PILOT SCALE EXTRACTION OF CRUDE BROMELAIN FROM PINEAPPLE MORRIS AND MD2 CULTIVAR USING HOLLOW FIBER NANOFILTRATION MEMBRANE

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ABSTRACT

Pineapple (Ananas comosus) is rich with nutrients, incredibly juicy and has been linked to many health benefits. Pineapples are not only taken fresh but they have been commercialized in the canning industry. However, in the canning industry only the flesh is utilized and the rest of the pineapple (50 wt %) such as the core, stem, peels and crown are discarded as waste. In the extraction of bromelain which is a vital proteolytic enzyme, the whole pineapple including its peels, core, stem and crown can be utilized. Bromelain is very valuable and different applications require different bromelain purity; therefore, the extraction and purification technique can be designed depending on the purpose of the bromelain. In this study, nanofiltration (NF) membrane process is used to extract bromelain of Morris and MD2 cultivar from the flesh, crown, stem, core and peel of pineapples. The different parts of the pineapple were crushed to extract the juice. The juice was then centrifuged and the supernatant was then passed through a hollow fiber NF. Finally, the retentate from the different pineapple parts which contain high amounts of bromelain were freeze-dried and quantified. Casein digestion unit method was used to determine the bromelain activity. The amount of bromelain activity increased after every process especially after freeze-drying. The bromelain content in retentate was also analysed using high performance liquid chromatography. Findings revealed that retention time peak of the bromelain appeared within 1 min and bromelain content was found to be highest in peel > flesh > stem > core for both pineapple species. The extracted bromelain solution was then freeze dried and maltodextrin was added so as to ensure encapsulation stability. The concentration of the maltodextrin added varied ranging from 2 – 10 % concentration (w/w %). Water was eliminated during freeze-drying, but unfortunately, bromelain powder with lower maltodextrin concentration (2 – 8 %) were found to be sticky compared to the highest concentration at 10 % which showed an amorphous glassy maltodextrin microstructure (i.e., "bromelain powder"). Upon crushing, the maltodextrin microstructure yielded a free-flowing powder with bromelain enzyme activity of 412.42 CDU/mg. As for the microbiological quality of the bromelain powder, it is safe to be consumed because pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella and Eshrerichia coli* were absent in the core and flesh bromelain powder. Enterobacteria and other gram-negative bacteria were less than 10 MPN/ml whereas bacteria count and fungi count was 3.9 x 10^4 CFU/ml and 6.5 x 10^1 CFU/ml for core bromelain powder and 5.1 x 10^4 CFU/ml and 7.1 x 10^1 CFU/ml for flesh bromelain powder, respectively. 2,2′-diphenyl-1-picrylhydrazyl test results showed the presence of anti-oxidant activity of bromelain was 8.75 % and 11.23 %, respectively for core and flesh pineapple. Meanwhile the anti-inflammatory activity was also detected in the extracted bromelain from the core (50 %) and flesh (45 %) of pineapple. Profitability analysis of the crude bromelain powder plant estimates the rate of return is 34 % and the payback period for the company to start earning profit is 2.92 years. In terms of price, the lowest price in the market for 30 tablets in a bottle was RM 39.14. While the product from this process can be sold at the price of RM 35 for 30 capsules in a bottle, which is cheaper.

