

AQUEOUS ETHANOL *Ananas comosus* PEEL EXTRACT AS THE BIOACTIVE
INGREDIENT IN AN OIL-IN-WATER NANOCREAM FOR TOPICAL
DELIVERY

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DEDICATION

This thesis is dedicated to my special one, papa and mama.

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ABSTRACT

The polyphenols-rich *Ananas comosus* peels extract (AcPE) can be developed as an alternative topical nanocream for the cosmeceutical industry. Also, active ingredients of nano formulated products are proven more effective in penetrating the stratum corneum of the human skin. However, there are yet no published works or known formulations of the AcPE for topical skin use. Therefore, the present study proposed the formulation of an AcPE nano cream (AcPENc) to explore further applications of the pineapple biomass to benefit humankind. This study optimized an integrated high-energy method using ultrasonic assisted extraction (UAE) for responses, high yield, and high total phenolic content (TPC) of AcPE. While the UAE gave an insignificant effect on the extraction yield ($p > 0.05$), the optimum conditions for the highest TPC extracted from AcPE were successfully identified, namely ultrasonication time (5 min), solvent ratio (50 %), amplitude (65 %), and liquid-solid ratio (35:1 mL/g). The characterization of AcPE revealed an adequate antioxidant capacity for the total flavonoid content (TFC), 1,1- diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) with corresponding values of 1146.86 mg QE/g, 11.83 mg/mL, and $1578.07 \pm 25.96 \mu\text{M Fe}^{2+}/100\text{g DW}$. High-Performance Liquid Chromatography (HPLC) analysis showed the presence of polyphenols in AcPE, with catechin as the major flavonoid, followed by quercetin and gallic acid. Next, the study successfully formulated the oil-in-water (O/W) AcPENc using integrated low- and high-energy methods of phase inversion temperature (PIT) and ultrasonication. The D-Optimal Mixture design (MED)-assisted optimization of AcPENc yielded a stable formulation comprising olive oil (1 %), grapeseed oil (12 %), Tween 80 (12.63 %), and water (74.37 %). The particle size and polydispersity index (PDI) of the optimized nano-cream (OPT-AcPENc) were $187.37 \pm 0.81 \text{ nm}$ and 0.248 ± 0.017 , respectively. Most importantly, the OPT-AcPENc formulation exhibited characteristics that supported its long-term stability for storage, with a zeta potential value of $-35.40 \pm 2.71 \text{ mV}$, with a pH value of 4.56 ± 0.01 . These attributes agree well with the recommended range for effective topical delivery of the active ingredients through human skin. The high conductivity ($1346.67 \pm 5.77 \mu\text{S/cm}$) of the OPT-AcPENc verified its O/W characteristics, while the rheological data proved the cream's shear-thinning (pseudoplastic) behavior. The OPT-AcPENc transmission electron microscopy micrograph revealed spherically shaped nano-sized particles of $< 350 \text{ nm}$. The OPT-AcPENc was stable against high centrifugal force, mechanical shock, light test, and heating-cooling cycles in the stability study. Results of the accelerated stability test under three different incubation temperatures (4 ± 2 , 25 ± 2 , $50 \pm 2 \text{ }^\circ\text{C}$) for 6 weeks revealed $25 \pm 2 \text{ }^\circ\text{C}$ to be the best storage temperature for the OPT-AcPENc. The suitability was evident from OPT-AcPENc's stability in pH, particle size, and PDI, without phase separation. Relevantly, the OPT-AcPENc was safe from microorganisms and heavy metal contamination and fulfilled the prerequisite of the National Pharmaceutical Regulatory Agency (NPRA) Malaysia for cosmetic products. Sensory evaluation by 47 consumer panelists through the scoring method revealed that the OPT-AcPENc was well accepted. The cream was favored for its color, fragrance, spreadability, adsorption, film/coating, and non-irritancy, as proven in the radar plot. Thus, the present study conclusively demonstrated the potential of AcPE as a nano cream for topical human skin use in the cosmeceutical industry.

