TAILORING STRINGINESS PROPERTIES OF COMPOSITE FLOUR PASTE FOR POTENTIAL APPLICATION IN NON-DAIRY IMITATION CHEESE: EFFECTS OF FLOUR CONSTITUENT RATIOS AND GLYCEROL MONOSTEARATE CONCENTRATION

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by

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LIST OF SYMBOLS

K	Consistency coefficient
°C	Degree Celsius
δ	Delta
n	Flow behaviour index
g	Gram
g	Gravitational force
Hz	Hertz
h	Hour
G”	Loss modulus
mL	Millilitre
mL/min	Millilitre per minute
mm	Millimetre
mm/s	Millimetre per second
min	Minute
N	Newton
mol/L	Number of moles per litre
Pa	Pascal
s^{-1}	Per second
%	Percentage
s	Second
$\dot{\gamma}$	Shear rate
σ	Shear stress
G’	Storage modulus
M_w	Weight average molecular weight

w/w	Weight per weight
σ_o	Yield stress

LIST OF ABBREVIATIONS

DMSO	Dimethyl sulfoxide
GPC	Gel permeation chromatography
GF	Glutinous rice flour
GMS	Glycerol monostearate
LVR	Linear viscoelastic region
PDI	Polydispersity index
RF	Rice flour
TS	Tapioca starch
3D	Three-dimensional

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PENGUBAHSUAIAN SIFAT KEBOLEHREGANGAN PES TEPUNG KOMPOSIT UNTUK APLIKASI BERPOTENSI DALAM KEJU TIRUAN BUKAN TENUSU: KESAN NISBAH KOMPONEN TEPUNG DAN KEPEKATAN GLISEROL MONOSTEARAT

ABSTRAK

Keju mozzarella secara umumnya mempunyai sifat kebolehregangan yang tinggi, namun sering kali sukar untuk ditiru dalam keju berasaskan tumbuhan. Sifat kebolehregangan ini telah dihasilkan menggunakan tepung komposit dan dikaji melalui ujian frekuensi, tingkah laku aliran, dan analisis kebolehregangan. Dengan tujuan untuk menghasilkan sifat kebolehregangan, kanji ubi kayu dan tepung pulut telah dicampurkan dalam tiga nisbah yang berbeza: 1:2, 1:1 dan 2:1. Ketiga-tiga sampel ini menunjukkan nilai G', G", tan δ dan parameter lengkung menaik yang serupa tetapi kadar pembentukan semula struktur yang berbeza. Ini dapat dilihat dari parameter lengkung menurun dan sifat tiksotropi. Jarak regangan adalah baik apabila kanji ubi kayu menjadi komponen utama. Dengan penambahan tepung beras, G' dan G" meningkat untuk semua tiga nisbah. Tingkah laku aliran juga banyak terjejas. Analisis kebolehregangan menunjukkan bahawa tepung beras meningkatkan ketegaran permukaan dan kecerunan menaik sementara penurunan ketebalan regangan diperhatikan. Kesan tepung beras terhadap jarak regangan dipengaruhi oleh nisbah kanji ubi kayu kepada tepung pulut, dengan regangan yang lebih panjang apabila nisbah berat komponen adalah sama atau melebihi tepung pulut. Ini disebabkan oleh peranan rantai amilosa berberat molekul kecil dalam tepung pulut yang bertindak sebagai pemanjang rantai. Hubungan antara kualiti dan kuantiti regangan dengan pengubahsuaian struktur telah ditentukan berdasarkan korelasi Pearson. Jarak regangan sebagai fokus utama dalam sifat kebolehregangan telah dipertingkatkan lagi dengan penggunaan gliserol monostearat (GMS) pada kadar 1, 2, dan 3% berdasarkan berat kering kanji menggunakan formulasi yang paling disyorkan. Pengkompleksan GMS dengan amylosa menggalakkan ikatan hidrogen, dengan ketara meningkatkan G', ketegaran permukaan, dan kecerunan menaik tepung komposit. Jarak regangan telah diperbaiki kerana bahagian hidrofobik GMS yang tidak berkompleks menghalang interaksi antara dan intra molekul kanji, memudahkan pergerakan molekul kanji semasa diregangkan. Kepekatan 2% GMS adalah kepekatan kritikal untuk meningkatkan kebolehregangan. Kajian ini menunjukkan bahawa tepung beras dan GMS adalah penting untuk menghasilkan rangkaian kebolehregangan dalam sistem berasaskan kanji.

TAILORING STRINGINESS PROPERTIES OF COMPOSITE FLOUR PASTE FOR POTENTIAL APPLICATION IN NON-DAIRY IMITATION CHEESE: EFFECTS OF FLOUR CONSTITUENT RATIOS AND GLYCEROL MONOSTEARATE CONCENTRATION

