INFLUENCE OF GLUCONO-DELTA LACTONE ON STARCH-PLANT PROTEIN INTERACTIONS FOR IMITATION CHEESE FORMULATION

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by

A'FIRAH BINTI MOHD SAKRI @ SHUKRI

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To those who believed in me when I struggled to believe in myself,

My beloved parents, Mohd Shukri and Marzizah,

My siblings, Akif, Taariq, and Ummu Habibah,

And my AK Mohd Nazrin,

Your faith, love, and support have been the foundations upon which I build my dreams.

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LIST OF SYMBOLS

°C	Degree Celsius
%	Percentage
>	More than
<	Less than
a_W	Water activity
g	Gram
g	Gravity
G’	Storage modulus
G”	Loss modulus
ml	Millilitre
tan δ	Tan delta
Kg	Kilogram
µm	Micronmetre
mm	Millimetre
nm	Nanometre
mA	Milliampere
kV	KiloVolt
Hz	Hertz
Å	Armstrong
w/v	Weight per volume
w/w	Weight per weight
s^{-1}	Per second

LIST OF ABBREVIATIONS

GDL	Glucono-delta-lactone
GPC	Gel Permeation Chromatography
h	Hours
min	Minutes
OPI	Oat Protein Isolate
PPI	Pea Protein Isolate
rpm	Revolutions per minute
RVA	Rapid Visco Analyser
s	seconds
SEM	Scanning Electron Microscopy
SPI	Soy Protein Isolate
TPA	Texture Profile Analysis
TS-PPI	Tapioca Starch-Pea Protein Isolate
WHC	Water holding capacity
XRD	X-ray Diffraction

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PENGARUH GLUCONO-DELTA-LAKTON PADA INTERAKSI KANJI-PROTEIN TUMBUHAN TERHADAP FORMULASI KEJU TIRUAN

ABSTRAK

Permintaan terhadap produk berasaskan tumbuhan sebagai alternatif kepada produk tenusu haiwan semakin meningkat, didorong oleh pertimbangan kesihatan, alam sekitar dan etika. Bagi menangani cabaran untuk meniru ciri-ciri fungsi dan deria keju tenusu yang unik, kajian ini dijalankan bertujuan untuk menyelidik kesan pengaruh penggunaan glucono-delta-lactone (GDL) sebagai asidulan gred makanan terhadap ciri-ciri matriks kanji–protein tumbuhan, sebagai asas dalam pembangunan keju tiruan berasaskan tumbuhan dengan sifat fizikokimia dan fungsian yang sama dengan keju tenusu. Bagi menetapkan penanda aras formulasi keju tiruan, lapan jenis keju komersial berbentuk blok telah dianalisis. Keju-keju ini menunjukkan variasi kandungan protein (19.0%–32.6%), lemak (22.0%–38.0%), dan kandungan lembapan (33.34%–49.33%) yang memberi kesan langsung terhadap sifat fungsinya. Mozzarella menunjukkan faktor keperangan tertinggi (130%) serta sifat kebolehrenggangan yang paling baik disebabkan proses *pasta filata*, manakala Parmesan yang telah mengalami penuaan lebih lama menunjukkan tekstur paling keras dan kebolehcairan yang paling rendah. Analisis reologi menunjukkan titik cair antara 52 hingga 58 °C untuk keju semula jadi dan tertangguh sehingga 70 °C bagi keju proses. Dalam fasa seterusnya, tiga jenis kanji berbeza sumber (ubi kentang, ubi kayu dan jagung) telah diubah suai menggunakan GDL dan kesannya telah dikaji. Pengasidan oleh GDL memberi perubahan ketara terhadap berat molekul, ciri kelikatan, sifat aliran, sineresis gel, dan sifat penghabluran, bergantung pada jenis sumber kanji. Kanji ubi kayu dikenal pasti sebagai bahan paling sesuai untuk aplikasi keju tiruan disebabkan sensitiviti sederhana

terhadap asid, kelikatan yang stabil, sineresis rendah (10–13%), kecenderungan retrogradasi yang rendah, dan tekstur gel yang dikehendaki. Dalam fasa ketiga, tiga jenis protein tumbuhan terasing (soya, kacang pis dan oat) telah dikaji di bawah pengasidan GDL. Protein kacang pis terasing (PPI) dipilih berdasarkan kandungan protein yang tinggi (80.37%) dan keupayaan pembentukan gel yang baik dalam keadaan berasid. Apabila digabungkan dengan kanji ubi kayu dan 0.1% GDL, formulasi kanji ubi kayu-protein kacang pis terasing (TS-PPI) dengan 14% PPI membentuk gel yang kuat, elastik dan homogen. Analisis reologi, tekstur dan mikrostruktur mengesahkan kebolehcairan yang baik (titik silang G′-G″ pada 60 °C), kebolehrenggangan yang boleh diterima (>207 mm), dan struktur matriks yang homogen, menyerupai sifat cair dan renggang keju tenusu. Berdasarkan penemuan tersebut, keju tiruan TS-PPI telah dibangunkan dan menghasilkan keju tiruan separa keras dengan kandungan lembapan (57.57%) dan karbohidrat (22.60%) yang tinggi, kandungan protein yang sederhana (15.33%) dan rendah lemak (2.53%). Walaupun kebolehcairannya lebih baik berbanding keju proses, ciri fungsi lain masih rendah berbanding keju tenusu kerana ujian reologi tidak menunjukkan titik silang G′-G″ semasa pemanasan dan jarak renggangan menurun kepada 180.24 mm. Teksturnya juga lebih lembut daripada keju tenusu. Penilaian deria menunjukkan tahap penerimaan sederhana (skor min: 4.42/7), dengan tekstur lembut dan rasa asing daripada protein kacang pis dikenal pasti sebagai aspek utama yang perlu ditambah baik. Walaupun terdapat beberapa kekangan, kajian ini memperluas pemahaman saintifik tentang peranan GDL dalam mengubah sifat fisikokimia matriks kanji–protein tumbuhan. Penemuan ini membuka laluan bagi inovasi lanjutan dalam pembangunan alternatif produk tenusu berasaskan tumbuhan bagi memenuhi permintaan pasaran yang semakin berkembang dalam industri makanan.

