CHARACTERIZATION, EXTRACTION OPTIMIZATION AND MICROENCAPSULATION OF BIOACTIVE COMPOUNDS FROM THE CELLULASE-TREATED MD2 PINEAPPLE [*ANANAS COMOSUS* L. (MERR)] PEEL

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By

NUR LIYANA BINTI NORDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the requirement for the Degree of Doctor of Philosophy

January 2024

IPPH 2024 4

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January 2024

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Pineapple waste are often discarded during processing and fresh consumption. Often, these waste would accumulate and decompose in landfills. The biodecomposition of pineapple wastes led to the release of methane gas which causes global warming. Alternatively, pineapple waste could be utilized as a functional Halal ingredient to overcome disposal issues. Thus, this study aimed to develop a powder ingredient from the enzyme-treated MD2 pineapple peel extract as a Halal component following the specific objectives; 1) to characterize the phenolic and volatile compounds from MD2 pineapple peel and core extracts, 2) to study the effect of pectinase and cellulase in the extraction of phenolics and volatile compounds from MD2 pineapple peel extracts, 3) to study the extraction process parameters by optimization using response surface methodology (RSM) on MD2 pineapple

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peel extracts 4) to study the effect of carrier agents and drying techniques in the encapsulation of extracts on its functional properties. Enzyme treatments were performed on MD2 pineapple peel under the influence of different enzyme concentrations (0-1.5% (v/v), and solid to solvent ratios (1:1-1:8 g/mL). In order to identify the kinetic parameters, second-order kinetic modelling was evaluated. Then, the identification of different phenolic fractions was carried out using acid-alkali hydrolysis. To further optimize, response surface methodology was employed to evaluate the optimal conditions of the extraction process. The optimized cellulase-treated MD2 pineapple peel extract was encapsulated by both spray-drying and foam-mat drying. Bioactive volatile compounds were identified in the extracts and microencapsulated cellulase-treated MD2 pineapple peel extract powder. The MD2 pineapple peel extracts were observed to show substantially greater ($p<0.05$) amounts of total phenolic (TPC) and flavonoid (TFC) compounds than the core extract. Furthermore, peel extract showed improved antioxidant activity, with an IC_{50} of 0.63 mg/mL in comparison to the core. Meanwhile, bioactive volatile compounds identified were 5-(Hydroxymethyl) furan-2-carbaldehyde (aldehyde), 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (DDMP), 1,2-benzenediol, 1,4-benzenediol, n-decanoic and n-Hezadecanoic acids. The effect of enzyme-assisted extraction on phenolics showed that the extraction process improved at a treatment of 1.5% (v/v) cellulase concentration, and 1:4 g/mL of solid to solvent ratio, respectively. The maximum yield of phenolics obtained by cellulase extraction was 8.72 mg GAE/g per dry weight basis (DW) using second-order kinetic modelling (R^2 = 0.9189). When compared to non-enzyme treatment, insoluble-bound phenolics (IBP) extraction increased

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considerably ($p<0.05$) by 99.79% following the cellulase treatment. Bioactive volatile compounds present comprised of benzophenone, beta-sitosterol, gamma-sitosterol, stigmast-8(14)-en-3 beta.ol and phenol, 2, 4-bis (1,1-dimethyl ethyl). Through optimisation of cellulase-assisted extraction process using RSM, parameter conditions obtained were 48.21°C and 132 min of extraction temperature and time, respectively. Meanwhile, during the encapsulation process, spray-drying showed good physical properties. Both drying techniques showed good encapsulation efficiency (EE%) of above 89% with spray-drying showing the highest solubility (75.42 – 98.53%). Encapsulation of IBP and bioactive volatile compounds using 10% GA comprised of 2-Methoxy-4-vinylphenol and Phenol, 2,4-bis(1,1-dimethylethyl). In conclusion, an improved extraction process utilizing cellulase and optimization enhanced the release of phenolics from MD2 pineapple peel extracts. Alternatively, the presence of esters and aldehydes detected in the extracts and microencapsulated powder could replace synthetic flavouring agents such as vanillin extracted using alcohol. Meanwhile, the presence of fatty acids and sterols could replace fatty acids derived from pork fat. Furthermore, microencapsulated cellulase-treated MD2 pineapple peel extract powder rich in insoluble-bound phenolics, and bioactive volatile compounds may find applications as ingredients in Halal additives, pharmaceutical and cosmetic products.

