MICROWAVE PASTEURISATION FOR RETENTION OF BROMELAIN AND VITAMIN C IN PINEAPPLE JUICE

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To my beloved parents, husband and friends.

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ABSTRACT

The health benefits of bromelain and vitamin C attract a lot of interest from the consumers and food processing industries. Since it was known that conventional thermal treatment causes an adverse effect towards nutritional value in fruit juices, therefore a novel thermal processing such as microwave can become a potential alternative for juice pasteurisation. The main objective of this study is to evaluate the effect of microwave and conventional thermal pasteurisation on the quality of pineapple juice that includes bromelain proteolytic activity, vitamin C contents, microbial contents, colour and pH. The growth of microorganisms and changes in all parameters during storage period of 30 days were also studied. The pineapple juice sample was heated to 60°C on a hot plate with a stirrer and was held for 5 minutes in conventional thermal treatment. For microwave sample it was heated with an operating power of 900 W until the temperature reached 60°C and was held for 5 minutes. It was shown that microwaved samples had significantly ($p<0.05$) retained a higher amount of bromelain and vitamin C compared to conventional thermal treatment. The residual percentage of bromelain and vitamin C in microwaved juice were 87.36 $\pm$ 3.15% and 90.87 $\pm$ 1.13% whereas for conventional treated samples the values were reduced to 70.83 $\pm$ 7.01% and 74.72 $\pm$ 0.37%, respectively. At the end of the storage period, approximately 50% retention of bromelain and vitamin C were found in microwave treated juice. The degradation of these nutrients were rapid in conventional heated sample during storage. Both degradation of bromelain and vitamin C in microwaved and conventional pasteurised juices followed first order kinetic model. The k values of bromelain degradation were 0.0175 $\pm$ 0.0002 day^{-1} (microwave) and 0.0322 $\pm$ 0.0027 day^{-1} (conventional) while k values for vitamin C degradation were 0.0182 $\pm$ 0.001 day^{-1} (microwave) and 0.0257 $\pm$ 0.001 day^{-1}. The shelf life of microwaved samples was 22 days compared to only 14 days for conventional pasteurised pineapple juice. No significant changes detected in pH values after treatment and storage, however, the differences in colour was well visible after 30 days in both pasteurised juices. Therefore, it can be concluded that microwave treatment is a potential alternative for fruit juice pasteurisation especially for small scale industrial application.

ABSTRAK

Kebaikan bromelain dan vitamin C telah banyak menarik minat pengguna dan industri pemprosesan makanan. Umumnya, pempasteuran secara konvensional memberi kesan buruk terhadap nilai pemakanan dalam jus buah-buahan. Oleh itu, pempasteuran baru menggunakan gelombang mikro boleh dijadikan sebagai alternatif untuk pempasteuran jus buah-buahan. Tujuan kajian ini adalah untuk menilai kesan pempasteuran menggunakan gelombang mikro dan secara konvensional terhadap kualiti jus nanas seperti perbezaan kandungan *bromelain*, vitamin C dan mikrob serta warna dan pH. Pertumbuhan mikrob dan perbezaan dalam semua parameter selepas 30 hari juga dinilai. Sampel jus nanas telah dipanaskan sehingga 60°C menggunakan *'hotplate stirrer'* dan dibiarkan selama 5 minit untuk pempasteuran konvensional. Manakala untuk sampel gelombang mikro, ia dipanaskan dengan kuasa operasi 900 W sehingga mencapai 60°C dan dibiarkan selama 5 minit. Keputusan menunjukkan sampel gelombang mikro dapat mengekalkan kandungan *bromelain* dan vitamin C yang lebih tinggi berbanding sampel yang dipasteur secara konvensional. Peratusan *bromelain* dan vitamin C selepas pempasteuran dalam sampel gelombang mikro adalah 87.36 ± 3.15% dan 90.87 ± 1.13% manakala dalam sampel yang dirawat konvensional ialah 70.83 ± 7.01% dan 74.72 ± 0.37%. Gelombang mikro dapat mengekalkan 50% *bromelain* dan vitamin C selepas 30 hari. Walau bagaimanapun, kemerosotan nutrien ini lebih cepat dalam sampel dipanaskan konvensional sepanjang tempoh 30 hari. Kedua-dua degradasi bromelain dan vitamin C dalam jus yang dipasteur secara gelombang mikro dan konvensional adalah mengikut model kinetik pertama. Nilai k untuk degradasi bromelain ialah 0.0175 ± 0.0002 $hari^{-1}$ (ketuhar gelombang mikro) dan 0.0322 ± 0.0027 $hari^{-1}$ (konvensional) manakala nilai k untuk degradasi vitamin C ialah 0.0182 ± 0.001 $hari^{-1}$ (ketuhar gelombang mikro) dan 0.0257 ± 0.001 $hari^{-1}$. Sampel gelombang mikro mempunyai jangka hayat yang lebih lama iaitu 22 hari berbanding 14 hari untuk sampel yang dipasteur secara konvensional. Tiada perbezaan ketara pada pH selepas dipasteur dan penyimpanan tetapi perbezaan warna yang ketara dapat dilihat dalam kedua-dua sampel selepas penyimpanan. Kesimpulannya, pempasteuran menggunakan gelombang mikro adalah alternatif yang baik untuk diaplikasikan dalam industri kecil-kecilan.