ABSTRACT

Stringiness is a common functional property of mozzarella cheeses, but often less successful to be imitated in plant-based cheeses. Stringiness properties were tailored using composite flour and were investigated on frequency sweep, flow behaviour and stringiness analysis. With the aim of delivering stringiness property, tapioca starch and glutinous rice flour were blended in three different ratios: 1:2, 1:1 and 2:1. These three samples exhibited similar G', G", tan δ and upward curve parameters but different structural reformation rate. This is observable from the downward curve parameters and thixotropy. The stretch distance was good when tapioca starch is the major constituent. Upon the addition of rice flour, G' and G" increased for all three ratios. Flow behaviour also was largely affected. Stringiness analysis demonstrated that rice flour increased the surface stickiness and upward gradient whereas a decrease in stretch thickness was observed. The effect of rice flour on stretch distance was influenced by the ratio of tapioca starch to glutinous rice flour, with an extension of stretch when the weightage of components was equal or exceeded by glutinous rice flour. This is attributed to the role of small molecular weight of rice amylose chains in rice flour acting as chain extender. The relationship of quality and quantity of stretch with low and high structural deformation was established based on Pearson correlation. The stretch distance as the main interest of stringiness properties was further enhanced using glycerol monostearate (GMS) at 1, 2 and 3% dry starch basis using the most preferred formulation. Complexation of GMS with amylose promoted hydrogen bonding, significantly increased the G', surface stickiness and upward gradient of composite flour. Stretch distance was improved as the hydrophobic part of uncomplexed GMS prevent inter- and intra- molecular interactions of starch, facilitating the gliding of starch molecules when stretched. The concentration of 2% GMS was the critical concentration for enhancing stringiness. This study showed that rice flour and GMS are crucial for imparting stringiness network in tapioca starch-glutinous rice flour system.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Cheese is a popular food that creates a sense of comfort to many people. It can be enjoyed on its own or used as an ingredient in a wide range of dishes. Cheese is a dairy product made by curdling milk, typically from cows, buffalo, goat, or sheep. The production of different types of cheese, such as Brie, Parmesan, Mozzarella, and Cheddar, involves typical manufacturing techniques that developed distinctive cheese flavour profile, textures, and colours, that offering a unique culinary experience to those eating the foods cooked or garnished with cheese. Mozzarella cheese ranked top in popularity among all types of cheeses attributed by its unique functionalities, including high melt ability, stringiness and oiling off (Mohd Shukri *et al.*, 2022). Stringiness, also known as stretchability or extensibility, is a desirable functional property of mozzarella cheese, commonly used in pizza and lasagna. Mozzarella cheese is uniquely characterized by its exceptional stringiness. It is the result of a typical manufacturing process known as "pasta-filata" during mozzarella cheese manufacturing. Pasta-filata is a process of repeated action of stretching, folding and kneading of cheese curd to align protein molecules into specific molecular alignments within a cheese matrix (McMahon & Oberg, 2017). With repeated kneading, casein molecules align into fibrous structure collecting fat globules in between protein strands. This structure of alternate columns of proteins and fat of mozzarella cheese impart melt flow and stringiness properties. The stringiness of cheese remains after being heated and pulled by maintaining the balance between melting and strength of protein-protein interactions.

Presently, the demand for vegan cheese and plant-based cheese is escalating due to several reasons, including population with lactose intolerance problem, significant carbon footprint contribution by the dairy industry, increased concerns about animal welfare, as well as the growing population of vegans, vegetarian and flexitarian. The base of dairy products is milk, which sourced from cow, goat or buffalo. Livestock farming is one of the major contributors of greenhouse gas emissions, mainly methane and nitrous oxide by enteric fermentation, manure management and the use of fertilizers. With increasing demand from consumers, cheese production is foreseen to be increased, which will then cause adverse climate changes, contributing to global warming issues. Due to the concern on carbon footprint and animal welfare, consumers are shifting towards flexitarian or vegan diet, especially among the youngsters. Moreover, dairy products are unfriendly towards lactose intolerance population.

Total or partial substitution of milk component with non-dairy ingredient produce imitation cheese (Bi *et al.*, 2016; Omrani Khiabanian *et al.*, 2020). The development of imitation cheese aims to mimic or improve the functional properties, in terms of texture, shredability, stretchability, meltability, colour, oiling off and browning, as well as nutritional properties. Among the various characteristics of cheese, stringiness is the most difficult characteristic to be duplicated in vegan cheese or plant-based cheese. According to Mohd Shukri *et al.* (2022), imitation cheeses have minimal or zero melt and very limited stringiness property. When casein is the top contributor of stringiness, vegan mozzarella cheese without the use of casein failed to deliver stringiness property. Achieving stringiness in imitation cheese is particularly challenging due to the limited research on this attribute when none or little successful development of stringy imitation cheese. The lack of standardized measurement techniques for stringiness further complicates the issue. From the literature, parameters used to describe stringiness property of a sample are varied and ambiguous, because the experimental settings used were not standardized (Bi *et al.*, 2016; Feng *et al.*, 2021; Fife *et al.*, 2002; Guinee *et al.*, 2015; Li *et al.*, 2019a, Salunke *et al.*, 2022). Existing measuring techniques have shortcomings. Consequently, cross-study data analysis and comparison is difficult, thus slowing the development process of vegan cheese and plant-based cheese. Among all, cheese extensibility rig emerges as a more promising tool for both qualitative and quantitative assessment of stringiness.

Considerable effort has been invested in creating cheese alternatives that replicates the desirable stretchy and stringy texture of natural cheese. However, majority of the studies reported negative outcomes on stringiness property when dairy components were substituted with non-dairy protein, oil or starch. This problem is especially noticeable in completely dairy-free cheese. Despite the growing popularity of vegan cheese, achieving an ideal stringiness remains under-explored. To the best of my knowledge, little or no vegan cheeses with stringiness comparable to mozzarella cheese are successfully produced, highlighting a research gap in developing this texture using plant-based ingredients. This presents a significant research opportunity to explore new formulations that could enhance stringiness in vegan cheese. Out of all the plant-based ingredients, starch stands out as a commonly used ingredients in imitation cheese development due to its ability to act as texture and structure provider (Bi *et al.*, 2016; Dharaiya *et al.*, 2019; Tuntragul *et al.*, 2010). Among various starches, tapioca starch has the ability to gelatinize into a stretchy, malleable mass. Mattice and Marangoni (2020) highlighted the potential of tapioca starch to impart stringy texture in plant-based cheese though the stretch distance achieved was not satisfactory. Meanwhile, glutinous rice flour also shown promise in delivering stringiness, particularly when used to produce stretchy waxy rice cakes (Rath, 2014; Sasaki *et al.*, 2013). On the other hand, Butt *et al.* (2020) developed imitation mozzarella cheese by including rice starch and observed improved meltability with starch addition. The selection of starches should consider amylose content as amylose is the network-forming molecules.