ABSTRAK

Ekstrak kulit *Ananas comosus* (AcPE) yang kaya dengan polifenol boleh dibangunkan sebagai nanokrim topikal alternatif untuk industri kosmetik. Bahan aktif produk nano yang diformulasikan juga terbukti lebih berkesan untuk menembusi stratum korneum kulit manusia. Walau bagaimanapun, masih belum ada kerja yang diterbitkan atau formulasi AcPE yang diketahui untuk kegunaan topikal di kulit. Oleh itu, kajian ini mencadangkan formulasi nanokrim AcPE (AcPENc) untuk meneroka penggunaan lanjutan biojisim nanas untuk memberi manfaat kepada manusia. Kajian ini mengoptimumkan kaedah tenaga tinggi bersepadu menggunakan pengekstrakan berbantuan ultrasonik (UAE) untuk tindak balas, hasil yang tinggi, dan kandungan fenolik total (TPC) AcPE yang tinggi. Sementara UAE memberikan kesan yang tidak ketara terhadap hasil pengekstrakan ($p > 0.05$), keadaan optimum untuk penghasilan TPC tertinggi yang diekstrak dari AcPE telah berjaya dikenal pasti iaitu masa ultrasonikasi (5 min), nisbah pelarut (50 %), amplitud (65 %), dan nisbah cecair-pepejal (35:1 mL/g). Pencirian AcPE menunjukkan keupayaan antioksidan yang mencukupi untuk kandungan flavonoid total (TFC), 1,1- difenil-2-pikrilhidrazil (DPPH), dan kuasa antioksidan penurunan ferrik (FRAP) dengan nilai sepadan 1146.86 mg QE/g, 11.83 mg/mL, dan $1578.07 \pm 25.96 \mu\text{M Fe}^{2+}/100\text{g DW}$. Analisis kromatografi cecair prestasi tinggi (HPLC) menunjukkan kehadiran polifenol di dalam AcPE dengan katekin sebagai flavonoid utama, diikuti oleh quercetin dan asid galik. Seterusnya, kajian ini berjaya memformulasi AcPENc minyak-di dalam-air (O/W) menggunakan kaedah bersepadu tenaga rendah dan tinggi bagi suhu penyongsangan fasa (PIT) dan ultrasonikasi. Pengoptimuman AcPENc berbantuan reka bentuk D-Optimal Mixture (MED) menghasilkan formulasi stabil yang terdiri daripada minyak zaitun (1 %), minyak benih anggur (12 %), Tween 80 (12.63 %) dan air (74.37 %). Saiz zarah dan indeks polipenyserakan (PDI) nanokrim yang dioptimumkan (OPT-AcPENc) masing-masing adalah $187.37 \pm 0.81 \text{ nm}$ and 0.248 ± 0.017 . Yang paling penting, formulasi OPT-AcPENc mempamerkan ciri yang menyokong kestabilannya untuk penyimpanan jangka masa panjang, dengan nilai keupayaan zeta $-35.40 \pm 2.71 \text{ mV}$ dan nilai pH 4.56 ± 0.01 . Sifat-sifat ini sangat sesuai dengan julat yang disyorkan untuk penyampaian topikal bahan aktif melalui kulit manusia dengan berkesan. Kekonduksian tinggi ($1346.67 \pm 5.77 \mu\text{S/cm}$) OPT-AcPENc mengesahkan ciri O/W bahan itu, sementara data reologi membuktikan kelakuan penipisan ricih (pseudoplastik) krim. Mikrograf mikroskopi elektron penghantaran OPT-AcPENc mendedahkan zarah bersaiz nano berbentuk sfera $< 350 \text{ nm}$. OPT-AcPENc stabil terhadap daya empar yang tinggi, kejutan mekanikal, ujian cahaya, dan kitaran pemanasan-penyejukan dalam kajian kestabilan. Hasil ujian kestabilan dipercepatkan di bawah tiga suhu inkubasi yang berbeza (4 ± 2 , 25 ± 2 , $50 \pm 2 \text{ }^\circ\text{C}$) selama 6 minggu menunjukkan $25 \pm 2 \text{ }^\circ\text{C}$ sebagai suhu penyimpanan terbaik untuk OPT-AcPENc. Kesesuaiannya terbukti daripada kestabilan OPT-AcPENc dalam pH, saiz zarah, dan PDI, tanpa pemisahan fasa. Berkaitan dengan itu, OPT-AcPENc adalah bebas daripada pencemaran mikroorganisma dan logam berat serta memenuhi prasyarat Agensi Peraturan Farmasi Nasional (NPRA) Malaysia untuk produk kosmetik. Penilaian deria oleh 47 ahli panel pengguna melalui kaedah penilaian skor menunjukkan bahawa OPT-AcPENc diterima dengan baik. Krim ini digemari kerana warna, keharuman, kemampuan penyebaran, penjerapan, filem/salutan, dan tiada kerengsaan, seperti terbukti dalam plot radar. Dengan demikian, kajian ini secara muktamad menunjukkan potensi AcPE sebagai nanokrim untuk kegunaan topikal kulit manusia dalam industri kosmetik.