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LIST OF ABBREVIATIONS

CVDs	-	cardiovascular diseases
E. coli	-	Escherichia coli
MPIB	-	Malaysian Pineapple Industry Board
MTX	-	methotrexate
NADPH	-	nicotinamide adenine dinucleotide phosphate
OFAT	-	one-factor-at-a-time
PCA	-	plate count agar
PME	-	pectin methyl esterase
PRO	-	proteolytic
ROS	-	reactive oxidant species
TA	-	Titratable acidity
TCA	-	trichloroacetic acid
TMB	-	total mesophilic bacteria
TPC	-	total plate counts
TSS	-	Total soluble solid
Y&M	-	yeast and mould

LIST OF SYMBOLS

ΔE*	-	total colour difference
*a**	-	measure of chromaticity
*b**	-	measure of yellowness
°C	-	degrees Celsius
cm	-	centimeter
GHz	-	gigahertz
g	-	gram
I. U	-	International Units
L*	-	measure of lightness
M	-	molar
mg	-	milligram
MHz	-	megahertz
min	-	minutes
mL	-	milliliter
mM	-	millimolar
µg	-	microgram
µL	-	microliter
nm	-	nanometer
ppm	-	parts per million
s	-	seconds
W	-	Watt

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Pineapple (*Ananas comosus L.*) is one of the most important tropical fruit that is commercially consumed fresh and as a processed product. Pineapple is cultured in many countries such as Malaysia, Philippines, Thailand, Costa Rica, Indonesia, Chile, Brazil and India. Different variety of pineapple has a different composition of nutrients. Pineapple varieties that are mainly cultured in Malaysia are Morris, Sarawak, Gandol, Maspine, N36 and Josapine. Pineapple can be produced into several products such as canned pineapple slices, dried pineapple, pineapple juice concentrate and pasteurised pineapple juice. Pasteurised pineapple juice is one of the important pineapple commodities among pineapple products (Hounhouigan *et al*., 2014).

Pineapple plant contains an abundant amount of bromelain enzyme which possess many beneficial properties for human health. Bromelain is a protein digesting enzyme (Sew *et al*., 2014). It can be used for therapeutic purpose such as reducing inflammation, treating indigestion, acts as an anti-tumour agent, supports skin debridement of burns and prevents aggregation of blood platelets (Tochi *et al.*, 2008; Abdul Majid *et al.*, 2008; Sew *et al*., 2014; Chakraborty *et al*., 2016). It is also known as a meat tenderizer (Ketnawa & Rawdkuen, 2011).

Besides bromelain, this fruit can also be a good source of vitamin C in human diet. Vitamin C is crucial for formation of antibodies, synthesis of collagen and bones and absorption of iron (Uckiah *et al*., 2009). Previous study had showed that inadequate intake of vitamin C can lead to a risk of hypertension, stroke, cancers and cataracts (Higdon, 2006). Besides, this nutrient is important for reducing a risk of coronary heart disease (Higdon, 2006; Laorko *et al*., 2013). The most natural way to consume these nutrients is by consuming fresh pineapple or fresh pineapple juice (Abdul Majid *et al.*, 2008).

However, fresh fruit juice has a high probability of spoilage due to microorganisms from the environment hence pasteurisation of fruit juice is needed to meet the safety requirements in the industry (Shamsudin *et al*., 2014). Thermal and non-thermal processes are usually applied to ensure the safety of processed juice (Goh *et al.*, 2012).

Pineapple juice is commercially processed using conventional thermal pasteurisation. Generally, the process uses a temperature that is lower than boiling point of water for a particular period of time to treat the juice and inactivate microbial contents in the juice (Abdul Majid *et al.*, 2008). The process is important as the edible part of pineapple fruit (60% of the fresh fruit) has high water content which is 85% and this can inhibit the growth of microorganisms and yeast (Hounhouigan *et al.*, 2012).

Thermal pasteurisation can also extend the shelf-life of pineapple juice. However it causes a significant decrease of bromelain enzyme and vitamin C content in pineapple as these compounds are sensitive to heat (Sriwatanapongse *et al.*, 2000; Vikram *et al.*, 2005). Besides, storage time and temperature also affect the degradation of vitamin C and bromelain contents. It has been proven that increasing temperature and storage time causes a general decrease in content for both compounds (Abdul Majid *et al*., 2008; Uckiah *et al*., 2009; Goh *et al*., 2012; Chia *et al*., 2012; Laorko *et al*., 2013; Sew *et al*., 2014).

In order to produce a highly nutritious fruit juice, a potential alternative thermal pasteurisation was introduced, which is microwave pasteurisation. Microwave pasteurisation has been reported to be effective in maintaining the quality of fruit juices and enhancing the inactivation of microorganisms which resulted in an extended shelf life (Demirdöven & Baysal, 2015). It also reduces a significant period of time during the process thus lowers the risk of losing essential nutrition in the juice (Benlloch-Tinoco *et al*., 2015). Microwave energy generates heat inside the food volumetrically unlike thermal pasteurisation. Thus, heating rate becomes faster and it has a higher thermal efficiency.

