MICROEMULSION BASED COATINGS WITH CALCIUM AND ANTIOXIDANT FOR REDUCING CHILLING INJURY OF *Ananas comosus*

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DEDICATION

This humble works is specially dedicated to:

My beloved parents, Hj Dolhaji and Hjh Zaiton..
My other half, Mr Nur Halis Isa and my kids Nurin and Iqbal.
All families and fellow friends

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ABSTRACT

Ananas comosus generally known as pineapple is one of Malaysia's commodity and commercially cultivated across various region in Malaysia. A high-quality pineapple is valued from both its internal and external quality attributes. The standard postharvest practice and storage by refrigeration is recommended for perishable commodities which includes pineapple. However, cold storage causes physical and chemical deterioration known as chilling injury (CI). Although certain pineapple cultivar had been genetically developed with resistance toward CI, the findings of this study showed that CI affected quality attributes of all cultivars tested i.e. *Morris* (*Queen*), *Josapine* (hybrid of *Spanish* and *Smooth Cayeen*) and *MD-2* (hybrid of *Smooth Cayeen*), regardless of their genetic makeup. Therefore, this research aims to develop a stable and edible microemulsion loaded with calcium chloride and clove essential oil which objectively to minimize the CI and maintain the quality attributes of pineapple both chemically and physically. An optimized microemulsion formulation with particle size of 203.22 nm, zeta potential (ζ- potential) of +3.65 mV and emulsion stability index (ESI) of 24.1 was obtained by applying 0.01% w/w whey protein isolate, 0.13% w/w carboxymethyl cellulose at pH 4.96. This optimized condition and formulation was obtained via response surface methodology. Characterization of optimum microemulsion was conducted morphologically and physically, and a stable microemulsion was observed for 30 days in response of size particle, ζ-potential and ESI. In overall, microemulsion coating performance results suggest that this coating significantly improved most of the quality attributes, antioxidant activity and storage capacity of the pineapples up to 30 days of storage at 4 ± 2 °C. Coated pineapples sustained up to 21 days with minimum effects of CI as compared to control which only capable to tolerate up to 7 days. A significant decrease in calcium content was found based on soluble calcium content and stained calcium localization qualitatively proven the occurrence of CI. The orthogonal testing to support this finding was by GC-MS primary metabolite analysis, in which coated pineapple showed greater shifts in metabolism by presence of distinctive amino acids and significant higher level of organic acids, soluble sugar and soluble alcohol as indicator of response to chilling stress and adaptive mechanisms.

ABSTRAK

Ananas comosus atau lebih dikenali sebagai nanas di Malaysia adalah tanaman komoditi dan ditanam secara meluas di Malaysia. Buah nanas berkualiti tinggi dinilai dari aspek kualiti luaran dan dalamnya. Amalan piawai bagi lepas-tuai dan penyimpanan secara sejuk adalah disarankan bagi tanaman komoditi yang mudah rosak termasuk buah nanas. Walaubagaimanapun, penyimpanan sejuk boleh menyebabkan kerosakan dan kecederaan secara fizikal dan kimia yang dikenali sebagai kecederaan dingin (CI). Walaupun sesetengah kultivar nanas secara genetiknya dibangunkan dengan ciri-ciri ketahanan terhadap CI, kajian ini menunjukkan CI memberi kesan terhadap kualiti kesemua kultivar nanas yang diuji iaitu Morris (*Queen*), Josapine (hybrid *Spanish* dan *Smooth Cayenne*) dan MD-2 (hibrid *Smooth Cayenne*) tanpa mengambilkira ciri-ciri genetiknya. Maka, kajian ini bertujuan membangunkan mikroemulsi stabil dan boleh dimakan serta mengandungi kalsium klorida dan minyak pati cengkih untuk meminimumkan CI dan mengekalkan kualiti fizikal dan kimia yang terbaik bagi buah nanas. Formulasi mikroemulsi optimum dengan saiz partikel 203.22 nm, potensi zeta (ζ-potensi) +3.65 mV dan indeks kestabilan emulsi (ESI) 24.1 telah diperoleh dengan menggunakan 0.01 % w/w protein whey terasing, 0.13% w/w selulosa karboksimetil pada pH 4.96. Keadaan dan formulasi optimum ini diperoleh melalui kaedah gerakbalas permukaan. Pencirian mikroemulsi optimum daripada aspek morfologi dan fizikal telah dilakukan dan mendapati mikroemulsi adalah stabil bagi tempoh 30 hari melalui penilaian saiz partikel, ζ-potensi dan ESI. Secara keseluruhannya, hasil prestasi salutan mikroemulsi menunjukkan bahawa penyalutan dapat meningkatkan sebahagian besar sifat kualiti, aktiviti antioksidan dan kapasiti penyimpanan nanas sehingga 30 hari pada suhu 4 ± 2 °C. Buah nanas dengan salutan mikroemulsi terbukti dapat bertahan sehingga 21 hari dengan kesan minimum CI berbanding tanpa salutan yang hanya mampu bertahan selama 7 hari. Penurunan ketara kandungan kalsium telah diperoleh berdasarkan kandungan kalsium terlarut dan tanda tompokan kalsium setempat yang dibuktikan secara kualitatif oleh kewujudan CI. Kajian ortogonal untuk menyokong dapatan kajian ini dilakukan dengan analisa GC-MS metabolit primer. Analisa menunjukkan terdapat anjakan yang besar pada metabolisma dengan kehadiran amino asid tersendiri dan kandungan asid organik, gula terlarut dan alkohol terlarut yang lebih tinggi sebagai tindakbalas terhadap tekanan kesejukkan dan mekanisma penyesuaian