Keywords: enzyme-assisted extraction; bioactive compounds; foam-mat drying; MD2 pineapple peel; spray-drying

SDG: GOAL 12: responsible consumption and production; GOAL 13: climate action

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Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENYIFATAN, PENGOPTIMUMAN PENGEKSTRATAN DAN MIKROENKAPSULASI KOMPOUN BIOAKTIF DARIPADA KULIT NANAS [*ANANAS COMOSUS* L. (Merr)] YANG DIRAWAT SELULAS

Oleh

NUR LIYANA BINTI NORDIN

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Sisa nanas sering dibuang semasa pemprosesan dan penggunaan segar. Selalunya, sisa ini akan terkumpul di tapak pelupusan sampah dan terbiodekomposisi. Biodekomposisi sisa nanas membawa kepada pembebasan gas metana yang menyebabkan pemanasan global. Sebagai alternatif, sisa nanas boleh digunakan sebagai bahan bernilai tinggi untuk mengatasi isu pelupusan. Justeru, kajian ini bertujuan untuk membangunkan ramuan serbuk daripada ekstrak kulit nanas MD2 yang dirawat enzim sebagai komponen Halal mengikut objektif khusus; 1) Untuk mencirikan sebatian fenolik dan meruap daripada kulit nanas MD2 dan ekstrak teras, 2) Untuk mengkaji kesan pektinase dan selulase dalam pengekstrakan fenolik dan sebatian meruap daripada ekstrak kulit nanas MD2, 3) Untuk mengkaji parameter proses pengekstrakan secara pengoptimuman menggunakan RSM

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pada ekstrak kulit nanas MD2 4) Untuk mengkaji kesan agen pembawa dan teknik pengeringan dalam pengkapsulan ekstrak terhadap sifat fungsinya. Rawatan enzim telah dilakukan pada kulit nanas MD2 di bawah pengaruh kepekatan enzim yang berbeza (0-1.5% (v/v)), dan nisbah pepejal kepada pelarut (1:1-1:8 g/mL). Untuk mengenal pasti parameter kinetik, pemodelan kinetik peringkat kedua dinilai. Kemudian, pengenalpastian pecahan fenolik yang berbeza telah dijalankan menggunakan hidrolisis asid-alkali. Untuk mengoptimumkan lagi proses pengekstrakan, Metodologi permukaan tindak balas digunakan untuk menilai keadaan optimum. Ekstrak kulit nanas MD2 yang dirawat selulase yang dioptimumkan telah dikapsulkan dengan pengeringan semburan dan pengeringan tikar buih. Sebatian meruap bioaktif telah dikenalpasti dalam ekstrak dan kulit nanas MD2 yang dirawat dengan selulase mikroenkapsul. Ekstrak kulit nanas MD2 diperhatikan menunjukkan jumlah sebatian fenolik (TPC) dan flavonoid (TFC) yang lebih besar ($p<0.05$) berbanding ekstrak teras. Tambahan pula, ekstrak kulit menunjukkan aktiviti antioksidan yang lebih baik, dengan IC_{50} sebanyak 0.63 mg/mL berbanding dengan teras. Sementara itu, sebatian meruap bioaktif yang dikenal pasti ialah 5-(Hydroxymethyl) furan-2-karbaldehid (aldehid), 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (DDMP), 1, Asid 2-benzenediol, 1,4-benzenediol, n-decanoic dan n-Hezadecanoic. Kesan pengekstrakan berbantukan enzim ke atas fenolik menunjukkan bahawa proses pengekstrakan bertambah baik pada rawatan 1.5% (v/v) kepekatan selulase, dan 1:4 g/mL nisbah pepejal kepada pelarut, masing-masing. Hasil maksimum fenolik yang diperoleh melalui pengekstrakan selulase ialah 8.72 mg GAE/g setiap asas berat kering (DW) menggunakan pemodelan kinetik tertib kedua