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LIST OF ABBREVIATIONS

BTX	-	Botulinum Toxin
HRT	-	Hormone Replacement Therapy
AcP	-	<i>A. comosus</i> Peels
AcPE	-	<i>A. comosus</i> Peels Extracts
AcPP	-	<i>A. comosus</i> Peels Powder
AcPENc	-	AcPE Nano Cream
OPT-AcPE	-	Optimized AcPE
OPT-AcPENc	-	Optimized AcPENc
UAE	-	Ultrasonic-Assisted Extraction
O/W	-	Oil-in-Water
W/O	-	Water-in-Oil
TPC	-	Total Phenolic Content
RSM	-	Response Surface Optimization
TFC	-	Total Favonoid Content
DPPH	-	1,1-Diphenyl-2-Picrylhydrazyl Radical Scavenging Activity
FRAP	-	Ferric Reducing Antioxidant Power
HPLC	-	High-Performance Liquid Chromatography
PIT	-	Phase Inversion Temperature
PDI	-	Polydispersity Index
TEM	-	Transmission Electron Microscopy
DW	-	Dry Weight
SC	-	Stratum Corneum
SG	-	Stratum Granulosum
SS	-	Stratum Spinosum
SB	-	Stratum Basale
UV	-	Ultraviolet
BTX-A	-	BTX- subtype A
MU	-	Mouse Units
FDA	-	Food and Drug Administration

LD ₅₀	-	50 % Lethal Dose
RF	-	Radiofrequency
CPI	-	Consumer Product Inventory
HLB	-	Hydrophilic-Lipophilic balance
T80	-	Tween 80
XG	-	Xanthan Gum
RAPEX	-	Rapid Alert System
EC	-	European Commission
Phy-Et	-	Phenoxyethanol
US-EPA	-	US Environmental Protection Agency
SCCS	-	Scientist Committee on Consumer Safety
ANSM	-	French National Agency for Safety of Medicines and Health Products
GA	-	Gallic Acid
GRAS	-	Generally Recognized as Safe
USFDA	-	United States Food and Drug Administration
HPH	-	High-Pressure Homogenizer
DLS	-	Dynamic Light Scattering
SEM	-	Scanning Electron Microscopy
AFM	-	Atomic Force Microscopy
COLIPA	-	European Cosmetic Toiletry and Perfumery Association
CTFA	-	The Cosmetic, Toiletry and Fragrance Association
ANVISA	-	Brazil National Health Surveillance Agency
NPRA	-	National Pharmaceutical Regulatory Agency
Fe	-	Iron
Zn	-	Zinc
Pb	-	Lead
Hg	-	Mercury
As	-	Arsenic
Cd	-	Cadmium
ASTM	-	American Society of Testing and Material
BBD	-	Box-Behnken Design
CCD	-	Central Composite Design

