STRUCTURAL CHARACTERIZATION AND FUNCTIONAL PROPERTIES OF SALT-GELATINIZED STARCH AND ITS POTENTIAL APPLICATION AS PACKAGING MATERIALS

WANG LI

UNIVERSITI SAINS MALAYSIA

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STRUCTURAL CHARACTERIZATION AND FUNCTIONAL PROPERTIES OF SALT-GELATINIZED STARCH AND ITS POTENTIAL APPLICATION AS PACKAGING MATERIALS

by

WANG LI

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LIST OF SYMBOLS

cm	Centimeter
cP	Centipoise
a*	Color channel of redness-greenness
b*	Color channel of yellowness-blueness
L*	Lightness
ΔE	Total color difference
°	Degree
°C	Degree Celcius
+	Addition
—	Subtraction
÷	Division
×	Multiply
MPa	Megapascals
g	Gram
G′	Storage modulus
G″	Loss modulus
η*	complex viscosity
h	Hour
–OH	Hydroxyl
<	Less than
≤	Less than or equal
mL	Milliliter
mm	Millimeter
d	Day
min	Minute

s	Second
L	Litter
mol/L	Mole per litter
>	More than
≥	More than or equal
nm	Nanometer
%	Percentage
±	Plus-minus
rpm	Revolutions per minute
θ	Theta
~	Tilde
T	Transmittance
Tg	Transition temperature
tan δ	loss tangent
V	Volume
λ	Wavelength
W	Weight
w/w	Weight per weight
wt. %	Weight percentage
w/v	Weight per volume
N	Newton

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
Ca	Calcium
$CaCl_2$	Calcium dichloride
$MgCl_2$	Magnesium dichloride
NaI	Sodium iodide
C_2H_5OH	Ethanol
$C_3H_8O_3$	Glycerol
CS	Chitosan
Cl	Chlorine
Cu	Copper
HCl	Hydrochloric acid
Na	Sodium
NaOH	Sodium hydroxide
NaCl	Sodium chloride
OP	Oxygen permeability
OPY	Oil permeability
PVA	Polyvinyl alcohol
SEM	Scan electron microscope
WVP	Water vapor permeability
XRD	X-ray diffraction
CLDs	Chain length distributions
Tc	Conclusion gelatinisation temperature
Tg	Glass transition temperature
To	Onset gelatinisation temperature
Tp	Peak gelatinisation temperature
ΔH	Gelatinisation enthalpy
ABTS	2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
DPPH	2,2-Diphenyl-1-picrylhydrazyl

RS	Rice starch
POV	Peroxide value
AV	Acid value
TBARS	2-thiobarbituric acid reactive substance
TCA	trichloroacetic acid
EDTA	ethylenediaminetetraacetate
TBA	2-thiobarbituric acid
MDA	malonaldehyde
TS	Tensile strength
EAB	Elongation at break
AA	Ascorbic acid
CG	$CaCl_2$-gelatinized without glycerol
CGG	$CaCl_2$-gelatinized with glycerol
HGG	Heat-gelatinized with glycerol
Eq.	Equations
NIRS	Native indica rich starch
NGRS	Native glutinous rice starch
RVA	Rapid viscos analyzer
AAC	Apparent amylose content
PV	Peak viscosity
CPV	Cool paste viscosity
SB	Setback
HAS	High amylose starch
DPn	Number average degree of polymerization
DPw	Weight average degree of polymerization
Mn	Number average molecular mass
Mw	Weight average molecular mass
CMC	Carboxymethyl cellulose
NS	Native potato starch

DS	Dialdehyde starch

LIST OF APPENDICES

The primiary experiment of rice starch salt-gelatinization

PENCIRIAN STRUKTUR DAN SIFAT FUNGSIAN KANJI TERGELATINISASI-GARAM DAN POTENSI PENGGUNAANNYA SEBAGAI BAHAN PEMBUNGKUSAN

ABSTRAK

Penggunaan bahan pembungkus berasaskan petroleum yang meluas mengakibatkan Kesan buruk kepada alam sekitar, melalui sisa plastik yang meluas, pencemaran mikroplastik, dan penggunaan tenaga yang tinggi. Ini telah mendorong pencarian alternatif bagi bahan pembungkus terbiodegradasi. Kanji telah muncul sebagai sumber boleh diperbaharui yang menjanjikan untuk filem pembungkusan. Walau bagaimanapun, proses gelatinisasi konvensional yang memerlukan suhu ≥ 80°C boleh menjejaskan bahan tambahan termosensitif (cth., antioksidan dan antimikrob) dan selalunya menghasilkan filem dengan kepekaan air yang tinggi dan sifat mekanikal yang lemah. Kajian ini membangunkan filem berasaskan kanji beras melalui gelatinisasi akibat garam pada suhu bilik (25°C) dan menilai kesesuaiannya untuk aplikasi pembungkusan makanan. Dalam Fasa 1, tiga garam logam (NaI, $CaCl_2$, dan $MgCl_2$ pada 4mol/L) dibandingkan untuk keupayaan mereka untuk mendorong gelatinisasi kedua-dua kanji beras indica dan pulut. Analisis pembelauan sinar-X mengesahkan bahawa rawatan dengan $MgCl_2$ dan $CaCl_2$ menghasilkan gelatinisasi lengkap, manakala rawatan NaI menghasilkan kanji dengan kelikatan paling rendah disebabkan oleh penembusan ion I^- yang cepat dan degradasi granul yang progresif. Pati $MgCl_2$-gelatin mempamerkan modulus penyimpanan akhir tertinggi (G'), kira-kira 10 kali ganda daripada kanji yang dirawat $CaCl_2$, dan mencapai puncak G' pada sekitar 25 minit. Walau bagaimanapun, pembengkakan butirannya yang meluas membawa kepada gel yang sangat likat dan padat yang menimbulkan cabaran untuk penuangan

filem seragam. Sebaliknya, gelatinisasi yang disebabkan oleh $CaCl_2$ menghasilkan gel dengan kelikatan sederhana, perkembangan struktur yang pesat (dalam masa 5 minit), dan gel yang lebih sesuai merujuk kepada keseimbangan antara kekuatan mekanikal, kelikatan, dan kemudahan pembentukan filem, menjadikan kanji bergelatin $CaCl_2$ lebih praktikal untuk pemprosesan selanjutnya. Dalam Fasa 2, filem yang disediakan melalui $CaCl_2$-gelatinisasi dibandingkan dengan yang dihasilkan oleh gelatinisasi haba konvensional. Filem $CaCl_2$ mempamerkan struktur mikro yang licin, padat dan seragam, dengan ketebalan berkurangan (0.34mm), ketransmisian cahaya yang lebih tinggi (75.13%), dan kekuatan tegangan 2.96 MPa. Selain itu, gliserol, yang digunakan sebagai pemplastis, adalah lebih serasi dengan kanji bergelatin $CaCl_2$, menghasilkan filem dengan pemanjangan yang dipertingkatkan dengan ketara semasa pecah (249.7%), hampir lima kali ganda daripada filem bergelatin haba (53.8%). Dalam Fasa 3, penggabungan agen penguat, kitosan dan polivinil alkohol (PVA), telah diterokai untuk menambah baik sifat mekanikal dan penghalang. Walaupun kitosan membawa kepada filem dengan ketelusan rendah (12.73%) dan kebolehtelapan wap air yang tinggi (16.75×10-12g/cm·Pa·s), PVA meningkatkan kekuatan tegangan dengan ketara (sehingga 4.12MPa), penghantaran cahaya (81.05%) dan prestasi penghalang. Dalam Fasa 4, asid askorbik (AA) telah ditambah kepada komposit kanji-PVA untuk membangunkan filem bioaktif untuk pembungkusan minyak bunga matahari. Ujian oksidatif dipercepatkan pada 50°C untuk 50d menunjukkan bahawa filem yang dimuatkan AA mempamerkan aktiviti antioksidan yang kuat (97.22% penghapusan radikal DPPH), nilai asid dan peroksida yang lebih rendah, dan keterlarutan air yang tinggi (95.03%), menunjukkan peningkatan biodegradasi dan kesesuaian untuk aplikasi makanan berlemak. Secara kolektif, penemuan ini mencadangkan bahawa filem kanji beras yang dihasilkan melalui $CaCl_2$-gelatinisasi，dengan gliserol sebagai pemplastis,