INFLUENCE OF GLUCONO-DELTA-LACTONE ON STARCH-PLANT PROTEIN INTERACTIONS FOR IMITATION CHEESE FORMULATION

ABSTRACT

The demand for plant-based alternatives to animal-based dairy products, fueled by health, environmental, and ethical considerations, has prompted research into the development of imitation cheese. Addressing the challenge of mimicking the unique functional and sensory attributes of natural cheese, this study aims to investigate the influence of glucono-delta-lactone (GDL) as a food-grade acidulant on starch-plant protein matrix characteristics, as a foundation for developing a plant-derived imitation cheese with physicochemical and functional properties comparable to dairy cheese. To establish benchmarks for imitation cheese formulation, eight widely consumed commercial cheese blocks were analysed. The cheeses exhibited variations in protein (19.0%-32.6%), fat (22.0%-38.0%), and moisture (33.34%-49.33%), contributing to differences in functional properties. Mozzarella showed the highest browning factor (130%), the best stretchability due to pasta filata process, while Parmesan, due to extended ageing, was the hardest and least meltable. Rheological analyses revealed melt transitions between 52 to 58 °C in natural cheeses and a delayed crossover at 70 °C in processed cheese. Expanding the investigation, three starches from different sources (potato, tapioca, and corn) were modified using GDL and the effects have been investigated. Acidification by GDL caused significant alterations in molecular weight, pasting properties, flow behaviour, gel syneresis, and crystallisation properties depending on the types of starch sources. Tapioca starch was chosen as the best option for imitation cheese applications due to its moderate sensitivity to acid, stable viscosity, low syneresis (10–13%), low tendency for retrogradation, and desirable gel texture. In the third phase, three plant protein isolates (soy, pea, and oat) were treated with GDL, and pea protein isolate (PPI) was chosen due to its high protein content (80.37%) and superior gel-forming ability under acidic conditions. When combined with tapioca starch at 0.1% GDL, the resulting tapioca starch-pea protein isolate (TS-PPI) matrix with 14% PPI formed a strong, elastic, and homogeneous gel network. Rheological, textural, and microstructural analyses confirmed good meltability (G′-G″ crossover point at 60 °C), acceptable stretchability (>207 mm), and homogenous matrix at this concentration, simulating cheese-like melt and stretch behaviour. Based on the findings, the TS-PPI imitation cheese was formulated, producing a semi-hard imitation cheese with high moisture (57.57%), carbohydrate content (22.60%), moderate protein content (15.33%), and low fat (2.53%). While meltability was superior to processed cheese, other functional attributes remained inferior to natural cheese, as rheological tests showed no G′-G″ crossover during heating, and stretch distance declined to 180.24 mm. The texture was softer than dairy counterparts. Sensory evaluation showed moderate acceptance (mean score: 4.42/7 for overall acceptability), with soft texture and mild off-flavour from pea protein being key areas needing improvement. Despite limitations, this study advances our understanding of the role of GDL in modulating the physicochemical properties of starch-plant protein matrices, paving the way for further innovation in the development of dairy alternatives in the food industry to meet evolving market demands.

CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter provides a brief introduction to the study by first discussing the background and context, followed by the research problem, the importance of conducting the research, the research objectives, and the research protocol.

1.2 Research Background

Cheese is a dairy product made from the curds of milk that have been acidified, coagulated, pressed, and separated from the whey. It comprises proteins and fat from milk. Because of its flavourful and distinct taste, cheese has become very popular worldwide, although it mostly originates from the West and Mid-West. Even Asians are now enhancing the taste of their food with cheese. Within dairy products, cheese is experiencing an increasing demand and is the most consumed dairy product after milk (Çakır & Torusdağ, 2024; González-García *et al*., 2013; Röös *et al*., 2018). Global cheese production is estimated to reach approximately 22.2 million tons, with the European Nation (EU) remains the largest producer, contributing nearly 47% of global output, which is about 10.5 million tons. The United States of America (USA) follows, producing around 6.45 million tons (29% of total output). Other notable producers include Russia, Brazil, and New Zealand (USDA Foreign Agricultural Service, 2024). In Malaysia, the cheese market is currently valued at approximately US$480 million in 2025, with a projected annual growth rate of over 7% through 2030. Per capita cheese revenue is about US$13.34, corresponding to an average consumption of around 0.6 kg per person per year (Statista Market Insights, 2025).

There are thousands of varieties of fresh and aged cheese made in a variety of countries using milk from different animals as well as other raw materials using a variety of production and ageing processes (McSweeney *et al*., 2004). Cheese also exists in diverse forms, from blocks and individual slices to cubes and even string, making it a preferred ingredient in many food applications such as toppings, fillings, and sprinkles. Different types of cheese vary in terms of nutritional qualities, but all types are rich sources of calcium, protein, phosphorus, Vitamin A, and Vitamin B12 (Velciov *et al*., 2018)

In recent years, however, there has been an evolving trend towards the production of plant-based imitation cheese made from only plant ingredients. The Good Food Institute in Washington D.C., reported that the growth of imitation cheese sales from 2019 to 2020 was 42%, with US$270 million dollars being sold in the USA alone in 2020 (Grossmann & McClements, 2021). In 2025, the plant-based imitation cheese market in Malaysia generated approximately US$ 11.45 million and is projected to grow at 4.64% annually through 2029, indicating strong market and sustained demand (Statista Market Inisghts, 2025).

As the number and quality of imitation cheese continued to increase, consumers acceptance and adoption of these products were also increased (Grossmann & McClements, 2021). Nevertheless, designing an imitation cheese that can precisely mimic the full range of physicochemical, sensory, and nutritional attributes of natural cheese is extremely challenging due to the plant's compositional and structural complexity (Bachmann, 2001; Grossman & McClements, 2021; Kamath *et al.,* 2022).