ABSTRAK

Nanas (Ananas comosus) kaya dengan nutrien, mengandungi banyak air dan dikaitkan dengan banyak manfaat kesihatan. Nanas tidak hanya diambil segar tetapi telah dikomersialkan melalui industri pengetinan. Namun, dalam industri pengetinan hanya isi nanas yang digunakan manakala sisa nanas (50 % berat) seperti empulur, batang, kulit dan jambul dibuang sebagai sampah. Dalam penghasilan bromelain yang merupakan enzim proteolitik penting, seluruh nanas termasuk kulitnya, empulur, batang dan jambul boleh digunakan. Bromelain sangat berharga dan aplikasi yang berbeza memerlukan tahap ketulenan bromelain yang berbeza; oleh itu, teknik pengekstrakan dan penulenan dapat direka bergantung pada tujuan bromelain. Dalam kajian ini, proses membran nanofiltrasi (NF) digunakan untuk mengekstrak bromelain daripada nanas jenis Morris dan MD2 daripada bahagian isi, jambul, batang, empulur dan kulit nanas. Bahagian nanas yang berlainan ini dihancurkan untuk mengeluarkan jusnya. Jus kemudian diempar dan supernatan kemudian diproses melalui NF. Akhirnya, *retentate* daripada bahagian nanas yang berlainan yang mengandungi sejumlah besar bromelain dikering bekukan dan diukur. Kaedah unit pencernaan kasein digunakan untuk menentukan aktiviti bromelain. Jumlah aktiviti bromelain meningkat selepas setiap proses terutama selepas pengeringan beku. Kandungan bromelain dalam *retentate* juga dianalisa menggunakan kromatografi cecair berprestasi tinggi. Hasil kajian mendapati bahawa puncak masa pengekalan bromelain muncul dalam 1 minit dan kandungan bromelain didapati paling tinggi pada kulit> isi> batang> empulur untuk kedua-dua spesies nanas. Larutan bromelain yang diekstrak kemudian dikering beku dan maltodekstrin ditambahkan untuk memastikan kestabilan pengkapsulan. Kepekatan maltodekstrin yang ditambahkan dibezakan antara 2 – 10 % kepekatan (w / w %). Air disingkirkan semasa pengeringan beku, tetapi sayangnya, serbuk bromelain dengan kepekatan maltodekstrin yang lebih rendah (2 – 8 %) didapati melekit dibandingkan dengan kepekatan tertinggi pada 10 % yang menunjukkan struktur mikro maltodekstrin berkaca amorfus (iaitu, "serbuk bromelain") Setelah dihancurkan, struktur mikro maltodekstrin menghasilkan serbuk bebas dengan aktiviti enzim bromelin sebanyak 412.42 CDU/mg. Mengenai kualiti mikrobiologi serbuk bromelain, ianya selamat dimakan kerana patogen seperti *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* dan *Escherichia coli* tidak ada dalam serbuk bromelain yang diperolehi daripada empulur dan isi. Enterobacteria dan bakteria gram-negatif lain kurang dari 10 MPN / ml sedangkan kiraan bakteria dan kulat adalah masing-masing 3.9 x 10^4 CFU / ml dan 6.5 x 10^1 CFU / ml untuk serbuk bromelain empulur dan 5.1 x 10^4 CFU / ml dan 7.1 x 10^1 CFU / ml untuk serbuk bromelain isi. Hasil ujian 2,2′-difenil-1- pikrilhidrazil menunjukkan kehadiran aktiviti antioksidan bromelain adalah 8.75 % dan 11.23 %, masing-masing untuk empulur dan isi nanas. Sementara itu, aktiviti antiradang juga dikesan pada bromelain yang diekstrak dari empulur (50 %) dan isi (45 %) nanas. Analisis keuntungan kilang serbuk bromelain mentah menganggarkan kadar pulangan pada 34 % dan tempoh pembayaran balik bagi syarikat untuk mula memperoleh keuntungan adalah 2.92 tahun. Dari segi harga pula, harga terendah di pasaran untuk 30 biji sebotol ialah RM 39.14. Manakala produk daripada proses ini boleh dijual pada harga yang lebih murah RM 35 untuk 30 kapsul dalam satu botol.

