DEVELOPMENT AND RELEASE MECHANISM OF THE SELECTED ACTIVE COMPOUNDS ENCAPSULATED POROUS SAGO STARCH FOR ACTIVE PACKAGING APPLICATIONS

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by

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Special dedications

My Hero

Hj. Latip bin Hj. Idris

"Study hard"

These were your last words to me. I hold them close to my heart, and they continue to guide me every step of the way.

I love you and miss you deeply, Dad.

Al-Fatihah.

My 'Cinta Hati'

Hjh. Noraini binti Nor

Your love, support, and prayers have been my greatest strength.

May Allah SWT bless you with happiness, good health, and a long, blessed life.

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"I never dreamed of success; I worked for it."

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LIST OF SYMBOLS

%	Percentage
°C	Degree Celsius
G	Gram
Mg	Milligram
Ml	Milliliter
µM	Micromolar
ppm	Parts per million
m^2	Meter square
cm^3	Centimeter cube
h	Hour
L	Lightness
A	Redness
B	Yellowness
mmHg	Vapor pressure
W	Weight
Eq. wt	Equivalent weight
V	Volume
N	Normality
$Å^2$	Polar surface area
N	Newton
A	Surface area of film
Q	Quantity
T	Time of storage

l	Thickness
Ps	Saturated vapor pressure
α	Alpha
β	Beta
T	Temperature
ΔT	Gelatinization temperature range
ΔH	Gelatinization enthalpy
Δd	Correlated to dispersion forces
Δp	Correlated to polar forces
Δh	Correlated to hydrogen bonding
Fdi	Component group contributions of dispersion forces, $(MJ/m^3)^{1/2}$. mol^{-1}
Fpi	Component group contributions of polar forces, $(MJ/m^3)^{1/2}$. mol^{-1}
Ehi	Component group contributions of hydrogen bonding, J/mol
V	Molar volume of the compounds

LIST OF ABBREVIATIONS

IUPAC	International Union of Pure and Applied Chemistry
FAO	Food and Agriculture Organization of the United States
Control	Ultrasonic and enzyme treatment
MWV 40	Microwave heat moisture pre-treatment combine with ultrasonication and enzyme hydrolysis
HMT 105	Oven dry heat-moisture pre-treatment combine with ultrasonication and enzyme hydrolysis
FT	Freeze-thaw pre-treatment combine with ultrasonication and enzyme hydrolysis
SEM	Scanning electron microscopy
DSC	Differential scanning calorimetry
TGA	Thermogravimetric analysis
SPME	Solid phase microextraction
GC-MS	Gas chromatography-mass spectrometry
GC-FID	Gas chromatography flame ionization detector
TSS	Total soluble solid
TA	Titratable acidity
RH	Relative humidity
PLA	Polylactic acid film
PBS	Polybutylene succinate film
TC	Trans-cinnamaldehyde
Thy	Thymol
EA	Eugenyl acetate

Mix	Mix compounds
Expose	Package without lid and active sachet
WAS	Close package without active sachet
PLA_Native_TC	Close package with PLA sachet film consists of trans-cinnamaldehyde encapsulated in native sago starch
PLA_Native_Thy	Close package with PLA sachet film consists of thymol encapsulated in native sago starch
PLA_Native_EA	Close package with PLA sachet film consists of eugenyl acetate encapsulated in native sago starch
PLA_Native_Mix	Close package with PLA sachet film consists of mix compounds encapsulated in native sago starch
PLA_FT_TC	Close package with PLA sachet film consists of trans-cinnamaldehyde encapsulated in porous sago starch
PLA_FT_Thy	Close package with PLA sachet film consists of thymol encapsulated in porous sago starch
PLA_FT_EA	Close package with PLA sachet film consists of eugenyl acetate encapsulated in porous sago starch
PLA_FT_Mix	Close package with PLA sachet film consists of mix compounds encapsulated in porous sago starch
PBS_Native_TC	Close package with PBS sachet film consists of trans-cinnamaldehyde encapsulated in native sago starch
PBS_Native_Thy	Close package with PBS sachet film consists of thymol encapsulated in native sago starch
PBS_Native_EA	Close package with PBS sachet film consists of eugenyl acetate encapsulated in native sago starch

PBS_Native_Mix	Close package with PBS sachet film consists of mix compounds encapsulated in native sago starch
PBS_FT_TC	Close package with PBS sachet film consists of trans-cinnamaldehyde encapsulated in porous sago starch
PBS_FT_Thy	Close package with PBS sachet film consists of thymol encapsulated in porous sago starch
PBS_FT_EA	Close package with PBS sachet film consists of eugenyl acetate encapsulated in porous sago starch
PBS_FT_Mix	Close package with PBS sachet film consists of mix compounds encapsulated in porous sago starch

LIST OF APPENDICES

PEMBANGUNAN DAN MEKANISME PELEPASAN SEBATIAN AKTIF TERPILIH YANG DIENKAPSULASI DALAM KANJI SAGO BERLIANG UNTUK APLIKASI PEMBUNGKUSAN AKTIF

ABSTRAK

Sebatian aktif memainkan peranan penting dalam keberkesanan pembungkusan aktif, namun sering terdedah kepada penguraian di bawah keadaan persekitaran yang ekstrem, sekali gus mengehadkan fungsinya. Kajian ini bertujuan membangunkan pembawa berliang berasaskan kanji sagu yang direka bentuk bagi melindungi sebatian tersebut serta membolehkan pelepasan terkawal dalam aplikasi pembungkusan aktif**.** Gabungan rawatan awal pembekuan dan pencairan, rawatan ultrasonik, serta rawatan enzimatik (FT) telah menghasilkan pembentukan struktur liang yang ketara pada permukaan granul kanji sagu. Entalpi gelatinisasi menurun daripada 3.32 J/g bagi sampel asli kepada 2.98 – 3.13 J/g bagi sampel yang dirawat, kesan daripada pembentukan liang. Analisis termogravimetri menunjukkan trend yang sama, di mana suhu degradasi bagi sampel asli menurun daripada 340 °C kepada 332 – 334 °C selepas pengubahsuaian. Peningkatan luas permukaan dalam sampel FT meningkatkan keupayaan penyerapan air dan minyak masing-masing sehingga 37.04% dan 108.74%. Variasi dalam pembentukan liang memberikan kesan yang signifikan terhadap kecekapan pengenkapsulan, dengan kaedah FT menunjukkan kecekapan pengenkapsulan tertinggi bagi semua sebatian, iaitu antara 0.41 hingga 9.86% bagi sebatian individu, dan antara 21.8 hingga 45.99% bagi campuran sebatian, berbanding kanji terubah lain. Sampel dengan kecekapan pengenkapsulan tertinggi telah dipilih, manakala kanji asli digunakan sebagai kawalan. Spektroskopi FTIR mengesahkan pengenkapsulan sebatian aktif. Ramalan pelepasan berdasarkan HSP telah disahkan