Starch extracted from plants is one of the most popular polysaccharide-based ingredients used in imitation cheese with particular importance for nutritional and functional properties, especially as a texture modifier (Walkenström & Hermansson, 1998). Commonly, potato, tapioca, and corn starch are being used in imitation cheese formulations (Grossman & McClements, 2021). During the production of starch-based imitation cheese, starch is subjected to heating and shearing. As the starch granules are heated with water, various molecular and structural changes take place, causing the starch granules to swell and create a sticky paste (Biliaderis, 2009; Carlstedt *et al.,* 2015). Upon cooling, starch gels experience significant deformations that may results in irreversible changes (Tabilo-Munizaga & Barbosa-Cànovas, 2005). This is the reason most imitation cheese made from starch has a hard texture, less meltability, and less stretchability (Bi *et al*., 2016; Kiziloz *et al*., 2009; Mounsey & O'riordan, 2001; Mounsey & O'Riordan, 2008).

Therefore, various methods have been researched to modify the texture of starch-based foods. Among them, acidification of starch has been investigated by adding organic acids such as acetic acid, lactic acid, malic acid, and citric acid. These organic acids can help to reduce the hardness, adhesiveness, elasticity, and cohesiveness of starch gels, making them suitable for creating beneficial functional properties of natural cheese (Majzoobi *et al.,* 2014; Karma *et al.,* 2022; Zambelli *et al.,* 2018). However, a novel ingredient of organic acid, such as glucono-delta-lactone (GDL) which has a Generally Recognized as Safe (GRAS) status, can also act as an acidulant and coagulant, but has not been used to alter the functional properties of starch. While other organic acids impart a sour taste to food products, GDL was believed to have less tartness and a mild sweet taste (Ramachandran *et al.,* 2006). As a result, this study utilized GDL in starch modification.

The incorporation of plant proteins is another alternative to improve the final properties of starch-based imitation cheese. The majority of plant proteins used in plant-based products, such as soy, pea, and oat proteins, are globular proteins that differ from milk proteins. These globular proteins can form gels at high temperatures due to intra- and intermolecular disulphide bonds, as well as hydrophobic interactions between non-polar amino acids group (Chang *et al.,* 2016). The structure of these gels is dependent on pH and ionic strength (Fernández Sosa *et al.,* 2023; Gibis *et al*., 1989). Thus, acidification using GDL can produce a desirable texture of imitation cheese that is block-forming but can be melted and stretched when heated.

Many studies have been conducted to investigate how starch and plant protein ingredients can be used in imitation cheese (Ferawati *et al.,* 2021; Grasso *et al.,* 2022a; Li *et al*., 2024; Mehfooz *et al*., 2021), but the effects of acidification on starch-plant protein matrix characteristics have yet to be studied. Taking these aspects into account, the current study sought to investigate the effect of lowering the pH of the starch-protein matrix on the chemical composition, physical properties, and sensory attributes of imitation cheese. Specifically, the study aimed to develop a newly formulated imitation cheese with a firm yet sliceable texture, a yellowish color, desirable browning upon heating, good meltability and stretchability, and a homogenous block structure without phase separation. The knowledge gained from this work may help modulate the formulation and improve the texture of new gel-like imitation cheese food products.

1.3 Problem Statement

Cheese as a versatile food ingredient, has seen a significant increase in its use in food products, especially as toppings on pizzas and pastas, fillings in bakery products, and a flavourful ingredient in burgers and sandwiches. However, the price of cheese has increased in recent years due to a short supply of milk production and insufficient in quantity, especially in developing and third-world class countries. This gradually priced out the lower-income groups. The meticulous process and long period of time spent making and storing cheese also increased its price and prevented it from meeting continuously increasing consumer demands (Aliwarga *et al*., 2017; Kiely *et al.*, 1991). Thus, the global food industry is shifting to plant-based ingredients, which are cheaper and have fewer complex production processes. Substituting milk with lower-priced vegetable sources that can be processed in a very short period of time may be a possible solution to this economic problem (Fox *et al.,* 2017; Guirguis *et al.,* 1985), allowing everyone to enjoy its delicious flavour. A cheaper alternative could also benefit the food industries, such as pizza-making and fast-food chains, which use cheese extensively in their food products (Chavan & Jana, 2007; Fox *et al*., 2017).

Besides that, cheese production has been said to have a great environmental impact and is unsustainable (Palmieri et al., 2017). Livestock rearing causes pollution, greenhouse gas emissions, water use, and habitat and biodiversity loss (Poore & Nemecek, 2018; Willett *et al.,* 2019). Existing research indicates that reducing the consumption of animal-based food products in human diets can have a positive effect on environmental sustainability, including reductions in greenhouse gas emissions and pollution (Clark *et al.,* 2019; Willet *et al.,* 2019). Thus, the food production system must be changed in order to achieve the United Nations Sustainable Development Goals (UN SDGs) for sustainable consumption and production (SDG#12).

In addition, there has been a monumental shift in the behavioural landscape of consumers nowadays towards health awareness, influencing buying patterns of cheese. Imitation cheese made from plant sources can be introduced into the market and is suitable for people who do not want to consume saturated fats and high-cholesterol foods, which are generally high in natural cheese. Unsaturated fats and proteins from plants have been associated with positive health outcomes such as controlling body weight and reducing the risk of heart and artery disease (Chen *et al.,* 2017; Fardet & Boirie, 2014; Kong-Chan *et al.,* 1991; Schwingshackl *et al.,* 2017). Furthermore, imitation cheese is also suitable for vegan community that do not consume animal-based products. Therefore, the production of healthier imitation cheese by using plant-based ingredients instead of animal fats that are high in calories and saturated fats can be a solution to this problem.