PVA sebagai agen penguat, dan AA untuk bioaktiviti， menawarkan alternatif yang menjanjikan, mampan untuk minyak pembungkusan dan produk makanan berlemak, mengimbangi kecekapan dan prestasi pemprosesan.

STRUCTURAL CHARACTERIZATION AND FUNCTIONAL PROPERTIES OF SALT-GELATINIZED STARCH AND ITS POTENTIAL APPLICATION AS PACKAGING MATERIALS

ABSTRACT

The widespread use of petroleum-based packaging materials has resulted in adverse environmental impacts, through widespread plastic waste, microplastic pollution, and high energy consumption. This has prompted the search for alternative biodegradable packaging materials. Starch has emerged as a promising renewable resource for packaging films. However, conventional gelatinization processes requiring temperatures of ≥ 80 °C can compromise thermosensitive additives (e.g., antioxidants and antimicrobials) and often yield films with high water sensitivity and weak mechanical properties. This study develops rice starch-based films through salt-induced gelatinization at room temperature (25 °C) and evaluates their suitability for food packaging applications. In Phase 1, three metal salts (NaI, $CaCl_2$, and $MgCl_2$ at 4 mol/L) were compared for their ability to induce gelatinization of both indica and glutinous rice starch. X-ray diffraction analysis confirmed that treatments with $MgCl_2$ and $CaCl_2$ resulted in complete gelatinization, while NaI treatment produced starch with the lowest viscosity due to rapid I^- ion penetration and progressive granule degradation. $MgCl_2$-gelatinized starch exhibited the highest final storage modulus (G'), approximately 10 times that of $CaCl_2$-treated starch, and achieved peak G' at around 25 min. However, its extensive granule swelling led to highly viscous, cohesive gels that posed challenges for uniform film casting. In contrast, $CaCl_2$-induced gelatinization produced gels with moderate viscosity, rapid structural development (within 5 min), and a better suitability for gel formation, referring to a balance between mechanical strength, viscosity, and

ease of film formation, making $CaCl_2$-gelatinized starch more practical for further processing. In Phase 2, films prepared via $CaCl_2$-gelatinization were compared with those produced by conventional heat gelatinization. The $CaCl_2$ films exhibited a smooth, compact, and uniform microstructure, with reduced thickness (0.34 mm), higher light transmittance (75.13%), and tensile strength of 2.96 MPa. Moreover, glycerol, used as a plasticizer, was more compatible with $CaCl_2$-gelatinized starch, resulting in films with a markedly enhanced elongation at break (249.7%), nearly fivefold that of heat-gelatinized films (53.8%). In Phase 3, the incorporation of reinforcing agents, chitosan and polyvinyl alcohol (PVA), was explored to improve mechanical and barrier properties. While chitosan led to films with low transparency (12.73%) and high water vapour permeability (16.75×10^{-12}g/cm·Pa·s), PVA significantly improved tensile strength (up to 4.12 MPa), light transmittance (81.05%), and barrier performance. In Phase 4, ascorbic acid (AA) was added to the starch-PVA composite to develop a bioactive film for sunflower oil packaging. Accelerated oxidative testing at 50 °C for 50 d demonstrated that AA-loaded films exhibited potent antioxidant activity (97.22% DPPH radical scavenging), lower acid and peroxide values, and high water solubility (95.03%), indicating enhanced biodegradability and suitability for fatty food applications. Collectively, these findings suggest that rice starch films produced via $CaCl_2$-gelatinization, with glycerol as a plasticizer, PVA as a reinforcing agent, and AA for bioactivity, offer a promising, sustainable alternative for packaging oil and fatty food products, balancing processing efficiency and performance.

CHAPTER 1

INTRODUCTION

1.1 Research background

Petroleum-derived plastic packaging makes a significant contribution in everyday life, present in various products including food containers, beverage bottles, and plastic utensils. Nearly 400 million tons of petroleum-derived plastic packaging are produced annually, with 40% of its production allocated for single-use applications. Disposal of these plastic packaging after minimal use resulted in substantial environmental issues. Certain plastic packaging may require centuries to degrade, during which it might inflict significant environmental harm. The proliferation of wasted plastics has consequently emerged as a global concern (Al Rayaan, 2021; Chia et al., 2020). In addition to environmental deterioration, petroleum-derived plastic packaging poses a significant risk to human health. Recent studies have identified hazardous constituents of non-biodegradable plastics in human organs, tissues, and cells (Danopouloset al., 2022; Napper & Thompson, 2023; Yin et al., 2021). Alarmingly, microplastics have been detected in human breast milk and semen (Montano et al., 2023; Ragusa, 2022).

Meanwhile, the energy-intensive manufacturing procedures for plastic packaging adversely affect global environmental quality (Napper & Thompson, 2023; Shahzad, 2020). Therefore, it is imperative to develop an eco-friendly material as an alternative to plastic packaging to reduce the impact of plastic pollution on the environment. Adopting biodegradable alternatives, such as starch-based films, plant-derived polymers, and reusable packaging, is essential. Biodegradable food packaging refers to materials designed to naturally decompose into non-toxic components, such as water, carbon dioxide, and organic matter, through microbial activity under specific

environmental conditions. These materials are typically sourced from renewable resources such as starch, cellulose, plant-derived polymers (e.g., polylactic acid), or proteins (e.g., gelatin or casein). Biodegradable food packaging lowers carbon footprints, supports a circular economy, and collectively addresses the ecological and health hazards associated with plastic pollution, paving the way for more sustainable solutions to environmental challenges. Numerous nations have offered relevant policies to safeguard the ecological environment and human health. The European Strategy for Plastic in a Circular Economy stipulates that by 2030, all plastic packaging in the EU shall be reused or recycled in a cost-effective manner (Berriman, 2021). Thus, the advancement and manufacturing of biodegradable packaging have emerged as critical research priorities in the domains of food, medicine, and materials.

