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Starch-based food matrices containing protein: Recent understanding of morphology, structure, and properties

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Abbreviations

CLSM	Confocal laser scanning microscopy
DSC	Differential scanning calorimetry
FTIR	Fourier-transform infrared
G'	Storage modulus
G''	Loss modulus
HMT	Heat-moisture treatment
pI	Isoelectric point
RS	Resistant starch
RVA	Rapid visco-analyzer
SAXS	Small-angle X-ray scattering
SEM	Scanning electron microscopy
TGase	Transglutaminase
T_p	Peak temperature
WHC	Water-holding capacity
XRD	X-ray diffraction
ΔH	Enthalpy change

Abstract

Background: Starches and proteins are two major types of biopolymer components, especially in many flour (starch)-based foods consumed worldwide, which provide energy and nutrition needed by the human body. In many such starch-based matrices (the main structural component of such foods), proteins and their interactions with starches greatly influence the matrix structure and properties. Studying the different roles played by proteins (endogenous and exogenous) in various starch-based food systems can provide a frame of reference for the design and production of improved starch-based food products with tailored properties and desirable nutritional functions.

Scope and approach: Significant efforts have recently been made to tailor the morphology, structure, and properties of many starch-based food systems, and thus to design various starch-based food products with satisfactory attributes. This review surveys the latest literature on starch-based matrices containing proteins. Discussed are the influences of proteins and their interactions with starches on the morphologies and structures (e.g. short- and long-range orders) of starch-based matrices, as well as on their pasting, thermal, rheological, textural, sensory, and digestive properties. Also, current understandings of structure–property links are presented, along with their implications on the production of various starchy foods (e.g. pastas, breads, cakes, and biscuits), including gluten-free versions.

Key findings and conclusions: Proteins in many starchy food matrices can encapsulate the starch phase (or be adsorbed on its surfaces) on a micron scale, and thereby interact with starch chains via both non-covalent (e.g. hydrogen bonding, hydrophobic, and electrostatic) and covalent bonds (e.g.

via Maillard reactions). These facts and protein features (e.g. hydration and gelation abilities) can play major roles in inhibiting starch retrogradation (the reassembly of cooked starch chains into ordered structures) and in regulating various other properties of such starch-based matrices, including viscosity, transition temperatures, moduli, hardness, sensory, digestibility, and shelf-life. Despite the fact that the current literature presents considerable information on the structure–property relationships of many different starch-based matrices and their applications in the processing of various starchy foods (e.g. pastas, noodles, and biscuits), it is still highly necessary to define more comprehensive correlations among starch–protein interactions, starch-protein matrix structures, and the resulting properties of such food products.

Keywords: Starch–protein interactions; Starch-based food processing; Starch structure; Food textural properties; Food sensory properties; Starch digestibility; Gluten-free products

1. Introduction

Many foods, especially flour (starch)-based ones, contain mainly two types of biopolymers, namely, starches and proteins. Such foods, primarily starch-based matrices containing protein, are consumed worldwide, serving as vital sources of energy and nutrition that maintain the health and functioning of the human body (Wang, Zhang, Wang, Ai, & Xiong, 2020; Yang, Zhong, Douglas Goff, & Li, 2019). Traditionally, such foods are prepared with relatively common ingredients containing starches and endogenous proteins (e.g. wheat or other cereal flour), through different types of processing. Among these products, bread, pasta, and pizza are enjoyed by Western people, while steamed bread, noodles, and rice are consumed by people in the East (Li, Zhu, Guo, Brijs, & Zhou, 2014). To meet consumer demand for improving diet quality and food functions, many high-quality flours and supplements (e.g. proteins, food gums, and fatty acids) have been developed and used in starchy foods (Li, Zhu, et al., 2014). Addition of exogenous proteins to improve the quality of starch-based products has been widely practiced, due to the safety, health, and numerous sources of these proteins. In this regard, although starches and proteins exist widely in many natural foods, it can be necessary to recombine them or incorporate exogenous proteins during processing, in order to obtain food products with desired properties (Li & Huang, 2015). For example, the incorporation of protein into gluten-free food results in a continuous protein phase and a crosslinked structure, leading to increased elastic modulus (Ronda, Villanueva, & Collar, 2014; Villanueva, Ronda, Moschakis, Lazaridou, & Biliaderis, 2018).