melalui analisis GC-FID-SPME. Pemkuktian konsep menunjukkan kesan yang signifikan terhadap kehilangan berat tomato ceri, hasil daripada interaksi antara jenis kanji, polimer, sebatian aktif dan tempoh penyimpanan. Semua bungkusan yang mengandungi kanji sagu berliang yang dienkapsulasi di dalam uncang PLA menunjukkan kehilangan berat kurang daripada 2% dalam tempoh 20 hari, berbanding sampel WAS dan PBS. Sebatian aroma seperti heksanal dan 6-metil-5-hepten-2-on dibebaskan dengan corak pelepasan yang serupa, namun menunjukkan intensiti yang berbeza. Secara keseluruhan, selepas menjalani rawatan awal pembekuan-pencairan, rawatan ultrasonik dan proses enzimatik, kanji sagu berliang menunjukkan potensi yang tinggi sebagai pembawa bagi sebatian aktif dalam pembungkusan makanan, khususnya apabila digunakan bersama bahan uncang berasaskan PLA

DEVELOPMENT AND RELEASE MECHANISM OF THE SELECTED ACTIVE COMPOUNDS ENCAPSULATED POROUS SAGO STARCH FOR ACTIVE PACKAGING APPLICATION

ABSTRACT

Active compounds play a critical role in the effectiveness of active packaging but are often vulnerable to degradation under harsh conditions, limiting their functionality. This study aims to develop a sago starch-based porous carrier designed to protect these compounds and enable their controlled release in active packaging applications. The combination of freeze thaw pre-treatment, ultrasonic, and enzyme (FT), resulted in significant formation of pore structures on the sago starch granule surface. The enthalpy of gelatinization decreases from 3.32 J/g for native samples to 2.98 – 3.13 J/g for treated samples due to pore formation. Thermogravimetric analysis showed a similar trend, with the native sample's temperature dropping from 340 °C to 332 – 334 °C after modification. Increasing the surface area in FT samples significantly enhances water and oil absorption capacity by up to 37.04% and 108.74% respectively. Variations in pore formation significantly affected encapsulation efficiency, with FT demonstrating the highest encapsulation efficiency across all compounds, ranging from 0.41 to 9.86% for individual compounds, and 21.8 to 45.99% for mixed compounds, in comparison to other treated starches. The sample with the highest encapsulation efficiency was selected, with native starch as control. FTIR confirmed active compound encapsulation. Release prediction by HSP was validated using GC-FID-SPME. Proof-of-concept revealed significant effects on cherry tomato weight loss from interactions among starch type, polymer, active compound, and storage duration. All packages with encapsulated porous starch in PLA sachets lost less than 2% weight

over 20 days compared to WAS and PBS samples. Hexanal and 6-methyl-5-hepten-2-one released aroma compounds with similar patterns but different intensities. After freeze-thaw pre-treatment, ultrasonic treatment, and enzymatic processes, porous sago starch shows promise as a carrier for active compounds in food packaging, especially with the use of PLA as a sachet material.

CHAPTER 1

INTRODUCTION

1.1 Research backgrounds

Active packaging is a packaging that has extended applications which include not only containing or protecting food from physical damage, but also preserving the quality, safety, and prolonging the shelf life of food products. The packaging material contains active compounds that can interact with the product or surrounding environment by releasing or absorbing them (European Commision, 2009). Interaction of active compounds determine the active packaging type, active releasing or non-migratory (scavengers/ absorber) (Jiang et al., 2023). Today, active packaging is one of the emerging technologies and innovations that help in reducing food loss and food safety issues.

The development of an active packaging system comprises three key elements: i) the active compound, ii) the packaging materials serve as a direct carrier of the compound (when the active compound is directly incorporated into the material) or as an indirect carrier for the active compounds (by containing external carriers, generally polysaccharide-based, that have been encapsulated with the active compounds), and iii) the food that needs to be protected.

Active compounds are a key figure in active packaging systems. A variety of active compounds, such as cinnamaldehyde, thymol, eugenol, carvacrol, curcumin, α-tocopherol, ethyl lauroyl arginate, trans-2(E)-hexenal, silver-kaolinite, have been incorporated directly or indirectly into packaging materials to fulfil various functions such as antimicrobial and/or antioxidant releaser, ethylene and/or other gases scavengers (Dairi et al., 2019; Álvarez-Hernández et al., 2021; Heras-mozos et al.,

2021; Li et al., 2021; Sun et al., 2021; Li et al., 2021; Nur Hanani et al., 2022; López-Gómez et al., 2023; Dong et al., 2024). Despite their importance, active compounds are particularly susceptible to degradation and volatility, thereby reducing their efficacy and posing challenges in managing their release or scavenging mechanism (Mukurumbira et al., 2022). Incorporating active compounds directly into packaging materials can be problematic due to the molecular structural instability they may exhibit, such as tautomerism observed in compounds like citral, eugenol, and limonene. This instability has the potential to affect their mechanism of action. Additionally, immiscibility within the polymer matrix can affect release kinetics depending on the compound's and the packaging matrix's polarity. Thermal sensitivity can also result in rapid degradation (Chen et al., 2019; Chen et al., 2020; Fonseca et al., 2021; Jiang et al., 2022). To overcome the limitations, polysaccharides such as chitin, chitosan, alginate, carrageenan, pectin and starch are widely used as carrier materials to encapsulate and protect the active compounds from degradation (Ghandehari-alavijeh et al., 2024).

