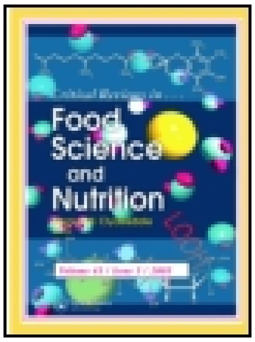

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REVIEW

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Exploring the potential of polysaccharides or plant proteins as structuring agents to design cheeses with sensory properties focused toward consumers in East and Southeast Asia: a review

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ABSTRACT

The focus of the global cheese industry on accessing new markets for cheese is currently driving a greater need for innovation in cheese products. Research to date suggests that, for example, East Asian consumers prefer cheeses that have a soft texture, with mild and milky flavors. Strategies for achieving such cheese characteristics are reviewed in this article. For example, incorporation of polysaccharides into cheese results in cheese with higher moisture levels and softer textures; this also results in modification of other properties such as adhesiveness, meltability and flavor release. Hydrated polysaccharides may be considered as filler particles within cheese matrices, and therefore filled gel models with suitable filler particles can be used to establish the effect of filler volume, size and surface properties on the fractural and rheological properties of cheese matrices, thus guiding the use of polysaccharides. Addition of plant proteins such as soy and pea protein can also result in cheeses with softer texture. Furthermore, it has been suggested that heat-induced gelation of soy or pea protein with casein results in a gel structure consisting of two independent protein gels, thus facilitating the design of bespoke structures by adjusting the ratio of the two proteins. Finally, it is proposed that incorporation of ingredients with sensory properties familiar to East and Southeast Asian consumers and with the capacity to achieve bespoke textures offer potential for the development of cheese products for consumers in these markets.

KEYWORDS

Cheese; East and Southeast Asian markets; Cheese structure; polysaccharide; plant protein; emulsion gel

Introduction

While the traditional diets of some Asian countries, such as India, contain cheese, cheese is not a traditional component of cuisines in most East and Southeast Asian countries, but it is gaining in popularity as consumers in these regions are living in an increasingly modern or westernized lifestyle, and as people appreciate its nutritional value. Japan has the most mature dairy market among East and Southeast Asian economies, attributed to the high income of Japanese consumers and their long exposure to westernized dairy products (Campo and Beghin 2006). This is also reflected in their consumption of cheese; Japan is considered to be the first Asian country in the region to significantly adopt cheese in its dietary intake, as per-capita consumption of cheese in the country increased rapidly since the 1960s (Campo and Beghin 2006), much earlier than for other countries in the region. Thus, its market development is of interest. The per-capita consumption of cheese in Japan increased from <0.1 kg per year in 1963 (Campo and Beghin 2006) to 2.6 kg per year in 2018 (CLAL 2019 Source:FAO, Eurostat). The increase in consumption was mainly attributed to rising individual income, favorable food policies that lower the price and increase availability of dairy products including cheese, and changing taste and lifestyle (Campo and Beghin 2006). A large increase in cheese consumption has also been observed in South Korea, where the annual per-capita consumption of cheese has tripled from ~0.9 kg in the beginning of this century (LAN 2017) to 3.19 kg in 2018 (CLAL 2019 Source:FAO, Eurostat); the highest per-capita cheese consumption in Asia.

China offers a significant opportunity if the same growth pattern of cheese consumption occurs, considering the huge population base of approximately 1.4 billion. Although China is already the world's fifth largest cheese importer (OECD and FAO 2018), the annual per-capita consumption is as low as 0.1 kg (CDIC 2017). Such a low consumption can mainly be attributed to the unfamiliarity of cheese; however, this situation is likely to change as young people are gaining more experience with cheese through conventional western foods and more innovative products and fusion foods, such as cheese tea (tea drinks covered with a cream and cream cheese mix) and rice cakes with cheese filling (Euromonitor International 2018). Other factors, such as

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rising income, increasing demand for convenient and healthy food, and a change away from traditional lifestyles, also increase the popularity of cheese products in China (Rong et al. 2019). It is estimated that the retail value of cheese in China will increase at an annual rate of 12% during the forecast period of 2019–2024 (Euromonitor International 2019). Similar trends are also reported in many other Southeast Asian countries such as Indonesia (Euromonitor International 2020a) and Malaysia (Euromonitor International 2020b).

Nutrition is also an important factor contributing to the growth of cheese in Asian markets. A high prevalence of lactose intolerance in Asian populations limits the consumption of milk as a nutritional source (Goh, Said, and Goh 2018); however, cheese, in particular hard cheeses, which contain little or zero lactose (Monti et al. 2017), provides an alternative nutritional source for lactose-intolerant individuals. The composition of cheese varies with type, but generally cheese is high in fat and saturated fat. Previous concerns regarding possible negative health implications of cheese consumption due to the high saturated fat content have prompted research focused on reducing fat in cheese. However, recent research (Nilsen et al. 2015; Raziani et al. 2016; Feeney et al. 2018) has suggested that consumption of saturated fat in cheese does not lead to increased levels of blood triglycerides and cholesterol levels, possibly due to the "cheese matrix" effect. The protein content of cheese ranges from 3% to 40%, depending on the variety of cheese, and several bioactive peptides, resulting from proteolysis during cheese production and ripening, have been shown to have health benefits, such as antioxidant, anti-inflammatory and antihypertensive effects (O'Brien and O'Connor 2017). Cheese also contains considerable amounts of bioavailable calcium, and 50 g of semi-hard or hard cheese can provide one third to half of the daily calcium intake requirement (1200 mg). Cheese is also a good source of phosphorus, zinc and magnesium, while vitamins A, B_2, B_6 and B_{12} are also abundant in cheese (Walther et al. 2008). Furthermore, cheese is an ideal food for fortification with vitamin D, due to its high fat content.

Cheese undergoes continuous structural changes during oral processing, during which dynamic perceptions of flavor and texture are observed (Khanal et al. 2020). Thus, cheese with different structures can result in different oral processing patterns and in different patterns of flavor and aroma release, which in turn are strongly correlated with flavor and texture perception (Pascua, Koc, and Foegeding 2013). Nondairy polymers have been shown to be promising ingredients to attract water, and to participate in and manipulate cheese structure, either by interacting with casein micelles *via* electrostatic interaction or by inducing micro-phase separation (Corredig, Sharafbafi, and Kristo 2011). It is therefore proposed that flavor and texture attributes appealing to Asian consumers could potentially be created by modulating cheese structures. This review initially profiles the preferences of Asian consumers for cheese products, and then focuses on how nondairy polymers may be incorporated into cheese manufacture processes to achieve flavor, texture and other properties that may be desired by such consumers.

Understanding the preference of Asian consumers for cheese products

Processed cheeses often have milder flavors compared to natural cheeses, and can also provide greater versatility in terms of flavor, texture and functionalities, and are therefore the most popular cheese type in China (Rong et al. 2019). Natural cheese, on the other hand, offers unique fermented flavors and diverse textures (Kilcawley 2017), and is perceived as a healthier option than processed cheese by consumers (Euromonitor International 2019). The strong flavor of natural cheese is thought to be the most significant barrier to average Chinese consumers (Euromonitor International 2019). However, there is limited information available defining which specific flavor attributes of natural cheeses are considered too strong, unacceptable, or simply not familiar to Chinese consumers.