($R^2 = 0.9189$). Jika dibandingkan dengan rawatan bukan enzim, pengekstrakan fenolik terikat tidak larut (IBP) meningkat dengan ketara ($p<0.05$) sebanyak 99.79% selepas rawatan selulase. Sebatian meruap bioaktif hadir terdiri daripada benzofenon, beta-sitosterol, gamma-sitosterol, stigmast-8(14)-en-3 beta.ol dan fenol, 2, 4-bis (1,1-dimetil etil). Melalui pengoptimuman proses pengekstrakan berbantukan selulase menggunakan RSM, keadaan parameter yang diperolehi ialah 48.21°C suhu dan 132 min masa pengekstrakan. Sementara itu, semasa proses enkapsulasi, pengeringan semburan menunjukkan sifat fizikal yang baik. Kedua-dua teknik pengeringan menunjukkan kecekapan enkapsulasi yang baik (EE%) melebihi 89% dengan pengeringan semburan menunjukkan keterlarutan tertinggi (75.42 – 98.53%). Enkapsulasi IBP dan sebatian meruap bioaktif menggunakan 10% GA terdiri daripada 2-Methoxy-4-vinylphenol dan Phenol, 2,4-bis(1,1-dimethylethyl). Kesimpulannya, proses pengekstrakan yang lebih baik menggunakan selulase dan pengoptimuman meningkatkan pembebasan fenolik daripada ekstrak kulit nanas MD2. Sebagai alternatif, kehadiran ester dan aldehid yang dikesan dalam ekstrak dan serbuk mikroenkapsul boleh menggantikan agen perasa sintetik seperti vanillin yang diekstrak menggunakan alkohol. Sementara itu, kehadiran asid lemak dan sterol boleh menggantikan asid lemak yang diperoleh daripada lemak babi. Tambahan pula, serbuk ekstrak kulit nanas MD2 yang dirawat dengan selulase mikroenkapsul yang kaya dengan fenolik terikat tidak larut, dan sebatian meruap bioaktif mungkin boleh digunakan sebagai bahan dalam bahan tambahan Halal, produk farmaseutikal dan kosmetik.

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Kata kunci: pengekstrakan bantuan enzim; Kulit nanas MD2; fenolik terikat tidak larut; sebatian meruap; pengeringan semburan; pengeringan tikar buih

SDG: penggunaan dan pengeluaran yang bertanggungjawab (12); tindakan iklim (13)

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ACKNOWLEDGEMENTS

Foremost, I am grateful to Allah S.W.T, the Almighty, for giving me the strength and courage to complete this PhD journey. I am most indebted to my advisor, Assoc. Prof. Dr. Rabiha and co-advisor, Assoc. Prof. Dr. Noranizan Mohd Adzahan for providing guidance, encouragement and motivation throughout the experimental work, publishing manuscripts and thesis writing. Also, to mention my former advisor, Prof. Jamilah Bakar, for her endless contribution of expertise and motivation to this research journey.

I am thankful to my family who are the number one supporter: Suhail bin Sharir (my husband), Nuwairah Binti Suhail (daughter), Prof. Nordin bin Saad, Pn. Rozita bt. Hasan, Zawani, Nadhirah, Syahmi, Hakim, and my in-laws.

I am grateful to the science officers (En. Hamizan, Pn. Suraya, En. Amran, En. Syawal, Pn. Hajar, Pn. Haslina, Pn. Zaffan and Pn. Azamimah) for their generous support in allowing me to conduct experimental work in Food Processing Laboratory (Food 5), Food 3, and Innovation Laboratory (IPPH). Finally, to all my postgraduate friends throughout the batch of 2016 to 2023 whom I learnt, through thick and thin, provided with constructive feedback and endless motivation; Naqiyah bt. Azmi (IPPH), Wan Hasyera Wan Omar (IPPH), Nor Adilah Abdullah (Food 5, FSTM), Dr. Ee Su Chee (Food 5, FSTM), Fatin Saiyidah (Food 5, FSTM), Dr. Rodhiah (Unisel), Maryam A'dilah Zainal Ariffin (FSTM), Dr. Afidah (Food 5, FSTM), Dr. Foong Han Lyn (Food 5, FSTM), Dr. Siti Zaharah Rosli (MSU), Anis Asyila (Food 4) and others.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 13 June 2024

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LIST OF ABBREVIATIONS

TPC	Total phenolic content
TFC	Total flavonoid content
DPPH	2,2-diphenyl-1-picryl- hydrazyl-hydrate
IC_{50}	Half maximal inhibitory concentration
FP	Free phenolic
EP	Esterified phenolic
GP	Glycosylated phenolic
IBP	Insoluble-bound phenolic
SSR	Solid-to-solvent ratio
GAE	Gallic acid equivalent
QE	Quercetin equivalent
DW	Dry weight
RSM	Response surface methodology
PCA	Principal component analysis
MD	Maltodextrin
GA	Gum Arabic
SD	Spray drying
FMD	Foam-mat drying
EE (%)	Encapsulation efficiency (%)
AI	Astringency index
SI	Sweetness index
FRAP	Ferric reducing antioxidant power
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AAE	Ascorbic acid equivalent
TE	Trolox equivalent
CE	Catechin equivalent

