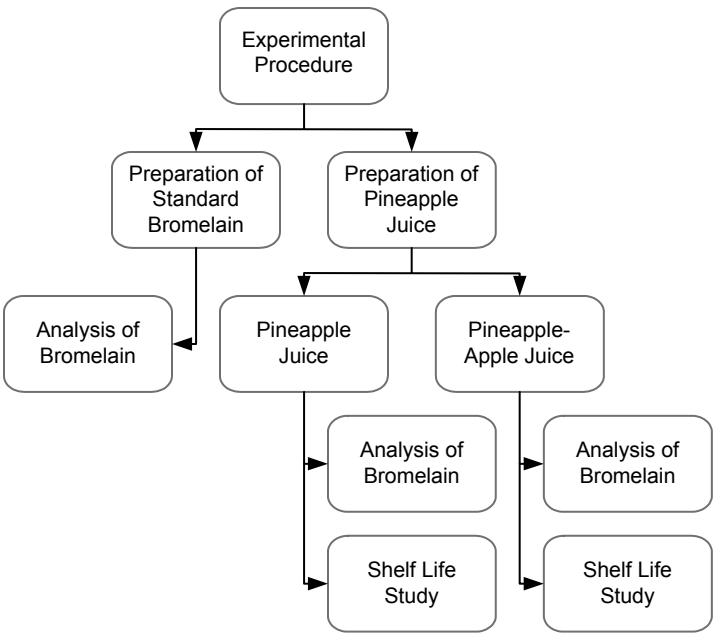

Figure 1 Process flow diagram for the experiment

2.2 Sample Preparation

Pineapples cores were collected from Pineapple Cannery of Malaysia Sdn Bhd at Pekan Nenas, Johor. The pineapple cores were cut and blended. The juice then divided into two parts. First part was used as control samples. The second part was added with apple with ratio one liter to five apples. Both type of samples (pineapple juice and pineapple-apple juice) were then filtered using filter paper Whatman No 1 into separate glass container. Next, the samples were aliquot into sterile PET bottle and capped. Each bottle contains 200 ml of samples and ready for heat treatment. Samples were prepared fresh daily for the experiment.

2.3 Bromelain Standard Curve

Standard bromelain was used as reference to estimate bromelain concentration in pineapple juice. The formula for Bromelain Standard Curve is presented in Table 2. Bromelain solution was read using HPLC (Perkin Almer). Type of column used was TSK Gel at UV detection at 280 nm. The elution used were buffer solution which contain 20 mM sodium phosphate and 0.5 M sodium chloride and phosphate solution which contain only 20 mM sodium phosphate. The standard curve of Bromelain Standard was plotted and used as reference for Bromelain quantification in the samples (Figure 2).

Table 2 HPLC analysis for various standard bromelain concentrations

Bromelain Concentration (%)	**Area $\times 10^4$ (uV.sec)**
0	0
1	6.18
2	285.44
4	525.87
6	1005.28
8	1338.14
10	1641.96

2.4 Thermal Treatment

Both samples were subjected to thermal treatment at various heating temperature and holding time. The samples were placed in water bath that was already heated up to appropriate temperature as required for the experimentation.

2.5 Samples Analysis

Samples analysis was divided into two sections. First section was to analyse bromelain concentration after heat treatment and second section was to analyse samples shelf life.