Starch based carriers and their derivatives, including β-cyclodextrin, starch nanostructures, and porous starch, have great potential for protecting active compounds through encapsulation techniques. Researchers have expressed great interest in the development and application of porous starch as an active compound carrier. Porous starch is defined as a modified starch with pore structures distributed across the granule and extending into the center cavities without altering the granule structures (Zhang et al., 2012). The unique structure contributes to the large surface area hence, providing space to accommodate the active compounds within the starch granule (Qi & Tester, 2019). This provides protection and assist in the controlled release of the encapsulated compounds (Digaitis et al., 2022).

Currently, corn starch is the primary derivative in all studies on porous starch controlled release-active packaging, which is still limited to a few food applications, such as ground pork (Sun et al., 2021), seabass (Wang et al., 2022), and bread (Ju et al., 2019; Ju et al., 2020a; Ju et al., 2020b). Corn derivatives are currently much preferable for producing porous starches due to their easily modifiable granules. To date, the development of porous starch from a readily available and inexpensive source, sago starch (*Metroxylon sagu*), as an active compound carrier in active packaging, remains scarce.

Furthermore, among all food products, fresh produce is the most perishable due to its active metabolic rate function even after harvest or post-handling, necessitating additional effort for quality protection and shelf-life extension. Temperature control and packaging strategies, particularly active packaging, are important components of effective fresh produce management. Based on recent active packaging research on cherry tomatoes (Liu et al., 2024; Wan et al., 2024), strawberry (Fu et al., 2024), chicken meat (Nandi & Guha, 2024), minced beef (Elhadef et al., 2024), fresh pork (Ibeogu et al., 2024), grass carp fillets (Dong et al., 2024), and shrimp (Khan et al., 2024; Riahi et al., 2024), most acknowledges that active packaging helps to maintain product quality, reduce microbial contamination, and extend food shelf life.

Therefore, the objectives of this study were i) to produce porous sago starch as an alternative active compound carrier using microwave, heat moisture or freeze-thaw pre-treatment methods in combination with ultrasonic and enzymatic treatments, and investigate its physicothermal properties, ii) to investigate the release mechanism of porous sago starch encapsulated active compounds (*i.e.,* trans-cinnamaldehyde, thymol, eugenyl acetate, and thereof combination) using Hansen solubility parameters and release experiment, and iii) to investigate a proof-of-concept for the use of porous

sago starch in an active packaging system designed for fresh produce applications, using cherry tomatoes as a model. The development of porous sago starch carriers is expected to show considerable potential as a carrier for active compounds in food packaging applications.

1.2 Problem statement

Active packaging is an innovation that is used to tackle the problem related to the food shelf life and food protection which is not provided by the traditional packaging. Active packaging is a packaging that has been incorporated with active compound(s) within the packaging material. Active compounds are widely known for their antifungal or antioxidant properties, but they are extremely susceptible to harsh environmental conditions. Several studies have discovered that encapsulation by starch-based carriers such porous starch have the potential to protect and control the release of active compounds.

However, the development of porous starch from high availability, sustainable and cost-effective sources, which is sago starch, is an area of research that remains underexplored. In addition, sago starch is naturally construct with smooth and compact surface, contribute to the lack of comprehensive understanding on the modification development, encapsulation ability and the release properties of porous sago starch as active compounds carrier. Current research on porous sago starch-based controlled release-active packaging has been limited to several food applications, hindering its broader potential.

This gap in knowledge presents a significant opportunity to investigate the feasibility, mechanisms, and benefits of using porous sago starch in active packaging, particularly its ability to encapsulate and release active compounds in a controlled

manner. Addressing these limitations can lead to advancements in sustainable packaging solutions and could lead to new possibilities for reducing the dependency on synthetic active compounds carrier and contributing to environmentally friendly alternatives.

1.3 Objectives

The general aim of this study is to produce a sago starch-based porous carrier that enable protection and controlled release of active compounds for active packaging application. This study has been divided into three main objectives as below:

1. To produce porous sago starch as an alternative active compound carrier using microwave, heat moisture or freeze-thaw pre-treatment methods in combination with ultrasonic and enzymatic treatments and investigate its physicothermal properties.
2. To investigate the release mechanism of porous sago starch encapsulated active compounds (*i.e.*, trans-cinnamaldehyde, thymol, eugenyl acetate, and thereof combination) using Hansen solubility parameters (HSP) and release experiment.
3. To investigate a proof-of-concept for the use of porous sago starch in an active packaging system designed for fresh produce applications, using cherry tomatoes as a model.

1.4 Thesis organization

Minimizing food losses is a critical concern, particularly as the number of individuals experiencing hunger continues to increase gradually each year. This project aims to investigate the possibility of porous sago starch as an alternate carrier for active

packaging application, with the goal of reducing food losses. The organization of this thesis is structured according to the active packaging development process, outlined as follows:

Chapter 1: Introduction

This introduction chapter introduces the main concepts of active packaging, active compound, and porous starch. The gap and research problem that prompted the development of this study were highlighted together with the specific objectives for each stage of the research.

Chapter 2: Literature review

This chapter focuses on the principles and mechanisms pertaining to porous starch development and application. It is divided into two parts:

Part 1: Modification methods toward the production of porous starch: A review. (This part has been published in Critical Reviews in Food Science and Nutrition: Hj. Latip, D. N., Samsudin, H., Utra, U., & Alias, A. K. (2021). Modification methods toward the production of porous starch: A review. Critical reviews in food science and nutrition, 61(17), 2841-2862.

Part 2: Porous starch: Flavor carrier, controlled delivery and food packaging applications.

Part 1 provides an in-depth review of the modification methods used to make porous starch, which include several techniques and starch sources. Part 2 extensively broadens the review, focusing on the principles and mechanisms of porous starch as an active compound carrier. The layout of these two subtopics is straightforward, with a focus on essential details for the porous starch area.