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of variance
AOS	-	Active oxygen species
APX	-	Ascorbate peroxidase
BH	-	Black heart
CAT	-	Catalase
CCD	-	Central composite design
CEO	-	Clove essential oil
CI	-	Chilling injury
CMC	-	Carboxymethyl cellulose
CRD	-	Complete randomized design
DPPH	-	2,2-diphenyl-1- picryl-hidrazil
EL	-	Electrolyte leakage
ESI	-	Emulsion stability index
ESI	-	Emulsion stability index
FAMA	-	Federal agricultural marketing authority
FRAP	-	Ferric reducing antioxidant power
GAE	-	Gallic acid equivalent
GC-MS	-	Gas chromatography mass spectrometry
IB	-	Internal browning
MARDI	-	Malaysian Agricultural Research and Development Institute
MPIB	-	Malaysia pineapple industrial board
MT	-	Metric tons
pI	-	Isoelectric point
POD	-	Peroxidase
ppm	-	Part per million
PPO	-	Polyphenol oxidase
RH	-	Relative humidity
ROS	-	Reactive oxygen species
RSM	-	Response surface methodology
SOCST	-	Sub-optimum cold storage temperature

TEM	-	Transmission electron microscope
TPC	-	Total phenolic compound
TPTZ	-	2,4,6-tripyridyl-s-triazine
TSS	-	Total soluble solids
TTA	-	Titratable acids
UV	-	Ultra violet
WPI	-	Whey protein isolate

LIST OF SYMBOLS

o/w	-	Oil in water
ζ	-	Zeta
v/v	-	Volume per volume
w/v	-	Weight per volume
rev/min	-	Revolutions per minute
μM	-	Micro meter
Δ	-	Delta
w/w	-	Weight per weight
m/z	-	Mass-to-charge ratio
r	-	Radius
D, d	-	Diameter
Pa/s	-	Pascal per second
cP	-	Centipoise
Θ	-	Contact angle
mg/mL	-	Milligram per millilitre
β	-	Beta
δ	-	Delta
U	-	Enzymatic unit
nm	-	Nanometer
N	-	Newton
Ca	-	Calcium
P	-	Phosphorus
Fe	-	Iron
Na	-	Sodium
K	-	Potassium
mM	-	Millimolar
rpm	-	Rotation per minute
L∗	-	Lightness
a∗	-	Redness
b*	-	Yellowness

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Pineapple (*Ananas comosus*) is the most widely cultivated species from family of *Bromeliaceae,* and this perennial species contributes to over 20% of the world production of tropical fruits. It is reported that 70% of the pineapple is consumed as fresh fruit in producing countries (Tassew, 2014). Within two decades from the period of 1990 to 2012, the global increase in production has been observed to be consistent where the number was doubled from 11.84 million metric tons (MT) to 23.33 million MT (Lobo and Yahia, 2017). A survey by CBI in 2013 have shown, pineapple was the highest fresh fruit consumed by European countries from year 2006 to 2009 and the number was projected to keep growing. As world market demand for fresh whole pineapple is continuously growing, the pineapple exporters are keen and expected to practice a high standard postharvest operation plus integration of current developed technology to ensure the high-quality pineapple production to shelf (Lobo et al., 2017).

Effective postharvest handlings and operations of tropical fruits are very crucial start from harvesting, in-field handlings, packing-house operation plus supply chain to the market (Kamol et al., 2014; Mirza, Senthilkumar and Singh, 2016). In recent years, effort has been made for the assurance of high quality product to market as pineapple were represented by its flavour, juiciness, texture, and vitamin C and fibre content (Paull and Chen, 2003; Abdullah, 2011). A high-quality pineapple is considered from both its internal and external quality attributes. The external attribute is measured by size and weight, and scaled by appearance whereas internal attribute is quantitated by its chemical characteristics, and qualitatively evaluated by its colour and textures (Suksawat, 2015).