In this study, the optimum conditions for microwave pasteurisation on pineapple juice will be determined. Effect of microwave treatment and conventional thermal treatment on nutritional value of pineapple juice such as bromelain and vitamin C will also be evaluated. Morris pineapple variety is chosen for juice production based on its suitability for juice production as stated by Malaysian Pineapple Industry Board (MPIB). The study will compare bromelain and vitamin C content as well as the shelf life of pineapple juice between both pasteurisation methods in terms of kinetic modelling throughout a storage period of 30 days. This study would be beneficial for providing an alternative juice pasteurisation for industrial application.

1.2 Problem Statement

It is known that fresh pineapple juice contributes a high nutritional value. The main nutrients in pineapple juice are bromelain enzyme and antioxidants such as vitamin C. Recent researches about the advantages of bromelain and vitamin C highlights the significance of these components in human body (Higdon, 2006; Abdul Majid *et al*., 2008; Chakraborty *et al.*, 2016).

Bromelain can aid digestion, act as an anti-inflammation agent, nasal decongestant, anti-tumour agent and reduce swelling after surgery while vitamin C is known for its importance in repairment of tissues and human growth, formation of antibodies and helps in biosynthesis of collagen. Optimum consumption of bromelain and vitamin C can be obtained from fresh pineapple juice. However fresh pineapple juice has a short shelf life, typically a few days if it is refrigerated. This is due to the high water content that makes the pineapple juice prone to microorganisms. Therefore, conventional thermal treatment which is one of a pasteurisation method is used commercially in industrial application to extend the shelf life of pineapple juice.

Although the conventional thermal treatment method can increase its shelf life, it leads to a degradation in nutritional value of pineapple juice especially bromelain and vitamin C based on previous studies (Sriwatanapongse *et al*., 2000; Goh *et al*., 2012). Hence, an alternative pasteurisation method using microwave technology was proposed in this study.

Previous studies of microwave pasteurisation on other fruit juices were reported abundantly and proves that this method is efficient in extending the shelf life of fruit juices and at the same time maintaining its nutritional value (Igual *et al*., 2010; Abdullah *et al*., 2013; Benlloch-Tinoco *et al.*, 2014a; Benlloch-Tinoco *et al.*, 2015, Bevilacqua *et al.*, 2015). However, no published study has been found on microwave effect towards bromelain and vitamin C contents in pineapple juice.

This study is important to determine the quality of pineapple juice pasteurised using microwave technology and compare the effect towards bromelain and vitamin C contents in pineapple juice for both methods. It will indicate the benefits of microwave pasteurisation method in pineapple juice industry as consumers are demanding a high quality product.

1.3 Objectives of the Study

The main objective of this study is to test the performance of microwave in maintaining the quality and nutritional values and at the same time, enhancing the inactivation of pathogen microorganisms in pineapple juice. The specific objectives of this study are as follows:

1) To optimise the processing conditions for microwave pasteurisation of pineapple juice
2) To evaluate the storage stability of pasteurised pineapple juice and obtain the degradation rate of the parameters studied

1.4 Scope of Study

This study elucidated the utilisation of microwave technology for pasteurisation of pineapple juice which reduced the losses of bromelain and vitamin C contents and enhanced the destruction of microorganisms. In achieving the desired objectives, the scopes of this study were drawn as the following:

1) Conducting evaluation and optimisation of microwave processing conditions which include heating temperature, microwave power and holding time. The range of temperatures used were 50-70°C while for microwave power were 500-900 W. Holding time were varied from 0 to 8 minutes. This optimisation was performed using one-factor-at-a-time (OFAT) method.

2) Evaluation and comparison of bromelain and vitamin C contents as well as microbial analysis of microwaved juice were conducted to identify the best processing conditions for microwave pasteurisation.

3) Comparison of the effects of microwave treatment and conventional thermal pasteurisation on the physicochemical properties of pineapple juice. The physicochemical properties that were studied are bromelain and vitamin C contents, microbiological analysis, changes of colour and pH.

4) Examination of storage stability of pasteurised pineapple juices through microbiological and physicochemical changes in pineapple juice. The shelf-life of pasteurised pineapple juice was determined based on the microbial counts in the juice. The storage stability was examined based on the degradation rate of bromelain and vitamin C contents.

1.5 Significance of Study

Although pineapple juice is one of the important commodities to national economy, a product of highly nutritious pineapple juice is yet to be fully accomplished. Therefore, this study is carried out to determine whether microwave pasteurisation will produce a better juice product with high contents of bromelain and vitamin C compared to traditional method.

The outcome of this study aims to contribute to a highly nutritious juice for the consumers as nowadays, the consumers are demanding a health and high quality products. If the demands increases this will also affect the economic growth of fruit juice industry which will improve the social life of farmers. Besides, this study

provides a faster alternative treatment for pineapple juice pasteurisation for small industrial application and it can contribute to energy conservation by using this novel thermal technology.

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