Chapter 3, 4, and 5: Discussions

Chapters 3, 4, and 5 correspond to the three objectives of this study. These three chapters explain the study's progress, starting with the development of porous starch, then understanding their release characteristics, and finally packaging application. Adequate justification for each finding is offered accordingly.

Chapter 3:

Chapter 3 explains and justifies the selection procedures employed to generate the porous sago starch, which represents the primary objective of the study. This section focuses on pore development without altering the sago starch granule structure, which is an important feature as the active compound carrier in this study. This part help in understanding the relationship between different treatment methods and morphological formation on sago starch granules. This chapter discusses the relationship between pore forms and physicochemical qualities, as well as oil and water absorption properties. Based on the findings, the following chapter examines deeper into the encapsulation efficacy of various active compounds, as well as the headspace release properties of the produced porous sago starch, to better understand their mechanism of action as an alternative active compound carrier. (This part has been published in Journal of Food Measurement and Characterization: Hj. Latip, D. N., Samsudin, H., & Utra, U. (2024). Morphology and physicochemical properties of porous sago starch carriers produced by a variety of combination pre-treatment techniques, 18, 9545–9559.

Chapter 4:

Chapter 4 narrows down the selection of produced porous sago starch from various treatments to those with the maximum encapsulation efficiency. This narrows the

inquiry to finding the best porous sago starch production method. This chapter demonstrates how pore development, encapsulation efficiency, and headspace release control interact in porous sago starch. Understanding these properties is crucial for advancing the development of active compound carriers for practical applications. Further exploration of the practical utilization of porous sago starch as an active compound carrier is demonstrated in the following chapter, as a proof-of-concept approach.

Chapter 5:

Chapter 5 presents a proof-of-concept for the use of encapsulated porous sago starch in active packaging applications involving actual food models, specifically cherry tomatoes. This study intends to reveal insights for better implementation by investigating its performance inside active packaging systems as well as its impact on a selected food model. Understanding its strengths and limitations offers the framework for improving its application, which includes promising advances in porous sago starch production for active packaging.

Chapter 6: Conclusion

This chapter present a comprehensive conclusion on each stage of this study including the porous sago starch development, release properties and the application part. Brief limitations and recommendations for improving this study are also addressed.

CHAPTER 2

LITERATURE REVIEW

2.1 Modification methods toward the production of porous starch: A review

2.1.1 Introduction

Porous starch is a starch modification product that consists of abundant pores distributed on the starch granules and extended toward the central cavities, without changing the granular structure (Zhang et al., 2012; Gao et al., 2013b; Veelaert, 2014). These pores provide space to accommodate small molecules within the starch granules. A pore can be defined as an opening in a surface with a depth that exceeds half of its entry width (Sujka & Jamroz, 2010). A porous starch structure is also described as a honeycomb-like-structure (Jyothi et al., 2010; Wu et al., 201; Wang et al., 2012), or sponge-like-structure (Belingheri et al., 2015a) as visualized in Figure 2.1. The International Union of Pure and Applied Chemistry (IUPAC) classifies pores into three main groups according to their diameter size: micropores (less than 2 nm); mesopores (2 to 50 nm); and macropores (more than 50 nm) (Sujka & Jamroz, 2010). In addition, micropores can be divided into two subgroups: ultramicropores (less than 0.7 nm) and supermicropores (0.7 nm to 2 nm) (Fannon et al., 1992; Sujka & Jamroz, 2010). Generally, porous starch can be produced by three common modification methods: enzymatic treatment, chemical/physical treatment or by combining the enzyme with chemical/physical treatments (Zhang et al., 2012; Gao et al., 2014; Guo et al., 2015; Majzoobi et al., 2015; Wang et al., 2016; Li et al., 2017; Deladino et al., 2017; Jung et al., 2017; Wang et al., 2017; Zhao et al., 2018; Xie et al., 2019; Keeratiburana et al., 2020; Wu et al., 2020).

A number of studies have developed porous starch from various sources of starches such as, cereals, roots, legumes, and palm (Whistler, 1991; Ishii et al., 1999; Rizzi, 2000; Uthumporn et al., 2010; Uthumporn et al., 2012; Zhang et al., 2012; Gao et al. 2013b; Li et al., 2013; Dura et al., 2014; Veelaert, 2014; Deladino et al., 2015; Christina & Lee, 2016; Wang et al., 2016b; Benavent-Gil & Rosell, 2017; Sujka, 2017). Wheat, corn, rice, and sorghum are cereals; cassava and taro are roots; mung beans and green pea are legumes; and sago starch is derived from palm (Karim et al., 2008; Zeeman, et al., 2010). Different sources of starches have dissimilarities in terms of their natural morphology and molecular structure, influencing the properties and the modification results (Leofanti et al., 1998; Nor Nadiha et al., 2010).

The formation of pore structure on the starch granule provides large surface area and high pore volume, which allow porous starches to be utilized in numerous applications (Wu et al., 2011), including as a flavor carrier (Golovnya et al.,1998; Wang et al. 2009; Belingheri et al., 2012; Belingheri et al., 2015a; Belingheri et al., 2015b; Christina & Lee, 2016), drugs carrier (Malafaya et al., 2001; Kundu et al., 2010; Ali et al., 2013; Gao et al., 2013a; Pawar et al., 2017), heavy metal absorber (Ma et al., 2015), probiotics carrier (Puupponen-Pimiä et al., 2002; Li et al., 2016), composite absorber in water treatment (Xu et al., 2017), plant oil carrier (Glenn et al., 2010), fluid absorber for skin care applications (Bazin & Barresi, 2005), malodor absorber (Rizzi, 2000), and antioxidant carrier (Deladino et al., 2015; Teixeira et al., 2015).

The uniqueness and the advantages of porous starch have driven more studies in this area with a focus on understanding the physicochemical properties, improvement of the porous structure, development of porous starches from new sources and their

applications. This review explores the potential and established modification methods to produce porous starch. The morphologies of porous starches will also be discussed.