MED	-	Mixture Design
TPTZ	-	2,4,6-Tri(2-pyridyl)-s-triazine
GSO	-	Grapeseed Oil
OO	-	Olive Oil
ANOVA	-	Analysis of Variance
CV	-	Coefficient of Variation
LOF	-	Lack of Fit
Na ₂ CO ₃	-	Sodium Carbonate
NaNO ₂	-	Sodium Nitrite
AlCl ₃	-	Aluminum Chloride
NaOH	-	Sodium Hydroxide
PPE	-	Percentage Prediction Error
MLB	-	Modified Letheen Broth
MLA	-	Modified Letheen Agar
PDA	-	Potato Dextrose Agar
TSB	-	Trypticase Soy Broth
HCl	-	Hydrochloric Acid
EY	-	Extraction Yield
MRA	-	Multiple Regression Analysis
LA	-	Linoleic Acid
3D	-	Three-Dimensional
ACD	-	ASEAN Cosmetic Directive
TEWL	-	Transepidermal Water Loss

LIST OF SYMBOLS

%	-	Percentage
w/w	-	Weight by weight
α	-	Alpha
β	-	Beta
nm	-	Nanometre
°C	-	Degree of Celsius
ft	-	Feet
g	-	Gram
kg	-	Kilogram
μ g	-	Microgram
μ m	-	Micrometre
μ S/cm	-	Micro siemens per Centimetre
mV	-	Millivolt
cfu/g	-	Colony-Forming Unit per Gram
mL	-	Millilitre
ppm	-	Parts per Million
h	-	Hour
kHz	-	Kilohertz
W	-	Watt
rpm	-	Revolutions per minute
s	-	Seconds
min	-	Minute
mL/g	-	Millilitre per Gram
mg/mL	-	Milligram per Millilitre
w/v	-	Weight per Volume
v/v	-	Volume per Volume
μ L	-	Microliter
mM	-	Millimolar
mg GAE/g	-	milligrams of gallic acid equivalent per gram
mg QE/g	-	milligrams of quercetin equivalent per gram

μM of Fe^{2+} /100 g - micromolar of Fe^{2+} equivalent to 100 grams
keV - Kiloelectron Volt
kcal - Calorie

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Aging is a natural phenomenon that transpires over every growing human being. The aging process of the human skin involves a complex biological progression where it is prejudiced by a combination of endogenous or intrinsic (genetic, cellular metabolism, hormone, and metabolic processes) and exogenous or extrinsic (chronic light exposure, chemicals, toxin) factors (Ganceviciene *et al.*, 2012, Baumann, 2018). Thus, to remain in an ageless beauty may prove a little challenging for the large population, considering the surrounding environment is becoming increasingly polluted. The elevated existence of pollutants in the environment, the consumption of unhealthy foods, and the sedentary lifestyle are causes of major concerns. The two are much to be blamed for the accelerated aging process in humans (Hirlekar and Patil, 2013).

Many consumers have since turned to various commercial over the counter skin rejuvenating creams or serums. In contrast, certain people have even resorted to more drastic measures such as plastic surgery, Botox shots, injection of Botulinum Toxin (BTX), Hormone Replacement Therapy (HRT) (Ganceviciene *et al.*, 2012). Even certain unscrupulous manufacturers take advantage of the high consumer demand by incorporating questionable synthetic skin rejuvenating active ingredients in their cosmeceutical formulations. Such chemicals are favored as they are considerably cheaper than the natural ones. This is a particularly disturbing trend in the cosmeceutical industry as it appears that consumer safety has become secondary over profit-making. Some reports even claimed that certain substances have contributed to unusual bouts of poor health among certain users. There are claims that some substances are carcinogenic and increase the possibility of contracting breast

cancer in women, trigger allergic reactions, and hazardous to the ecology (Chen, 2009, Rasanayagam *et al.*, 2015).