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LIST OF ABBREVIATIONS

ALE	-	Pineapple (Ananas comosus) Leaf Extract
ATPS	-	Aqueous Two-Phase System
BHA	-	Butylated Hydroxy-anisole
BHT	-	Butylated Hydroxytoluene
BSA	-	Bovine Serum Albumin
$C_2H_3NaO_2$	-	Sodium acetate
$C_4H_4Na_2O_6$	-	Sodium tartrate
$C_7H_4N_2O_7$	-	3, 5 - Dinitrosalicylic acid
CAGR	-	Compound Annual Growth Rate
CD44	-	Hyaluronan Receptor
CDU	-	Casein Digestion Unit
CFU	-	Colony Forming Unit
CH_3COOH	-	Acetic acid
CH_3OH	-	Methanol
COM	-	Cost of Manufacture
CWM	-	Crude Waste Mixture
DC	-	Direct cost
DMSO	-	Dimethyl sulfoxide
DNS	-	Dinitrosalicylic Acid
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
DTAB	-	Dodecyl Trimethyl Ammonium Bromide
EA	-	Enzyme activity
EC. 3.4.22.32	-	Bromelain from Pineapple Stem
EC. 3.4.22.33	-	Bromelain from Pineapple Fruit
EDTA	-	Ethylene diamine tetra-acetic acid
FAO	-	Food and Agriculture Organization
FAOSTAT	-	Food and Agriculture Organization of the United Nations
FC	-	Fixed cost
FDA	-	Food and Drug Administration

GC	-	Gas Chromatography
GDP	-	Gross Domestic Product
GDU	-	Gelatine Digestion Unit
GE	-	General expenses
H_2O	-	Water
HCl	-	Hydrochloric acid
HF	-	Hollow Fibre
HPLC	-	High Performance Liquid Chromatography
HNO_3	-	Nitric Acid
IFN	-	Interferon-gamma
IL	-	Ionic Liquid
LC	-	Liquid Chromatography
MD	-	Maltodextrin
MF	-	Microfiltration
MPIB	-	Malaysian Pineapple Industry Board
MRFR	-	Market Research Future
MW	-	Molecular Weight
MWCO	-	Molecular Weight Cut-Off
NaCl	-	Sodium chloride
Na_2HPO_4	-	Anhydrous disodium phosphate
$NaPO_4$	-	Sodium phosphate
Na_2O_2	-	Sodium peroxide
Na_2SO_4	-	Sodium sulphate
Nano-TiO_2	-	Titanium dioxide nanoparticles
NaOH	-	Sodium hydroxide
NF	-	Nanofiltration
$(NH_4)_2SO_4$	-	Ammonium Sulfate
NO	-	Nitric Acid
NSAIDs	-	Non-Steroidal Anti-Inflammatory Drugs
OD	-	Optical density
PBP	-	Payback Period
PC	-	Paper Chromatography
PC	-	Protein content

PEG	-	Polyethylene Glycol
PES	-	Polyether Sulfone
PGE2	-	Prostaglandin E_2
RM	-	Ringgit Malaysia
RME	-	Reverse Micellar Extraction
RMS	-	Reverse Micellar System
ROI	-	Return on Investment
RPM	-	Rotation per Minutes
SA	-	Specific activity
SPIONs–Br–FA	-	Superparamagnetic Iron Oxide Nanoparticles–Bromelain–Folic Acid
TA	-	Titratable Acidity
TCA	-	Trichloroacetic Acid
Tg	-	Low Glass Transition Temperatures
TLC	-	Thin-Layer Chromatography
TMP	-	Trans-Membrane Pressure
TNF	-	Tumor Necrosis Factor Alpha
TSS	-	Total Soluble Solid
UF	-	Ultrafiltration
USD	-	United State Dollar
ZrO		Zirconium Oxide

LIST OF SYMBOLS

A	-	Membrane area
A_{IT}	-	Annual income taxes
°Brix	-	Degree Brix
°C	-	Degree celcius
C_{AC}	-	Auxiliary facility cost
C_{CF}	-	Contingency fee
C_{FC}	-	Fixed capital cost
C_{OL}	-	Operational labor cost
C_{RM}	-	Raw material cost
C_{TBM}	-	Total bare module cost
C_{TCI}	-	Total capital investment cost
C_{TM}	-	Total module cost
C_{UT}	-	Utilities cost
C_{WC}	-	Working capital cost
C_{WT}	-	Waste treatment cost
CDU/mg	-	Casein Digestion Unit per milligram
CDU/ml	-	Casein Digestion Unit per millilitre
CFU/ml	-	Colony-Forming Unit per millilitre
cm	-	centimetre
FCI	-	fixed capital investment cost
g	-	gram
GDU/ml	-	Gelatin Digestion Unit per millilitre
h	-	hour
J	-	Filtrate flux rate
kDa	-	kilo Dalton
kg	-	kilogram
kg/h	-	kilogram per hour
kW	-	kilowatt
kW/h	-	kilowatt per hour
L	-	litre