Starch, a prevalent renewable resource, has emerged as a viable raw material to produce biodegradable packaging materials. Starch-based films, comprising approximately 50% of the biofilm market, provide an environmentally sustainable alternative to conventional plastics (Tapia-Blácido et al., 2022). Starch gelatinization is a crucial process for the creation of starch-based films, characterized by irreversible order-to-disorder transitions during both thermal and non-thermal processing in the presence of water (Liu et al., 2023). During heat- gelatinization, starch granules absorb water and swell irreversibly when heated beyond their gelatinization temperature. The thermal energy disrupts the intermolecular hydrogen bonds between amylose and amylopectin chains in the semi-crystalline granule structure. This leads to the loss of crystalline regions, leaching of amylose into the surrounding water, and formation of a viscous paste (Woodbury et al., 2023). Heat-gelatinization is the most traditional and widely used method to produce starch-based film. In the presence of high temperature, shear force, and plasticizer, starch can be converted into thermoplastic starch (La

Fuente et al., 2022). However, the heat-gelatinization of starch has numerous drawbacks, including elevated energy usage, constrained regulation of the gelatinization process, diminished compatibility with other constituents, and deterioration in its functional attributes such as mechanical strength. Consequently, salt-gelatinization can be employed to mitigate these disadvantages.

Salt-gelatinization occurs through electrostatic interactions between salt ions and the hydroxyl groups of starch molecules, which change the water structure. Due to the low volatility and high diffusivity of salt, it can transform starch into plasticized starch (Liang et al., 2024; Vinkx et al., 2022; Zhiguang et al., 2023). Certain salts at specified concentrations, such as $CaCl_2$, $MgCl_2$, and $ZnCl_2$ at 4 mol/L, have been shown to effectively induce starch gelatinization at room temperature (Y. Li et al., 2020; Shang et al., 2019). These salts possess low volatility and strong diffusivity, enabling them to penetrate the granules, disrupt hydrogen bonds, and enhance the availability of hydration sites with water, hence facilitating starch swelling and gelatinization. At the molecular level, salt ions interact with starch chains by neutralizing hydroxyl groups, breaking internal hydrogen bonds, and restructuring the surrounding water layer. Such electrostatic and hydration effects promote gelatinization in the absence of high heat, enabling starch to hydrate and swell in a controlled manner without thermal degradation. Unlike heat-induced gelatinization, which disrupts the granular structure randomly and may cause chain breakage, salt-induced gelatinization is gentler and better preserves thermosensitive additives (e.g., antioxidants). This method fosters the formation of uniform and amorphous starch matrices suitable for film formation and hydrogel applications (Chakraborty et al., 2022; W. Wang et al., 2017).

Starch-based films are produced solely from starch or in combination with other biopolymers (La Fuente et al., 2022; Nawab et al., 2017; Saberi et al., 2017).

Pure starch films are fragile and demonstrate inadequate tensile characteristics. In addition, starch-based films are recognized for two primary disadvantages: increased hygroscopicity and diminished mechanical characteristics (Choo et al., 2021; F. Wang et al., 2023). To tackle these issues, incorporation of plasticizers or biopolymers is often used to enhance the performance of starch-based films. The addition of plasticizers and biopolymers improves the flexibility, integrity, and processability of starch film, thus producing a high-quality elastic film. Both plasticizers and biopolymers can disturb the crystalline structure of starch during pasting, resulting in amorphous products. Typical plasticizers utilized in starch-based films are water, glycerol, sorbitol, and polyethylene glycol (Gao et al., 2021; La Fuente et al., 2022; Nordin et al., 2020; Suderman et al., 2018). However, excessive plasticizer content can lead to poor adhesion between the plasticizer and starch matrix, adversely affecting the film's mechanical properties. Hence, studying the suitable type of plasticizer or biopolymers and knowing the optimal addition level are crucial to ensure the quality of films produced.

During the development of starch-based films via heat-gelatinization, the starch solution must be heated to a minimum temperature of 80℃ to achieve thermoplastic starch, which is essential for successful film production. However, heat gelatinization of starch has several drawbacks, including high energy consumption, limited process control, and reduced compatibility with heat-sensitive additives. Furthermore, exposure to elevated temperatures can degrade bioactive compounds and diminish the functional properties of the resulting films, such as mechanical strength, barrier performance, and antioxidant activity. Certain salts can facilitate starch gelatinization at room temperature (25℃), converting it into amorphous substances, which is a viable approach for producing starch-based films without or with low energy

consumption. Jane (1993) discovered that 3 mol/L $CaCl_2$ facilitated maize starch gelatinization at room temperature, as evidenced by differential scanning calorimetry (DSC). Meanwhile, Zhiguang et al. (2023) demonstrated that 4 mol/L $MgCl_2$ completely gelatinized potato starch, forming a gel at room temperature within 3 h, starting at the periphery of starch particles, corroborating the findings of Haixia et al. (2022). In addition, Lai et al. (2000) reported that $MgCl_2$ enhanced the dissolution of significant quantities of starch molecules in potato starch granules, producing a potent gel at room temperature, whereas $CaCl_2$ released fewer starch molecules, resulting in a weaker gel. However, there has been scant research on the utilization of these salts as pasting agents in the preparation of starch-based films.

The salts used in this study, NaI, $CaCl_2$, and $MgCl_2$, were selected based on their ionic characteristics. Divalent cations such as Ca^{2+} and Mg^{2+} have higher charge density, promoting stronger ionic interactions with starch chains. These ions can disrupt the hydrogen bonding network and alter the hydration structure around starch granules more effectively than monovalent cations like Na^+. Furthermore, iodide ions, due to their large ionic radius and polarizability, influence the structure of surrounding water, facilitating granule swelling and film formation. This theoretical basis supports the exploration of ion-specific effects on gelatinization efficiency and film performance.

Rice starch was chosen as the raw material due to its widespread availability, high purity, low protein/lipid content, and favorable film-forming properties. Two rice varieties were selected, high-amylose rice and waxy rice, to investigate how differences in amylose content affect gelatinization behavior, interaction with salts, and final film characteristics. The hypothesis is that high-amylose rice will form

stronger, more rigid films due to greater molecular entanglement, while waxy rice may offer improved flexibility due to its amylopectin-rich nature.