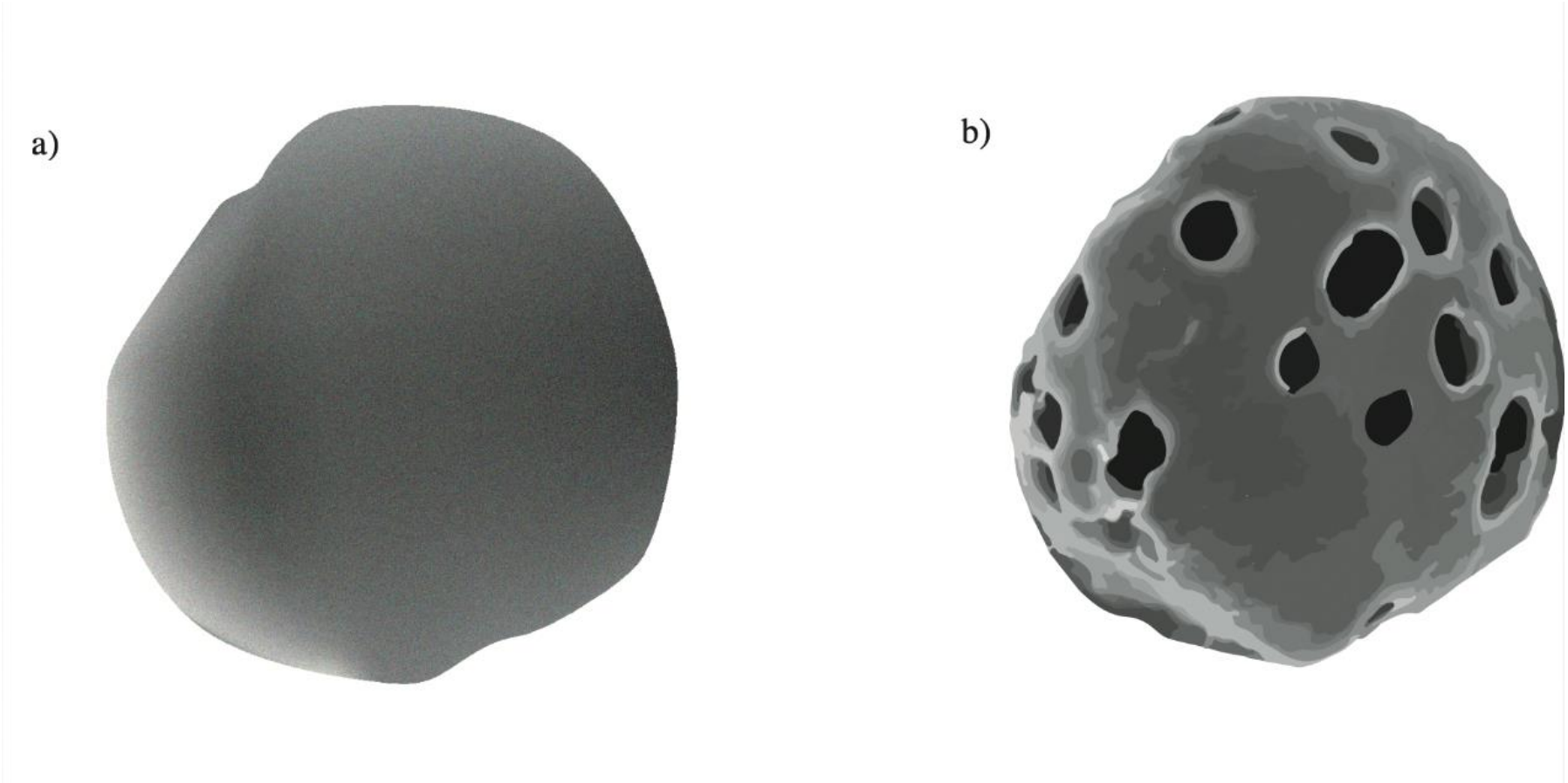

Figure 2.1 a) Native starch granule, b) Porous starch granule.

2.1.2 Porous starch preparation

The preparation of porous starch can be carried out by enzymatic or chemical/physical methods or combination thereof (Figure 2.2). Although enzymatic hydrolysis alone is capable of producing porous starch, its efficiency can further be improved when combined with physical (*e.g.,* ultrasonication, heat treatment, high hydrostatic pressure) or chemical (*e.g.,* esterification, cross-linking, etherification) treatments (Qian et al., 2011; Wu et al., 2011; Zhang et al., 2012). The combination of two methods is known as dual modification. The successful production of porous starch does not rely solely on the type of treatments, as additional factors affect production, such as origin of the starch, type and concentration of enzyme, hydrolysis conditions such as temperature, pH buffer, and hydrolysis time (Matsubara et al., 2004; Wang et al., 2016b; Benavent-Gil & Rosell, 2017).

2.1.2(a) Enzyme treatment

Enzyme treatment helps to produce pores without changing the shape of starch granule (Takashi et al., 2014). In this treatment, the pore production is resulted from the hydrolysis of starch granules by amylolytic enzymes in controlled conditions. Five patterns of pore formation have been identified in enzyme-treated starches: pin-holes, sponge-like erosion, numerous medium-sized holes, distinct loci leading to single holes in individual granules, and surface erosion (Evers et al., 1971). α-amylase, β-amylase, glucoamylase and cyclodextrin glycosyltransferase are the commonly used enzymes in porous starch preparation. Each enzyme can be classified into several groups, including endoamylases, exoamylases, and transferases; according to their site and bond hydrolysis (Table 2.1). α-amylase is classified as an endoamylase because it

specifically hydrolyzes the inner and linear part of α,1-4 glycosidic bonds (Jung et al., 2017).