Given the undesirable effects of synthetic active compounds on human skin, the quest for safer, naturally sourced alternatives as ingredients in skin rejuvenating cosmeceutical products warrants commercial consideration. Moreover, the ever-growing market for skin rejuvenating products would resonate well with the increasing consumer demand for natural plant-based bioactive compounds as the main ingredients to maintain good skin conditions (Aburjai and Natsheh, 2003, Mukherjee *et al.*, 2011). In this perspective, the study believes that the search for novel skin rejuvenating bioactive compounds can first be explored in agricultural or food-based industrial biomass. The amount of discarded biomass in Malaysia has substantially risen to ~1.2 million tonnes/year, consistent with brisk developments in the agricultural sector alongside growth in the global population. The high biomass turnover from the manufacturing processes contributes to the high volume of waste. It poses serious environmental challenges, such as requiring larger landfills and eventually contributing to the greenhouse effect as the biomass decomposes. Rather than scouring the depths of forests for exotic plants and exacerbating the fragile ecosystems' overexploitation, the biowastes accrued from agricultural biomass may be a promising and sustainable source of skin rejuvenating bioactive compounds.

In this milieu, such a source is the peels of pineapple that are discarded by food manufacturers. The scientific name for pineapple, the *Ananas comosus* (L.) Merr is a herbaceous and perennial monocot of the Bromeliaceae family. Generally, ~50 % (w/w) of pineapple's total weight comes from pineapple wastes *viz.* peel, core stem, crown, and leaves. The *A. comosus* peels (AcP), in particular, is inherently loaded with polyphenols, specifically catechin, quercetin, and gallic acid, likely useful as the active ingredients in skin rejuvenating formulations (Li *et al.*, 2014). These are groups of substances with excellent extractable radical scavenging capability (Ribeiro *et al.*, 2015b). For instance, Freitas *et al.* (2015) reported eight different carotenoids *viz.* α -carotene, β -carotene, β -cryptoxanthin, lutein, lycopene, neoxanthin, violaxanthin, zeaxanthin. Li *et al.* (2014) also stated gallic acid, catechin, epicatechin, and ferulic acid are the major polyphenolic compounds in AcP. Hence, the *A. comosus* peels

extracts (AcPE) may have useful application as an active ingredient in skin rejuvenating formulation, justifiable by their reported biological activities of phenolic compounds *viz.* anti-inflammatory and anti-cancer (Murakami *et al.*, 2015). Thus, the extraction of AcPE can be improved through an ultrasonic-assisted extraction (UAE) technique. The UAE technique's versatility to extract plant-based extracts relies on the cavitation process, where bubbles generated by the acoustic waves shaped and imploded, then disintegrating the plant material's cells. Thus, more polyphenolic compounds are liberated from the cells and extracted, while advantageously reducing extraction time and requiring less use of organic solvents (Esclapez *et al.*, 2011, Gil-Chávez *et al.*, 2013, Živković *et al.*, 2018).

Nano formulated active ingredients in cosmeceuticals are proven more effective given their better penetrative power into the human skin. Aside from improving active ingredients' stability, the outlook of nano formulated products are also more appealing (Mu and Sprando, 2010, Lohani *et al.*, 2014, Garces *et al.*, 2018). While designing functional cosmetics that promote human health, it is necessary to examine the release of active ingredients to a specific location under the skin layer. Such nano-delivery systems have been developed to efficiently carry the active ingredients across the skin with reduced adverse effects on the human. As a matter of fact, plant-based ingredients in the form of nano cream-emulsified forms are more effective for topical transmission across the skin barrier (Tadros *et al.*, 2004, Solans *et al.*, 2005, Gutiérrez *et al.*, 2008, Rai *et al.*, 2018). Literature has shown that nanoemulsions, or in actual fact, are mini emulsions either comprised of fine oil-in-water (O/W) droplets or water-in-oil (W/O) dispersion with droplet sizes typically below 300 nm but can range up to 500 nm (Lovelyn and Attama, 2011, Hardas and Brin, 2017, Singh *et al.*, 2017). Most importantly, transmembrane delivery of active ingredients in the form of nano-cream is advantageously more practical. The minute size of the bioactive particulates promotes better penetration of the active ingredients through the skin (Zainol *et al.*, 2015).