Although imitation cheese has been widely produced and sold in the market, it often has low or no protein content when compared to dairy-based cheese (Craig *et al*., 2022; Grasso *et al*., 2021a; Mattice & Marangoni, 2020). Furthermore, they also have inferior functional properties such as undesirable texture, low meltability and stretchability, unpleasant flavour and taste, inability to be sliced and shredded, and are sticky and clumpy following processing. Matching the functionality of casein, the primary milk protein in cheese using plant-based proteins has proven to be extremely challenging (Bachmann, 2001; Fox *et al*., 2017). Casein forms a unique, heat-stable, three-dimensional protein network during cheese making, which entraps fat and water. Its amphiphilic nature and micellar structure allow for stable emulsification and consistent texture, even under thermal stress (Kayihura, 2024). Matching this complex functionality using plant proteins, which differ in molecular structure, solubility, and emulsifying behavior, has proven to be extremely difficult. Many plant-based cheeses contain a high amount of plant-based solid fat to mimic cheese meltability but are unable to obtain the stretch and flow characteristics (Mattice & Marangoni *et al*., 2020). Hence, this study finds suitable ingredients and formulations to develop imitation cheese with higher protein content, better texture, and functional properties.

Plant-based ingredients, such as starches and proteins, emerge as promising ingredients for imitation cheese formulations due to their functional and nutritional profiles. Nevertheless, mixing starch and protein together will cause water competition and affect the structural properties of the system (Zhang *et al*., 2021). Innovative techniques can be introduced to manipulate starch-protein interactions. Thus, the incorporation of GDL as a slow-acting acidulant presents an opportunity to enhance the texture and flavour of plant-based cheese. Unlike rennet, which is an enzymatic coagulant that cleaves κ-casein to form a firm curd in dairy cheese, GDL induces gelation through a gradual hydrolysis to gluconic acid, which slowly reduces the pH of the system. As the pH drops, proteins in the system approach their isoelectric point, leading to reduced solubility and increased protein-protein interactions, which contribute to the formation of a firmer and cohesive gel network (Alavi *et al.,* 2020). This gradual acidification process is desirable because it promotes a controlled gel formation, allowing better water retention, smoother texture, and enhanced structural integrity (Adams *et al.,* 2009; Li *et al.,* 2023). GDL also influences flavour development. The slow release of acid imparts a mild and slightly tangy taste, avoiding the harsh sourness often associated with direct acidification.

Despite its potential, the effects of GDL on the characteristics of the starch-plant protein matrix, which are crucial for imitation cheese development, have yet to be fully explored. Thus, this research focused on investigating the influence of GDL on starch-plant protein matrix characteristics and their implications for the development of desirable quality and sensory properties in imitation cheese.

1.4 Significance of Study

The study is significant in providing an understanding of the underlying mechanisms affecting the physicochemical properties of commercial cheese through standardized and consistent analytical methods, which is vital in gaining the information that can drive product development and innovation of plant-based cheese products tailored to consumers' specific preferences and market demand. Furthermore, the use of GDL to modify the starch-plant protein matrix lowers environmental footprints and significantly contributes to the goal of promoting sustainability. By incorporating plant-based ingredients into the imitation cheese formulation, this study focuses on producing high-protein, low-fat, and allergen-free imitation cheese with desirable functional characteristics that can fulfill the consumer demand for healthier food options. Additionally, food manufacturers can have a better understanding of how to enhance their applicability in starch-based foods, especially starch-containing plant-based foods for vegetarians and vegans.

1.5 Research Objectives

1.5.1 General Objective

The overall objective of this research is to produce a plant-based imitation cheese by developing a starch-plant protein matrix with GDL incorporation, which has the similar characteristics to natural cheese, such as block-forming properties, firm texture, good slice-ability, good melt-ability, and good stringiness.

1.5.2 Specific Objectives

The specific objectives of the research conducted in this study are:

1. To examine the physicochemical properties such as chemical composition, pH, water activity, colour and browning, meltability, viscoelasticity behaviour, texture, slice-ability, and stretchability of eight types of block-type cheeses (i.e Parmesan, Gouda, Emmental, Cheddar, Havarti, Processed, Edam, and Mozzarella) which are commercially available on the market using suitable analytical methods.

2. To evaluate the influence of varying concentrations of GDL (0%, 0.05%, 0.1%, and 0.5%) on the physicochemical properties (pH, pasting properties, flow characteristics, texture, syneresis, molecular weight distribution, and crystallinity) of three types of starches (potato, tapioca and corn) using appropriate analytical techniques.
3. To identify the textural, rheological, and microstructural changes in starch-plant protein matrix treated with 0.1% GDL concentration using instrument-based analysis.
4. To assess the physicochemical, rheological, textural properties, and consumer acceptability of the developed block-type imitation cheese from starch-plant protein with GDL as acidulant through instrumental analysis and sensory analysis involving 50 untrained panellists, respectively.

1.6 Research Protocol

This study is divided into four phases, each addressing a practical aspect of the research in accordance with the specific objectives outlined. The first phase focused on investigating the physicochemical properties of commercial block-type cheeses. Eight variants of commercial block-type cheeses (Parmesan, Gouda, Emmental, Cheddar, Havarti, Processed, Edam, and Mozzarella) were collected and analysed.

Chemical composition such as moisture content, protein, fat, carbohydrate, pH, and water activity were assessed. The colour before and after heating was examined using a colourimeter, and the browning factor was calculated. Meltability was measured using a modified Schreiber test and rheological analysis was performed with a rheometer. Texture Profile Analysis (TPA), slice-ability, and stretchability were evaluated with a texture analyser. The overall methodology is summarised in the flowchart presented in Figure 1.1.

The second phase studied the properties of different types of starches under the influence of GDL at varying concentrations. Potato, tapioca, and corn were treated with GDL at concentrations of 0%, 0.05%, 0.1%, and 0.5%. The effects of GDL on the molecular weight, pasting properties, flow behaviour, gel syneresis, and crystallisation properties of potato, tapioca, and corn starches were investigated. From the data obtained, one of the starches was chosen to be added to the plant protein which is the tapioca starch because it is less susceptible to GDL and has a lower tendency of retrogradation in high dosage application.