Temperature during storage by refrigeration is critical parameters in postharvest handlings for maintaining the freshness of the fruit during supply-chain. Pineapple is non-climacteric fruit which must be harvested ripe since the ripening process does not take place after harvest. Low temperature storage has many beneficial effects to pineapple fruits where it reduces respiration rate (Benítez *et al.*, 2012; Sánchez *et al.*, 2012), delay compositional breakdown of macromolecules, and control development of microorganisms (Kader, 2013). However, pineapple has high moisture content, hence influences an increase of deterioration rate cause by the metabolic reaction of fruit soon after harvest. This may be the key for an area of development which open for researcher to further explore and provide solutions to the industry. In real scenario at domestic and commercial scale practices, maintaining a low temperature during storage is the main common factor considered by consumer and marketer in single management approach used to prolonged shelf life of fruits and vegetables. The low temperature is an effective means for keeping horticultural commodities at high post-storage quality (Hong *et al.*, 2013).

Based on given facts and current practices driven by standard postharvest practices, storage by refrigeration is recommended for storage perishable commodities which includes pineapple. However, chilling injury (CI) is a major problem for tropical fruits during storage which has been recognised as unique abiotic stress impact on crop plant physiology when expose to low but above freezing temperature. This fundamental understanding on direct impact of cool storage practices may not be exceptional case for pineapple as well. In addition, among other postharvest disorder of pineapple is internal browning (IB) which cause by CI attracts most attention from researchers around the world as most pineapple varieties are susceptible to CI and IB (Abdullah, 2011). This physiology disorder will further cause severe economic losses to pineapple producing countries (Hong *et al.*, 2013; Zhou *et al.*, 2014b). Chilling injury (CI) will cause symptom on internal tissues of pineapple such as pale, watery and IB especially at the flesh surrounding the core. On top of that, research on maintaining postharvest fruits and vegetables is limited (Zhang, Zhang and Yang, 2015).

In order to provide a solution and mechanism of protection for pineapple from chilling stress, the application of microemusion coating has been proposed in this study. Coating is defined as application of thin layer edible material on product surface to create a modified atmosphere which provides optimal functionality to products and increase their market value (McHugh and Senesi, 2000; Salvia-Trujillo et al., 2015). Moreover, films and coatings have gained an interest in the field of food preservation due to the promising results and commercial interest (Galus and Kadzińska, 2015). Coatings on fruits during refrigeration storage possible to control moisture transfer, respiration rate, oxidation process and extend shelf life (Dhall, 2013). In addition, coating may serve as a carrier of active compound such as antioxidant, flavouring agent, colouring agent, growth regulator and microbial inhibitor (Valencia-Chamorro *et al.*, 2011).

An effort to establish a delivery system for active compounds has driven the direction of research on colloidal system such as nanoemulsion and microemulsion technology in edible coatings. By terminology, microemulsion is thermodynamically stable isotropic liquids formed by mixing oil, water, and surfactants together. It is also stable under a specific combination of compositional and environmental conditions and can be formed by mixing water, oil and surfactants with external energy, such as stirring or heating (Rao and McClements, 2011). Recent studies have shown the potential application of essential oil in microemulsions as antioxidants to improve food quality (Hamed and Edris, 2012).

Essential oil, have been traditionally used to extend food shelf life due to their antioxidant and antimicrobial activity. These niche attributes and increasing demand from the society for natural food active ingredients options have led to an interest in considering of essential oils as potential alternative to eliminate the additional chemical and additive used for emulsion formulation. Essential oil with strong flavour, antioxidant and antimicrobial are plant secondary metabolites with high lipophilic characteristic. In this study, clove essential oil (CEO) as anti-oxidative active ingredient being incorporated in the microemulsion formulation as the model of insoluble oil phase. Incorporating the essential oil in food system is limited by its poor water solubility and volatile nature, however microemulsion colloidal system could

enhance this limitation (Ma and Zhong, 2015). Hence, it is important to understand the major components influencing the formulation of food emulsions and their effects on physical properties to obtain delivery systems based on small-sized emulsions with long-term stability (Arancibia, Zúñiga and Matiacevich, 2016).