1.2 Problem Statement

Considering that skin aging is an inevitable process in humans, and the long-term use of synthetic chemicals in skin rejuvenating beauty products could be hazardous to consumers' health. Hence, the development of alternative creams derived from natural, i.e., plant-based active ingredients, may prove useful in averting the above-said issue. Herein, the study proposed the preparation of an O/W nano formulated polyphenolic extract of *AcPE*, containing catechin, quercetin, and gallic acid as anti-oxidants in the topical rejuvenation of the human skin.

The study believes the naturally anti-oxidants rich *AcPE* can effectively scavenge the reactive oxygen chemical species in the human skin. In doing so, this can delay the effects of physiological aging on the human skin. The *AcPE* is rich in different kinds of carotenoids and vitamins A and E, too (Erukainure *et al.*, 2011, Hossain and Rahman, 2011, Barbulova *et al.*, 2015). It is pertinent to indicate here that there are yet any studies on the nano formulated *AcPE*, i.e., catechin, quercetin, and gallic acid, as active ingredients for skin rejuvenating cream. As a matter of fact, previous cosmeceutical formulations have focused mainly on the proteolytic enzyme bromelain (Ketnawa *et al.*, 2010, Ketnawa *et al.*, 2011, Spir *et al.*, 2015). Besides being highly loaded in natural anti-oxidants, information on the potential use of *AcPE* remains rather limited (Ketnawa *et al.*, 2012).

There are a few questions regarding the formulation of active ingredients-loaded in cosmeceuticals products specifically in terms of stability, such as (i) chemical, (ii) physical, and (iii) microbiological, that needed clarification to ensure efficacy in delivering the active ingredients to the target skin layers. The study believes that reconstruction of the *AcPE* into a nano cream carrier system may answer these questions. Hence, this is the first study detailing the applicability of *AcPE*, i.e., catechin, quercetin, and gallic acid, particularly the hydrophilic fractions from *AcP* biomass, for the purpose above. Notably, *AcPE*-loaded nano cream of catechin, quercetin, and gallic acid would permit better topical and safer controlled release of the ingredients (Donsi *et al.*, 2011, Casanova and Santos, 2015).

1.3 Objectives of Study

The objectives of this research are as follows:

1. To identify the optimized UAE of *AcPE* for improved extraction yield and total phenolic content (TPC) and characterize the optimum *AcPE* (OPT-*AcPE*) for anti-oxidant properties.
2. To screen and optimize *AcPE* nano cream (*AcPENc*) formulation using a combination of high and low energy methods.
3. To perform physicochemical properties and stability evaluations on the optimized *AcPENc* (OPT-*AcPENc*).
4. To conduct a sensory evaluation on the OPT-*AcPENc* using a scoring method.

1.4 Scopes of Study

This study has four phases, with the first phase involving the proximate analysis of the *AcP*. The study then carried out the response surface optimization (RSM) of variables for the protocol of the UAE of *AcPE*. In the optimization study, the response was to attain a high extraction yield and phenolic content of the anti-oxidants in *AcPE*. Four relevant parameters, *viz.* effect of sonication time, solvent ratio (ethanol: water), amplitude, and liquid-solid ratio, were selected for the experimental UAE optimization assessment. Then, this study investigated polyphenolic compounds' anti-oxidative properties in the OPT-*AcPE*. In this part of the work, the assessed parameters were total flavonoid content (TFC), 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH), and ferric reducing anti-oxidant power (FRAP). The characterization of OPT-*AcPE* involved reverse-phase High-Performance Liquid Chromatography (HPLC).

In the second phase, the study screened and optimized the *AcPE* nano-cream (*AcPENc*) preparation via a combination of high and low energy methods, utilizing ultrasonic bombardment and phase inversion temperature (PIT). The screening process screened for oil amount, surfactant: water amount, and xanthan gum amount. The range of parameters was chosen by monitoring the quality of the formed *AcPENc* for texture, particle size, polydispersity index (PDI), stability, and pH. D-optimal Mixture Design was used to optimize the protocol to prepare the *AcPENc*. The optimization was focused on optimizing four formulation relevant parameters, namely, the olive oil amount (A), grapeseed oil amount (B), surfactant amount (C), and water amount (D) for responses, particle size (R_1), and PDI (R_2).