L/h	-	Litre per hour
mM	-	millimolar
M	-	molar
m	-	metre
m^2	-	metre squares
m^3	-	Metre cubes
mg	-	milligram
mg/L	-	Milligram per litre
mg/ml	-	Milligram per millilitre
ml	-	millilitre
ml/min	-	millilitre per minute
mol/L	-	moles per litre
mmol/L	-	millimoles per litre
MPN/ml	-	most probable number per millilitre
nm	-	nanometre
Pa	-	pascal
RM/m^3	-	Ringgit Malaysia per metre cubes
T	-	Process time
U/ml	-	Unit per millilitre
V	-	Volume of filtrate generated
V_c	-	absorbance of control
V_t	-	absorbance of the test sample
vol%	-	volume percent
w/w %	-	mass percentage of the solute in solution
wt%	-	weight percent
µm	-	micrometre
µg	-	microgram
µg/ml	-	microgram per millimetre

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CHAPTER 1

INTRODUCTION

1.1 Overview Background

Pineapple (Ananas comosus L. Merr) is a tropical fruit of the Bromeliaceae family, comprising more than 2,000 species, and the third most widespread tropical fruit behind banana and citrus (Jaji *et al.*, 2018). Pineapples are used in canning industry and also juice products. There are massive pineapple plantations in Asia (Thailand, Philippines, Malaysia, China, and India) as well as South-Central America (Brazil, Costa Rica). According to FAO, among the world's pineapple producers, Brazil is the largest, followed by Thailand, Philippines, and Costa Rica. Malaysia is also listed as a pineapple producer in the world especially in the Asia region and the state that produces most of the pineapple is Johor. In 2016, approximately 0.27 million tons of pineapples (almost 70 %) were produced in Johor (MPIB, 2016).

The Morris cultivar is most extensively planted in Malaysia, followed by Josapine, Sarawak, Gandol, N36 and latest MD2 varieties. For the export of fresh pineapples, the N36 and Josapine cultivars are grown. Grown for both the domestic market along with exportation to Singapore, the Morris cultivar is used for fresh market, while the Gandol and N36 varieties are produced for canning (Othman *et al.*, 2011). The MD2 (or Gold) cultivar has gained recognition on the international market for its excellent taste, balance between sweetness and acidity, and juicy texture (Žemlička *et al.*, 2013). Firm, fully ripe and evenly matured pineapples are usually selected for processing as they have strong flavor, better color and high product yield. Pineapple can be processed into various products including pulp, juice, beverages, jam, confectionery jelly, canned in syrup, fruit roll/leather, breaded, dehydrated candied, pickle, sauce, fruit fillings and powder. In Malaysia, ‘Morris’, ‘Sarawak’ and ‘Josaphine’ are recommended for fresh consumption whilst ‘Gandul’ and ‘Maspine’ are recommended for processing, especially for canned slices and breaded fruits (Zainun, 2011).

For the growth of the pineapples, the crown is removed and hormones are applied, so that the size of the pineapples can be increased. Even, during the pineapple processing for canned pineapple or pineapple juice, the crown is cut-off before peeling. The same step goes to the stem, and the core is removed for further processing. From the total pineapple produced, 70 % is consumed fresh while the balanced is processed into juice, canned slices, crush (solid pack) and chunks (Nor *et al*., 2017). Basically, pineapples industries create almost 50 % (w/w) wastes (peel, core, stem and crown) of total pineapple weight. This means, increasing pineapple productions also increases the pineapples wastes. These wastes will cause serious environmental problems due to waste disposal failure. Therefore, the waste must be evaded from being a part of world's pollution. Fortunately, potential source of valuable components like bromelain enzyme has been identified from pineapple waste (Nor *et al*., 2015), hence research have been made to utilize bromelain from all parts of pineapple so as to increase value to its industries.