Starch-based composite films possess excellent oxygen barrier capacities, essential for inhibiting oxidative processes in food products (Freitas et al., 2023). The antioxidant capacity can be improved by incorporating natural antioxidant compounds that serve as oxygen or radical scavengers (Nikmanesh et al., 2023). For example, ascorbic acid (AA) derived from mango pulp, anthocyanins derived from blueberries, purple cabbage, and mulberries, carvacrol nanoemulsions, and red pitaya peel extract have been incorporated in starch-based composite films, demonstrating remarkable antioxidant properties (Kan et al., 2022; Kong et al., 2020; Liu et al., 2017; Qin et al., 2021). Among these additives, AA has emerged as a versatile antioxidant, exhibiting the ability to scavenge free radicals, chelate metal ions, and inhibit lipid oxidation. These properties are particularly valuable in preserving fat-rich foods, such as sunflower oil, which contains over 89% unsaturated fatty acids that are highly susceptible to rancidity (Zhang et al., 2024; Brewer, 2011). AA-incorporated active films have demonstrated broad applicability in extending the shelf life of fat-rich foods, including nuts, palm oil, and snacks (Haq et al., 2015; Souza et al., 2011; Ribeiro et al., 2021). Notably, Souza et al. (2011) found that AA added to starch-based films, exhibited excellent antioxidant effects during palm oil storage, resulting in a 36.12% reduction in peroxide index compared to the control ($p < 0.05$). It is widely recognized that AA is a thermosensitive antioxidant that undergoes degradation at ≥ 40°C. Bajer & Burkowska-But (2022) emphasized that AA was introduced into a pre-prepared mixture only after it was cooled to 35 ℃ to prevent degradation. Kowalczyk (2018) similarly demonstrated that AA was added to a 5% (w/w) oxidized potato starch solution after it was heated at 90 °C for 30 min and subsequently cooled to 40 °C. In

these approaches, film-forming solutions must often be cooled to ≤ 40 °C following gelatinization before antioxidants can be incorporated, leading to a complex, multi-step process that increases production costs and limits scalability (Pereira et al., 2022; Qin et al., 2021).

The use of salt-gelatinization provides a simpler, more sustainable alternative by preserving antioxidant activity without the need for a heating-cooling cycle, thus making the process more industrially feasible. Previous studies have examined salt-gelatinized starch; few have focused on its application in antioxidant packaging or explored multiple rice varieties in combination with specific salts. This research provides both mechanistic insights and practical applications for low-energy, active packaging development.

1.2 Problem statement

Petroleum-derived plastics, which predominate the food packaging sector, pose significant environmental and health hazards to humans. The use of biodegradable starch-based films has gained attention, especially for their prospective antioxidant and antibacterial characteristics. Starch is a viable renewable, naturally biodegradable polymer capable of substituting traditional packaging materials, owing to its diverse plant sources, low cost, superior biodegradability, and film-forming capabilities (Jiang et al., 2020; Savadekar & Mhaske, 2012; Tapia-Blácido et al., 2022). Starch gelatinization is a crucial technique for the preparation of starch-based films. This produce conventionally necessitates heating starch to a temperature over 80 °C to produce thermoplastic starch (Nayak et al., 2014; Perin & Murano, 2017; Wang et al., 2018). Nonetheless, heat-gelatinization has some drawbacks, such as the necessity for a heating apparatus, a convoluted process, elevated energy consumption, and

antioxidant component degradation resulting from high temperatures. Natural antioxidant constituents, including anthocyanins (Kan et al., 2022; Liu et al., 2017; Qin et al., 2021), carvacrol nanoemulsions (Kong et al., 2020), and red pitaya peel extract (Liu et al., 2017), exhibit a lack of heat resistance and are susceptible to oxidation and decomposition at temperatures exceeding 40℃. Consequently, the thermoplastic starch must be cooled to below 40 ℃ before the incorporation of the thermosensitive active component, thereby augmenting energy consumption and preparation duration. Thus, the development of antioxidant starch-based films at room or low temperatures (≤ 40℃) is a potential method, minimizing energy usage and safeguarding thermosensitive compounds.

Reports indicate that starch can be gelatinized with certain salts at room temperature (25℃) to form a starch gel (Ai & Jane, 2015b; Haixia et al., 2022; Li et al., 2015; Wang et al., 2017). Salt-gelatinization presents a distinct advantage over traditional heat-gelatinization, including zero energy consumption, simplified operations devoid of heating apparatus, preservation of nutrients, and enhanced antibacterial properties. The mechanism of salt gelatinization is contributed by the structure-breaking and structure-forming effects of salts on water, as well as the electrostatic interaction between starch and ions (Li et al., 2020; Nicol et al., 2018). Factors impacting the process and mechanism of salt-gelatinization generally include the type of salt, its concentration, the quantity of charge, charge density, charge polarity (positive or negative), and pH level. This process is of salt gelatinization generally considered a form of cold gelatinization, sharing similarities with alkali- and solvent-induced gelatinization. Like alkaline treatment (e.g., NaOH), which disrupts hydrogen bonding and promotes granule swelling (Xie et al., 2006), or solvents such as DMSO and DMF that solubilize starch via dipole interactions (Jane, 1993), salt ions facilitate

gelatinization through ionic hydration and charge screening effects. However, the mechanisms are ion-specific and distinct from chemical degradation seen in alkali treatment. Despite extensive research and experimental observation, significant uncertainties remain regarding starch salt gelatinization, including the detailed process, and physicochemical properties of starch gels prepared with salts. Moreover, studying the potential of certain salts at specific concentrations to function as pasting agents for achieving total starch gelatinization at room temperature is valuable.

Although starch-based films have emerged as promising candidates for eco-friendly packaging, their performance remains constrained by inherent material limitations and processing conditions. Pure starch films exhibit brittleness, inferior tensile properties and significant hygroscopicity, which restricts their potential applications (Domene-López et al., 2019; Gómez-Aldapa et al., 2020; Choo et al., 2021; Hu et al., 2016; Wang et al., 2023). The incorporation of plasticizer or other biopolymers helps to improve the quality of starch-based films (Patil et al., 2021; Perdana et al., 2021; Sapper et al., 2019; Yurong & Dapeng, 2020). Plasticizers like glycerol, ethylene glycol, and sorbitol are primarily organic liquids characterized by small molecular size and high boiling points, which diminish hardness and brittleness while enhancing ductility and elongation at break in starch-based films (La Fuente et al., 2022; Suderman et al., 2018). Understanding the effect of plasticizer on salt - gelatinized film is essential and helps, as it aids in optimizing the film's mechanical properties, enhancing its flexibility, and improving its overall stability.

Natural compounds possessing antioxidant capabilities included in starch-based packaging films demonstrate considerable promise in mitigating food oxidation and prolonging shelf-life (Valdés et al., 2014). These compounds may serve as alternatives to synthetic antioxidants due to their biodegradability and overall safety

(Dainelli et al., 2008). However, most natural compounds lack in heat -resistance, resulting in diminished oxidative activity or decomposition at temperatures above 40 ℃. A multitude of studies have indicated that the starch/PVA film-forming solution must be cooled to 40 ℃ before the incorporation of natural antioxidant extracts, including anthocyanins (Kan et al., 2022; Qin et al., 2021), carvacrol nanoemulsions (Kong et al., 2020), red pitaya peel extract (Liu et al., 2017), and ascorbic acid (Bajer & Burkowska-But, 2022; dos Santos Garcia et al., 2017; Kowalczyk et al., 2018; Pereira et al., 2022). This is particularly critical for preserving thermosensitive antioxidant compounds like ascorbic acid, which are highly sensitive to thermal processing. Mild salt-induced gelatinization offers a pathway to retain antioxidant activity while forming structurally robust films.