Starch is a widely used food ingredient due to its affordability and versatility. Different types of starch possess distinct functional characteristics. Native starches often could not provide desired functionalities in many food products which limits their applications in food industry. Due to significant characteristics contributed by different types of starch, blending native starches is a feasible alternative to produce eco-friendly vegan food through modification of starch functional properties in food without any chemicals used. In addition, the use of native starches instead of modified starch in food products is more consumer friendly due to the trend of clean label food. Several works demonstrated significant effects on the quality of extruded snack, noodle and cookies when different varieties of starch were blended at different ratio (Bhattacharyya *et al.*, 2006; Dilek & Bilgiçli, 2021; Noda *et al.*, 2006; Olawoye & Gbadamosi, 2020). The application of starch blend of two or more types of starches in food is challenging as the optimization of desired functional properties involves usage of the types of starches and the mixing ratio. Hence, blending different types of starch could boost their performance in providing desirable characteristics of imitation cheese.

Emulsifier addition in starch-based food is common. Starch gelatinization, pasting, and retrogradation behaviour could be affected by the addition of emulsifier thereby altering the functions of starches in food. Studies had been done on the effects of emulsifiers such as glycerol monostearate, stearic acid, lauric acid and diacetyl tartaric esters of mono- and diglycerides on noodle, wonton wrap, pancake and bread (Kang *et al.*, 2021; Kaur *et al.*, 2005; Prakaywatchara *et al.*, 2018; Yamashita *et al.*, 2020). Emulsifiers often added as additives in starch-based food aiming to inhibit retrogradation to maintain the textural properties upon prolonged storage. This inhibiting effect largely depending on the type and level of emulsifier added. Glycerol monostearate (GMS) with saturated carbon chain often presented good anti-retrogradation effect (Wang *et al.*, 2022a; Yang *et al.*, 2017). Kang *et al.* (2021) observed softer bread texture after one week storage as GMS addition slow down staling of bread.

Stringiness is a unique rheological property of mozzarella cheese. However, stringiness is less given attention in imitation cheese development as it is too difficult to be imitated as well as the lack of standardized measuring method. Starch possesses inherent properties that can contribute to stringiness, yet its potential in this regard remains largely unexplored. Although glycerol monostearate is often used in starch system for anti-retrogradation, its function as stringiness enhancer has not been studied. In this study, tapioca starch, glutinous rice flour and rice flour will be blended at different ratio to investigate the rheological properties in fabricating the stringiness of mozzarella cheese. In addition, the effect of glycerol monostearate on the rheological and stringiness properties will be studied.

1.2 Rationale and Hypothesis of Study

Plant-based alternatives to dairy products have become commonplace, with consumers expecting comparable or superior quality. Mozzarella cheese, renowned for its excellent stringiness, has proven challenging to replicate in vegan mozzarella cheese. This is because stretchability tends to be compromised when the milk components are substituted with non-dairy ingredients. This key characteristic is vital to consumer satisfaction but remains elusive in plant-based alternatives. Consequently, the development of a diverse vegan cheese market was hindered, thereby limiting consumer options and slowing market growth.

Starch is a cost-effective and versatile ingredient to mimic cheese’s characteristics. Surprisingly, despite the potential, starch remains largely unexplored as a primary ingredient in imitation cheese development. This presents a captivating opportunity for exploration, as the incorporation of starch into imitation cheese formulations could lead to the development of novel products with improved properties. This research gap is particularly intriguing given starch's versatility and widespread use in various food applications. Delving deeper into functionality of starch could open a new avenue for developing plant-based cheese that have similar or even surpass the functional properties of their dairy counterparts. Hence, starch potentiality to mimic cheese’s properties should be further investigated to deliver better functionality of non-dairy imitation cheese.

Despite the potential in delivering stringiness, starch is incapable to achieve desired stretch length of Mozzarella cheese, limiting its application in imparting stringiness in vegan cheese. This is attributed to the molecular structure of gelatinized starch. The amylose and amylopectin molecules form a cohesive network through extensive hydrogen bonding, both within and between chains. It is postulated that the stretching of starch paste mimics the mechanism that of casein network in natural mozzarella cheese. When the starch paste is stretched, the molecules slide past each other until the intermolecular hydrogen bonds breaks, leading to a fracture. However, the stretchability is limited as the movement of the molecules is hindered by physical entanglements within the network.

The blend of tapioca starch, glutinous rice flour, and rice flour is hypothesized to enhance molecular gliding and promote a stringier texture in starch-based pastes. Tapioca starch, known for its high amylopectin content, forms a smooth, elastic gel, facilitating better molecular movement. Glutinous rice flour, being predominantly amylopectin, is expected to contribute to a cohesive, stretchy network. Meanwhile, rice flour, which contains shorter amylose molecules, may act as a chain extender during stretching by promoting inter- and intra- molecular interactions. These shorter amylose chains could enhance the flexibility of the network, allowing the molecules to stretch further before breaking. Additionally, the inclusion of glycerol monostearate (GMS) as an emulsifier is believed to play a role in spacing the starch molecules apart, reducing the overall cohesiveness of the network. This spacing effect could further enhance molecular gliding, reducing resistance during stretching and contributing to improved stretchability and stringiness of the paste. However, this combination of starches and GMS has not been studied or reported elsewhere, presenting a research gap worth further investigation.

1.3 Objectives

The primary objective of this study is to enhance the stringiness properties of a composite flour made from tapioca starch and glutinous rice flour by incorporating rice flour and glycerol monostearate (GMS).

The specific objectives are:

i. To elucidate stringiness properties of tapioca starch and glutinous rice flour blends at varying ratios in terms of rheological properties.

ii. To assess the impact of rice flour on the rheological properties of tapioca starch and glutinous rice flour composites.

iii. To investigate the effects of different concentrations of glycerol monostearate (GMS) on the rheological and stringiness properties of the composite flour.