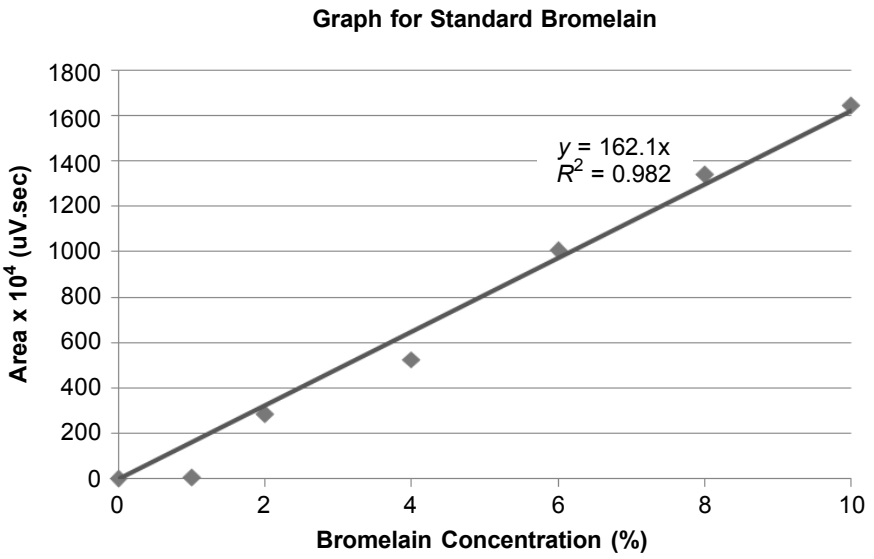

Figure 2 The standard curve of bromelain standard

2.6 Analysis on Bromelain Concentration

After thermal treatment, 2 ml of sample was taken for analysis. The samples were centrifuged to remove precipitate at 10 000 rpm for 5 minutes at 25 °C. Bromelain concentration was analysed using HPLC analysis.

2.7 Samples Shelf Life Analysis

The shelf life study was done under two conditions. First at room temperature, 27 °C and second, kept in a fridge with temperature 5 °C. The changes in taste, smell and colour were used as indicator of spoiled juice.

2.8 Relative Decrease in Bromelain Calculation

Relative decreasing of bromelain concentration was determined from this equation.

$$\text{Relative decreasing of bromelain} = 100\% - \frac{[A-B]}{A} \times 100\%$$

A = Bromelain concentration at holding time = 0
B = Bromelain concentration at holding time = t_i
t_i = 10, 20, 30...

3.0 RESULTS AND DISCUSSION

3.1 Analysis of Bromelain Concentration in Pineapple-Apple Juice Complex at Different Thermal Treatment

In this experiment, the samples (pineapple-apple juice complex) and its control (pineapple juice) were subjected to different temperatures and holding times. Significant changes in bromelain concentration were observed when samples were exposed to different temperatures with different holding times.

Figure 3 shows the bromelain concentration in samples exposed to 40 °C for various holding time from 0 to 60 minutes. The control sample shows significant decrease in bromelain concentration with the increased in holding time. While in treated samples, the bromelain concentration was stabilized when subjected to longer holding time for 50 and 60 minutes. This profile support the work of Liang *et al.* [2] where they reported the addition polyphenols compound can extent length of heating time without denature the bromelain. The polyphenols in apple juice could have contributed to this profile.

We extended our investigation to see any changes in bromelain concentration profile when exposed the samples to higher temperature of 50 °C. Figure 4 shows more promising profile than Figure 3. In this experiment, we found that treated samples we almost constant in its bromelain content when we exposed them to