In the third phase, the study explored the effects of GDL on plant proteins and the starch-plant protein matrix. The proximate composition and isoelectric point of plant protein isolates from soy, pea, and oat were determined. Plant proteins were then mixed with different concentrations of GDL (0%, 0.05%, 0.1%, and 0.5%) and observed for their effects on pH, water holding capacity (WHC), and temperature sweep of plant protein pastes for gelation. One of the plant protein isolates which is the pea protein was selected to be mixed with tapioca starch at a 0.1% GDL concentration to form starch-plant protein matrix as pea protein forms firm gel. The pea protein concentration was varied (10%, 12%, 14%, and 16%). The effects of protein concentrations on hardness, frequency sweep, temperature sweep, stretchability and microstructure of starch-plant protein matrix were investigated.

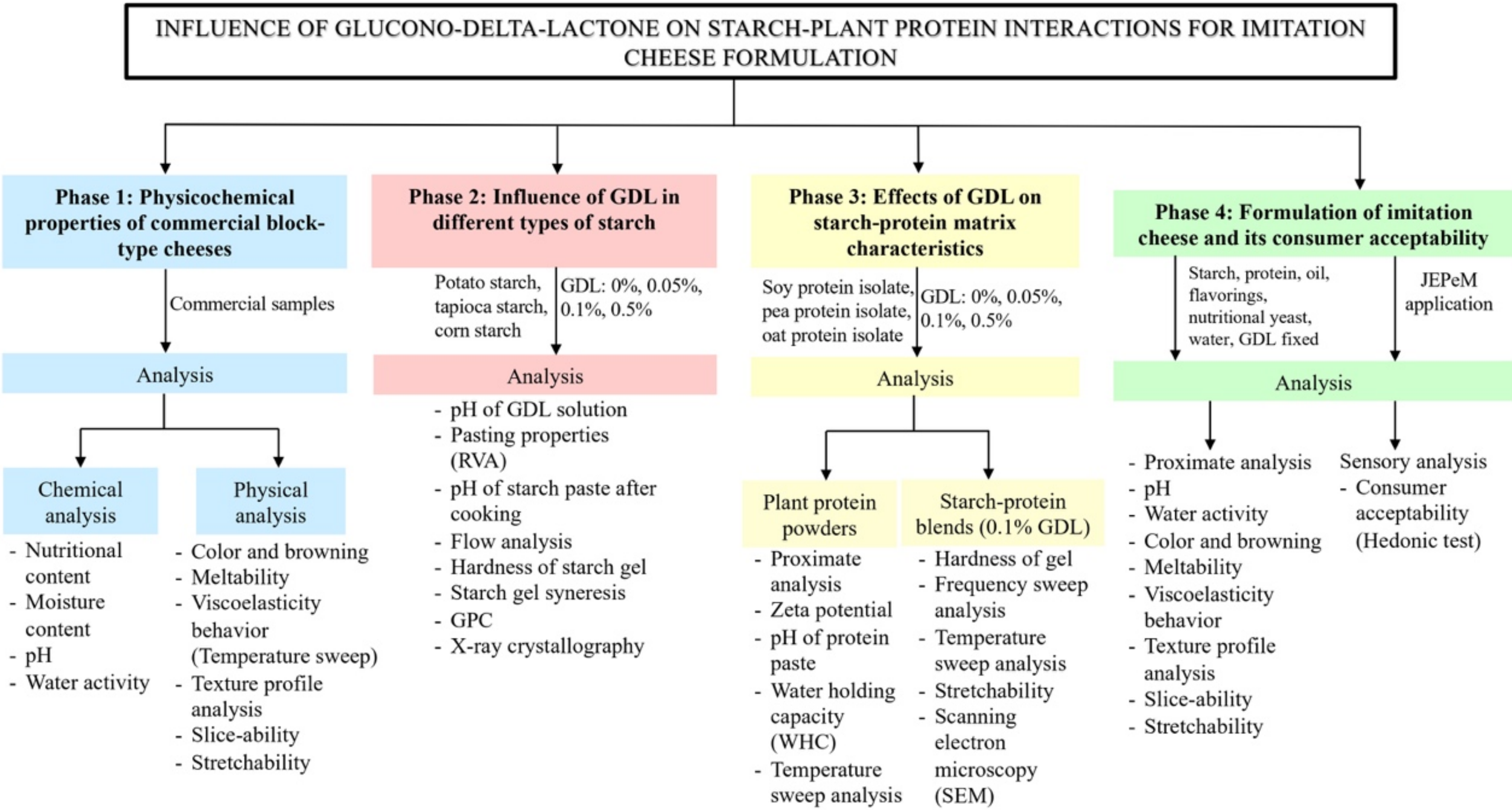

Figure 1.1: Flowchart of the research methodology

The final phase focused on developing plant-based imitation cheese using tapioca starch and pea protein. The chemical composition and physical characteristics of the imitation cheese and its sensory acceptability were assessed. The characterization includes proximate composition, pH, water activity, colour and browning, meltability, rheology, TPA, slice-ability, and stretchability. The data obtained were then compared with those of commercial cheeses. Sensory evaluation was conducted with 50 panellists to assess sensory attributes such as appearance, texture, colour, aroma, taste, and overall acceptability using a 7-point hedonic scale, with commercial Cheddar cheese as a control. Through this comprehensive approach, this research seeks to contribute valuable insights into the potential of plant-based ingredients in imitation cheese, ultimately supporting the development of healthier and sustainable dairy alternatives.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter provides an overview of previous research on cheese, imitation cheese, starch, vegetable proteins, and glucono-delta-lactone (GDL). The purpose of this review is to summarise current progress in understanding plant-based ingredients, such as starch and vegetable proteins, as animal substitutes in the production of imitation cheese.