Calcium as a major regulatory ion in horticultural crops involved in gene expression by response of wide arrays of biotic and abiotic sources. In addition, calcium plays a fundamental role in plant growth, development and important mineral element that regulates fruit quality, specifically, maintenance of fruit firmness, a decrease in postharvest decay and incidence of physiological disorders (Aghdam *et al.*, 2013). Incorporation of calcium in postharvest pipelines to enhanced calcium concentration in pineapple fruits have been reported previously by researchers for better understanding fruit calcium physiology (Hewajulige *et al.*, 2003; Youryon and Wongs-Aree, 2015; Youryon *et al.*, 2018), it is also suggested the possibility of suppressing CI symptoms by increasing the calcium concentration in the fruit of chill-sensitive pineapple varieties

1.2 Problem Statement

Concern have been reported by several researcher as Malaysian pineapple continuously struggling to develop its export market and ability to remain competitive suffered tremendous setback (Abdullah and Atan, 1996; Hassan, Othman and Siriphanich, 2011) and according to Othman et al. (2011), research and development must be carried out more aggressively in the field of agronomy, machinery and postharvest management that produce high quality, disease-resistant, and longer shelf-life pineapple. Moreover, small-scale pineapple farmers have been encouraged to focus on fresh pineapple production apart from processed downstream product due to its high value at international market (Raziah, 2009).

The major drawback for world market of fresh pineapple is the postharvest storage shelf life. Application of cool storage is required to assure the freshness and prolong the shelf life of pineapple, but it face challenges by the development of CI (Youryon et al., 2011; Nukuntornprakit et al., 2015). The recommended storage temperature of ripe and under ripe pineapple for export is 10ºC (Jobling, 2002), but in real scenario in supply chain, the pineapple is prepared, shipped and stored below 10ºC (Cruz and Garcia, 2005). This sub-optimal temperature may cause physiological injuries (Maharaj and Sankat, 1990). As on today, very few studies have been published regarding the influence of lower temperature on quality of harvested pineapple fruit (Hong et al., 2013; Nukuntornprakit et al., 2015). Although the metabolic dysfunction changes in pineapple have been well documented, the current understanding of CI of pineapple is still incomplete (Luengwilai, Beckles and Siriphanich, 2016). Moreover, current postharvest practices on Malaysian fresh pineapples for export are focusing on application of fungicide without any physiological treatment proven effective for CI.

1.3 Objective of Study

The objectives of this research are as the follows:

(a) To determine the quality attributes by physical and chemical properties of pineapple cultivars with different low temperature resistance towards chilling injury (CI).

(b) To study and optimize microemulsion edible coating formulation for pineapple coating.

(c) To evaluate the performance of microemulsion edible coating towards CI and the primary metabolite changes on reducing CI during sub-optimum cold storage of pineapple.

1.4 Scope of Study

To achieve the objectives, the scopes of study are stated as follow:

(d) Investigation on the effects of sub-optimal temperature storage on physical, chemical, antioxidant activity and capacity; and antioxidant related enzyme activities quality attributes of 3 pineapples cultivars (cvs.) (*Morris*, *Josapine* and MD2 cvs.) with different low temperature resistance by weekly basis (up to 28days)

(e) Determination optimized factors of; WPI, CMC and pH using response surface methodology for formulation of oil in water (o/w) and characterization with response to particle size, ζ-potential and emulsion stability index (ESI).

(f) Evaluation of the performance of microemulsion coating towards chilling injury other quality attributes and primary metabolite identification.

1.5 Hypothesis and Significance of Study

It is hypothesized that edible coated pineapple with calcium and antioxidant-loaded microemulsion could potentially be an effective solution to prevent chilling injury (CI) disorder cause by low temperature storage. This eventually will improve the physiochemical characters of pineapple leads to high quality pineapple attributes internally and externally. In addition, the positive impact on quality factors will be evaluated majorly by the final content of calcium distribution, antioxidant activity and capacity and by primary metabolite compound analysis in pineapple.

High quality pineapple that meets the criteria of export market, need an efficient postharvest storage strategy. The potential of microemulsion as coating material served as carrier of antioxidant and calcium in this study may provide significant impact on effective formulation of edible coating for application on pineapple. Edible coating with functional properties on fruits is a promising advantage for postharvest storage method.

The development of effective methods to maintain post-harvest quality of pineapple fruit has been widely reported such as heat, chemical treatment and controlled atmosphere. These preservation technologies in combination with low temperature storage could exert a better beneficial effect on quality maintenance than those applied alone. In addition, enrichment of the microemulsion with calcium also proposed in this study, thus addressing this challenge shall revive and boost the existence of Malaysian pineapple industry and give a competitive challenge to the world market through credits on local postharvest technology development.

Premium Fruits and Vegetables are one of the Entry Point Project (EPP) in Agriculture sector under Malaysia Economic Transformation Programme (ETP) with the aims to export local premium fruits and vegetables to Middle East and Europe. Thus, addressing the Malaysian pineapple postharvest challenge in this study shall create a niche and competitive advantages for Malaysia to stand firm at international agri-business based on locally developed technology.

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