The choice of rice starch—particularly from high-amylose and waxy rice—provides a model system for evaluating the influence of amylose content on salt-gelatinization efficiency and film functionality. The use of different salts (e.g., NaI, $CaCl_2$, $MgCl_2$) with varied ionic characteristics allows the investigation of specific ion effects on starch structure disruption, gelation, and film properties. These insights are necessary to develop low-energy, effective packaging solutions that maintain bioactivity and mechanical integrity. Thus, a systematic investigation is required to elucidate the mechanisms of salt-induced starch gelatinization and optimize the conditions for producing high-performance bioactive packaging films using rice starch and ascorbic acid.

1.3 Objectives

The research aims to develop bioactive films via salt-gelatinization as packaging materials for commercial sunflower oil. The specific objectives of the

research work include:

i. To study the effect of different salts and starch types (with different amylose content) on the starch pasting properties, morphology, structure, and rheological properties of salt-gelatinized rice starch.

ii. To compare the mechanical, moisture barrier, morphology, and appearance of salt-gelatinized and heat-gelatinized rice starch-based films.

iii. To determine the effect of reinforcement agents (chitosan and PVA) on the mechanical and barrier properties of salt-gelatinized rice starch-based composite films.

iv. To evaluate the efficiency of ascorbic acid incorporated into salt-gelatinized rice starch-based films in preserving the quality of commercial sunflower oil during storage.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice Starch

Rice starch, a carbohydrate polymer extracted from rice grains, is increasingly used in various industries such as food, medicine, and packaging materials due to its unique physical behaviors and functionalities. In food applications, rice starch serves as a thickener, colloid stabilizer, gel-forming agent, water-retaining agent, adhesive, polishing agent, or coating (Hermansson & Svegmark, 1996; Kennedy & Panesar, 2006). In packaging applications, rice starch has shown excellent film-forming properties due to its molecular structure. When processed, rice starch can form flexible, transparent films with a smooth texture that are suitable for various applications. Rice starch-based films exhibit good tensile strength and flexibility, which contributes to a balance between strength (to protect the contents) and flexibility (to conform to different shapes) (Aghazadeh et al., 2018; Ramos Da Silva et al., 2022). Additionally, rice starch has superior compatibility with other biodegradable materials, such as polyvinyl alcohol, which can be modified to improve their properties, like moisture resistance, mechanical strength, and barrier properties.

2.1.1 Composition and structure of rice starch

2.1.1(a) Composition of rice starch

Native rice starch consists primarily of two α-glucans: lightly branched amylose and highly branched amylopectin (Takeda et al., 1989). Amylose is a chain molecule composed of α-D-glucose connected by D-1-4 glycosidic bonds (shown in Figure 2.1), forming a right-handed helical structure. Each helical cycle contains 6 glucose groups with a pitch of 10.6 Å. The structural characteristics of amylose are

similar to wheat and corn starch, but its molecular chains are much shorter compared to potato starch and cassava starch. The number average degree of polymerization (DPn) of rice amylose is 920–1100, the weight average degree of polymerization (DPw) is 2750–3800, and the number average molecular mass (Mn) is 1.5×10^5–3.8×10^5, and Mw/Mn is 1.2–3.6. The average molecular chain length is 250–320. Amylose has a small amount of branching, and each branching amylose molecule has 2.3–4.5 branches, with each branch chain length of approximately 17.3–20.9 glucose units (Hizukuri et al., 1989). The type of rice influences the content of amylose in rice starch, for example, the amylose content of indica rice is generally 25.4%±2.05%, Japonica rice has an amylose content of 18.4%±2.7%. In contrast, glutinous rice has almost zero amylose content (0.98%±1.51%).

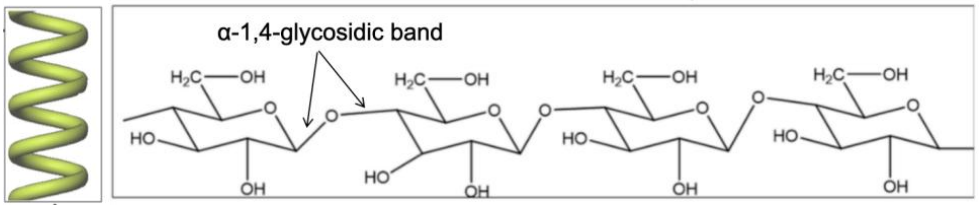

Figure 2.1 Single helix structure of amylose. (Source: Amaraweera et al., 2021)

Meanwhile, amylopectin is considered the main factor in forming the shape and structure of starch granules. It is a highly branched macromolecule, with branched chains on the chain and glucose units separated by α-1-4 glycosidic bonds connected to form its main chain, with branched chains connected by α-1-6 glycosidic bonds linked to the main chain and branching points α-1-6 glycosidic bonds (shown in Figure 2.2) account for 4% to 5% of the total glycosidic bonds.

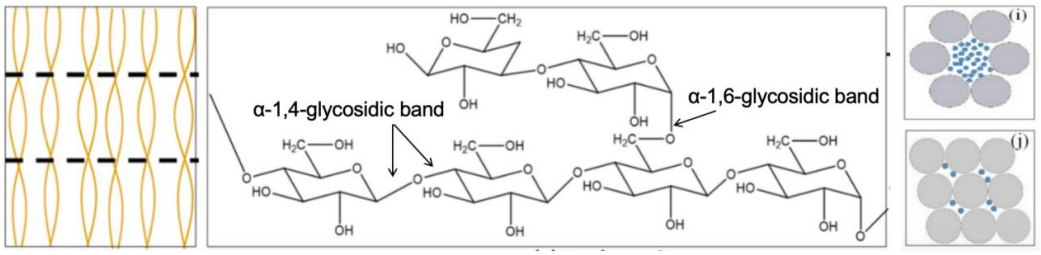

Figure 2.2 Double helix structure of amylopectin and vertical view of the double helix clusters (i-A and j-B type). (Source: Amaraweera et al., 2021)

The various chains of amylopectin can be further divided into the main chain (C-chain), inner chain (B-chain), and outer chain (A-chain), as shown in Figure 2.3. The A chains (unbranched) are linked to B chains and do not carry any other chains; the B chains (B1–B4) carry one or more A chains and/or B chains; and the C chain has the reducing end of the molecule. Rice amylopectin has an average degree of polymerization (DP) reported to be from 2700 to 12000 and an average chain number (NC) of 128–586. Compared to low-amylose rice, high-amylose indica rice reportedly had a lower average DP. The average chain length (CL) of amylopectin was 18–22, 15–18, and 17–20 for indica, japonica, and waxy rice, respectively.