2.2 Cheese

Cheese, a fermented dairy product, has been consumed since antiquity and historically served as a mean to preserve milk's nutritional value in the absence of refrigeration, ensuring stable food supply (Tunick, 2014). Produced by coagulating milk to separate soft white curds from yellowish liquid of whey, cheese-making process allows the transformation of perishable milk into a stable, nutrient-dense food source. The curds are then processed, moulded, and aged, resulting in a wide array of cheese varieties distinguished by flavour, texture, aroma, and form whereby their characteristics were influenced by milk composition, processing techniques, and maturation conditions (Fox & Guinee, 2013; Papademas & Bintsis, 2017).

Over the past 4000 years, cheese has been recognised as an essential dietary component, especially in Europe, with milk from cows, sheep, goats, buffalo, and other mammals being used (Salque *et al.,* 2013). However, the globalisation of food cultures has expanded cheese acceptance and consumption to non-traditional markets such as

Asia, driven by the adoption of Western dietary patterns (Lipoeto *et al*., 2013; OECD/FAO, 2020).

Cheese's versatility, rich nutrient profile, and diverse sensory attributes have made it a staple ingredient in global cuisine. It is available in various forms, including blocks, slices, shreds, spreads, and dips to suit different culinary applications. Among these, block cheese represents the largest market segment in the United States (Fortune Business Insights, 2024), primarily due to its practicality for transport, storage, uniform structure, and extensive applications in food service (Homwongpanich et al., 2025). Block cheese can be shredded, sliced, cubed, or melted, offering flexibility across a range of recipes. Its cost-effectiveness and extended shelf life further enhance its appeal in both household and commercial settings.

Today, modern cheese production is shaped by many factors such as milk type, animal diet, butterfat content, pasteurisation, and the specific microorganisms used in fermentation, processing, and ageing (Johnson, 2017). The rising demand for specialty, reduced-fat, and plant-based cheeses reflects broader shifts toward health-conscious and sustainable consumption among consumers. This trend emphasises the importance of cheese not only as a food, but also as a scientific and economic resource, indicating the need for continued research into its health, production, and sustainability implications.

2.2.1 Chemical Composition and Structure of Cheese

Cheese is a complex dairy product composed primarily of moisture, protein, and fat, with minor constituents such as calcium (calcium phosphate), salt (sodium chloride) and other dissolved solutes. Its structure is often described as an emulsion-gel, where para-casein micelles form a porous matrix cross-linked by calcium and hydrophobic interactions, while water, fat globules, bacteria, and serum with dissolved solutes such as lactic acid, residual lactose, soluble salts and peptides are all interspersed in the matrix, as shown in Figure 2.1 (Guinee, 2016; Hickey *et al.* 2015; Lamichhane *et al.* 2018; Vogt *et al.,* 2015). The amount of each cheese composition varies by type, depending on milk composition and its pre-treatment, cheese production conditions, maturation conditions, and cheese preparation before consumption (Feeney *et al.,* 2021).

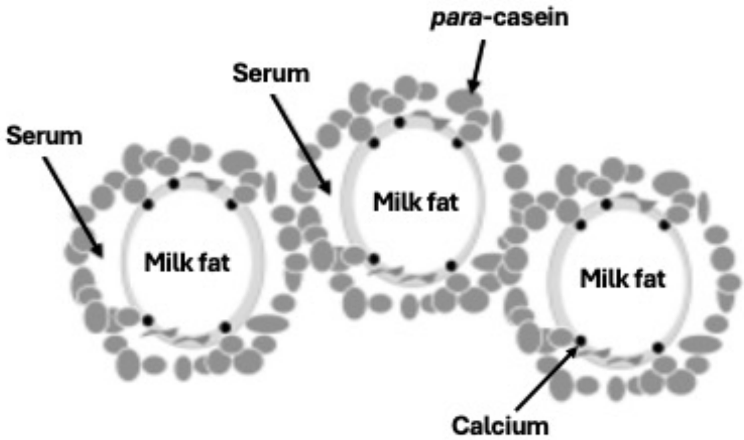

Figure 2.1: Illustration of para-casein micelles that form aggregates in cheese (Johnson, 2000).

During cheese production, casein micelles which are made up of four polypeptides, consisting of αS1-, αS2-, β-, and κ-caseins are destabilised through acidification (using starter cultures or food grade acidulants) or enzymatic coagulation (rennet), leading to curd formation via aggregation and gelation (Feeney *et al.,* 2021; Robitaille *et al.,* 2004). Casein micelles have a strongly negative surface charge, causing them to repel each other. This repulsion can be reduced either by lowering the pH to the isoelectric point through acid addition or by enzymatic action using rennet, which hydrolyses κ-casein into para-κ-casein, leading to micelle destabilisation and curd formation (Robitaille et al., 2004). Both acidification and rennet addition promote casein aggregation, forming a protein network that traps water and fat, resulting in the gel-like structure of cheese (Dalgesh & Corredig, 2012; Guinee, 2016; Lucey, 2002). This network contributes to the texture, meltability, and flow properties of the final product (Lucey *et al.,* 2003; Udayarajan *et al.,* 2005). Figure 2.2 illustrates the aggregation of casein micelles through acid and rennet addition.