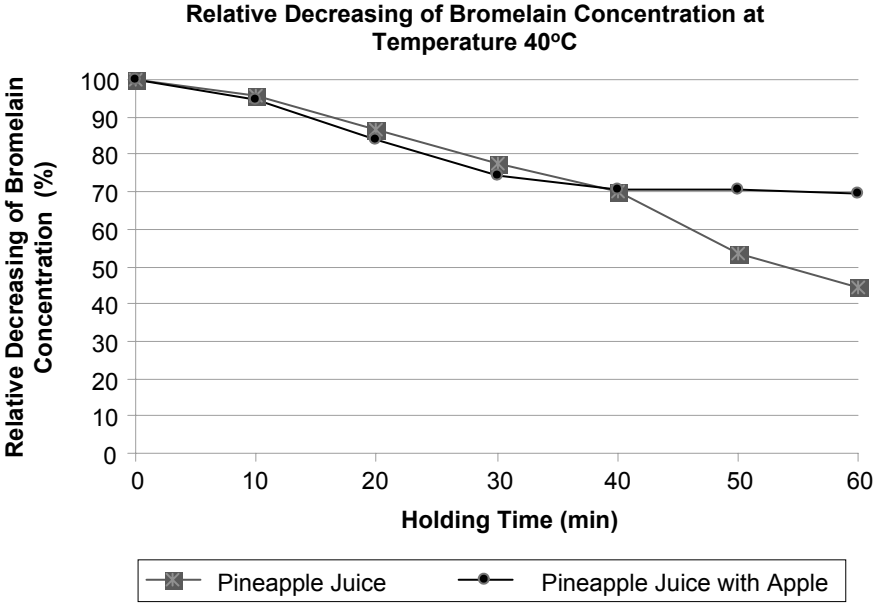

Figure 3 Relative decrease in bromelain concentration exposed to 40 °C at different holding time

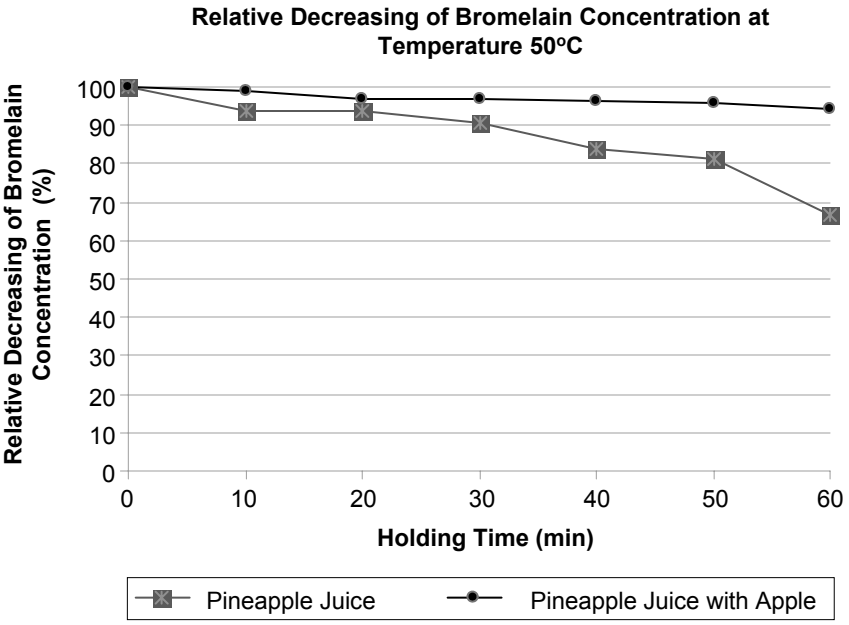

Figure 4 Relative decreasing of bromelain concentration at temperature 50 °C

50 °C for different holding time. It seems that bromelain concentration could be retained in the treated juices at 50 °C even though it has been exposed to this temperature for one hour. In contrast, untreated juice shows significant reduction in bromelain concentration exposed to 50 °C for 10 to 60 minutes.

Further expose of samples to temperature of 70 °C and 80 °C shown less effectiveness of apple juice in protecting the denaturation of bromelain as presented in Figure 5 and Figure 6 respectively. However we could see that holding time contributed in bromelain concentration constant. As we can see in Figure 5, when we heated the samples at 70 °C with holding time of 30, 40 and 50 minutes, we found that bromelain concentration was unchanged compared to control samples. However samples exposed to this temperature with longer holding time (one hour) reduced its bromelain concentration in both control and treated samples. Similar pattern was observed in samples exposed to 80 °C with holding time of 30 and 40 minutes (Figure 6).

The bromelain were optimally active at 60 °C [7,8] and about 55 °C [2]. Proteolytic activity was consistently lost when bromelain solutions were heated at 100 °C [9]. From the findings showed that heating to 70 °C or above resulted in degradation of full-length bromelain. Loss of immunoreactivity correlated with loss of protein consistenr with protein degradation. When bromelain was affinity-bound to a Sepharose matrix precoupled with the lectin concanavalin A (Con A) exhibited more resistant to thermal inactivation as evident from the retention of over 50% activity after incubation 60 °C for 100 min compared to 20% retained by native