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RE	Rutin equivalent
DW	Dry weight
FW	Fresh weight
FAOSTAT	Food Agriculture and Organization Statistics
GMO	Genetically Modified Organisms
JECFA	Joint FAO/WHO Expert Committee on Food Additives
FCC	Food Chemical Codex
HCl	Hydrochloric acid
EDTA	Ethylenediaminetetraacetic acid
NaOH	Sodium Hydroxide
EC	Enzyme concentration
EA	Activation energy
ES	Enzyme substrate
GC	Gas Chromatography
GSC	Gas Solid Chromatography
GLC	Gas Liquid Chromatography
HPSME-GCMS	Headspace solid-phase microextraction-Gas Chromatography
HPTLC	High-performance thin-layer chromatography
UV-Vis	Ultraviolet-visible
AR	Analytical grade
TSS	Total soluble solids
DF	Dilution factor
DMHF	2,5-dimethyl-4-hydroxy-3(2H)-furanone
HMF	5-hydroxymethyfufural
DDMP	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one

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CHAPTER 1

INTRODUCTION

1.1 Background

In Halal *Thoyyiban* concept, chemical ingredients in products should abide by Islamic law and contain no porcine and alcohol residues (Hashim & Mat Hashim, 2013). The term Halal *Thoyyiban* (good), is concurrent which is permitted by Islamic law, clean, and poses no health risk (Sugibayashi et al., 2019; Che Man & Sazili, 2010). Substantial awareness of the origin has occurred in recent years for Halal ingredients from plant-based constituents (Borhan, 2021). The is due to the nature of the ingredient, which allows industries to manufacture Halal chemical products (Sugibayashi et al., 2019).

Fruit peel extracts contain significant bioactive compounds such as phenolic acids, flavonoids, antioxidants, and volatiles constituents (Mohamad et al., 2019). Phenolic acids may find applications as anti-inflammatory, antioxidant, anticancer, antimicrobial, and neuroprotective properties in food preservatives and skin-care products (Kumar & Geol, 2019). Phenolic fractions are categorized into three types: soluble-free, soluble-conjugate (esterified and glycosylated), and insoluble-bound (Shahidi & Hossain, 2023). It has been found that insoluble-bound phenolics (IBPs) are high in antioxidant activities, anti-inflammatory, anti-cancer, probiotic, antidiabetic and antiobesity and prevent cardiovascular disease (Wang et al., 2020; Shahidi & Yeo, 2016). IBPs

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is located in the protective layer of plants with total phenolics exceeding 70% in peels, hulls, seed coats, and leaves (Shahidi & Hossain, 2023).

According to Xiao et al. (2021), fruits contain a considerable amount of volatile compounds. They can be found in hundreds of fresh and processed fruits and vegetables (Reshna et al., 2022). Pineapple fruit contains a considerable amount of bioactive volatile compounds comprising methyl-2-methylbutanoate, methyl hexanoate, methyl-3-(methylthiol)-propanoate, methyl octanoate, 2,5-dimethyl-4-methoxy-3(2H)-furanone, δ-octalactone, 2-methoxy-4-vinyl phenol, and δ-undecalactone (Lasekan & Hussein, 2018); and methyl-2-methylbutyrate, methyl-2-methylbutyrate, 3-(methylthio)propanoic acid ethyl ester, ethyl hexanoate and decanal in Tainong No.6 variety (Zheng et al. 2012). These compounds provide potential applications as flavouring agents and biological active properties.

By the year 2029, the Asian and American areas will produce 33 million tonnes of pineapples globally (Garcia et al., 2021). Only half of the projected 1.45 million tonnes of imported pineapples in Europe were utilised, with the remainder heading into the production of juice, canned fruit, crystallised fruit, and dried snacks (Campos et al., 2020). Twenty percent of pineapples of good grade are for fresh and canned consumption, while incorrect handling and storage cause postharvest losses. (Chaurasiya & Hebbar, 2013). About 30% (w/w) of the initial weight of the fruit is consumed (Roda & Lambri, 2019). However, pineapple by-products have gained interest as a high-value ingredient due to their therapeutic and bioactive compound properties such as

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being high in antioxidants, bromelain, organic acids, enzymes and many more (Abraham et al. 2023).