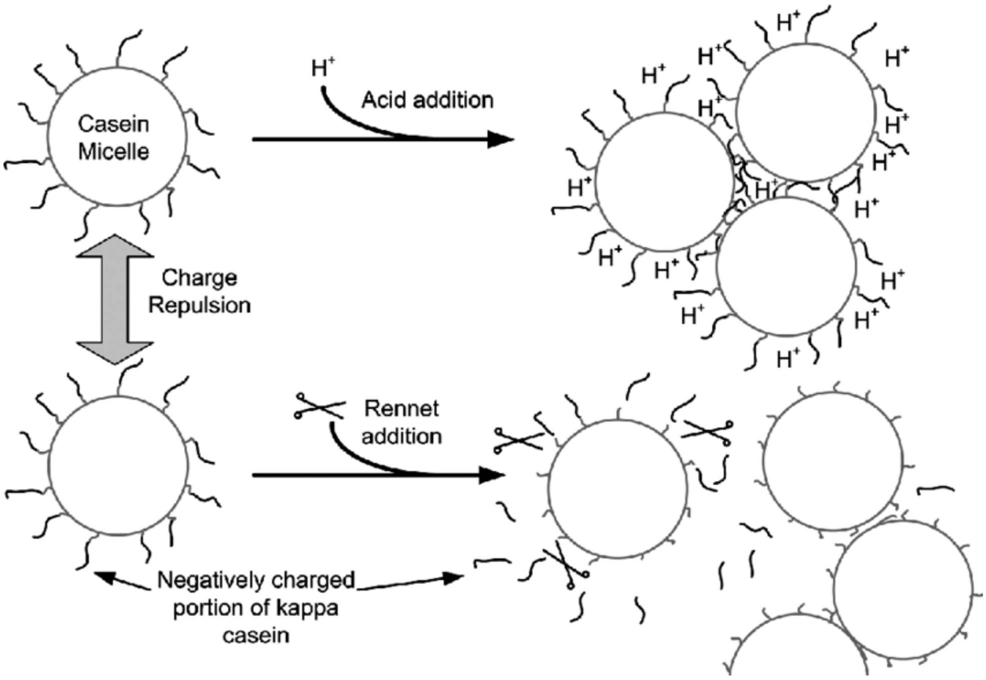

Figure 2.2 : Schematic diagram illustrating how casein micelles forms aggregates after the addition of acid and rennet (Carr, 2010).

Whey proteins, accounting for approximately 20% of total milk protein, are largely removed with the whey but may be retained in small amounts within the curd (Kindstedt, 2013). These proteins are made up of globular proteins such as β-lactoglobulin (~50% w/w), α-lactalbumin (~20% w/w), lactoferrin, immunoglobulins (~10% w/w), bovine serum albumin (~6% w/w), and other minor protein or peptide components, including lactoperoxidase, lysozyme, glycomacropeptides, lactose, and minerals, contributing minor nutritional and functional properties (Jenkins & McGuire, 2006; Malacarne *et al.,* 2002). Milk fat, containing over 400 fatty acids with diverse saturation levels, including saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA), with cis and trans configurations and straight or branched carbon chains, plays a key role in cheese texture, meltability, free oil formation, and mouthfeel (Ma *et al.,* 2013). In cheese, fat globules are entrapped in the protein network in various forms which are intact (spherical and covered with native membrane), aggregated (clumps), coalesced (spherical but larger than typical milk fat globules), elongated (in pasta-filata cheese) and nonglobular, depending on the processing methods such as scalding, cheddaring, hot water stretching, and pressing (Lamichhane *et al.,* 2018). The complexity of milk fat gives its unique properties, distinguishing it from other natural fats (Jensen, 2002; Månsson, 2008; Parodi, 2004).

Other critical components include lactose (about 5% of milk), which is mostly removed during whey separation, but the remaining are further fermented by lactic acid bacteria (LAB) into lactic acid during ageing, reducing its content and enhancing tolerability for lactose-intolerant individuals (Kindstedt, 2013). Calcium and phosphate, especially in the form of colloidal calcium phosphate, stabilise the casein network and influence firmness and meltability depending on pH and ion availability (De Kruif & Holt, 2003; Guinee, 2016; Swaisgood, 2003). Salt (sodium chloride), added primarily for flavour and preservation, also promotes protein-water and protein-protein interactions, affecting texture, rheological, cooking properties, and shelf life (Guinee, 2004; Lamichhane *et al.,* 2018).

Nutritionally, cheese is dense in high-quality protein, essential fatty acids, calcium, magnesium, phosphorus, and vitamins A, D, B2, B12, and K2, supporting muscle repair, bone health, vision, and cardiovascular and neurological systems. Some varieties also offer probiotic benefits through live *Lactobacillus* spp. cultures.

However, concerns remain over its high saturated fat, cholesterol content, and sodium which may contribute to chronic diseases such as obesity, heart disease, high blood pressure, and some cancers if consumed excessively (Parodi, 2004). In response, industry innovations focus on reformulating cheese with reduced fat and salt using ingredients like inulin, konjac glucomannan, modified starches, or salt replacers such as magnesium chloride, potassium chloride, and calcium salts, while maintaining texture and flavour (Fan & Picchioni, 2020).

The structure of cheese is determined by the assembly and interactions of its components within the matrix, influenced by processing, which in turn affects its characteristics and functionality. For instance, stretching during Mozzarella production creates fat and serum channels between parallel casein fibres, whereas the cheddaring step in Cheddar results in a continuous protein casein gel network interspersed with irregular, relatively large fat globules (Auty *et al.,* 2001; Vogt *et al.* 2015). These structural variations, determined by both cheese composition and processing, are crucial in determining the texture, meltability, and overall functionality of cheese. Understanding these interactions is essential for optimising production and developing cheese products with targeted properties with improved sensory attributes, nutritional quality, and consumer acceptance.

2.2.2 Classification of Cheese

Cheese exists in thousands of varieties worldwide, differing in shape, form, texture, flavour, and production method, yet all originate from the same primary ingredient, which is the milk. The classification of cheese remains complex and inconsistent, with no one universal system adopted. Traditionally, cheeses are categorised based on milk source, fat content, texture, production method, and country of origin (Walter & Hargrove, 1972; Burkhalter, 1981). Among the most common classification criteria are milk source, texture, and ripening method.

Milk type plays a critical role in defining cheese quality and characteristics. Cow, goat, and sheep milk are commonly used, while buffalo and ewe milk have also been explored (Albenzio *et al.,* 2013; Barcenas *et al.,* 2004; Di Cagno *et al.,* 2003; Sameen *et al.,* 2008; Zicarelli, 2004). Differences in fat and casein content among milk types result in distinct flavour and texture profiles. Cow's milk typically produces sweet, creamy cheeses, goat's milk yields tangy, digestible cheeses with smaller fat globules and lower lactose, while sheep's milk contributes rich, nutty flavours due to its higher fat content. These compositional differences also affect cheese functionality. For instance, cow's milk cheese is preferred for pizza due to its superior meltability and stretchability (Bhattarai & Acharya, 2010; Jana & Tagalpallewar, 2017; Zedan *et al.*, 2014).