Pineapple is well known source of proteolytic enzyme called bromelain and can be found abundantly in fruit and stem of pineapple. Bromelain is a mixture of cysteine proteases found in the tissue of plant family Bromeliaceae obtained from pineapple. The proteolytic enzymes obtained from pineapple commercially available are stem bromelain and fruit bromelain. Nevertheless, pineapple wastes such as peel, core and crown were reported to contain bromelain as well (Banerjee *et al*., 2020; Guo *et al*., 2018; Nor *et al*., 2015; Yin *et al*., 2011; Ketnawa, Chaiwut and Rawdkuen, 2011; Zhang *et al*., 2010). This enzyme is being seen as valuable because of its incredible phytomedical effects. Bromelain exhibits antifibrinolytic (J. Han et al., 2020), anti-inflammatory (Adu and Mabandla, 2021), anti-cancer (Debnath *et al*., 2019), anti-oedema (Bhoobalakrishnan *et al*., 2021), anti-thrombotic and antidiabetic (Ajayi *et al*., 2021) properties. Besides that, bromelain is a promising postoperatively agent that decreases post-surgical discomfort and swelling. It is also known to have wound healing properties. Apart from medical perspective, various industries also use bromelain in food, textile and cosmetic industries (Manzoor *et al*., 2016, Soares *et al*., 2012).

In addition, bromelain also have massive benefits that contribute to many industries as well. Therefore, researchers have attempted several conventional extraction/purification procedure to attain highest purified bromelain at low cost (Arshad *et al*., 2014). Bromelain is expected to reach USD 1,055.1 million in market size by 2025 (CAGR 4.60 %), according to Market Research Future (MRFR). By 2025, it is anticipated that market demand will reach 580 tonnes per year, at a CAGR of 6.54 %. Globally, in 2020, bromelain production is about 87,268 metric tonnes per year. Bromelain's unique properties will cause its market to grow due to rising demand from diverse end-use industries. Furthermore, bromelain can be used in the production of dietary supplements and serve as a meat tenderizing agent, which is expected to raise demand for bromelain and boost growth on the global market. Demand for bromelain is increasing, especially since natural products are now widely used in industries across the board.

The extraction of bromelain from all parts of pineapple is gaining attention in view of environment issues as well as economical (Noaves *et al*., 2013). Even though the juice of pineapple can be easily extracted by ultrafiltration yet fruit bromelain is not commercially available compared to stem bromelain (Corzo, Waliszewski and Welti-Chanes, 2012, Pavan *et al*., 2012). There are several techniques such as lyophilization, centrifugation, ultrafiltration and as well as two-step fast protein liquid chromatography (FPLC) to extract the marketable bromelain which is mostly from stem of pineapple, (Manzoor *et al*., 2016). Additionally, numerous new purification methods have been applied to extract and purify the bromelain owing to increase interest toward bromelain. The methods include aqueous two-phase system (Coelho *et al*., 2013; Spir *et al*., 2015), membrane processes (Doko *et al*., 1991; Lopes *et al*., 2009; Nor *et al*., 2016), precipitation (Devakate *et al*., 2009, Soares *et al*., 2012), reversed micellar system (RMS) (Hebbar, Sumana and Raghavarao, 2008, Kumar, Hemavathi and Hebbar, 2011, Wan *et al*., 2016) and other chromatographic techniques (Yin *et al*., 2011, Soares *et al*., 2012).

An innovative technique to utilize the waste would be significant to overcome the pineapple waste disposal. Since the demand for bromelain worldwide is increasing, a feasible purification and extraction technique for this enzyme is important. The simple technique introduced may benefit the pineapple agroindustry because the industrial and agricultural wastes of pineapple (crowns, stems and leaves) which have an abundance of bromelain enzyme can contribute to gross domestic product (GDP) of the country. Usually, this waste has no proper destination and have not been used (Costa *et al*., 2014). Thus, the current study presents an innovative and practical technique for the extraction of bromelain from all pineapple parts (Morris and MD2) using nanofiltration as its main technique. Besides, the anti-oxidant, anti-inflammatory and microbiological analysis were evaluated to determine the characteristics of bromelain which was retained using the separation/extraction techniques.