However, several processing methods are required to ensure the stability of the bioactive compounds before being developed into a high-value ingredient. According to Costa et al. (2020), enzyme-assisted extraction has several advantages in environment, specificity, and superior quality over conventional extraction. The environmental advantages involve the use of non-toxic solvents which operate under mild temperature conditions and reduce extraction time. Meanwhile, the specific biomolecule is extracted by an enzyme to obtain a purer extract. The quality of the extract is superior in terms of no changes in the biochemical and biological properties of the bioactive compounds extracted. After obtaining the extract, the encapsulation of bioactive compounds is of upmost importance. Encapsulation ensures protection from environmental factors including light, moisture, oxygen, and prolonged shelf life (Marcillo-Parra et al. 2021; Nguyen et al., 2022). According to Navarro-Flores et al. (2020), microencapsulation of plant extracts with high antioxidant and phenolic compounds was to prevent degradation. Meanwhile, Marcillo-Parra et al. (2021) highlighted that encapsulation products provide adverse health benefits which have the potential as functional food ingredients.

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1.2 Problem statements

Pineapple resulted in 360 M € in projected economic losses of 60% (w/w) waste in Europe (Campos et al., 2020). In the processing of pineapples, peels are often discarded, which constitute around 5% of the original fruit (Kumar et al., 2017). In landfills, fruit peels would accumulate and have a detrimental effect on the environment. The biological degradation of trash results in a combination of dissolved and suspended particles that produce liquid leachate, which is hazardous to human health. (Nunes et al., 2009). Consequently, methane is produced, causing emissions of greenhouse gases. To minimise the carbon footprint, it is crucial to explore the possible utilisation of pineapple waste as a high-value ingredient.

As described earlier, the major portion of pineapple waste is comprised of peel. Therefore, several studies highlighted the characterization of different parts of pineapples with high phenolics, antioxidant activity and volatile compounds. Several studies in the peel of smotth cayenne (19.24.1 mg GAE/mg) and Tainung17 (20.5-24.3 mg GAE/mg) varied in their respective TPC contents (Huang et al., 2021). Meanwhile, the peel of Golden variety pineapple showed TPC of 30 mg GAE/g and 570 mg GAE/g for control and ultrasonic-treatments, respectively (Polaria et al., 2022). However, there is a lack of research on the exploration of the core and peel from pineapples. Therefore, exploration of the core and peel of MD2 pineapple variety should be further evaluated.

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Biologically active compounds derived from pineapple waste are by conventional extraction methods such as studies conducted by Azizan et al. (2022); Huang et al. (2021); Li et al. (2014). Organic solvents used in conventional extractions are such as methanol, ethanol, ethyl acetate, petroleum ether, hexane and many more which may pose a risk of toxicity (Rifna et al., 2021; Chemat et al., 2017; Apostolakis et al., 2014; Prado et al., 2015). Nevertheless, chemical solvents in extractions resulted in low yield, high extraction temperature and time which lead to degradation of bioactive compounds. Furthermore, almost all solvents are harmful to health if ingested or inhaled in excess (Joshi & Adhikari, 2019). Alternatively, the green extraction method could be used to recover the bioactive compounds from pineapple waste preventing degradation as well as improving the extraction yield.

Moving on now to consider the biostability and bioavailability of bioactive compounds where encapsulation plays a critical role. Bioactive compounds in extracts are sensitive to environmental factors such as pH, humidity, light, and temperature. In a research done by Luorenco et al. (2020), microencapsulation of pineapple peel extracts showed that bioactive compounds were extracted by ethanol prior spray-drying. Therefore, consideration of the green extraction of bioactive compounds from extracts and encapsulation may improve the functional properties and biological properties of the potential ingredient from pineapple waste.

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1.3 Significance of study

The significance of this study is the contribution to Halal Product Development and commercial application. Investigation of the extraction of phenolics and volatile compounds from MD2 pineapple peel will provide methodological information to researchers. Green chemistry/technique/technology is in demand due to its sustainability, solvent-free, advanced extraction method with increased extracts yield (Noore et al., 2021). Enzyme-assisted extraction is a method to extract high-value-added compounds (de Souza & Kawaguti, 2021). Enzyme-assisted extraction offers the benefit of excluding the need for very pure chemical solvents during extractions, non-toxic, performs in conditions of mild temperatures, is environmentally safe and has low capital investment (Peixoto Araujo et al., 2019; Alexandre et al., 2018). One way to achieve the stability of the extracts is through encapsulation to reduce microbial growth, improve powder quality, prevent bioactive compound degradation, prolong shelf life and provide convenient packaging, storage, and transportation (Domínguez-Rodríguez et al., 2021). The enzyme-treated pineapple peel extract powder may find commercial applications in the Halal *toyyiban* food products, cosmetics, and household care products.