Cheese can also be classified into different categories according to its their textures which is linked to the moisture content. According to the General Standard for Cheese (Codex Standard 283 – 1978) of Codex Alimentarius (2013), cheeses can be classified based on their percentage of moisture on a fat-free basis (MFFB), which reflects their firmness and ripening process. Table 2.1 below shows the classification of cheese based on firmness and ripening characteristics.

Table 2.1: Classification of cheese based on moisture on a fat-free basis (MFFB%) and ripening characteristics (Joint FAO/WHO Codex Alimentarius Commission, 2013).

Designation according to firmness and ripening characteristics		
According to firmness		According to ripening characteristics
Percentage moisture on a fat-free basis (MFFB%)	Designation	
<51	Extra-hard	Ripened
49-56	Hard	Mold ripened
54-69	Firm/Semi-hard	Unripen/fresh
>67	Soft	In brine

Cheese is commonly classified into four main categories based in moisture content which are soft, semi-hard, hard, and extra-hard cheese. Soft cheeses, with over 67% moisture, are typically unripen or ripen from the outside in, making them suitable for spreading but less ideal for cooking due to their high perishability. Semi-hard cheeses have a moisture content ranges from 54% to 69%, offering a smooth, easily sliceable texture and mild flavour resulting from shorter ageing periods. Examples of semi-hard cheeses include rindless Havarti, red paraffin wax coated Edam, and mouldy-coated Gorgonzola. Hard cheeses, such as Dutch Gouda, Norwegian Jarlsberg, and American Colby, contain 49% to 56% moisture and are firmer in texture, with applications ranging from grating and melting to direct consumption. Extra-hard cheeses, including Parmesan, Grana Padano, and Emmental, have less than 51% moisture and undergo extensive whey removal and ageing, often from 2 to over 36 months or even a longer period, resulting in a dense, grainy texture, and more concentrated flavour. Longer ageing periods reduce creaminess while enhancing sharpness, and higher ageing temperatures contribute to larger holes formed by starter cultures during fermentation.

Cheese can be classified as unripen or ripened (matured). Unripened cheese, such as Cottage, Cream, and Ricotta, is made from curd separated from whey without the use of ripening agents. On the other hand, ripened cheese undergoes ageing with selected strains of bacteria, moulds, yeasts, or combinations of these to develop cheese with distinct flavours, textures, and appearances. Bacterial-ripened cheeses include Cheddar, Parmesan, and Swiss, while mould-ripened examples include Roquefort, which is treated with *Penicillium roqueforti*.

In the early 20th century, processed cheese emerged as a new category, developed to extend the shelf life of natural cheese and find alternative uses for natural cheese that was difficult to sell (Kapoor & Metzger, 2008). It is made by blending natural cheeses of different ages and degrees of maturity with emulsifying salts and other dairy and nondairy ingredients, followed by heating and continuous mixing to create a uniform, consistent, and shelf-stable product (Guinee *et al.,* 2004; Kapoor & Metzger, 2008). The U.S Code of Federal Regulations (CFR) categorises processed cheese into three categories: Pasteurized Processed Cheese (PC), Pasteurized Processed Cheese Food (PCF), and Pasteurized Processed Cheese Spread (PCS). The popularity of Processed cheese on the market can be attributed to its affordability, versatility in cooking, convenience, and consistent quality, with ongoing innovations catering to diverse consumer preferences and dietary needs.

To explore the diversity of cheese characteristics and functionality, this study focuses on eight commercially available block-type cheeses which are Cheddar, Mozzarella, Edam, Emmental, Gouda, Havarti, Parmesan, and processed cheese. These cheeses were selected based on their market availability, diversity in ripening methods, and functional properties (Guinee, 2004; Fox *et al.,* 2017). Their selection also aligns with the cheese classification systems previously discussed and reflects their widespread application in food manufacturing, particularly in ready meals, bakery toppings, pizzas, and sauces. This representative range allows for a comprehensive assessment of functional attributes such as meltability, stretchability, texture, and slice-ability which are critical for guiding the formulation of imitation cheese products. Table 2.2 summarises the differences of eight types of commercial block-type cheeses selected for the study.

Table 2.2: Different types of commercial block-type cheeses.

Cheese Type	**Cheese Category**	**Processing Technique**	**Ripening Method**	**Notable Features**	**References**
Cheddar	Natural, hard	Cheddaring (curd stacking and turning)	Bacterial-ripened	Sharp flavour, firm and dense texture	Murtaza *et al.* (2014)
Mozzarella	Natural, semi-soft	Pasta filata (curd stretching in hot water)	Pasta-filata, unripened	High meltability and stretchability	Kindstedt (1999)
Edam	Natural, semi-hard	Washed curd technique	Bacterial-ripened	Smooth texture, mild flavour, lower fat than Gouda	Düsterhöft et al. (2017)
Emmental	Natural, hard	Cutting and cooking the curd at high temperatures, and Propionibacteria gas production	Bacterial-ripened	Large "eyes", elastic texture, nutty flavour	Çavuş, & Beykaya (2022)
Gouda	Natural, semi-hard	Washed curd technique	Bacterial-ripened	Creamy texture, buttery, slightly sweet flavour	Düsterhöft et al. (2017)
Havarti	Natural, semi-soft	Washed curd technique	Bacterial-ripened	Creamy texture, small irregular holes	Ritvanen et al. (2010)
Parmesan	Natural, extra-hard	High-heat cooking and long ageing	Long-aged (24–36 months)	Granular and brittle texture, strong umami flavour	Aliwarga et al. (2017)
Processed	Blended, pasteurised	Emulsification with emulsifying salts	Heat-treated, non-ripened	Consistent texture, long shelf life	Fox *et al.,* (2017)