The third phase encompassed the characterization of the physicochemical properties of the OPT-*AcPENc* involving: organoleptic evaluation, zeta potential, pH value, conductivity, rheology study, and lastly, a morphological study by Transmission Electron Microscopy (TEM). In response, a stability study was done to affirm the above-said tests. Stability study involved three different tests: physical (centrifugal and mechanical shock test), light and chemical (shelf test conducted at four different conditions: (1) room temperature (2) oven, (3) refrigerator, and (4) heating-cooling cycle). Other than that, this study embarked on safety evaluations: (1) microbial test and (2) heavy metal test of OPT-*AcPENc*.

In the final phase of the study, sensory evaluation was done on two sets of consumers, totaling 47 panelists, on the formulated OPT-*AcPENc* through the scoring method. Here, this study appraised on preferable nano cream profile among consumers other than provide a parametric benchmark to improve the OPT-*AcPENc* formulation. The outcome of the investigation was based on a given set of questionnaires to gauge consumer acceptance of the cream developed in this study.

1.5 Significance of the Study

Because this study aimed at developing an alternative O/W cream using plant-active ingredients from *AcPE*, thus the study could establish the best UAE technique

to extract high quantities of the bioactive compounds. Consequently, the O/W *AcPENc* developed here could serve as a steppingstone for further improvements in the utilization of pineapple biomass, namely, the exploration of other valuable uses of the peels, preferably for the benefit of humankind. This research also highlights the *AcPE* nano cream's topical applicability, containing catechin, quercetin, and gallic acid, as active ingredients to improve the human skin. It is pertinent to indicate that the findings on the physicochemical, safety evaluation, and sensory evaluation studies on the *AcPE* nano cream can further add to the body of knowledge on existing natural-based cosmeceuticals while being mindful of the environment and ecosystem issues.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

In this present research, a stable bioactive compound from agricultural or food-based industrial biomass of *AcPE* was successfully explored to develop an O/W nano cream formulation. Since this is the first attempt to develop a nanoemulsion containing *AcPE* for topical human skin use, the extraction and screening of formulated *AcPE* must be established. Herein, this research aimed to improve bioactive compounds extraction from *AcPE* while fulfilling the cosmeceutical formulation guidelines. The objectives of this research included that of (1) optimization of UAE and *AcPE* characterization, (2) screening of *AcPE* formulation, (3) physicochemical and safety evaluation of OPT-*AcPENc* as well as (4) sensory evaluation on *AcPENc* among the panelists.

This study successfully optimized the parameters of UAE through RSM to extract a better amount of yield and TPC from *AcPE*. While the UAE revealed successful extraction of *AcPE*, the BBD displayed the insignificant effect of UAE's parameter on yield. In contrast, UAE showed a significant effect on TPC, revealing the optimum condition for *AcPE*'s extraction suggested by RSM was 5 min sonication time, 50 % solvent ratio, 65 % amplitude, and 35:1 mL/g liquid-solid ratio. On top of that, TFC, DPPH, and FRAP assay revealed an acceptable amount of antioxidant capacity, thereby supporting the feasibility of the probe-UAE technique for extracting phenolics from *AcPE*. Besides, HPLC analysis revealed the presence of phenolic compounds in *AcPE* with catechin as the major flavonoid, followed by quercetin and gallic acid.

Other than that, this research successfully screened and optimized the parameters to formulate a stable O/W nano cream. It was affirmed that a combination

of high and low-energy techniques utilizing ultrasonic bombardment and PIT was appropriate to develop a stable O/W AcPENc. Based on D-optimal MED, it could be construed that the optimum formulation comprised of OO amount (1 %), GSO amount (12 %), T80 (12.63 %), and water (74.37 %) with a constant value of 10% AcPE, 2 % XG, 1 % phenoxyethanol and 2 % perfume oil. Regarding that, OPT-AcPENc reported exhibit low particle size (187.37 ± 0.81 nm) with desirable PDI and zeta potential values of 0.248 ± 0.017 and 35.40 ± 2.71 mV, respectively. TEM analysis illustrated OPT-AcPENc as a spherically shaped nano size. Also, the formulated nano cream demonstrated its suitability for human skin application with an acceptable pH value (4.56 ± 0.01). In contrast, a high conductivity value (1346.67 ± 5.77 μ S/cm) was observed; hence, verifying its O/W nature. Likewise, OPT-AcPENc proved its cosmeceutical suitability with a shear thinning and non-Newtonian behavior.