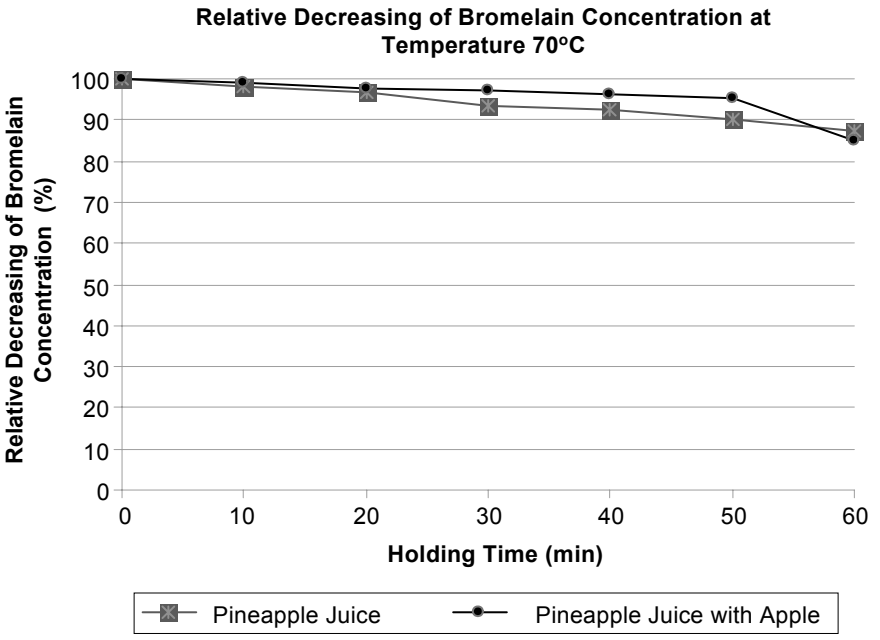

Figure 5 Relative decreasing of bromelain concentration at temperature 70 °C

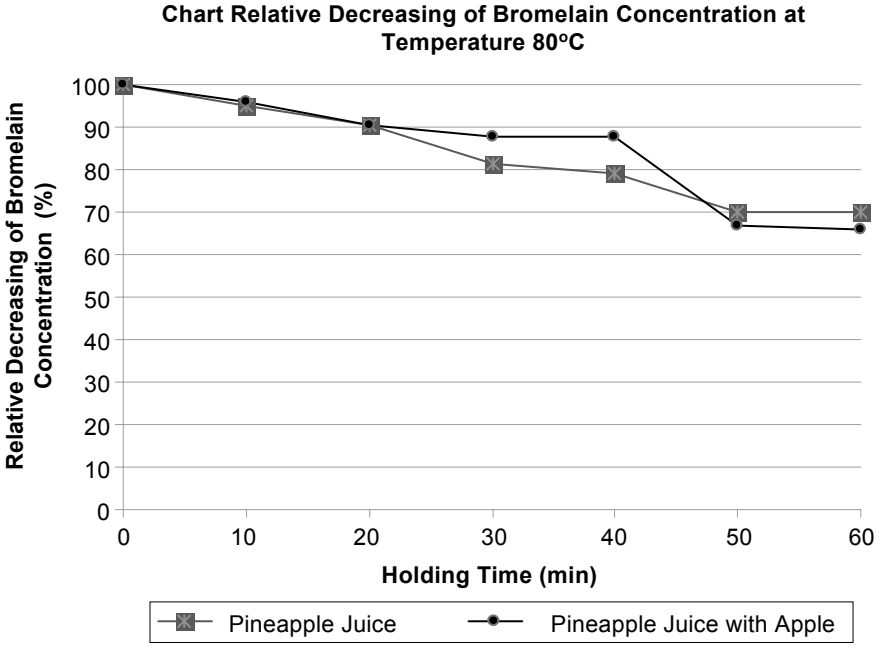

Figure 6 Relative decreasing of bromelain concentration at temperature 80 °C

bromelain enzyme [7]. The antioxidant activity in apple may account for its improved thermal stability. The free bromelain has much weaker antioxidant activity and tends to be easily oxidized and denatured.