1.2 Problem Statement

There are several cultivars in Malaysia like Morris which are used in canning industry and the waste are converted and sell as cheap fertilizer. Generally, about 50 – 60 % of fruit contributes to waste like core, peel, stem and crown. In addition, some popular species like MD2 which loved to be eaten fresh must be of a sufficient size and perfect for domestic and export industries. In cases when the fruits do not meet the specifications, they cannot be sold and will be discarded and wasted due to an oversupply. In general, Grade A pineapples are exported and the other grades are sold in local markets as fresh produce. In total, about 5 % of pineapples were discarded due to post-harvest deterioration and export regulations (Suhana *et al*., 2019). Therefore, it is important to convert them to valuable products. Fortunately, bromelain enzyme has been identified from pineapple waste as a potential source of valued materials. Basically, pineapple produces both the stem bromelain and fruit bromelain. But the stem bromelain has been studied extensively for commercial use, while studies on fruit bromelain are comparatively limited. Studies of fruit bromelain, however, are rather scarce since pineapples are typically consumed fresh, juiced or canned. Nowadays, canned fruit consumption has declined greatly, including consumption of canned pineapples. Therefore, the pineapple industry could benefit from fruit bromelain (Han *et al*., 2019). Thus, the selection of the Malaysian pineapple species was determined. Morris was selected because this species is not as sweet as the other type of pineapple species, thus they are not eaten fresh and mostly used in the canning industry and MD2 was selected because farmers cannot sell them at good price when they do not have right size in meeting the specification.

For separation and extraction methods, various techniques were applied in order to gain highest bromelain production such as reverse micellar extraction, precipitation, aqueous two-phase system and ultrafiltration. All of this procedure requires a number of process. Currently, the methods used is reverse micellar extraction which entangled only the protein of interest in the micelles. However, the disadvantage of this method is difficulty in retrieving target proteins from organic solvents containing surfactants. Besides, precipitation is another method used by adding salts, organic solvents non-ionic polymer as well as metals. Majority of the proteins will precipitate from the solution under saturation and further steps such as centrifugation and dialysis are required in order to remove excess salt. Furthermore, aqueous two-phase system (ATPS) is another method applied to achieve higher yield of bromelain. During ATPS, the presence of PEG can change the active site structure of the enzyme, thus the recovery enzyme is significantly high. However, the drawback of this method is difficulty in recovering target proteins from phase-forming polymers. Another current method used is membrane filtration process using ultrafiltration. The proteins are separated by the size of its molecular weight and the membrane will become clogged with increased protein loading.

Since commercial bromelain has a wide range of applications, it is very expensive, costing as much as US$ 2400/kg primarily due to its production costs. Therefore, the current focus in this field is to design an effective concentration and purifying process employing waste from the pineapple industry with the goal in reducing the number of procedures required to achieve the desired enzyme purity. Generally, bromelain do not necessitate high purity for most commercial applications (food, feed and fabrics) except for research, pharmaceuticals and medical field, therefore the enzyme is typically generated in large quantities as crude for requests in high-volume manufacturing (Illanes, 2008; Vasiljevic, 2019). Specialty enzymes to be used in medicine and research are usually required in high levels of purity and in rather small quantities, while enzymes used in the bulk production of food, feed, fabrics and fuel are usually produced as rather crude preparations in high tonnage. Hence, in this study polymeric hollow fiber nanofiltration membrane method with unique characteristics is proposed as a feasible separation technique to extract bromelain as a single step. Besides that, nanofiltration membrane method can be scaled up easily and offer continuous operation. Furthermore, this method attracts an immense attention in particular for the extraction of an enzyme in recent times.