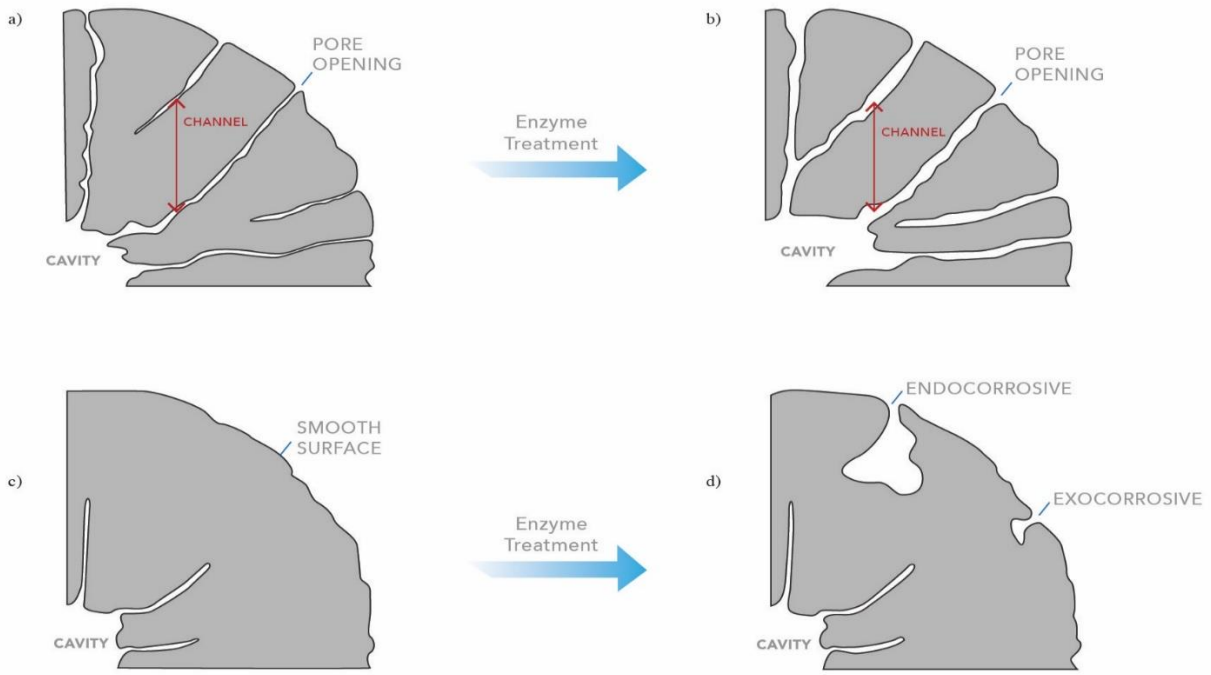

Figure 2.2 Mechanisms of enzyme treatment on starch granule. Starch granule with natural pore (a and b), Smooth and compact starch granule (c and d).

Meanwhile, β-amylase and glucoamylase are grouped as exoamylases, because both enzymes capable of hydrolyzing the external part of the starch molecular structure. These two enzymes have different modes of action; β-amylase specifically hydrolyzes the external part of linear α,1-4 glycosidic bonds, while glucoamylase hydrolyzes both α,1-4 glycosidic and α,1-6 glycosidic bonds (van der Maarel et al., 2002; Qian et al., 2011). A transferases-classified enzyme cleaves the α, 1-4 glycosidic bond, and transfer it to a glycosidic acceptor to produce a new glycosidic bond (van der Maarel et al., 2002). Cyclodextrin glycosyltransferase is classified as a transferases, since this enzyme cleaves the inner side of α-glycosidic bond to form a cyclic oligosaccharide that eventually cyclizes into cyclodextrin (van der Maarel et al., 2002; Dura & Rosell, 2016). α-amylase is the commonly applied enzyme compared to the others (*e.g.,* glucoamylase, cyclodextrin-glycosyltransferase and β-amylase) due to its effectiveness in hydrolyzing the α, 1-4 glycosidic bond, the main structure in the amorphous region (Horváthová et al., 2000; van der Maarel et al., 2002; Konsula & Liakopoulou-Kyriakides, 2004; Souza et al., 2019).

Table 2.1 Enzyme classification and bond cleavage in starch structure.

Enzyme classification	**Enzyme**	**Bond cleavage**	**Reference**
Endoamylases	α – amylase	α, 1-4 glycosidic bond (Inner part)	(Jung et al., 2017)
Exoamylases	β – amylase	Specifically α, 1-4 glycosidic bond (External part)	(Qian et al., 2011; van der Maarel et al., 2002)
	Glucoamylase	α, 1-4 & α, 1-6 glycosidic bond (External part)	(Qian et al., 2011; van der Maarel et al., 2002)
Transferases	Cyclodextrin glycosyltransferases	Cleave α, 1-4 glycosidic bond of donor group & transfer to glycosidic acceptor to produce new glycosidic bond	(Dura & Rosell, 2016; van der Maarel et al., 2002)

Over the past decades, most research into porous starch modification has emphasized the application of α-amylase and glucoamylase enzyme. Extensive research has been done for these enzymes in both single and combine applications. A variety of enzyme and hydrolysis conditions that have proven their potential to produce porous structure on the starch granules will be covered in the next section. Interestingly, different types of enzyme and hydrolysis conditions lead to different results in the porous structure of the starch granule (Table 2.2).

2.1.2(a)(i) Single enzyme treatment

Single enzyme treatment refers to a hydrolysis process conducted solely by a single type of enzyme. Commonly used enzymes for this type of treatment include α-amylase, glucoamylase and cyclodextrin-glycosyltransferase. The hydrolysis patterns of these enzymes will be discussed in the following subsection.

α-amylase

For single enzyme treatment, α-amylase has different mode of action for cereals, tubers, and palm sources. For corn starch, α-amylase manages to produce large and well-distributed pores on the starch granules regardless of treatment conditions (Chen & Zhang, 2012; Li et al., 2016; Benavent-Gil & Rosell, 2017). This could be explained by the presence of natural pits or pores on corn starch granule surface (Fannon et al., 1993; Huber & BeMiller, 1997; Uthumporn et al., 2012; Yussof et al., 2013; Deladino et al., 2017). Theoretically, natural pores, pits, cracked or damaged region will be the initial site for enzyme attack (Fannon et al., 1992; Fortuna et al., 2000; Thomson et al., 1994). The presence of natural pores and channels known as cavities facilitate the adsorption of the enzyme into the center of starch granule (Figure 2.3).