3.2 Shelf life Analysis of Treated Samples

The treated samples were the tested for its shelf life capacity. We have chosen two temperatures setting which are 5 °C and 27 °C to mimic daily and common juice storage environment in the fridge and room temperature respectively. Table 3 below represents data collected during the samples shelf life experiment.

Data for day of pineapple juice spoilage were shown in Table 3. From the table, day for both pineapple juice spoilt increase as temperature for thermal treatment increased. This indicates that thermal treatment extends the storage life of the juices. The shelf life of juice also increases when stored at lower temperature. The taste,

Table 3 Shelf life of samples a) pineapple juice and b) pineapple-apple juice complex exposed to different temperature and holding time

a)

	Sample Treatment Temperature(°C)							
Holding Time (min)	**40**		**50**		**70**		**80**	
Storage temperature	**5°C**	**27°C**	**5°C**	**27°C**	**5°C**	**27°C**	**5°C**	**27°C**
0	3	1	3	1	4	1	5	2
10	3	1	3	1	4	1	5	2
20	3	1	4	1	4	2	5	2
30	3	1	4	1	4	2	5	2
40	4	1	4	1	5	2	5	2
50	4	1	4	1	5	2	5	2
60	4	1	4	1	5	2	5	2

b)

	Sample Treatment Temperature(°C)							
Holding Time (min)	**40**		**50**		**70**		**80**	
Storage temperature	**5°C**	**27°C**	**5°C**	**27°C**	**5°C**	**27°C**	**5°C**	**27°C**
0	3	1	3	1	4	2	4	2
10	3	1	3	1	4	2	4	2
20	3	1	4	1	4	2	5	2
30	3	1	4	1	4	2	5	2
40	3	1	4	1	4	2	5	2
50	3	1	4	1	4	2	5	2
60	3	1	4	1	5	2	5	2

smell and colour were used as indicator of juice spoilage. The juice was considered spoiled when the taste change to bitter and the aroma of pineapple becoming less and with sour smell.

The observation of the shelf life of juice for this study focus on a) the temperature of treatment and b) temperature of storage in 5 °C and room temperature 27 °C. The parameters obtained were used to predict the stability the juices at different thermal treatment and different storage condition. The taste, smell and colour were used as indicator of juice spoilage. From the findings, it shows that pineapple juice which heat at 60 °C for 60 minutes and store at 5 °C would stay longer until 5 days compare to the others. We observed that untreated and phenolic treated juice with showed similar result. In this case, moderate thermal treatment with lower temperature extended the storage life at low temperature (in fridge condition).

Several treatments have been studied to maintain quality and extend shelf-life of juice by modifying parameters such as atmosphere, storage, packaging and refrigeration to slow undesirable quality [10]. During the storage various changes may occur. The appearances like taste, color, texture are important attribute for acceptance or rejection the fresh juice fruit by the consumer. According to previous study reported that shelf life testing of enriched apple juice during 4 days at 80 °C showed decreases in the antioxidant activity of 20-40% [11]. In their study also showed that during one month storage of apple juice without thermal treatment in a refrigerator or even room temperature will not lower concentration of the present polyphenolic antioxidant. Therefore, the phenolic compound in pineapple juice also helps to maintain the pineapple juice stabilisation. Despite in previous study reported cold storage may affect the antioxidant properties of apples [12].

4.0 CONCLUSIONS

Our study shows that the bromelain-polyphenol complex enhanced bromelain thermal stability and the shelf life of juice when stored at lower temperature. Polyphenol as antioxidant agent play importance role to stable the condition of the pineapple juice. The bromelain free tends to be easily oxidized and denatured without antioxidant agent. Further studies are needed to evaluate the behaviour study of polyphenols antioxidants from apple that mix in pineapple juice in different temperature of treatment and storage condition at normal and elevated temperatures.

ACKNOWLEDGEMENT

The authors are grateful to Cannery of Malaysia Sdn Bhd for supply of raw materials and Malaysia of Pineapple Institute Board by supporting this study.

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