This study also completed the stability study, which consists of; (1) physical (centrifugal and mechanical shock), (2) light, and (3) chemical (heating-cooling cycle and accelerated test). The system displayed a stable appearance with no phase separation during the physical and light tests as well as the heating-cooling cycle. During the accelerated test, it was found that the changes in particle size as a function of time at 4 ± 2 °C were affected by Ostwald ripening destabilization phenomenon. The nanoemulsion was destabilized at a high temperature of 50 ± 2 °C, showing phase separation at the end of storage time. The effect seen here was plausibly due to dehydration of T80 in the system. This, consequently, changed the curvature of the surfactant, and the spherical-droplet shape of the encapsulated active ingredients was disrupted. Despite that, this test uncovered the optimum storage temperature of OPT-AcPENc to be at room temperature (25 ± 2 °C). In fact, the suitability was evident from OPT-AcPENc's stability in pH, particle size, and PDI, without phase separation in this investigation. Noteworthy to mention, OPT-AcPENc was revealed to be safe from microorganism contamination and heavy metal exposure, fulfilling the requirement of NPRA Malaysia for any cosmetic products.

Additionally, the sensory evaluation revealed that OPT-AcPENc was preferred among the panellists regarding its color, fragrance, spreadability, adsorption, film/coating, and irritancy, based on the attained scale of 7 to 8. However, attributes

on consistency, skin feel, and residue were rated lower than the commercial cream, with a difference of 1.0. The findings revealed that the lowest evaluated factor on OPT-AcPENc was the thickness of nanoemulsion, with a collective scale of 6.0. This effect was plausibly due to the emulsion after-effects of the formulated composition. Still, findings of the evaluation thus could be used as a benchmark to certain attributes of the O/W OPT-AcPENc.

In view of the overall findings of this present research, the O/W AcPENc developed here has the potential application in the cosmeceutical industry, which focus on naturally derived plant active ingredients. Thus, the study's focus on exploring the AcPE's potential could further add to the body of knowledge on existing food by-products and natural-based cosmeceuticals.

5.2 Recommendations

Considering that this formulated of O/W *Ananas comosus* peels extract on skin rejuvenation is relatively nascent, the following recommendations for future research are suggested:

1. To determine the antioxidant capacity of the formulated nano cream. The test can gauge the capacity and stability of bioactive compounds after the formulation and prove to consumers what the products claimed in the research studies and marketing materials.
2. To incorporate stabilizer, polymer, and emollients in the formulation. As the present research discovered AcPENcs' imperfection regarding stability at accelerated temperature and texture attributes through sensory evaluation, it is recommended to upgrade the formulation to improve the stability and texture properties (feel particularly smooth and light on the skin) as well as maximize the skin benefits to consumers.
3. To carry out a further in-vitro study on the formulation, as topical semisolid is a complex formulation must carry bioactive compounds to the skin and the

underlying tissue. Thus, the optimization of drug release and skin penetration measurements *viz.* Franz diffusion using synthetic membrane and human skin, respectively, is suggested.

4. To study the in-vivo by instrumental measurement. The efficacy of non-invasively changes in skin barrier function such as epidermal hydration levels and transepidermal water loss (TEWL) evaluated on human volunteers is suggested. Besides, the correlation between in-vivo measurement and sensory evaluation can be investigated to accomplish cosmetic products with consumer-satisfying sensory attributes.
5. To perform molecular modeling and dynamic simulation for the formulation design. The use of computational tools could help explore the interactions between nanoparticles and the biological component of AcPE. It also could suggest avenues for understanding complex formulation design in less time with lower investments.

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