In recent years, there has been a research focus on the morphology, structure, and properties of starch-based matrices containing endogenous and/or exogenous proteins, aimed at providing a reference for the rational design and production of high-quality starch-based food products that meet the needs of diverse consumers. Also, researchers e.g. (Bhattarai, Dhital, & Gidley, 2016; Considine et al., 2011; Jekle, Mühlberger, & Becker, 2016; Kumar, Brennan, Mason, Zheng, & Brennan, 2017; López-Barón, Gu, Vasanthan, & Hoover, 2017; Witczak, Ziobro, Juszczak, & Korus, 2016) have carried out extensive studies on many different starches and proteins, which have provided a large amount of basic and important information for the understanding of mixed starch–protein matrices for food product development.

As shown in **Fig. 1**, starches and proteins have different functional properties that largely determine the processing, product quality, and nutritional properties of starch-based food matrices. Additionally, interactions between starches and proteins present in natural or processed food systems are often responsible for the structure and thus the properties and quality of such food products (Quiroga Ledezma, 2018). Therefore, a better understanding of protein inclusion and related interactions with starches can help to enable the achievement of desirable structural, textural, sensory, and digestive properties, and shelf stability, and can enable expanded applications of starch matrices, based on advanced food technologies. At present, the role of proteins in starch-based products has attracted extensive attention, with many studies specifically focused on the effects of proteins (endogenous and exogenous) on the structures and properties of starch-based food matrices. However, the results of such specific effects have not been systematically summarized.