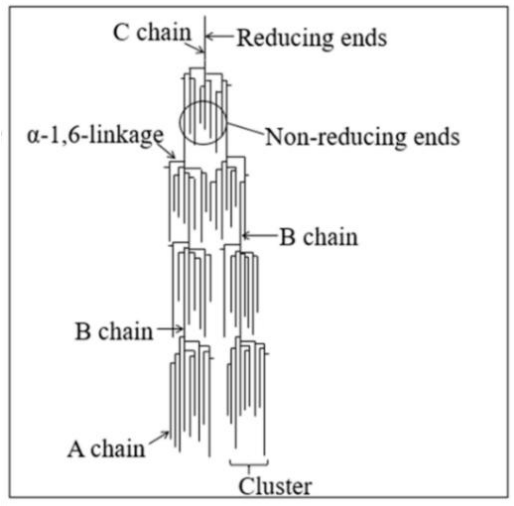

Figure 2.3 Cluster structure of amylopectin, with indication of A, B, and C chains in amylopectin. (Source: Amaraweera et al., 2021)

2.1.1(b) Structure of rice starch

Studies using the scanning electron microscope show rice starch exists in the form of granules. Compared to all known cereal grains, the diameter of rice granules is the smallest, with approximately 3μm to 8μm, most of which have smooth surfaces but angular and polygonal shapes. There is a significant difference in starch particle size among different varieties of rice. Generally, the starch granules of glutinous rice are larger than those of japonica and indica rice. Zhong et al. (2009) reported that the larger-sized starch granules were about 85% of the total volume of particles, and their peak heights had values ranging from 4.2 to 5.3 μm. Like other types of starch, rice starch granules are composed of branched starch molecules in a combination of dense crystalline and amorphous regions, with linear starch molecules doped in a spiral structure. Both amylose and amylopectin form divergent anisotropic and semi-crystalline structures in starch granules. External chains of amylopectin are organized into double helices and some form crystalline structures. Amylose is proposed to be more concentrated at the periphery or in the amorphous core area of the starch granules, although some molecules may be interspersed radially among the amylopectin clusters.

Native rice starch occurs mainly in the form of semi-crystalline granules, which have a very complex hierarchical structure (Wang & Copeland, 2015). The hierarchical structure of rice starch can be divided into five layers, including chain, crystalline, amorphous, and crystalline lamellae, growth rings, and granules, as shown in Figure 2.4.

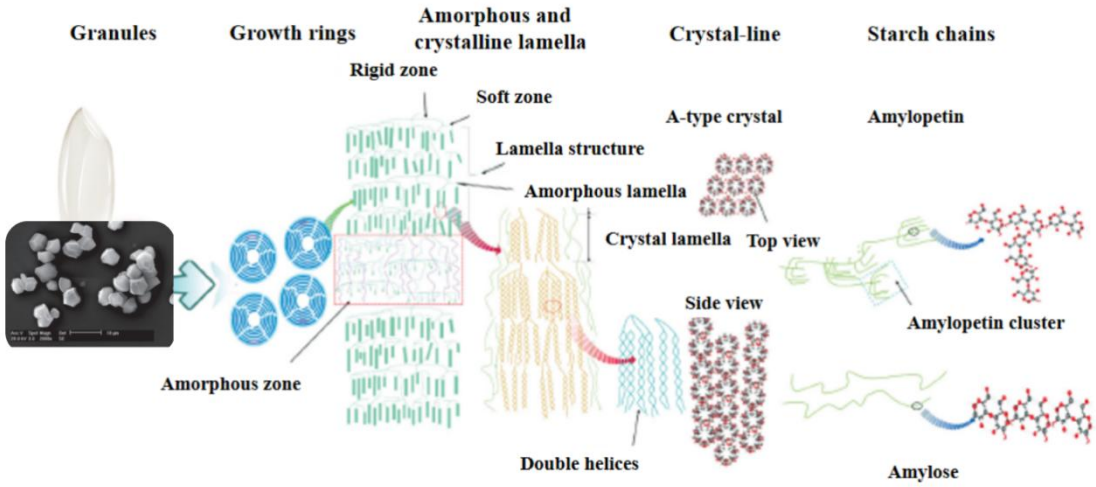

Figure 2.4 The hierarchical structure of rice starch. (Source: Wang & Copeland, 2015)

The α-D-glucose monomers are connected through α-(1, 4) glycosidic linkages and α-(1, 6) glycosidic linkages into individual starch chains, which are amylopectin and amylose molecules (0.1–1.0 nm). It was reported that the neighboring amylopectin branches with DP ~ 10–20 intertwine into double helices and stack together into semi-crystalline clusters (1–4 nm) (Bertoft, 2017). Depending on the packing patterns of the amylopectin double helices, rice starch has an A-type crystalline structure, as shown in the XRD structure pattern. The lamellar thickness in the semi-crystalline growth rings has been determined to be around 9–10 nm for various starches by small-angle X-ray scattering (SAXS) (Zhang et al., 2017). The semi-crystalline growth rings are further alternated with amorphous growth rings (120–150 nm) to form starch granules (2–100 μm). The multi-scale structures of granules and the changes they undergo during processing and storage are the major determinants of starch functionality and food quality (Li & Gong, 2020).

The amylose content in rice starch plays a critical role in determining the structural and mechanical characteristics of starch-based films. High-amylose rice starch (e.g., Indica, 20–30% amylose) forms films with greater tensile strength and

lower elongation due to enhanced molecular alignment and hydrogen bonding, resulting in denser, more rigid structures (Mali et al., 2002; Thakur et al., 2017). In contrast, glutinous rice, which is virtually amylose-free, produces weak, soft, and highly stretchable films with poor barrier properties due to its amorphous, branched amylopectin-dominant structure (Khunae et al., 2007; Bhat & Riar, 2017). Ramos da Silva et al. (2022) demonstrated that pigmented rice types (black, red, white) with varying amylose contents (18.6–25.7%) still produced flexible and homogeneous films. However, mechanical strength, transparency, and barrier properties positively correlated with amylose content. Thus, rice variety selection significantly affects the functional performance of starch-based films in packaging applications.

2.1.2 Physicochemical properties of rice starch

The composition and structure of rice starch, particularly amylose-to-amylopectin ratio, granule size, and crystallinity, strongly influence its physicochemical properties. High amylose leads to higher gelatinization temperature and firmer gels, while high amylopectin enhances viscosity and swelling (Y. Wang et al., 2024; C. Zhang et al., 2023). These characteristics affect solubility, retrogradation, and film-forming ability, which are critical for starch's performance in food and packaging applications.

2.1.2(a) Solubility properties

The solubility of starch is associated with its hydrophilicity and the ease of disruption of starch granules. Changes in the physical environment (e.g., high temperature, high pressure, specific salt concentration, or pH) and certain chemical modifications, such as phosphorylation, can enhance starch solubility in aqueous solvents. Generally, rice starch, like all cereal starch, is insoluble in neutral aqueous solutions such as cold water due to its compact granule structure. Additionally, amylose

and amylopectin are only lightly soluble, which makes it difficult to study starch's structure and use it in industry in water-based media. It has been found that corn starch can dissolve in dimethyl sulfoxide (DMSO) at room temperature, which is the most commonly used polar aprotic solvent (Han & Lim, 2004). DMSO disperses starch by acting as a hydrogen bond acceptor, disrupting inter- and intramolecular starch-starch and water-starch hydrogen bonds and replacing hydroxyl-hydroxyl hydrogen bonding with DMSO-starch hydrogen bonding (Zhong et al., 2006).