Zhang et al. (2011) conducted a consumer preference test among 217 Beijing youths (aged between 12 and 25) for seven cheese varieties, and descriptive sensory analysis was conducted by 7 trained panelists. Several descriptive attributes, including "bitter", "salty", "umami", "free fatty acid" flavors, and "firm" texture, were considered to be drivers of disliking. In contrast, descriptive attributes such as "milky", "soured milk", "sour flavor", and "slimy", "moist" and "rate of breakdown" were associated with liking of cheese. "Bitter", "umami", and "free fatty acid" flavors are associated with proteolysis and lipolysis during cheese ripening. These explained why two soft unripened cheeses (Cream cheese and Cottage cheese) received the highest scores among the 7 cheeses tested. Rong et al. (2019) also reported that the most preferred texture descriptors are "fine and smooth", and "soft", and the most preferred flavor descriptors are "milky" and "sweet", based on the results of an online survey regarding cheese among Chinese consumers ($n = 1260$). That suggested that Chinese people expect the flavor of cheese to be milky and the texture of cheese to be soft, such as in children's snack cheese, and that cheese in hamburgers and pizza are the main cheese formats which most people have experienced. However, this is not to say that all ripened cheese flavors are not accepted by Chinese consumers or that ripened cheese will not ever be accepted by Chinese consumers. For example, five different clusters of consumers were identified among Beijing youth, and each cluster showed different preferences toward cheese. It was reported that "nutty" flavor, as a typical ripened cheese flavor, was desired by a consumer cluster that consumed cheese more frequently (Zhang et al. 2011), suggesting that certain ripened cheese flavors might be appealing to certain Chinese consumer clusters.

The current preference for cheese in many Asian countries reflects that of Japanese consumers over a number of decades, where the low intensity flavor and the texture of processed cheese was considered more acceptable than natural cheese. However, natural cheese consumption and the

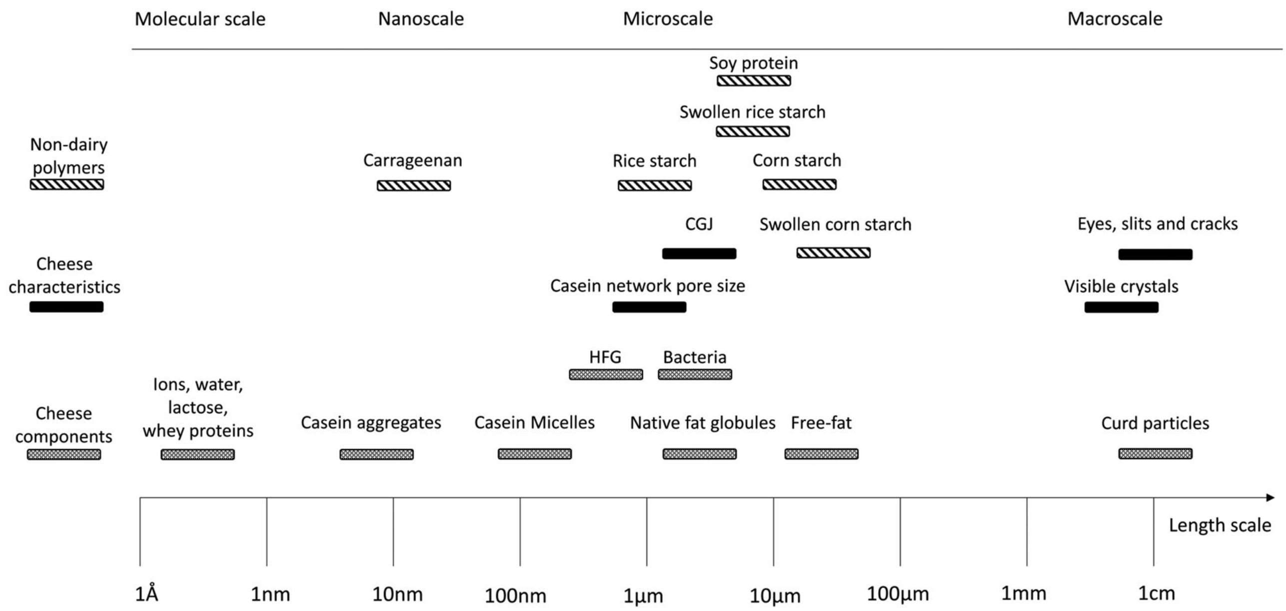

Figure 1. Length scales in cheese. HFG: homogenized fat globules. CGJ: curd granule junction. Adapted from (Lamichhane, Kelly, and Sheehan 2018).

acceptability of natural cheeses have increased over time in Japan, as a result of overall changes in dietary habits (Morita et al. 2015). Morita et al. (2015) identified that the aroma compound γ-dodecalactone (which contributes to milky flavor) was a key factor in overall liking of Cheddar cheese among Japanese consumers, and it was also shown that Japanese consumers prefer mild cheese flavor to characteristic cheese flavors. Go, Kim, and Chung (2017) applied check CATA (check-all-that-apply) methodology to understand sensory drivers that affect the liking/disliking of six different cheese samples among Korean consumers, with and without repeated exposure to these cheeses. It was found that the liking for some natural cheeses (Brie, Emmental, Gouda, and sharp Cheddar cheese) can increase over repeated exposure; the main positive attributes in earlier exposure sessions are flavor familiarity and soft texture, and selection frequencies of other terms such as "mild flavor", and "like with no particular reason" increased in later exposure sessions.

Future sensory research is still required to capture consumer preferences in evolving Asian markets, which include: evaluating cheeses with more diverse sensory attributes to screen for other potential sensory attributes that might be desirable or undesirable to Asian consumers; and exploring if changes in such sensory attributes can change the liking of cheese. In addition, it may be of benefit to assess the preference for different cheeses within the end use matrices, similar to the way that Mozzarella cheese is often assessed on prepared pizzas for sensory properties and functionalities (see for example, Banville et al. 2015), as cheese producers are also targeting food service opportunities by exploring cheese applications in traditional Asian cuisines, fusion foods, or as a complement to other food. Qualitative approaches are also recommended to understand the 'how', 'why', and 'what' aspects of consumer behavior regarding cheese products, providing insights into underlying motives and attitudes (Gambaro 2018).

Nonetheless, a pattern is emerging from the available data, suggesting that consumers in these Asian countries prefer cheeses that have mild and milky flavor and a softer texture (Zhang et al. 2011; Morita et al. 2015; Go, Kim, and Chung 2017; Euromonitor International 2019; Rong et al. 2019). It is important to consider what soft texture means. First bite firmness, which is often used in descriptive sensory analysis and is defined as the amount of force needed to bite through the sample (Zhang et al. 2011), is a good indicator of the hardness of a cheese. However, texture perception is a complex and dynamic process, and the structural breakdown pattern during oral processing is also important to consider. Cheeses with the same first bite firmness might have different overall perception of hardness, as changes in hardness during mastication differ between cheeses with different structures. Cheeses that are easier to be broken down during mastication will also be perceived as having a softer texture. This highlights the need for an understanding of how structural transformations during the entire process of oral processing affects texture perception (Foegeding, Stieger, and van de Velde 2017; Wee, Goh, Said, and Goh 2018).