CHAPTER 2

LITERATURE REVIEW

2.1 Cheese classification and foundations of imitation cheese development

Traditionally, cheeses are produced using dairy milk. The growing interest into imitation cheese is driven by sustainability, health considerations and cost-effectiveness which leads to the exploration in key ingredients that make these alternatives possible.

2.1.1 Classification of cheese

Cheese is a popular food that creates a sense of comfort to many people. It can be enjoyed on its own or used as an ingredient in a wide range of dishes. Aside from being a versatile ingredient, cheese is also an excellent source of protein and calcium. Cheese is a dairy product made by curdling milk, typically from cows, buffalo, goat, or sheep. The production of different types of cheese, such as Brie, Parmesan, Mozzarella, and Cheddar, involves typical manufacturing techniques that developed distinctive cheese flavour profile, textures, and colours, that offering a unique culinary experience to those eating the foods cooked or garnished with cheese.

Cheeses could be classified into natural cheese, processed cheese, and imitation cheese (Figure 2.1). Natural cheese is produced by coagulating dairy milk, resulting in a delightful blend of flavours and textures. A product is labelled as "cheese" when it meets specific regulatory guidelines, which vary depending on the type of cheese. For instance, the term "mozzarella cheese" can only be used when the product meets FDA standard as shown in Table 2.1 (FDA, 1993). Natural cheese which further grind, mix and heat with other dairy ingredients with or without emulsifier produces "processed cheese product". According to FDA (1993), processed cheese food contains a minimum of 51% cheese as an ingredient. On the other hand, imitation cheese or cheese analogue is a product that mimic properties of cheese but did not comprise of natural cheese as main ingredient. Imitation cheese could be produced using dairy-based individual ingredients, a mixture of dairy and plant-based ingredients, or only plant-based ingredients, which further categorised imitation cheese into dairy, partial dairy and non-dairy imitation cheese respectively.

Table 2.1 Classification of mozzarella cheese according to FDA (1993).

Type of mozzarella	**Moisture Content (%)**	**Fat in Dry Matter (%)**
Mozzarella	52 – 60	≥ 45
Low-moisture mozzarella	45 – 52	≥ 45
Part-skim mozzarella	52 – 60	30 – 45
Low-moisture part-skim mozzarella	45 – 52	30 – 45

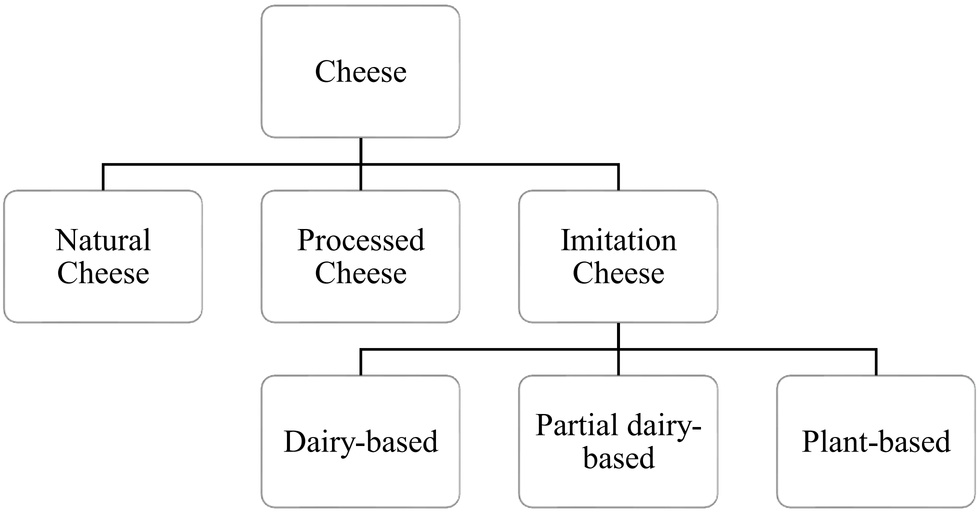

Figure 2.1 Classification of cheese.

2.1.2 Drivers in Imitation Cheese Development

Imitation cheese has gained significant attention in recent years due to various challenges faced by food industry, including sustainability, health considerations and cost-effectiveness. Dairy industry has been related to the emission of greenhouse gases, soil pollution and water pollution, which brings up the environmental concern of the production of dairy products (Clay *et al.*, 2020). According to Grossmann and

McClements (2021), transformation from consumption of dairy cheese to plant-based cheese aids in mitigating global warming due to lower carbon dioxide emissions. With the growing popularity for cheese, cheese analogue which utilizes non-dairy ingredients offer a promising solution in conserving environment without compromising the enjoyment of cheese. Besides, consumers' awareness about animal welfare, environmental impact, and personal health have fuelled the growth of plant-based diets. Developing innovative products using plant-based ingredients like imitation cheese cater to the growing vegan and flexitarian population while offering a familiar cheese-like experience. Imitation cheese also presents a viable option for lactose-intolerant individuals to enjoy the taste and texture of cheese without the associated health concerns.

Plant-based food showed a healthy market growth with steady expansion. The global plant-based food market is expected to project at 12.2 % at compound annual growth rate from 2023 to 2033 (Future Market Insights, 2023). According to Allied Market Research (2020), vegan cheese market is estimated to grow at compound annual growth rate (CAGR) of 15.5 % from 2021 to 2027. On the varieties of cheese, mozzarella helds the major share of 30.4% in 2019, and is expected to sustain as a major share in 2027. Imitation cheese provides a cost-effective alternative to conventional dairy cheese. By utilizing affordable plant-based ingredients and a relatively simple manufacturing process, imitation cheese is cheaper and more accessible. Additionally, it offers customizable convenience due to its flexibility in composition and nutrition. By reducing or eliminating dairy components such as milk fat and protein, manufacturers can create imitation cheeses that meet a wide variety of dietary preferences and needs. For example, a low-fat cheese analogue can be produced by replacing milk fat with polysaccharides. Furthermore, the diverse gelling properties of starches enable manufacturers to customize the meltability of imitation cheese products (Ahsan *et al.*, 2023; Bi *et al.*, 2016; Butt *et al.*, 2020).