The swelling and solubility behaviors of starch vary significantly with botanical origin, amylose and lipid contents, and amylopectin fine structure (Tester & Morrison, 1990). Amylose is known to inhibit granule swelling while amylopectin promotes granule swelling, due to the availability of more short chains, which provides less stability to the structure, thus resulting in higher swelling (Thakur et al., 2016). Wang et al. (2017) found that waxy maize starch showed higher swelling power and solubility than maize starch. Waxy maize starch is low in amylose and contains nearly 100% amylopectin. Amylopectin branches may possess more steric hindrance than amylose (Tester & Morrison, 1990), allowing water to be more easily absorbed by the starch granules. The granules in waxy maize starch may disintegrate easily when swollen, exhibiting higher swelling power and solubility. In contrast, maize starch granules are more rigid and not easily ruptured (Sandhu & Singh, 2007).

Previous research showed that starch film solubility was profoundly affected by the presence of amylose content (Chinma et al., 2013). Being linear, amylose possesses strong bonding forces in the starch granule and provides fewer OH groups for interaction with water. Due to intact granule aggregation and strong bonding forces, the free OH groups are unavailable to interact with water, hence lowering the solubility of composed films (Thakur et al., 2019). Mainly, amylopectin contributes to the swelling

of starch in rice, while amylose acts as a diluent. Lin et al. (2011) reported that the amylose content of indica rice starch (27.5%) was significantly lower compared to japonica rice starch (24.5%) of swelling power and solubility. Kong et al. (2015) and Cai et al. (2015) showed that rice starch with low amylose and glutinous rice starch had higher swelling capacity than high-amylose rice starches, with rice starch having low total amylose contents being less rigid and swelling freely when heated.

Overall, the solubility of rice starch is influenced primarily by its amylose-to-amylopectin ratio, crystalline structure, and granule morphology. Indica rice starch, which typically contains 20–30% amylose, exhibits lower solubility due to the linear amylose chains that resist water penetration and inhibit granule swelling (Zainab et al., 2024). In contrast, glutinous (waxy) rice starch, composed almost entirely of amylopectin, shows higher solubility and swelling power due to the highly branched molecular structure that facilitates water uptake (Chen et al., 2019).

2.1.2(b) Thermal properties

The thermal properties of starch slurry during heating are related to the granule size, swelling power/solubility, and the properties of the swollen granules and soluble materials of starch (Keawpeng & Venkatachalam, 2015). When starch is heated in excess water, the granules swell and burst, the semi-crystalline structure is lost due to the breakage of hydrogen bonds, and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin. The smaller amylose molecules start leaching out of the granule, forming a network that holds water and increases the mixture's viscosity, and finally generates a gel (Fariña et al., 2019; Martinez et al., 2018; Li et al., 2021), as shown in Figure 2.5. This process is known as starch heat-gelatinization, which irreversibly disrupts the molecular order within a starch granule (Bao & Bergman, 2024). Evidence of this loss of order can be seen in

irreversible granule swelling, loss of birefringence and crystallinity, and starch solubilization (Bao et al., 2021).

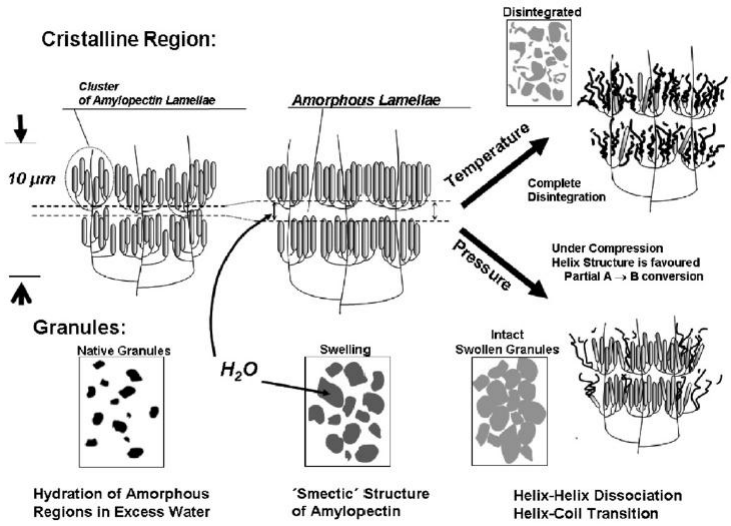

Figure 2.5 Schematic diagrams of starch granule gelatinization induced by either heat or pressure. (Source: Kim et al., 2012)

In general, starch heat-gelatinization can be measured by differential scanning calorimetry (DSC) and rapid visco-analyzer (RVA). DSC measures the range in transition temperature required for gelatinization to occur. Thermal properties typically reported using DSC include gelatinization onset (To), peak temperature (Tp), conclusion temperature (Tc), and enthalpy (ΔH), as shown in Figure 2.6, which are influenced by the molecular structure of amylopectin (unit chain length; CL, extent of branching, molecular weight, and polydispersity), starch composition (amylose to amylopectin ratio and phosphorous content), and granule architecture (crystalline to amorphous ratio). It is reported that the To, Tp, and Tc of gelatinization temperature (GT) of rice starch are in the range of 58.2-72.7 ℃, 63.3-77 ℃, and 69.9-85.5 ℃, respectively (Li & Gong, 2020). ΔH reflects the overall crystallinity of amylopectin, which has a value of 8-20 J g^{-1} (Cai et al., 2015; Kong et al., 2015). Compared to japonica rice starch, indica rice starch with a high apparent amylose content (AAC) has higher GT. Waxy rice can be categorized into high- and low-gelatinization temperature

(GT) classes (Bao et al., 2007). Wang et al. (2010) reported that the proportion of amylopectin chains with DP6-11 and DP12-24 was negatively and positively correlated with GT, respectively, which confirmed GT is significantly related to the chain-length distributions of amylopectin. Cheng & Bo (2020) reported that To, Tp, and maximum gelatinization rate were negatively correlated with the relative length of amylopectin short chains, based on the study of selected 14 rice samples. The glutinous rice starch shows the highest ΔH and the broadest Tc-To as 19.1 ℃ (Li & Gong, 2020). Most scholars commonly indicate that starch gelatinization temperatures are not affected by amylose chain-length distributions (CLD), but are controlled by the relative length of amylopectin short and medium chains.

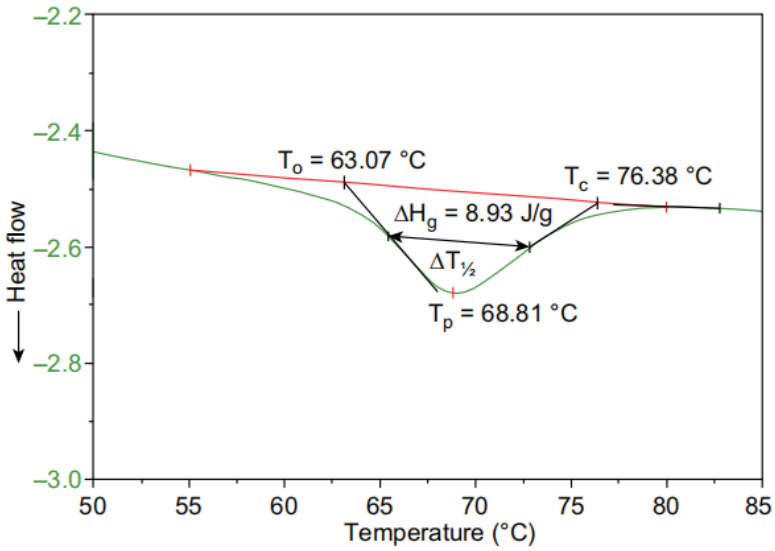

Figure 2.6 The thermal property of nonwaxy rice starch was determined by differential scanning calorimetry (DSC).