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1.4 Objectives

This study aimed to develop MD2 pineapple peel extract powder for application in food and non-food ingredients. Commercial microbial enzymes (cellulase and pectinase) were used to facilitate the process of phenolic and volatile compound extraction from MD2 pineapple peel extract. The recovery of different forms of phenolics and volatile compounds was evaluated. Through the optimisation of process parameters using response surface methodology (RSM), an optimum process condition was obtained. The encapsulated enzyme-treated MD2 pineapple peel extract was evaluated on its powder qualities. General research flow on objectives 1 to 4 is summarized in Figure 1.1.

The specific objectives covered are as follows:

1. To characterize the different phenolic fractions and volatile compound profiles in the MD2 pineapple peel and core extracts
2. To evaluate the effect of cellulase and pectinase in the extraction of phenolics, insoluble-bound phenolics and volatile compounds from MD2 pineapple peel extracts and kinetic model
3. To optimize the process parameters for cellulase-assisted extraction of phenolics from md2 pineapple peel extract and its volatile compounds
4. To evaluate the effect of carrier agents and drying techniques on the physicochemical, insoluble-bound phenolics and volatile compounds of cellulase-treated MD2 pineapple peel extract powders

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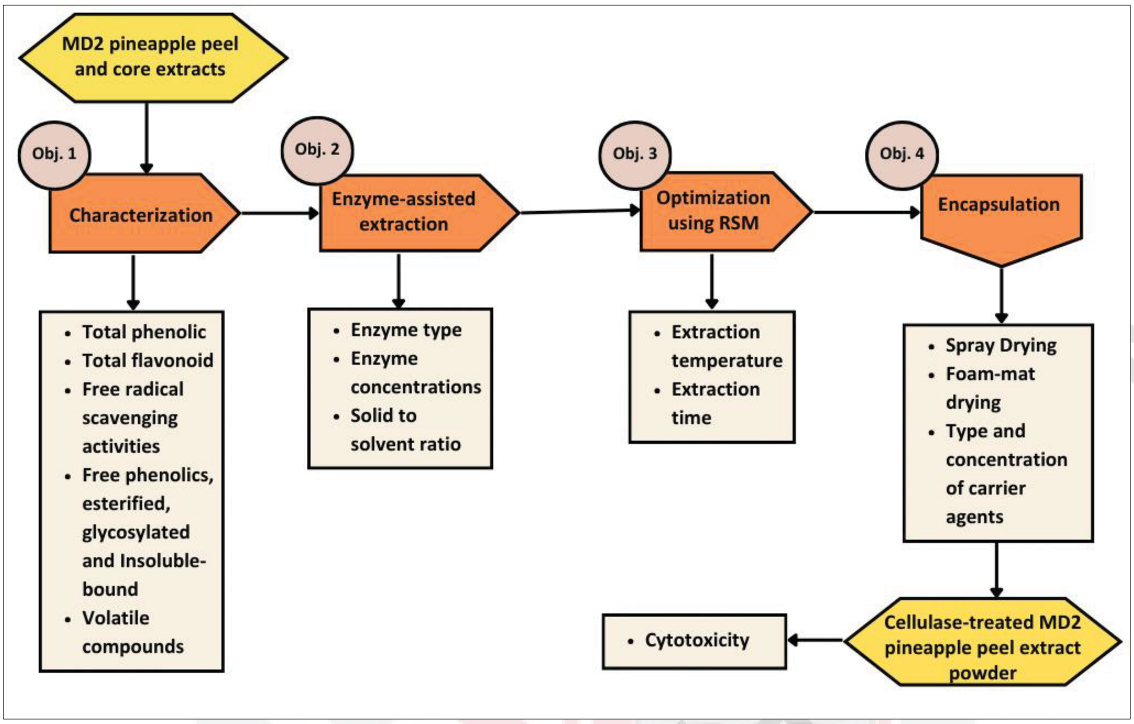

Figure 1.1 : Research flow diagram representing objectives 1 to 4

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