While imitation cheese is a valuable option for those seeking dairy-free or vegan alternatives, it often falls short in replicating the rich mouthfeel and functionality of traditional cheese. To meet consumer demand, the imitation cheese market is continually evolving, with manufacturers striving to enhance taste, texture, and functionality. As the market expands, further innovation and refinement will be necessary to provide consumers with cheese alternatives that offer the desired taste, functionality, and nutritional profile, catering to a wide range of dietary needs and preferences.

2.1.3 Key Ingredients of Imitation Cheese

The formulation of cheese analogues is distinguished by the inclusion or exclusion of dairy ingredients. Generally, casein is used in dairy-based imitation cheese to achieve expected cheese's functionality. Contrary, plant protein will be used in formulating dairy-free cheese analogue. When plant protein could not match the functionality of casein, other non-protein ingredients will be included to enhance quality and sensorial of cheese analogue.

2.1.3(a) Casein

The curdling of milk during cheese production is attributed to casein content in milk. Hence, casein or casein salt is a typical ingredient in the formulation of partial dairy imitation cheese to impart cheese's texture and functional properties. Partial dairy imitation cheese usually contains 5 to 29% of casein or casein salt (Ahsan *et al.*, 2023; Butt *et al.*, 2020; Kiziloz *et al.*, 2009; Mounsey & O'Riordan, 2008d; Noronha *et al.*, 2008b; Ørskov *et al.*, 2023). Other casein products used include micellar casein concentrate, which is produced through membrane filtration of skim milk, has the advantage in heat stability and high protein content (Hammam & Metzger, 2023; Salunke *et al.*, 2022). Other than using rennet-coagulated casein, Dharaiya *et al.* (2019) included acid casein in their formulation and observed imitation cheese produced using mixture of rennet and acid casein has better baking qualities.

2.1.3(b) Plant protein

In the case of dairy-free imitation cheese, the use of casein is prohibited. Hence, plant protein is often used to replace casein in imitation cheese production to act as structure provider and protein source. The plant protein used include chickpea protein concentrate, gluten, pea protein isolate, and soy protein isolate with the concentration range of 7 to 41% (Grasso *et al.*, 2022; Mattice & Marangoni, 2020; Moon *et al.*, 2021). The type and concentration of plant protein could influence the textural and functionality of plant-based cheese. According to Grasso *et al.* (2022), chickpea protein contributed to adhesiveness, springiness and cohesiveness, but did not impart meltability upon heating. Mattice and Marangoni (2020) attempted to deliver meltability in plant-based cheese and discovered that incorporating 30% of zein, a corn-derived protein, in replacement of starch and fat constituents, displayed melting effect due to the heat-weakened non-covalent interactions.

2.1.3(c) Plant-based milk

For dairy-free imitation cheese, plant-based milk is employed as the main ingredient in consideration of its nutritional advantages. Cheese produced from plant-based milk was reported as source of essential fatty acids, is rich in protein, and also contains fibre (Chumchuere *et al.*, 2000; Oyeyinka *et al.*, 2019; Zulkurnain *et al.*, 2008). Among the plant-based milk, soy milk has been extensively used in production of plant-based cheese alternative. Instead of using direct soy milk produced from soybeans, Chumchuere *et al.* (2000) reconstituted soy milk from low fat soy milk powder. In imitation cheese production, soy milk is made into soy solid via lactic acid fermentation by bacteria culture or coagulation using coagulant such as glucono-δ-lactone and calcium sulphate (Chumchuere *et al.*, 2000; Li *et al.*, 2013; Zulkurnain *et al.*, 2008). Li *et al.* (2013) adopted hybrid coagulation method to produce soy solid and resulted a stable homogeneous imitation cheese structure with an improved sensorial attribute. Furthermore, Oyeyinka *et al.* (2019) used blended milk in cheese analogue manufacturing and concluded that substituting 40% of soy milk with cashew nut milk produce cheese analogue with preferred flavour, colour and overall acceptability, in addition to increased protein content.

2.1.3(d) Fat

Fat content of cheese could range from 0.42 to 34.9% depends on type of cheese and the manufacturing method (Gunasekaran & Ak, 2002). Fat acts as a plasticizer in providing smooth homogeneous texture and flavour carrier, as well as a major contributor to meltability of cheese (Fox *et al.*, 2017; McMahon & Oberg, 2017). With the understanding in functional role of fat, it is not surprising that the quality of cheese is being compromised if milk fat is replaced with vegetable oil/fat. This is evidenced by Dinkci *et al.* (2011) who observed reduced meltability and textural attributes when milk fat is replaced with vegetable fat blend. Cunha *et al.* (2013) substituted butter oil with partially hydrogenated soybean fat or soybean oil in spreadable cheese analogue and resulted reduced meltablity, spreadability and overall sensory acceptability but greater hardness. Despite this deteriorated quality, vegetable oil is considered a healthier option compared to milk fat because vegetable oil benefits by reducing cholesterol content and improving fatty acid profile of imitation cheese as vegetable oil has healthier saturated to unsaturated fats ratio (Giha *et al.*, 2021; Lobato-Calleros *et al.*, 1997). Other examples of vegetable fat/oil used in imitation cheese are rapeseed oil, canola oil, sunflower oil, coconut oil, rice bran oil and palm oil. To achieve both cheese's quality and health benefit simultaneously, Moon *et al.* (2021) suggested oleogel inclusion in imitation cheese. They demonstrated improved textural and rheological properties of imitation cheese by using canola oil oleogel.