1.3 Research Objectives

The main objective of this study is to separate/extract crude bromelain from different parts of pineapple which include flesh, peel, core, stem and crown from two different species; Morris and MD2 using nanofiltration membrane on a pilot scale of 20L. In order to attain the main objective, the following issues need to be addressed:

i. To quantify the bromelain content of the juice obtained from the different parts of the fresh pineapples and also from pineapple wastes from the canning industry.

ii. To extract the crude bromelain using hollow fiber (HF) nanofiltration (NF) and determine its quality and quantity at every step of the process.

iii. To examine effect of maltodextrin as an encapsulation material to obtain crude bromelain powder and to study anti-oxidant, anti-inflammatory and effect of processing on the final quality of the powder.

iv. To determine the feasibility of the process using profitability analysis.

1.3 Scope of the Study

i. The study was limited to two Malaysian pineapple cultivars which is Morris and MD2 and also pineapple waste from canning industry.

ii. The effect of the pineapple maturity (fully ripened and partially ripened) and pineapple Morris with crown and no crown on bromelain content was investigated.

iii. The bromelain content of the different pineapple parts such as flesh, core, peel, stem and crown were determined and the bromelain enzyme activity was analyzed at every step of the process (after juicing, after centrifugation and after nanofiltration).

iv. The pineapple juice was characterized for pH, total sugar and total soluble solid (TSS) whilst the bromelain in the retentate samples from membrane were determined using HPLC analysis.

v. Molecular weight cut-off (MWCO) of the HF NF membrane was studied. Pressure, flowrate and permeation rate of the membrane process were determined and bromelain was extracted using the membrane process.

vi. Bromelain activity at every step of the process (after juicing, after centrifugation and after nanofiltration) were evaluated for the industrial pineapple waste (peel and core) and the stability was also analyzed.

vii. The storage stability of the crude bromelain obtained from both pineapple species was analyzed.

viii. Maltodextrin was used as an encapsulation material for bromelain powder. The percentage of maltodextrin was varied from 2-10 % w/v.

ix. The stability of the bromelain powder was analyzed at room, chiller and freezer temperature and anti-inflammatory and anti-oxidant test have been done on the crude bromelain powder.

x. Microbiological quality analysis was done for core and flesh crude bromelain powder.

xi. Finally, profitability analysis was performed taking into consideration all the relevant process and equipment involved.

1.5 Significant of the Study

The significant of the research is to produce crude bromelain not only from pineapple fruit but also for other parts of it such as core, peels, stems and crowns using a single step HF NF suitable for food and beverages application. The knowledge gained can help farmers improve their productivity and generate some revenue from the waste production thus reduce percentage disposal of pineapple waste and environmental problem. Instead of disposing the waste on land without any great value, production of bromelain can economically give values to the industry. Bromelain production has high demand as per 100 g of bromelain worth for $ (25-150) (Lakshminarasimaiah *et al*., 2014). Besides, pineapple can bring many benefits to industry, human health and other organisms especially from extraction of bromelain. In addition, production of bromelain may be useful in to food and pharmaceutical field. Bromelain obtained from the pineapple waste can be used as a diet supplementary in food industry and many other applications as well.

In industries, the selection of purification techniques should be anchored by consideration of practicality and efficiency when implemented on a large-scale operation. A low-cost and viable upscaling technique should be developed in view of the high cost of purification (Spir *et al*., 2015). In addition to its scalability, membrane filtration is a green technology that doesn't require chemicals, making it an attractive choice. Also, it is cost-effective compared to other methods of purification (Vasiljevic, 2019). Thus, the novelty of this study is the single step process of HF NF that can be implemented, capable of extraction crude bromelain and retain not only its activity but also its anti-oxidant and anti-inflammatory. This study also provides knowledge and information about the bromelain content of every part (flesh, peels, core, stem and crown) of Malaysian pineapple cultivar (Morris and MD2) which has never been done by other researchers. As for the chemical engineering aspect, the feasibility of the process was also determined using profitability analysis according to the data obtained in this study.

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