Table 2.2 Enzyme treatment methodology and morphological structure of produced porous starch.

Type of starch	Method / Treatment					Granule morphology / Structure	References
	Starch concentration	Type of enzyme	Enzyme concentration / ratio	Hydrolysis process	Drying treatment		
Corn	20% (w/v) of starch in 20 mM sodium acetate buffer (pH 4.0)	• Glucoamylase (AMG) • α-amylase (AM) • Cyclodextrine-glycosyltransferase (CG) • Branching enzyme (BE)	AMG: 5.5, 11, 16.5, 33 & 55 U/g AM: 5.5, 11, 16.5, 33 & 55 U/g CG: 0.1, 0.2, 0.3, 0.6 & 1.0 U/g BE: 500, 1000, 1500, 3000, & 5000 U/g	Shaking water bath: 50 rpm, 50°C, 2 hrs.	Freeze dryer	Pore size according to type of enzyme: AMG > AM > BE > CG Pore size according to concentration of enzyme: Pore size increases with increasing concentration of enzyme.	Benavent-Gil & Rosell (2017)
Corn	1%, 3% and 5% of starch in 50 mM acetate buffer (pH 4.8)	α-amylase (*Aspergillus awamori) KT-11,* (AM)	7.5 mU of AM	Incubate 37°C, 5 days.	Freeze dryer	* Results shown is for the 100 mg of starch in the buffer solution • AM produced pores on corn starch granule. • Prolonged hydrolysis time increased pore size. • 48 hrs treatment caused pores to merge together and formed a cave structure.	Matsubara et al. (2004)
Corn	5.5 mg of starch in 50 mM acetate buffer (pH 4.8)	• α-amylase (*Aspergillus*	1.0 mU of enzyme solution	Incubate 37°C, 72 hrs.	Freeze dryer	• α-amylase produced large pores on the starch granules	Matsubara et al. (2004)

		awamori) KT-11, (AM) • Glucoamylase (*Aspergillus awamori) KT-11,* (AMG)				compared to glucoamylase treatment. • Synergistic effect of both enzymes produced big holes and breakdown of the granules.	
Sago Waxy-corn Sweet-potato Tapioca Potato Rice Wheat	37.5 mg of starch in 50 mM acetate buffer (pH 4.8)	α-amylase (*Aspergillus awamori) KT-11,* (AM)	5.6 mU of AM	Incubate 37°C, 24 hrs.	Freeze dryer	• AM produced deep pores sago, waxy maize, rice and wheat starch granules. • AM produced shallow pores and surface erosion on the potato, tapioca and potato starch.	Matsubara et al. (2004)
Corn	25% (w/v) of starch in sodium acetate buffer (pH 4.6)	Glucoamylase (AMG)	11 GSHU/g starch (*GSHU: Granular starch hydrolyzing unit)	Stir at 50°C, 8 hrs.	Vacuum dryer	• AMG produced uniform pore size. • Prolonged hydrolysis time increased pore size.	Chen & Zhang (2012)
Corn	20% (w/v) of starch in sodium acetate buffer (pH 4.0) / NaH_2PO_4 buffer (pH 6.0)	• Fungal α-amylase (AM) • Glucoamylase (AMG)	• AM: (5 U/g) • AMG: (4 U/g)	Water bath shaker, 50 rpm, 50°C, 24 hrs.	Freeze dryer	• Different pH (without enzyme) did not significantly affect the morphology of the starch granules. • AM produced small pores compared to AMG.	Dura, Blaszczak, & Rosell (2014)
Corn	20% (w/v) of starch in sodium phosphate buffer (pH 6)	Cyclodextrin glycosyl-transferase (CGTase)	0.32 U of CGTase/g starch	Water bath shaker, 50 rpm, 50°C, 48 hrs.	Freeze dryer	• CGTase produced small pores on washed (in water) sample compared to non-washed sample.	Dura, Yokoyama, & Rosell (2016)

Corn	5% (w/v) of starch in PBS buffer	• Pancreatic α-amylase (PAM) • Pancreatin (Pc) • Fungal α-amylase (FAM)	0.5 U/ mg starch	Magnetic stirrer, 37°C, 2 hrs.	Vacuum-oven dryer, 40°C.	• Control sample (without enzyme) showed smooth granule surface. • PAM and Pc produced large and deep pores after 30 min enzyme treatment. • Efficiency of FAM treatment reduced after 30 min.	Li et al. (2016)
Corn	20% of starch in 20 mM sodium phosphate buffer at pH 6.0 (C6) / 20 mM sodium acetate buffer at pH 4.0 (C4)	Cyclodextrin-glycosyl-transferase (*Thermoanaerobacter* sp.) (CGTase)	0.32 U of CGTase/10 g starch	Water bath shaker, 50 rpm, 50°C, 24 and 48 hrs.	Freeze dryer	• Different pH (without enzyme) did not affect the morphology of the starch granules. • CGTase produced pores on the starch granules. CGTase C6 produced pores extensively compared to CGTase C4.	Dura & Rosell (2016)
Corn	5% of starch in 50 mM sodium acetate buffer α-amylase (AM): pH 5.8 Glucoamylase (AMG): pH 4.5 β-amylase (BAM): pH 5.2	• AM (*Bacillus lichemiformis*) • AMG (*Aspergillus niger*) BAM from barley	800 U/g of starch	Incubate, 30°C / 40°C, 8 hrs.	Freeze dryer	• Pore size according to type of enzymes after 8 hrs hydrolysis: AM > AMG > BAM • Prolonged hydrolysis time increased the pore size.	Jung, Lee, & Yoo (2017)
Waxy corn	25% of starch in distilled water	Commercial enzyme complex Distillase™ SSF (Genecor™)	15.0 µL of enzyme	Incubator shaker, 100 rpm, 30°C,	Oven dryer 50°C, 24 hrs.	• Untreated waxy corn starch had irregular polyhedral shape, smooth surfaces and	Malucelli et al. (2015)