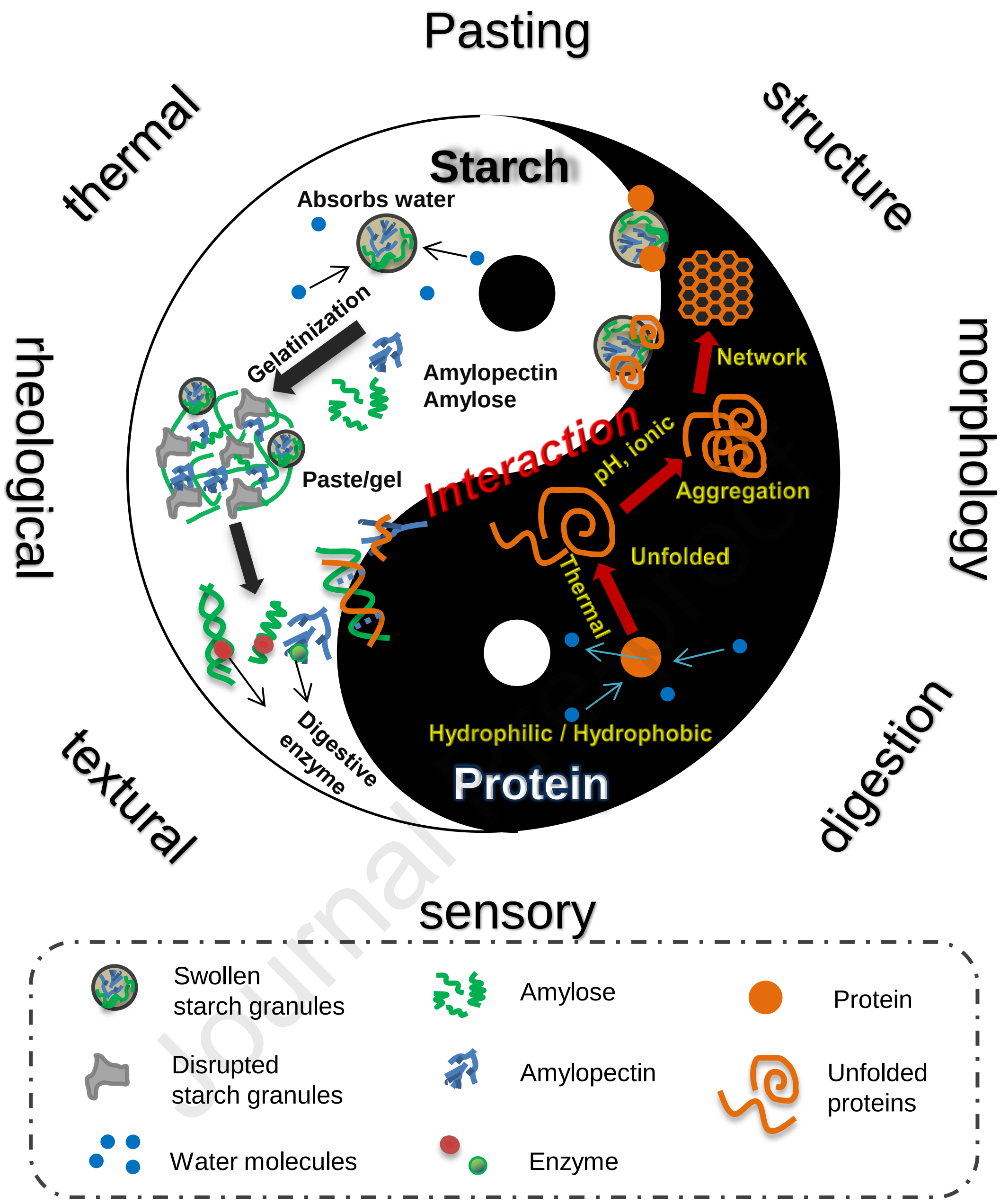

Fig. 1 Overview of the characteristics of mixed starch–protein matrices

Therefore, this review provides a survey of the latest developments in starch-based food matrices, with a particular focus on the impact of protein presence and resulting starch–protein interactions on the morphology and structure, as well as the physicochemical and digestive properties of such starch-based systems. Based on that focus, this review further discusses the structure–

property relationships and mechanisms of mixed starch–protein systems, as reported in the literature to date. Additionally, some possible hypotheses are proposed to describe the effects of proteins on the different properties common to starches, hypotheses that can guide the processing of such starch-based food systems. Furthermore, we suggest that this review can provide insights into the development of novel food systems based on starches and proteins.

2. Basic aspects of starch and protein

2.1 Starch

Starch, the major component of starch-based foods (e.g. noodles, pasta, and bread), is a glycemic carbohydrate in the diet (Svihus & Hervik, 2016; Wang & Copeland, 2013). Starch is typically composed of a mixture of amylose and amylopectin polymers (Barak, Mudgil, & Khatkar, 2014), and its multi-level structure (i.e. starch granules, crystalline, semi-crystalline, and amorphous lamellar structures) has been described extensively and reviewed in detail (Pérez & Bertoft, 2010; Quiroga Ledezma, 2018; Vamadevan & Bertoft, 2015; Wang & Copeland, 2013). The characteristic behaviors of starch (e.g. gelatinization and retrogradation) greatly affect the properties (e.g. structure, texture, and digestibility) of many starch-based food matrices (Toutounji et al., 2019; Wang & Copeland, 2013).

The properties of such starch matrices are often largely affected by hydrothermal treatments. During heating, starch granules absorb water and swell, and some starch molecules leach out, resulting in changes in the viscosity of such starch suspensions (Vamadevan & Bertoft, 2015). This process is called “gelatinization”, the process by which native starch granules lose their natural order and crystalline structure and become amorphous (i.e. water uptake, swelling, crystallite melting, the disruption of molecular order, and starch solubilization) (Wang & Copeland, 2013). With the application of constant heating and shearing, gelatinized starch forms a paste with certain rheological properties (Ai & Jane, 2015). Due to the rupture of starch granules, starch molecules in the starch paste are more easily bound to amylase enzyme, thereby accelerating their hydrolysis. After cooling and storage, some starch pastes can form gels and thereby lose their fluidity at appropriate concentrations, while others can remain more liquid-like (Ai & Jane, 2015). This starch gelation process is called “retrogradation” (aka recrystallization), and is due to chain rearrangement, including the formation of new double helices (Quiroga Ledezma, 2018; Wang, Li, Copeland, Niu, & Wang, 2015). Fu, Chen, Luo, Liu, and Liu (2015) systematically summarized the influencing factors (e.g. proteins, lipids, carbohydrates, and salts) of starch retrogradation. Furthermore, the retrogradation of starch results in considerable changes, such as increases in viscosity, opacity, and gel hardness, and phase separation of the polymers and water, and has a great impact on the texture of many starch-based food systems (Wang & Copeland, 2013). The effects of starch retrogradation on its digestion have been summarized by Toutounji et al. (2019), showing that this reordering of starch chains decreases starch digestibility and can increase the content of resistant starch (RS).