2.1.3(e) Starch

Despite protein, starch appears as the most used ingredients in formulating imitation cheese. Starch plays a pivotal role in partial- and non-dairy imitation cheese, commonly used as casein or/and fat replacer to build structure through the interactions with other components within cheese analogue. Based on Table 2.2, different types of starch with varies concentration have been incorporated into imitation cheese in the effort of imitating properties of cheese. Due to the challenges to act as casein-mimetic, starch is often used alongside with casein in the formulation for the best results.

The inclusion of starch influences the quality of imitation cheese. According to Ørskov *et al.* (2021), modified starch with emulsifying ability decreased size and fat droplet and impaired meltability of imitation cheese. The significant effects of starch on the imitation cheese's properties is attributed to the altered microstructure of casein-containing imitation cheese by starch inclusion. Swollen granules of gelatinized native corn starch formed coalesced starch mass in imitation cheese matrix and tend to disrupt the continuity of casein network (Noronha *et al.*, 2008a). This situation even worsens for pre-gelatinised corn starch because pre-gelatinised starch easily imbibed to free water at low temperature (Noronha *et al.*, 2008b). Resistant starch in contrast remains unswollen upon heating and has lower water holding capacity. With minimum interactions with casein, resistant starch embedded within casein matrix as small discrete particles hence allowed continuity of casein matrix (Noronha *et al.*, 2008a; Noronha *et al.*, 2008b). A different perspective was presented by Mounsey and

O'Riordan (2008b), which may be attributed to the varying types and levels of starch inclusion. They concluded that when native wheat starch is included at levels of 3 to 9%, it acts as a filler dispersed within casein matrix, resulting in the dominance of casein in determining the product's rheological properties at ambient temperatures. However, Ye and Hewitt (2009) suggested that native potato starch could act as filler at concentrations ≤4%. At concentrations above 4%, native potato starch formed a continuous network, creating a binary continuous phase of protein and starch. In bi-phase system, the thermodynamic incompatibility between starch and casein caused a rise in G' and a reduction in tan δ. Although starch shows promise in imitation cheese, the extent to which it can replace casein and fat without significantly compromising the product's attributes is often limited.

Imitation cheese provides a valuable alternative for those seeking dairy-free or vegan options, though it often falls short in replicating the rich mouthfeel and functionality of natural cheese. The use of starch in imitation cheese production has gained attention for its potential to deliver functional properties similar to those of conventional cheese. Lyu *et al.* (2023) proposed a starch-protein-oil gels using oxidized potato starch as main ingredient that could demonstrate melting properties and suitable to be used in vegan cheese. Studies also have demonstrated starch's ability to gel and even mimic fat perception (Table 2.2).

Table 2.2 Utilization of starch in imitation cheese and its effects on imitation cheese's quality.

Type of imitation cheese	Starch used	Percentage of starch (%)	Reason of starch inclusion	Effects on imitation cheese's quality	Reference
Partial dairy	Native potato starch; oxidized potato starch	1.3–4.98	To replace 15% casein or/and 5% fat	Meltability was not influenced by the addition of native starch when starch is used for combined casein and fat substitution, however, was slightly reduced by oxidized starch. Shredding and cutting of cheese was ease by the inclusion of oxidized starch as the elasticity of cheese was reduced.	Ahsan *et al.* (2023) and Ahsan *et al.* (2024)
Partial dairy	Maize resistant starch	7.3–17.3	To replace 20–100% fat	Total replacement of fat with resistant starch allowed microwave expansion producing healthy crispy snack containing functional fibre, had high protein and no fat.	Arimi *et al.* (2008)
Partial dairy	Rice resistant starch	2.4–12	To replace 10–50% fat	With increase resistant starch content, hardness increased, whereas cohesiveness, springiness, meltability and stretchability reduced. Resistant starch could be used to replace up to 30% of the fat in imitation cheese.	Bi *et al.* (2016)
Partial dairy	Native rice starch; pre-gelatinized rice starch	3.675	To replace 15% casein or 15% casein and 10% fat	The resultant imitation cheese was softer, more cohesive, and possessed improved melting properties.	Butt *et al.* (2020)
Partial dairy	Pre-gelatinized starch	2	As a binder	NA	Dharaiya *et al.* (2019)

Table 2.2 Continued.

Partial dairy	Maize resistant starch	8.9–18.2	To replace 38.7–100% oil	Without water competition with protein, resistant starch allowed plasticization effect of water to maintain good functionality of cheese.	Duggan *et al.* (2008)
Non-dairy	Yellow peas flour; faba beans flour	17.5	Provide nutritional value	The developed sliceable cheese analogue had high fibre content.	Ferawati *et al.* (2021)
Non-dairy	Chickpea flour	19.6–43.3	Provide nutritional value	The imitation cheese was not meltable. The higher starch content resulted harder cheese.	Grasso *et al.* (2022)
Partial dairy	Partially hydrolysed waxy maize starch	22–24.5	To replace 5–25% casein	The best meltability and textural properties of imitation cheese could be achieved by controlling the level of kappa-carrageenan and the extent of starch hydrolyzation.	Kiziloz *et al.* (2009)
Non-dairy	Corn and tapioca starch mixture	2.8–22.7	Stretchy in nature	The resultant cheese possessed slight stretch ability.	Mattice and Marangoni (2020)
Partial dairy	Granular resistant starch; retrograded resistant starch	5–12.5	To replace 30–75% hydrogenated oil	Hardness increased linearly with resistant starch content. Meltability was not affected by granular resistant starch but was decreased by retrograded resistant starch.	Montesinos-Herrero *et al.* (2006)
Non-dairy	Tapioca starch	4.47	NA	NA	Moon *et al.* (2021)
Partial dairy	Native maize starch; waxy maize starch; wheat starch; potato starch; rice starch	3	To replace 15% casein	Upon addition of starch, meltability and cohesiveness decreased. Wheat, potato and maize starch increased hardness, whereas opposite effect demonstrated by waxy maize and rice starch.	Mounsey and O'Riordan (2001)

Table 2.2 Continued.