Cheese structure

Cheeses have very complex and hierarchical structures, characterized at different length scales, ranging from molecular scale to macroscale, which are formed by interactions and spatial arrangements of different components such as protein and fat (Lamichhane, Kelly, and Sheehan 2018). How different molecules are arranged and interact with each other are dependent on the characteristics of the molecules (size, charge density, shape, stiffness, etc.), the environmental conditions (volume fraction of molecules, temperature, pH, ionic strength, etc.), the processing techniques (e.g., stretching in Mozzarella cheese), and biochemical processes (e.g., proteolysis, lipolysis).

Table 1. Impact of addition of polysaccharides into cheese matrices on texture.

Matrix	Polysaccharides and concentrations	Altered attributes resulting from the addition of polysaccharides	Analytical method used for texture	Reference
Fresh semi-hard model cheese	ι -carrageenan, low acyl gellan, and a mixture of κ -carrageenan and locust bean gum (50%:50%) (0.04%wt)	Higher moisture content Lower hardness Lower fracture stress Lower cohesiveness and spring index (low acyl gellan)	Uniaxial compression test and texture profile analysis	(Benjamin et al. 2018)
Low-fat white brined cheese	Basil seed gum (0.00012%wt and 0.00048%wt)	Higher moisture content throughout ripening Lower hardness at day 60 of ripening Lower adhesiveness	Texture profile analysis	(Baghdadi et al. 2018)
Low-fat Iranian white cheese	Guar gum (75-300 ppm)	Higher moisture content Lower storage modulus and fracture stress	Uniaxial compression test	(Lashkari et al. 2014)
Low-fat Cheddar cheese	Amidated, high-methoxy, and low-methoxy pectins (1.95%, 2.60%, and 4.55% w/v)	Higher moisture content Lower hardness, springiness	Texture profile analysis	(Ibanez, Waldron, and McSweeney 2016)
Low-fat Cheddar cheese	Exopolysaccharides produced by *Lactococcus lactis ssp. cremoris*	Higher moisture content and moisture in nonfat substances Lower hardness, fracture stress, springiness, and chewiness Higher adhesiveness Descriptive sensory analysis: Lower hardness Higher degree of breakdown Higher cohesiveness, adhesiveness, and smoothness	Texture profile analysis and descriptive sensory analysis	(Costa et al. 2010)
Low-fat Cheddar cheese	Sodium alginate (0.12%, 0.17%, 0.18%, 0.23%wt)	Lower hardness, chewiness, and gumminess	Texture profile analysis	(Khanal et al. 2018)
Low-fat Cheddar cheese	Sodium alginate (0.12%wt)	Increased preference for creaminess Lower coefficient of friction	Preference ranking test by a semi-trained panel and tribological measurement	(Khanal et al. 2020)
Low-fat and no-fat Mozzarella cheese	Konjac glucomannan (0.025%w/v)	Higher moisture content Lower firmness of grated Mozzarella	Texture analyzer with TTC spreadability Rig	(Dai et al. 2018)
Reduced-fat white brined cheese	β-glucan (0.4%wt)	Higher moisture content and moisture in nonfat substances Lower hardness and brittleness (fracture stress)	Uniaxial compression test	Kondyli et al. (2020)

Cheese can be described as having an emulsion-gel structure, in which caseins act as the main structural components to form a porous casein matrix, and localized domains of fat can be found interspersed in the matrix (Vogt et al. 2015). Other components, including water, minerals, bacteria, and dissolved solutes, are also interspersed in the cheese gel matrix (Lamichhane, Kelly, and Sheehan 2018). Cheeses with different structures have significantly different characteristics; for example, Mozzarella cheese contains fat and serum channels between parallel casein fibers, which results from stretching steps during processing, while Cheddar cheese has an isotropic casein gel network interspersed with fat globules (Vogt et al. 2015). Introduction of new polymer ingredients into cheese matrices will induce structural and sensory changes. The relative scale of principal components of cheese and selected nondairy polymers are shown in Figure 1. Methods commonly applied to analyze the structure of cheese at various length scales include rheology, differential scanning calorimetry, microscopy, and light-scattering (Everett and Auty 2008), while novel methods for measuring and visualizing microstructure were reviewed by El-Bakry and Sheehan (2014) and Verboven, Defraeye, and Nicolai (2017).

Use of polysaccharides as ingredients to modify cheese structure

Polysaccharides are traditionally used in certain dairy products as thickeners, stabilizers or gelling agents. Recently, polysaccharides (e.g., carrageenan, starch) have also been exploited in a wide variety of cheese matrices to evaluate their effect on coagulation, structure, mouthfeel, flavor, and functionality (Brown, McManus, and McMahon 2012; Benjamin et al. 2018). The use of polysaccharides in cheese was able to achieve a softer texture in different cheese matrices, which is preferred by Asian consumers (Table 1). The change toward a softer texture was mostly characterized by mechanical tests instead of direct sensory analysis in the listed studies. Rheological tests such as compression testing and texture profile analysis were often applied, since they give good indications to some sensory attributes and it is not always possible to carry out sensory analysis. A uniaxial compression test mimics the first bite of oral processing and generates parameters such as firmness and fracture stress, while texture profile analysis mimics the first two bites of oral processing and generates parameters such as firmness and adhesiveness (Joyner 2018). First bite sensory attributes such as hardness are generally well-correlated with rheological parameters such as firmness and fracture stress (Foegeding, Stieger, and van de Velde 2017). Casein mainly gives the cheese matrix its rigid structure, and therefore increased moisture levels in cheeses containing polysaccharides with high water-holding capacity (which is often accompanied by a decrease in relative casein concentration) usually results in lower hardness.

Springiness and chewiness of cheese are also influenced by the decrease in relative casein density. As less casein needs to be deformed per unit volume, cheese containing polysaccharides often has a lower springiness value. Chewiness is derived by multiplying values for hardness, cohesiveness, and springiness, and therefore cheese containing polysaccharides often has lower chewiness as well (Wee, Goh, Said, and Goh 2018). These parameters are correlated with breakdown attributes from sensory analysis. For example, the degree of breakdown, as measured by descriptive sensory analysis, is significantly higher in low-fat Cheddar cheese containing exopolysaccharides than in cheese without exopolysaccharides (Costa et al. 2010). The adhesiveness of cheese reflects moisture-casein matrix interaction, and addition of polysaccharides into the cheese matrix could increase water retention, moisture-casein matrix interactions, and adhesiveness (Costa et al. 2010).