Studies have shown that the rate and extent of starch gelatinization and retrogradation depend on multiple intrinsic and extrinsic factors (Toutounji et al., 2019; Wang & Copeland, 2013). Intrinsic factors include starch granule morphology, amylose/amylopectin ratio, and starch molecular structure. Extrinsic factors can include processing operations and conditions (e.g. thermal processing, extrusion cooking, and processing environment) and the presence of other constituents (e.g. proteins, lipids, and polysaccharides) (Singh, Dartois, & Kaur, 2010; Wang & Copeland, 2013; Wang, Li, et al., 2015).

2.2 Protein

Protein (endogenous or exogenous) is typically the second-highest component in many starch-based foods, at about 4–20% by weight (Baik, 2010; Ortolan & Steel, 2017; Storck et al., 2013). Therefore, protein can have an important impact on the quality of such starch-based food matrices. For example, wheat gluten protein can be a major determinant of wheat-based product quality, by affecting the water-holding capacity (WHC), cohesiveness, and viscoelasticity of wheat flour doughs (Wang, Jin, & Xu, 2015). Moreover, the quality and nutritional properties of gluten-free foods are often improved by the addition of exogenous, non-gluten proteins with certain functional properties (e.g. gelling ability and WHC) (Aryee, Agyei, & Udenigwe, 2018; M, 2017; Manoj Kumar et al., 2019; Phongthai, D'Amico, Schoenlechner, Homthawornchoo, & Rawdkuen, 2017; Ribotta & Rosell, 2010). An understanding of the functional properties of proteins is of great significance in their application in developing many starch-based food systems.

Gelation is an important behavioral characteristic of proteins, especially related to their elasticity and textural properties, and is often affected in food products (Aryee et al., 2018). Proteins can be induced to undergo gelation by heat, chemical (e.g. pH and salt ions), and/or enzymatic treatments (Nieto-Nieto, Wang, Ozimek, & Chen, 2015; Tarhan, Spotti, Schaffter, Corvalan, & Campanella, 2016). Protein gel formation involves protein unfolding, leading to the exposure of hydrophobic amino acid residues (Foegeding & Davis, 2011). Subsequently, unfolded molecules are irreversibly rearranged, associated, and aggregated by interactions such as disulfide interactions, hydrophobic interactions, hydrogen bonding, and van der Waals forces (Foegeding & Davis, 2011). If the protein concentration is high enough, a three-dimensional gel network can be formed (Foegeding & Davis, 2011). Furthermore, various studies have shown that proteins alone or combined with polysaccharides (e.g. inulin, native starch, or acetylated starch) can produce gel matrices with different microstructures, thereby improving their water-retention, rheological, and texture properties (Nieto-Nieto et al., 2015; Ren, Dong, Yu, Hou, & Cui, 2017; Ren & Wang, 2019; Yu, Ren, Zhao, Cui, & Liu, 2020).

WHC is directly related to the interactions between protein molecules and water. The WHC of proteins in starch-based food matrices can affect the distribution of water in such mixed systems, thereby modifying the interactions between other components and water molecules (Aryee & Boye, 2017; Pelgrom, Vissers, Boom, & Schutyser, 2013). In addition, protein aggregation is promoted in protein gels, and, as a result, protein–water interactions are limited, leading to a decrease in WHC (Nieto Nieto, Wang, Ozimek, & Chen, 2016). Protein gels that have low WHC may not be able to hold water effectively, thereby leading to low textural stability (Boye, Zare, & Pletch, 2010). Properties such as WHC can be affected by both intrinsic (e.g. protein structure, conformation, amino acid composition, hydrophobicity, and hydrophilicity) and extrinsic (e.g. pH, temperature, and ionic strength) factors (Aryee et al., 2018; Foegeding & Davis, 2011).