Partial dairy	Native wheat starch	3–9	To replace 15–45% casein	Meltability of imitation cheese was reduced. Wheat starch had limited effects on the rheology of casein matrix at temperature below 50 °C.	Mounsey and O'Riordan (2008b)
Partial dairy	Waxy rice starch; regular rice starch; high amylose rice starch; pre-gelatinised rice starch; crosslinked rice starch; cross-linked acetylated waxy rice starch	3	To replace 15% casein	Hardness of imitation cheese decreased with decreasing amylose content except for crosslinked starch. All starched impaired meltability of imitation cheese.	Mounsey and O'Riordan (2008c)
Partial dairy	Pre-gelatinised maize starch	3–9	To replace 15–45% casein	Less homogeneous protein matrix due to water competition by starch. Imitation cheese containing high levels of pre-gelatinised maize starch was resistant to flow at temperatures above 60 °C.	Mounsey and O'Riordan (2008d)
Partial dairy	Native corn starch; pre-gelatinised corn starch; resistant corn starch; waxy corn starch	1.9–9.9	To replace hydrogenated oil	Increasing starch content decreased water mobility, which was related to the increased hardness and decreased meltability of imitation cheese.	Noronha *et al.* (2008a)

Table 2.2 Continued.

Partial dairy	Native corn starch; pre-gelatinised corn starch; resistant corn starch; waxy corn starch	10	To produced fat-reduced imitation cheese without impairing the functionality	NA	Noronha *et al.* (2008b)
Partial dairy	Potato starch acetate; potato starch acetate with octenyl succinic anhydride	8–25	To replace 26.5–100% casein	The hardness and rigidity increased, while elasticity decreased with increasing starch concentration.	Ørskov *et al.* (2021)
Partial dairy	Native potato starch; acetate potato starch; oxidized potato starch	10	NA	NA	Ørskov *et al.* (2023)
Partial dairy	Native potato starch	3–10	To replace 13.6–45.5% casein	Properties of imitation cheese was less affected when starch concentration was ≤ 4%.	Ye and Hewitt (2009)

2.2 Defining stringiness in cheese

Generally, stringiness has been used vaguely as a descriptive term or a textural parameter in different fields. For example, “stringy” is used interchangeably with “fibrous” to illustrate long strands or fibres showed in a crop, meat or meat-based product (Chong *et al.*, 2020; Leighton *et al.*, 2010; Leksrisompong *et al.*, 2012; Oupadissakoon *et al.*, 2010; Paulos *et al.*, 2015; Popoola *et al.*, 2019; Sato *et al.*, 2018). A different definition has been provided for molten cheeses and liquid-like food products, like syrup, caramel, and sauce. For these semisolid or liquid samples, stringy is used to describe the phenomenon when a long strand is formed when the product is being pulled apart (Gulzar *et al.*, 2020; Mayhew *et al.*, 2018; Molina-Rubio *et al.*, 2010; Okonkwo *et al.*, 2021; Sikora *et al.*, 2007). Similar description is reported in Savouré *et al.* (2021), where slimy okra is said to be stringy when exhibiting the ability to resist plastic deformation without breaking. On the other hand, stringiness attribute also being applied in cosmetic products such as skin cream, lotion and hair care products (Churchill & Greenaway, 2018), in which stringiness is known when long continuous strand is observed upon stretching.

In the context of cheese, stringiness is a desirable attribute of a mozzarella cheese when it is used as a topping or filling. When mozzarella cheese is heated, the casein strand is able to extend and elongate, creating long strings. This property is also known as stretchability or extensibility. Stringiness is the results of the ability of a molten cheese to form continuous fibrous strands which elongate without breaking when pulling force is applied (Yang *et al.*, 2016). This melt and stretch performance is influenced by various factors, such as ingredients used and manufacturing procedures applied to make the cheese, as well as the temperature of the cheese during stretching (Chen *et al.*, 2009; Feng *et al.*, 2021; Li *et al.*, 2019b). The evaluation of stringiness could be categorized into stretch quantity and stretch quality, which represents the extent of stretch and the ease to form fibrous strands when continuous stress is applied, respectively (Dharaiya *et al.*, 2019). Stringiness, as a significant functional property of mozzarella cheese, is difficult to be measured, especially without a standardized and objective measurement technique.

To study cheese stringiness, one needs to observe the response of the cheese strands formed when the testing probe was withdrawn above the cheese surface and before all the elongated strings or sheets of the molten cheese completely broken. Due to the lack of measurement guidelines, cheese stringiness analysis is very subjective. Reddy and Seib (2000) considered stringiness property was exhibited when the length of the pulling strand achieved at least 10 mm before ruptured. On the other hand, cheese stringiness could be analysed qualitatively by checking its response towards the pulling force. When a hot mozzarella cheese is stretched, the thickness of the fibrous strands could vary from thread-like strings to extended sheet. Interestingly, Gulzar *et al.* (2020) determined the cheese stringiness quality by the number of strings formed whereas Fife *et al.* (2002) recorded the weight changes of cheese strands during pulling. On the other hand, Chen *et al.* (2009) presented a more comprehensive index to evaluate cheese stretch thickness and it was stated that a thicker strand would serve a better quality (Figure 2.2). It is worth noted that, with the introduction of cheese extensibility rig in the past decade, additional parameters have been used to further describe cheese stringiness quality objectively, which includes the resistance to extension and work done/strength of cheese strand during extension (Feng *et al.*, 2021; Mattice & Marangoni, 2020; McCarthy *et al.*, 2016; Salunke *et al.*, 2022).