While standard rheological tests can provide useful information about certain texture attributes, some more complex texture attributes such as creaminess perceived at the later stages of oral processing are harder to explain. Novel techniques, such as tribology which measures food friction and lubrication behavior (Joyner 2018), have been used to provide insight into these complex texture attributes, and will be of significant use in future studies, once correlations between these techniques and sensory attributes are better established. For example, Khanal et al. (2020) used tribology to explain why low-fat Cheddar cheese containing sodium alginate ranked higher in preference of creaminess compared to the low fat control. Depending on their individual specific properties and on concentrations used, polysaccharides will exert different influences on the various process steps of cheese manufacturing, and thus will impact on the final structure of cheese. These are discussed in the following sections.

Principles of interactions between polysaccharides, caseins, and the cheese matrix

Interactions between polysaccharides and proteins can be segregative or associative in nature (Corredig, Sharafbafi, and Kristo 2011). Associative interactions between polysaccharides and casein are usually electrostatic, where the oppositely charged sites interact to form complexes. Hydrogen bonds and hydrophobic interactions are also considered to play a role in stabilizing such complexes (Corredig, Sharafbafi, and Kristo 2011). Gelation of such complexes often results in gels with hindered casein-casein crosslinks. When mixing incompatible polysaccharides with casein, the polysaccharides can be excluded from the surface of the proteins due to incompatibility, resulting in an excluded (depletion) layer. The osmotic pressure generated by polysaccharides is lower in the depletion layer than in the bulk. Therefore, if the two particles meet because of Brownian motion, the available volume for polysaccharides increases, which then increases the systems' free energy, and aggregation of protein particles is promoted. Above a certain concentration, the system could become unstable and phase-separated, which is not ideal for cheesemaking. Below the critical concentration, cheese containing dispersed polysaccharide can be made with an inhomogeneous structure (polysaccharide-rich areas and casein-rich areas). Mechanisms of how polysaccharides may interact with casein and the casein matrix in cheese are illustrated in Figure 2.

Effect of polysaccharides on rennet coagulation

Coagulation is an essential step of cheese manufacture, where destabilization and coagulation of casein micelles occurs. Casein micelles are stabilized in two ways: (1) by electrostatic repulsion due to a surface zeta potential of about $-20\,\text{mV}$ at pH 6.7 and (2) steric repulsion because of the protruding C-terminal at the end of κ-casein (Fox et al. 2015). Rennet addition reduces surface zeta potential from $-20\,\text{mv}$ to $-10\,\text{mv}$ and removes the steric stabilizing layer of casein micelles by specifically hydrolyzing the Phe_{105}-Met_{106} bond of κ-casein. The addition of polysaccharides may interfere or enhance coagulation, depending on the concentration and molecular properties of the polysaccharide added.

When a non-interactive polysaccharide is added during the rennet coagulation process, protein aggregation caused by depletion flocculation occurs in combination with the aggregation process, often leading to an earlier onset of gelation and a stronger gel firmness. This is the case for many negative charged polysaccharides (Fagan et al. 2006) and uncharged polysaccharides (Tan et al. 2007; Mende, Jaros, and Rohm 2019). Mende, Jaros, and Rohm (2019) showed that dextran addition (1%-3% wt/wt) reduced rennet coagulation time and increased maximum gelation speed and gel stiffness. The presence of dextran also resulted in a more inhomogeneous gel microstructure, with larger pores and a higher degree of protein aggregation. Similar observations were made in rennet-induced milk gels containing other polysaccharides, such as gum acacia (1-3%), pectin (0.2%), and guar gum (0.025% and 0.05%), where the addition of these polysaccharides reduced rennet coagulation time and increased maximum gelation speed and gel stiffness (Fagan et al. 2006; Galante et al. 2017). In addition, the addition of these polysaccharides at these concentrations also changed the rennet gel from a homogeneous to a heterogeneous microstructure (Fagan et al. 2006; Tan et al. 2007; Galante et al. 2017).

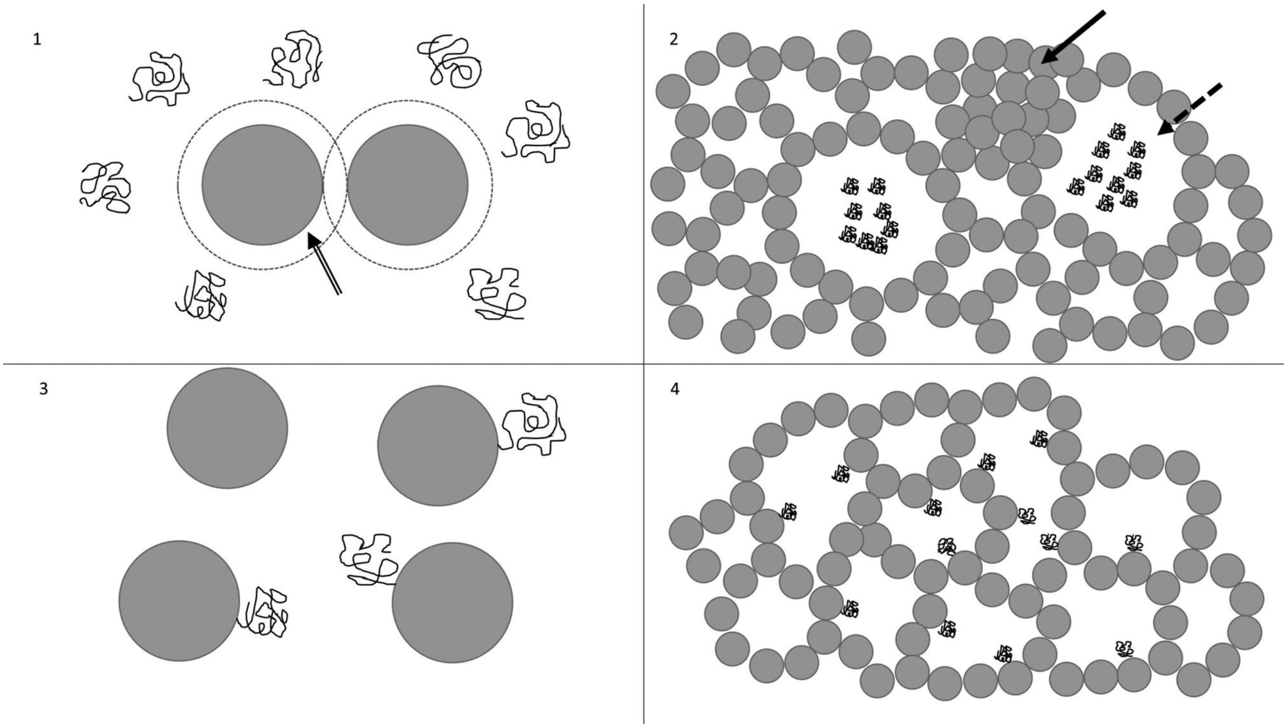

Figure 2. Interactions between polysaccharides, caseins, and cheese matrix. 1. Interaction between caseins and incompatible polysaccharides. Arrow indicates depletion layer. 2. Cheese matrix containing incompatible polysaccharides. Arrow on the top indicates area of greater casein aggregation. Dotted arrow indicates polysaccharide-rich serum area. 3. Interaction between polysaccharides and caseins through electrostatic interaction. 4. Cheese matrix containing interactive polysaccharides. The structure is characterized by reduced casein-casein coagulations due to the stabilizing effect of polysaccharides after it is electrostatically adsorbed onto caseins.