Note: To onset temperature; Tp peak temperature; Tc conclusion temperature; ΔH enthalpy of gelatinization; DT1/2 width at half peak height (Source: Bao et al., 2007).

Starch exhibits characteristic pasting viscosities when heated to the GT in excess water. The starch granule swells to several times its initial size because of the loss of the crystalline order and the absorption of water inside the granular structure. The pasting viscosity during swelling and gelatinization can be recorded using a rapid visco-

analyzer (RVA), which records the viscosity changes of starch in water caused by heating, holding constant for a time, and cooling, resulting in gelatinization curves, as shown in Figure 2.7. The native rice starch is insoluble in water at room temperature. When the temperature rises to ≥ 50°C, the starch granule absorbs a significant amount of water and swells to many times its original volume. This stage is known as the reversible water absorption stage of the starch granule. During this stage, the swollen starch particles compress each other, increasing in viscosity. As the temperature rises above the gelatinization temperature range of 60 to 80°C, the starch particles start to break down. Amylose and amylopectin begin to leach out of the granules. This leaching causes the viscosity of the starch solution to increase rapidly, ultimately reaching its peak viscosity. The temperature at which this rapid increase in viscosity occurs is identified as the gelatinization temperature. It is subjected to a constant high temperature (95°C) and mechanical shear force during the retention period of the test. This process further breaks up the starch particles and rearranges the starch molecules that enter the solution, thereby lowering the viscosity of the starch paste. As the temperature slowly drops in the later stages of the test, molecular rearrangement or polymerization takes place between starch molecules, especially between amylose molecules. This rearrangement leads to gel formation, causing an increase in viscosity, ultimately reaching the final viscosity.

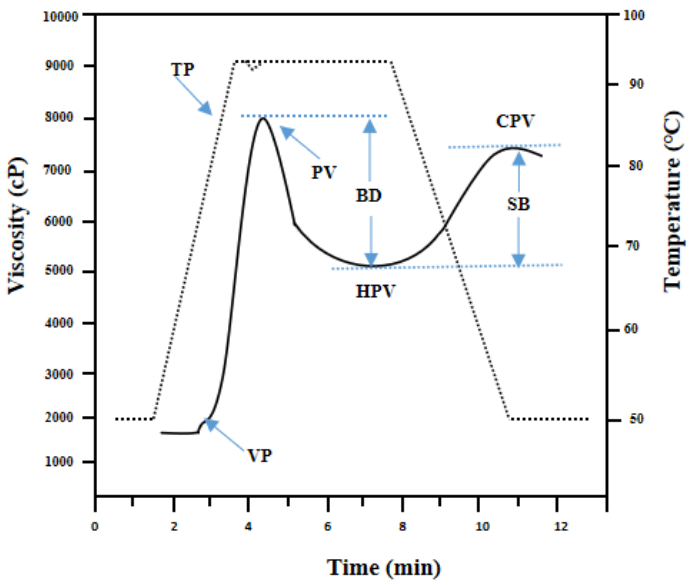

Figure 2.7 Pasting properties of indica rice starch measured with rapid visco-analyzer (RVA).

Note: TP temperature profile; VP viscosity profile; PV peak viscosity; HPV hot paste (trough) viscosity; CPV cool paste (end) viscosity; BD breakdown (PV-HPV); SB setback (CPV-HPV).

The slope of the initial increase in viscosity can reflect the degree of uniformity in the structure or composition of starch particles. A larger slope indicates that the starch particles have completed swelling within a smaller temperature range, indicating that the swelling behavior of the starch particles is uniform and consistent, further indicating that the structure and composition of the starch particles are relatively uniform.

Peak viscosity indicates the ability of starch molecules to bind to water, as branched starch molecules are more likely to form hydrogen bonds with water molecules. In contrast, straight-chain starch molecules are usually tightly packed and curled, easily forming intramolecular hydrogen bonds to prevent water molecules from entering. Therefore, the higher the content of branched starch in starch particles, the stronger the binding force with water, and the final peak viscosity is also higher. Figure 2.8 shows the glutinous type, which has a higher-branched starch of amylopectin having a higher peak viscosity than indica rich with lightly branched starch of amylose. The final viscosity indicates the strength of the gel formed after starch gelatinization. The

final viscosity of starch is large, which can form a strong gel. Conversely, it can form a weak gel or a thin gel.

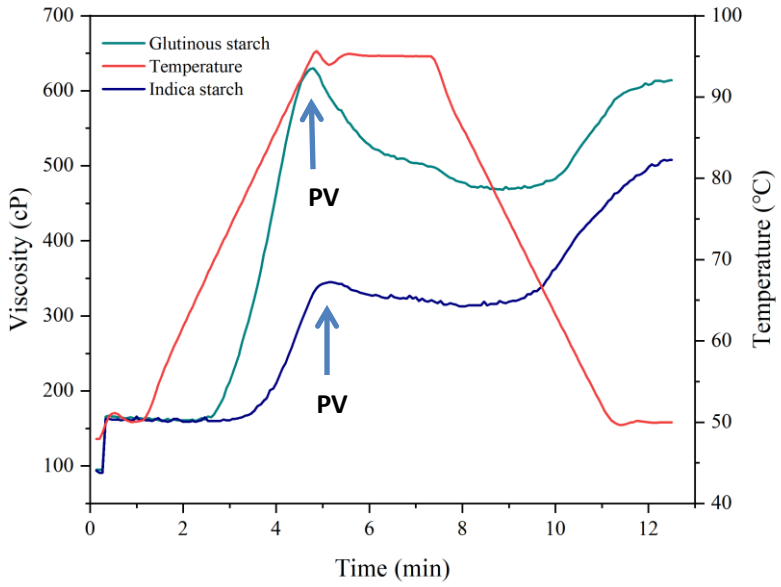

Figure 2.8 Pasting properties of glutinous and indica rice starch measured with rapid Visco-analyzer (RVA).

The structure of amylopectin strongly relates to the pasting parameters of rice starch when compared to AAC. Bhattacharya et al. (1999) found that the pasting viscosity analysis parameters of rice starch and AAC don't seem to be related. However, rice starches with similar AAC have very different pasting properties. It is generally accepted that amylopectin structure plays an important role in the pasting properties of rice starch. There is no significant correlation between chain length distribution (CLD) of amylopectin and pasting parameters such as peak viscosity (PV), cool paste viscosity (CPV), and setback (SB), as studied by Vandeputte et al. (2003a, 2003b, 2003c) with 15 types of rice starch. However, Tao et al. (2019) and Vamadevan et al. (2013) consistently reported that amylopectin fine molecular structures, such as chain-length distribution (CLD), could affect the starch gelatinization process. Amylopectin, including A (DP 6–12) and B1 (DP 13–24) chains, forms double helices and comprises