				12, 24, and 36 hrs.		some natural pores on the granules. • Commercial enzyme complex produced small pores on the starch granules. • Prolonged hydrolysis time caused more pronounced effect on the starch granule.	
Corn Tapioca Mung-Bean Sago	25% (w/v) in sodium acetate buffer	Stargen 001 (Genencor Int, Palo Alto,CA). (α-amylase: glucoamylase)	1% (w/v) to the weight of starch	Incubator shaker, 150 rpm, 35°C, 24 hrs.	Oven dryer, 40°C, 48 hrs.	• Pore productions according to the starch type: Corn starch > Mung bean > Cassava > Sago starch • Native corn and mung bean starch possess of natural pit holes on their granule surfaces. • Native cassava and sago starch did not show any natural pit holes or pores on their surfaces	Uthumporn, Zaidul, & Karim (2010)
Corn Rice Sago Tapioca Potato	1% w/v of starches in 50 mM universal buffer (pH 6.5)	α-amylase (*Bacillus aquimaris)* MKSC 6.2	150 µL of the enzyme	Incubator shaker, 37°C, 24 hrs.	Dryer, 35°C.	• α-amylase produced big pores on corn starch granules. • α-amylase produced small pores on rice starch granules. • α-amylase did not alter tapioca, sago and potato starch granule surface.	Puspasari et al. (2011)

Corn Potato	500 mg of starch: •*Porcine α-amylase:* Carbonate buffer (pH 7.0) •*Amylo-glucosidase:* 0.2M sodium acetate buffer (pH 6.0) *Samples were prepared according to their granule size: VS: Very small S: Small M: Medium L: Large VL: Very large	• Porcine α-amylase (PAM) • Glucoamylase (AMG)	•PAM: 250 U/ ml •AMG: 28 U / ml	Recipro-cating water bath 37°C, 85 rpm, 24 hrs. *Corn:* 8 hrs. *Potato:* 24 hrs.	Electrical oven 80°C, 12 hrs.	• Corn starch hydrolyzed easily compared to potato starch. • Small granule size hydrolyzed rapidly by the enzyme compared to large granule.	Dhital, Shrestha, & Gidley (2010)

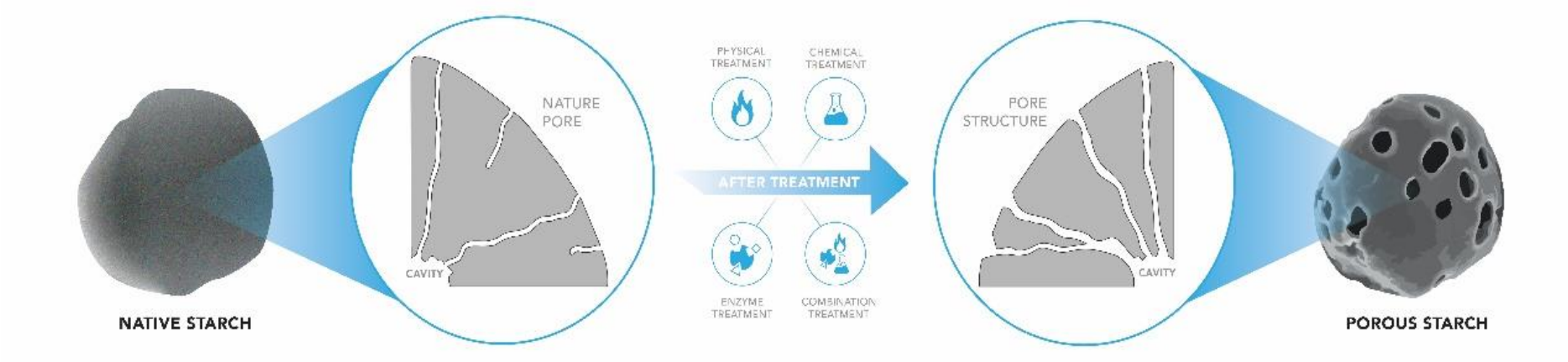

Figure 2.3 Mechanisms of modification treatments on porous structure development in starch granules.

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It is believed that enzyme attacks are initiated from the cavities outwards, enlarging the pore granules (Huber & Bemiller, 2000; Huber & BeMiller, 2001).

In contrast to corn starch, a single application of α-amylase shows a lower ability to hydrolyze tuber starches. As observed by Matsubara et al. (2004) and Puspasari et al. (2011), only slight fissures and shallow pores are produced on sweet potato, tapioca, and potato starch granules after 24 hours incubation with α-amylase derived from *Aspergillus awamori* and *Bacillus aquimaris,* respectively. Similarly, research conducted on tapioca, sweet potato and Peruvian carrot observed only slight degradation on the surface of the starch granules after 48 hours incubation with bacterial α-amylase (Rocha et al., 2010). These findings indicate that the hydrolysis of α-amylase on tuber starches only occurs by exocorrosion (Matsubara et al., 2004; Rocha et al., 2010). Exocorrosion occurs because of the limited area for the enzyme adsorption due to a smooth and compact granule surface. Hence, the enzyme action only occurs on the starch granule surface (Figure 2.3). Additionally, larger size of starch granule, and the presence of phosphate contribute to the reduction in enzyme susceptibilities of tuber starches (Goyal et al., 2005; Noda et al., 2008; Absar et al., 2009; Dhital et al., 2010; Schirmer et al., 2013; Qi & Tester, 2016).

Compared to corn and tuber starches, hydrolysis of α-amylase on sago starch granule produces a different type of pore structure. Instead of having well-distributed pores or honeycomb-like-structure, sago starch ends up with single deep round cavity structure as a result of enzyme hydrolysis (Wang et al., 1995; Cui & Oates, 1999; Yang et al., 2010). According to Cui and Oates (1999), this deep round cavity is formed from the complete digestion of the granule's inner part. As reported by (Govindasamy et al., 1992), α-amylase weakly hydrolyzes sago starch due to limited surface available for enzyme adsorption as native sago granule has a very smooth and compact surface. This