Polysaccharide concentration is important, as the gel stiffness decreased when the polysaccharide concentrations exceeded certain levels (e.g., guar gum >0.075%, pectin >0.3%), suggesting impaired casein-network formation (Fagan et al. 2006; Tan et al. 2007). If the concentration of incorporated polysaccharides continues to increase, phase separation could occur and the protein would no longer be the continuous phase in the gel (Fagan et al. 2006; Galante et al. 2017). It has been suggested that the rennet coagulation process of milk containing uncharged polysaccharide can be seen as a competition between protein aggregation caused by phase separation and by enzymatic activity. At lower polysaccharide concentrations, rennet-induced casein coagulation is predominant, whereas, at higher polysaccharide concentrations, the casein aggregation caused by depletion flocculation is predominant. This offers the opportunity to control rennet gel structure by modulating the two process, such as by changing polysaccharide or rennet concentrations (Mende, Jaros, and Rohm 2019).

The effects of carrageenans on rennet coagulation have been widely investigated because, unlike most negatively charged polysaccharides that only have a very weak interaction or do not interact with casein at close to neutral pH (casein micelles are also negatively charged overall), carrageenans can adsorb onto the positively charged patch located between residues 97 and 112 of κ-casein (Langendorff et al. 2000; Wang et al. 2014). Carrageenans are negatively charged polysaccharides consisting of galactose and 3-6 anhydro-galactose repeating units, which can be further categorized into three main forms based on the number of sulfate groups per disaccharide unit: one for κ-carrageenan, two for ι-carrageenan, and three for λ-carrageenan. Wang et al. (2014) proposed that κ-carrageenan, ι-carrageenan, and λ-carrageenan adsorb onto casein micelles by tail adsorption, flat adsorption, and ring adsorption, respectively (Figure 3). The adsorption of carrageenans to casein micelles generally has an inhibitory effect on rennet coagulation (increased coagulation time and decreased gel firmness), which has been demonstrated by multiple studies (Wang et al. 2014; Wang, Zhang, and Ren 2016; Benjamin et al. 2018). The effects can be attributed to factors such as changed surface charge, inhibited κ-casein hydrolysis, and self-association of the double helices of carrageenans (Wang et al. 2014).

Positively charged polysaccharides are not usually investigated in the context of rennet coagulation, because they can interact with casein *via* electrostatic interactions, resulting in formation of polysaccharide/casein complexes, or leading to precipitation. For example, it was shown that chitosan can interact with caseins and cause precipitation at low concentration due to the reduction of negative surface charges of caseins, while at moderate or high chitosan concentrations a stable complex of chitosan/casein is created, as chitosan is sufficient to cover casein surface, and the linear charge density of chitosan is an essential factor determining the critical concentration (Ding et al. 2019).

Effect of polysaccharides on syneresis and cheese moisture content

During cheese manufacture, rennet-induced gels are often cut into small curds (5-15mm), facilitating whey expulsion (syneresis). This process is complicated, and involves

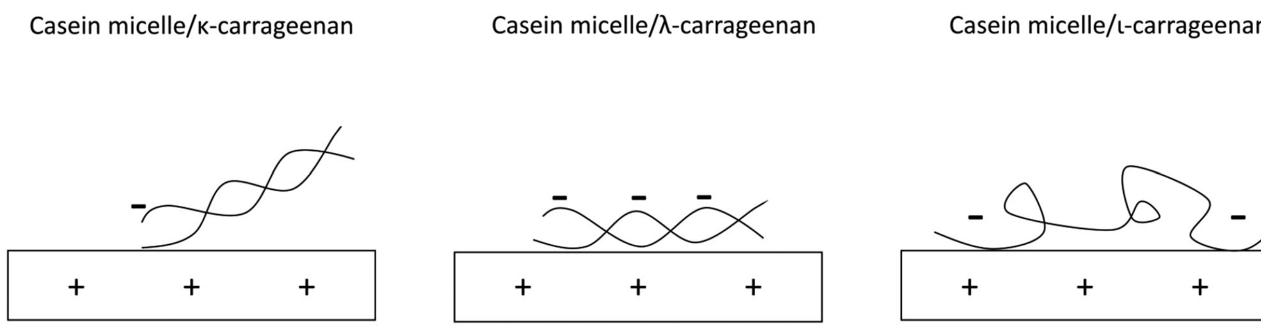

Figure 3. Proposed interactions between different types of carrageenans and casein micelles. Adapted from (Wang et al. 2014).

shrinkage and rearrangement of *para*-casein micelles (Fagan et al. 2017). The syneresis process is essential in controlling the moisture level of cheese, and also affecting parameters such as cheese yield, the loss of protein and fat in whey, and curd fusion during shaping and pressing (Fagan et al. 2017).

Arango, Trujillo, and Castillo (2013) investigated the syneresis kinetics of a rennet-coagulated gel containing inulin. They found that expulsion of whey from curds with and without inulin followed first order kinetics, and that the increase in inulin concentration decreased whey drainage rate but did not affect overall whey drainage. It was also shown that the tan δ value (where $\tan \delta = G''/G'$, indicating relative effects of viscous and elastic components) remained constant for all treatments after the gel hardness reached 12–15 Pa, and was independent of inulin concentration. This indicated that inulin did not influence the ability of gels to rearrange and shrink, and the inhibitory effect of inulin on syneresis was attributed to the water-holding capacity (Arango, Trujillo, and Castillo 2013). It is evident that polysaccharides can also absorb water and reduce syneresis in curd and cheese, resulting in cheeses with higher moisture and higher moisture in nonfat substance (MNFS) contents than the same cheese without polysaccharides (Brown, McManus, and McMahon 2012; Aminifar and Emam-Djome 2016; Benjamin et al. 2018), although the syneresis kinetics of rennet curds containing other polysaccharides have not been reported. The increase in moisture content is often accompanied by lower protein concentration (a key structural component of the gel network), resulting in cheese with a lower firmness than conventional cheese (Benjamin et al. 2018; Khanal et al. 2018). As an example, Benjamin et al. (2018) demonstrated that the addition of low acyl gellan to a fresh semi-hard model cheese resulted in cheese with higher moisture content, lower fracture stress, lower cohesiveness, and larger voids in the microstructure (increased pore size and pore volume) than the control cheese. It also significantly decreased the sensory firmness and elasticity compared to the control cheese. These changes are generally desired by Asian consumers, since a softer texture has been associated with liking of cheese (Zhang et al. 2011; Go, Kim, and Chung 2017).