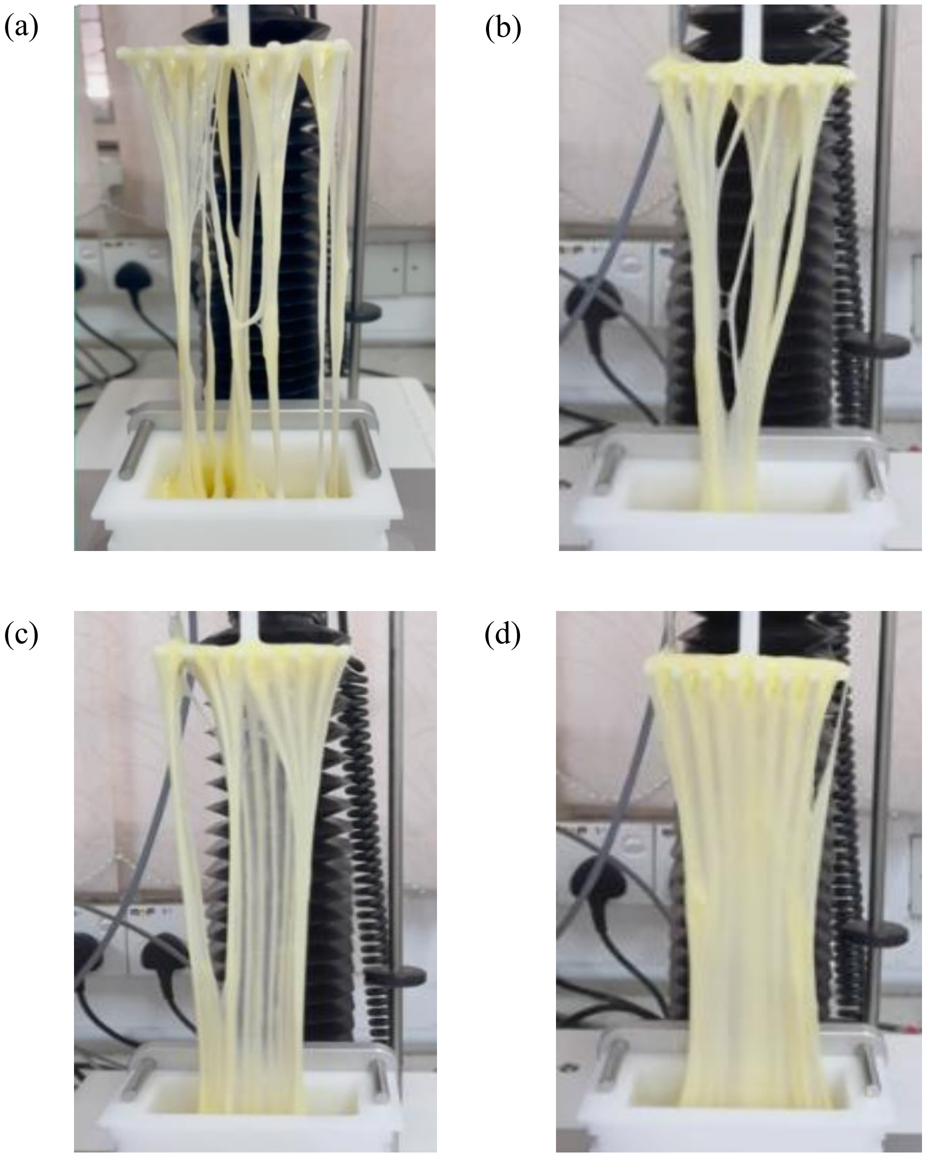

Figure 2.2 Cheese stretch formation are characterized as, (a) strings with very slight thickness (b) strings with slight thickness (c) thick strings (d) very thick sheet, when molten mozzarella cheese is being stretched using texture analyzer.

2.3 Measuring Technique of Cheese Stringiness

Stringiness is a critical and complex textural attribute. The relevance of stringiness attribute is subjective as unstandardized methods are employed for stringiness study. The importance of measurement technique, particularly for cheese, is well recognized as stringiness characteristics will ultimately determine the quality of cheese in applications as an ingredient. Difficulties have been encountered during the measurement of stringiness but continuous effort in development of the measurement technique have been made by the researchers since 1990's, and a holistic solution is yet to be discovered. Numerous measuring techniques of cheese stringiness are still adopted today regardless of their shortcomings. Custom-built stretch apparatus has been developed to tackle specific problems by controlling the experimental condition to collect further information on the textural properties of melted cheese. The indicator of stringiness should not be limited to stretch length, but several other testing parameters should also be analysed to provide an overall insight on stringiness property.

The most common stringiness measuring technique is tensile stretching (Table 2.3). Pizza stretch test used pizza or bread as a base, whereby an adequate amount of cheese is uniformly distributed on top of a pre-cut pizza, melted by subsequent baking process, following which the pizza is stretched apart. This uniaxial stretching measuring technique could be conducted using an apparatus controlled at a constant velocity or by hand stretching vertically or horizontally. Fork test is performed by stretching a molten cheese vertically using tines of a fork. In fork test, pizza is usually used as a base, cheese spread on the pizza will be melted before the fork tines are inserted into the molten cheese mass and lifted manually. According to Guinee and O'Callaghan (1997), disregard of the testing method, the amount of cheese spread on a pizza sample base is very crucial and it should not exceed 0.25 g/cm^2 to achieve a uniform cheese distribution. In addition, the testing temperature of molten cheese should be controlled to the best by setting a constant heating condition, including heating power, or heating temperature, heating time, and holding time of cheese before testing. Despite a stringent control over these parameters, the testing procedures itself may be a cause of uncertainties in the analysis. The fork test consists of several undefined variables, for instance, the direction and depth of fork insertion, the