Effect of polysaccharides on cheese ripening

Costa et al. (2010) studied the effect of exopolysaccharides (EPS) produced *by Lactococcus lactis* ssp. *cremoris* on cheese ripening, by comparing two batches of half-fat Cheddar cheese with single starters of isogenic strain (DPC6532 and DPC 3533), which only differed in their ability to produce EPS. It was shown that Cheddar cheese containing EPS had a higher moisture content, higher moisture in nonfat substances, and higher cheese yield than its counterpart. The higher levels of moisture also resulted in higher levels of primary proteolysis throughout ripening, which in turn resulted in a lower hardness and higher adhesiveness in the samples containing EPS. The flavor profile was also shown to differ between cheeses with and without EPS as determined by descriptive sensory analysis (Costa et al. 2010). In a study on addition of pectin as fat replacer, it was found that reduced-fat Cheddar cheese containing amidated pectin or low methoxy pectin had significantly lower levels of insoluble calcium after 180 days of storage than both reduced-fat and full-fat cheeses without pectin (Ibanez, Waldron, and McSweeney 2016), resulting in differences in texture and meltability. This was attributed to the possible formation of pectate (calcium-induced gelation mechanism of amidate and low methoxy pectin) in the casein matrix. Information on how different polysaccharides affect cheese ripening processes is limited and such mechanisms should be the focus of future investigations, as ripening is an essential step in both flavor and texture development.

Behavior of polysaccharides as filler particles in cheese matrices

As cheese may be considered an emulsion-gel structure (Vogt et al. 2015), the physiochemical properties of the emulsion-filled gel can be controlled by modulating the properties of the emulsion droplets and their interactions with gel matrices (Foegeding, Stieger, and van de Velde 2017). The presence of filler particles might increase or decrease the dynamic modulus (such as Young's modulus (Sala et al. 2009) and storage modulus (Vanvliet 1988; Rosa et al. 2006)) of the gel, depending on the modulus of the filler particles and the interaction between the filler particles and the gel network. In the case of an active filler (involving interactions between filler particle and gel matrix), the modulus of the emulsion gel is increased if G_f (the modulus of the filler particles)$>G_m$ (the modulus of the gel matrix), and *vice versa*. Alternatively, in the case of an inert filler (where the filler particle does not interact with the gel matrix), the presence of filler particles should always reduce the shear modulus, since they create voids in the matrix (Foegeding, Stieger, and van de Velde 2017; Koc et al. 2019). The concept of filler gel particles in emulsion filled gels has been validated in a range of protein gels (Sala et al. 2007), polysaccharide gels (Koc et al. 2019), and cheese (Yang et al. 2011; Barden et al. 2015; Thionnet et al. 2017).

The concept of hydrated hydrocolloids as filler particles in cheese has been raised (Barden et al. 2015; Thionnet et al. 2017), where cheese could be considered as the matrix in which the filler particles are dispersed. As an example, it was shown that sodium alginate gel particles, formed due to cross-linking by calcium ions present in, or added to, milk, act as hydrated filler particles in the protein network (Khanal et al. 2018). Similarly, other polysaccharides, such as low acyl gellan (Benjamin et al. 2018) and some starches (Brown, McManus, and McMahon 2012), also do not form the backbone of a gel matrix with casein, but mainly absorb water, only weakly interact with the casein matrix, and are primarily located in the serum voids, behaving in a similar way to filler particles in gel matrices. If hydrated polysaccharides are considered as filler particles, it is then possible to predict the physiochemical properties of cheese containing polysaccharides by applying an appropriate model. Thionnet et al. (2017) incorporated spherical glass beads of a controlled size and coating as filler particles into model Mozzarella cheese, to investigate the effect of particle volume fraction, size, and surface properties of the filler particles on fracture properties and microstructure of the cheese. It was concluded that a simple equation could be used to predict the effect of bespoke filler particle properties on the rheology of the cheese. However, the glass beads used had a significantly higher hardness than that of the matrix, and therefore were not representative of the properties of common hydrated polysaccharides.

Barden et al. (2015) incorporated hydrated dextrin beads into Cheddar cheese as a comparison to fat globules and concluded that cheese made using dextrin beads as filler particles behaves similar to cheese using fat as filler particles at cold temperature (where melting of fat does not occur), suggesting that it is possible to simplify the view of the system and establish filled gel models to help design cheese structure. The ideal substituted filler particles should melt or soften at higher temperature like fat. Low-fat cheeses often exhibit an undesirably firmer and more rubbery texture, with lower rates of breakdown during oral processing, compared to full-fat counterparts (Rogers et al. 2010; Cakir et al. 2012), which can be attributed to the denser protein network as a result of fat reduction. Additionally, fat contributes to other sensory characteristics, such as smoothness, cohesiveness, and creaminess, as fat is released from the casein gel matrix and melts during oral processing. Starches are commonly used fat-replacers, due to their ability to bind extra water during gelatinization, they contribute to fat-related texture sensations, and are relatively inexpensive and widely available (Diamantino et al. 2014). It was reported that the presence of different gelatinized starches in rennet-coagulated casein gels resulted in micro-phase-separated and less compact gel structures (Brown, McManus, and McMahon 2012), However, the location of different starches differed as observed by confocal laser scanning microscopy (CLSM). Waxy corn starch and waxy rice starch were primarily located at the protein surface, suggesting interactions with the surface of casein aggregates, while instant tapioca starch was homogeneously dispersed within the space it created in the casein network, very similar to fat globules in full-fat cheese, where fat globules are primarily located in the serum cavities in cheese and only weakly interact with casein matrix (Everett and Auty 2008). Modified maize starches (acetylated/adipate maize starch and hydroxypropylated/phosphate maize starch) have also been suggested as promising fat replacers in cheese, because they had a high retention rate in casein matrix, increased water retention, and disrupted the curd microstructure (Diamantino et al. 2019).

Konjac glucomannan has also been exploited as a fat replacer in many dairy gel systems because of its health benefits (Dai et al. 2018) and the ability to improve gel structure. Konjac glucomannan is a food polysaccharide extracted from the tubers of *Amorphophallus konjac*, which is composed of a linear chain of β-D-mannose and β-D-glucose residues at a ratio of 1.6:1, linked by β-(1,4) glycosidic bonds with random sugar residues distribution. A low degree of acetyl substitution at the C-6 position and short chain branches at the C-3 position of mannose are responsible for its solubility in water (Abhyankar et al. 2011). It was shown that konjac glucomannan could improve the stability of low-fat processed cheese (da Silva et al. 2016) and low-fat yoghurt (Dai, Corke, and Shah 2016); the lightness, moisture content, and firmness of low-fat Mozzarella with konjac glucomannan addition were closer to those of its full-fat counterpart compared to low-fat Mozzarella without konjac glucomannan (Dai et al. 2018).

In summary, the role of polysaccharides in cheese matrix might be simply viewed as that of inactive fillers. Similar to fat, they could also affect many sensory attributes of the matrix such as hardness, cohesiveness, and smoothness. Model systems could be applied to correlate changes in sensory attributes with attributes of the inactive fillers (e.g., particle volume fraction, size), which can then guide how polysaccharides could be used to achieve target sensory properties.

The incorporation of polysaccharides during other steps of cheese manufacture

The majority of the studies regarding the use of nondairy polymers as structuring ingredients in cheese investigated the effect of polymer addition prior to coagulation, and this has been shown to be effective in modifying cheese structure, even at relatively low concentrations. However, it is also possible to incorporate polysaccharides in other stages of the cheese manufacture process after rennet coagulation, such as during the stretching step of Mozzarella production. Xanthan gum has been shown to physically block coalescence of the casein network, resulting in increased stringiness and better consumer liking of flavor upon the mixing of xanthan gum with cheese curd during production of low-fat Mozzarella string cheese (Oberg, Oberg et al. 2015). Cankurt (2019) demonstrated an interesting approach to altering the properties of white cheese by adding polymers (guar gum, carrageenan, xanthan gum, and gelatin) into the brine. It was suggested that the stabilizing effect (increased viscosity of the brine) of these polymers (except guar gum) prevented cheese from absorbing excessive water from the brine, which resulted in cheese with a firmer texture.

It may also be possible to alter the structure of cheese by incorporating polymers during dry salting of curds, although no study has been published to date on this. This is based on the prediction that the polysaccharides will impact on curd fusion during the pressing/moulding and ripening processes. The benefit of incorporation of polysaccharides into cheese manufacture after whey drainage is the preservation of whey value, important for nutritional applications such as infant formula products.

Incorporation of plant proteins in cheese matrices

Plant proteins are gaining its popularity as ingredients in food formulations due to consumers' concerns about the environmental impact of production of animal protein, and also due to the perception of health benefits (Silva et al. 2019). Limitations exist to using only plant proteins in food systems due to their low solubility in water and some negative textural properties (such as lack of body). However, opportunities may arise when used in combination with milk proteins to create novel structures with favorable sensory and functional properties (Silva et al. 2019). Plant proteins, such as sesame protein, soy protein, and pea protein, have previously been incorporated into cheese matrices, in which changes to composition, sensory and textural properties, and microstructure were observed (Kumar et al. 2011; Lu et al. 2012; Ben-Harb et al. 2018).

Hsieh, Yun, and Rao (1993) showed that the addition of 2–3% soy protein to cheese milk resulted in Mozzarella cheese with a softer texture compared to the control, which could possibly be explained by the more open structure in cheese containing soy protein (Atia, Xia, and Zhang 2004). Similar findings have also been reported for low-fat Paneer cheese; Kumar et al. (2011) investigated the effect of soy protein as a fat replacer, and reported that the firmness of low-fat Paneer cheese with soy protein addition was lower than that without soy protein. It was suggested that soy protein might interrupt the gel structure in low- fat cheese matrices and could potentially act as a fat-replacer. However, sensory appearance and color were scored lower in cheese with soy protein addition, and soy protein concentrations over 0.2% imparted a bean-type flavor to the final product.

The beany or grassy flavor associated with plant proteins results from oxidation of unsaturated fatty acids during the manufacturing process or storage, which limits their use as structuring ingredients in dairy products. Indeed, it has been shown that the sensory flavor score of processed cheese decreased gradually as the level of plant protein (sesame protein, peanut protein, chickpea protein) increased (ElSayed 1997), and negative descriptors such as pea or earthy flavors have been associated with pea concentration in a model yoghurt gel (Yousseef et al. 2016). Many studies have focused on the flavor-binding mechanism of plant proteins (Wang and Arntfield 2017), and on methods to reduce the beany flavor, such as using solid dispersion based spray-drying (the dispersion of poor water-soluble ingredients in an amorphous matrix carrier through spray-drying which creates a dispersed state with improved solubility) and thermal treatment (e.g., blanching and grinding soybeans between 80 °C and 100 °C) (Jiang et al. 2016; Lan et al. 2019). Such studies can inform the design of novel foods using plant proteins, where impact on flavor is an issue.

Recent studies also investigated the gelation of mixture of plant proteins and milk proteins. Plant proteins such as soy proteins and pea proteins are globular proteins, which can be denatured and form gels upon heat, enzymatic (transglutaminase), or acid treatment (Ben-Harb et al. 2018). Ben-Harb et al. (2018) reported that enzyme-induced gelation of a pea protein and milk mixture, which was achieved by adding chymosin and transglutaminase to suspensions or emulsions, led to gels with a lower storage modulus compared to pure milk gels or pure pea gels. Heat-induced gelation of micellar casein and plant proteins (soy protein and pea protein) mixtures have been investigated in several studies (Ako et al. 2009; Beliciu and Moraru 2013; Mession, Roustel, and Saurel 2017; Silva et al. 2018; Silva et al. 2019). It was concluded that micellar casein does not crosslink with soy protein or pea protein upon heat gelation, unlike the crosslinking observed between micellar casein and whey protein upon heating. Silva et al. (2019) also suggested that individual proteins (casein and soy or pea protein) form gels independently in a mixture, and that gel stiffness is predominantly determined by the protein present in the highest concentration. The potential structure of heat-induced gelation of a mixture of plant and dairy proteins is illustrated in Figure 4. In addition, individual micellar casein gels and plant protein gels appeared to be more homogenous than the mixed gels, indicating potential micro-phase separation; however, further research is required to confirm this, since different proteins were not individually labeled in the CLSM analysis.

Heating of plant proteins also provides benefits in terms of inactivating physiochemical harmful substances, such as trypsin inhibitors and hemagglutinin, and reduce the associated undesirable odor (Nishinari et al. 2014). The fact that casein and plant protein form gels separately upon heating allows design of a more novel structures, the gel properties of which may be fine-tuned by adjusting the ratio of the different proteins (Silva et al. 2019).

Plant proteins are traditionally important protein sources and ingredients in Asian cuisines, such as in China, Japan and Korea, where they are used in various traditional foods, such as tofu (soybean curd), miso (fermented soybean paste), and natto (fermented soybeans covered with mucilaginous substance) (Nishinari et al. 2014). Therefore, incorporation of ingredients familiar to Asian consumers

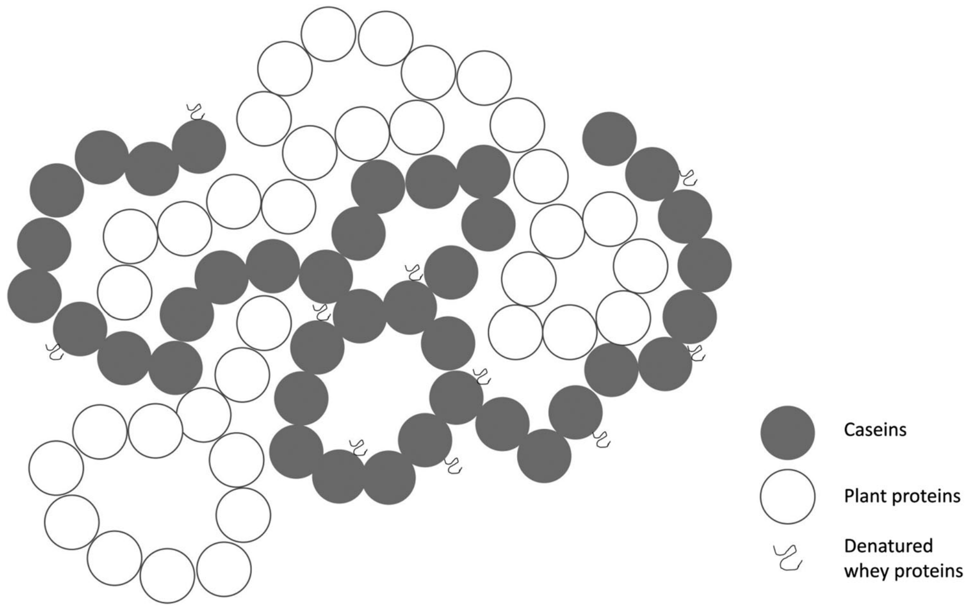

Figure 4. Structure of heat-induced gel of mixture of plant and dairy proteins.

such as soy protein into cheese might bring familiar flavors to cheese and increase acceptability. Such an approach has been studied in the context of sesame protein (Lu, Schmitt, and Chen 2010; Lu et al. 2012). Sesame protein isolate and sesame meal are used in salad dressings and flavoring ingredients for noodles, stir fries, and pastries in China (Lu, Schmitt, and Chen 2010), and therefore the potential use of sesame protein isolate in fresh cheese has been investigated (Lu, Schmitt, and Chen 2010; Lu et al. 2012) in an attempt to create a more acceptable cheese product for Chinese consumers. Lu, Schmitt, and Chen (2010) suggested that the sesame protein interacted with casein throughout the cheese-making process, as specific sesame protein isolate-gel clusters were observed on the surface of curd fractures, stacked or fused with casein micelles, or wrapped around casein gel strands. The addition of sesame protein was shown to accelerate proteolysis during the late stages of cheese aging, and cheeses with 4% and 8% of sesame protein addition were also shown to have a significantly increased rate of adhesiveness during ripening compared to control cheeses. In a later study (Lu et al. 2012), results from SEM and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed that adding calcium to sesame protein isolate/milk mixture facilitated sesame protein-milk protein interactions, resulting in a more compact and more regular cheese microstructure, suggesting that calcium played an important role in sesame protein/casein complex. However, no flavor compound analysis or consumer acceptance testing was reported.

Nutty flavor in cheese was shown to be desirable to certain consumer clusters among Chinese youth (Zhang et al. 2011), and was also shown to be an attribute selected by many Korean consumers as a reason for liking cheeses (Go, Kim, and Chung 2017). Nut-based ingredients, such as almond protein (Sathe and Sze 1997) and walnut protein (Mao and Hua 2012), were shown to have comparable functionalities to the more commonly used soy protein, and may have potential to be utilized in developing cheeses with novel characteristics and flavors which are appealing to groups among Asian consumers. To explore the opportunities of using such nut-based proteins in cheese, fundamental research is required concerning the inhomogeneous structure of these ingredients with casein during different process treatments (e.g., heat or rennet gelation), and their impacts on cheese flavor and texture.

Impact of polysaccharide and protein addition on cheese flavor

The effect of the addition of polymer ingredients on the composition, texture, and functionalities of cheese matrices is well documented, but less so for the influence on flavor profiles. The addition of polymer ingredients may also influence the flavor of cheese in two ways: (1) the modified composition and texture of cheese result in modification of flavor release; and (2) the inclusion of new flavor compounds into cheese. Benjamin et al. (2018) investigated the effect of various polysaccharides on the salt release of fresh model cheese as a strategy of salt reduction in cheese. Salt release from cheese depends on ion mobility through cheese pores and how the protein network changes under deformation. Compositional and structural modifications, such as increased moisture content, lower firmness, higher fracture strain, and more open microstructure with larger pore size, can result from the addition of different polysaccharides, and these changes also resulted in increased salt release (Benjamin et al. 2018). A previous study also showed a negative correlation between saltiness and sample firmness in model cheese (Panouille et al. 2011). Similar findings hold true for aroma release; Gierczynski, Guichard, and Laboure (2011) undertook a review of aroma perception in dairy products, and concluded that an increase in viscosity or firmness generally decreased aroma perception in dairy matrices.

Increased moisture levels of cheeses as a result of polysaccharide addition are likely to increase the retention of hydrophilic aroma compounds (Gierczynski, Guichard, and Laboure 2011). Major food components such as proteins, lipids, and carbohydrates can also interact with aromatic compounds, and so may also affect the release of typical aromatic compounds (Wang and Arntfield 2017). Fat is generally considered to play the most important role in aroma release, because it significantly affects the partitioning of hydrophobic aromatic compounds (Jelen 2011). Polysaccharides can form inclusion complexes with aromatic compounds, in which aromatic compounds are trapped, and are regarded as good flavor-carriers and as ingredients for flavor encapsulation. Because of the wide range of chemical structures that proteins can provide, they have the most diverse interactions with flavor compounds, including hydrophobic interactions, ionic bonds, and irreversible covalent linkages (Wang and Arntfield 2017).

Overall, the addition of polysaccharides and proteins that are familiar to Asian consumers offer significant opportunities to achieve desired textures, modify flavor, and bring flavors familiar to Asian consumers into cheese.

Conclusions

The significant potential in emerging markets in East and Southeast Asia has focused the global cheese industry toward the development of innovative cheese products with diverse sensory characteristics appealing to those consumers. The nutritional properties of cheese will also add to this appeal. Sensory studies showed that such consumers generally prefer cheeses that have soft (lower firmness, higher rate of breakdown, higher perceived moisture) textures, with mild and milky flavor, although a nutty flavor is also appealing to certain consumer clusters.

Incorporation of polymer ingredients may be used to create bespoke structures. Non-interacting polysaccharides (most negatively charged and uncharged polysaccharides) generally reduce rennet coagulation time, increase curd firming rate, and increase curd firmness. The mechanism is best described as a competition between depletion aggregation and rennet-induced coagulation; however, carrageenans are an exception, as they can adsorb onto the positive patch of κ-casein and inhibit rennet coagulation. Polysaccharides reduce syneresis mainly due to their ability to absorb water, resulting in cheese with higher moisture levels and softer texture, which are of interest to East and Southeast Asian consumers. It is likely that polysaccharides will also affect cheese ripening, although limited information is available. Other than higher moisture levels and softer textures, the addition of polysaccharides also modify other properties, such as color, adhesiveness, flavor release, and meltability (Ibanez, Waldron, and McSweeney 2016; Benjamin et al. 2018; Khanal et al. 2018). Regarding the role of hydrated polysaccharides as filler particles, model cheese systems containing suitable filler particles could be used to establish the effect of filler volume, size and surface property on fractural and rheological properties of cheese matrices, which can give clear guidance in terms of choosing the appropriate concentration and type of polysaccharides to achieve desired attributes.

Addition of plant proteins such as soy protein and pea protein can also result in cheeses with a softer texture (Hsieh, Yun, and Rao 1993; Kumar et al. 2011; Silva et al. 2018). Interestingly, it was suggested that heat-induced gelation of soy protein or pea protein with casein resulted in a gel structure consisting of two independent protein gels, allowing the design of bespoke structures by adjusting the ratio of the two proteins. Additionally, heat treatment also inhibits harmful substances and undesired odor associated with plant proteins.

Ingredients that are familiar to East and Southeast Asian consumers are proposed to have the most potential to be used in the development of cheese products in these markets, since this may incorporate familiar flavors into cheese, as well as modifying texture.

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