2.3 Interactions between starches and proteins

Both covalent-bonding and non-covalent interactions have been reported to exist between starches and proteins (Li, Wang, Chen, Yu, & Feng, 2018; Ren & Wang, 2019; Wang et al., 2021). Such interactions are affected by the intrinsic nature of the polymers (e.g. net charge, solubility, size, and weight ratios), protein/starch ratio, temperature, pH, and ionic strength (Heertje, 2014; Li & Huang, 2015; Warnakulasuriya & Nickerson, 2018; Wei & Huang, 2019). Moreover, the interactions between the starches and proteins present in many natural or processed food materials are responsible for the structures, properties (e.g. physicochemical and digestion), and quality of such foods (Quiroga Ledezma, 2018). Sometimes, these interactions may be more important than the physicochemical properties of the individual components. Therefore, a better understanding of such interactions can help to enable the achievement of desirable textural, sensory, and digestive properties of many starch-based food systems, especially those produced using advanced food processing techniques (Quiroga Ledezma, 2018).

2.3.1 Non-covalent interactions

Non-covalent binding is the most common type of interactions between starches and proteins, involving hydrogen bonding, hydrophobic interactions, electrostatic forces, ionic interactions, and van der Waals force (Li et al., 2018; Quiroga Ledezma, 2018; Wang, Appels, et al., 2017). The main types of interactions between starches and proteins are summarized in **Table 1**. It has been found that the tryptophan (Trp), tyrosine (Tyr), or phenylalanine (Phe) aromatic side chains of proteins can interact with starches by non-covalent binding (Li et al., 2018). Proteins contain many hydrophilic groups (e.g. ─COOH, ─NH_2, ─OH, and ─SH), all of which are capable of forming physical crosslinks with starches (Kumar et al., 2017; Zhu et al., 2020). In the past few years, the factors resulting in starch–protein interactions have been widely studied, and there is often a co-existence of multiple interactions such as hydrogen bonding, hydrophobic interaction, electrostatic interactions, and van der Waals forces (Joshi, Aldred, Panozzo, Kasapis, & Adhikari, 2014; Li et al., 2018).

Table 1 Overview of starch and protein interactions

System	Processing	Interactions	Reference
Lentil starch, lentil protein	Cooking	Non-covalent interactions (hydrophobic and hydrogen bonding) and covalent bonds	(Joshi et al., 2014)
Wheat starch, hydrolyzed pea protein	Extrusion	Hydrogen bonding	(López-Barón et al., 2018)
Corn starch, whey protein isolate	Cooking	Hydrogen bonding	(Yang et al., 2019)
Rice	Cooking	Hydrogen bonding (weak)	(Zhu et al., 2020)
Wheat flour, soy protein	Mixing	Hydrophobic	(Ryan & Brewer, 2007)
Waxy rice flour	Soaking	Hydrogen bonds and hydrophobic	(Li et al., 2018)
Waxy maize starch, caseinates	Mixing	Hydrophobic	(Kett et al., 2013)

Wheat starch, wheat gluten	Heating	Hydrophobic, hydrogen bonds	(Li et al., 2020)
Wheat starch, soy protein	Cooking	Hydrogen bonds	(Ribotta, Colombo, León, & Añón, 2007)
Damaged cassava starch, wheat gluten protein	Dough formation	Non-covalent bonds	(Liu et al., 2019)
Corn starch, hydrophilic protein	Dehydration	Hydrogen bonds	(Zeng et al., 2010)
Phosphate starch, casein	Heating, mixing	Electrostatic adhesion (main)	(Sun, Liang, Yu, Tan, & Cui, 2016)
Starch granules, soybean peptide	Mixing	Weak electrostatic interactions	(Chen, Luo, et al., 2019)
Starch ester of octenyl succinic, casein	Heating, mixing	Steric stabilization (main)	(Sun et al., 2016)
Potato starch, whey protein isolate	Acidic conditions	Electrostatic interactions	(Chen, Fang, Federici, Campanella, & Jones, 2020)
Indica rice starch (IRS), whey protein	Heating, mixing	Hydrophobic molecular interactions (main)	(Wang et al., 2021)

isolate (WPI), casein (CS)			
Indica rice starch (IRS), soy protein isolate (SPI)	Heating, mixing	Hydrophobic, hydrogen bonding, and electrostatic interactions	(Wang et al., 2021)

Hydrogen bonds are formed by the electrostatic attraction of negatively charged atoms to hydrogen atoms (Silverman & Holladay, 2014). The formation of hydrogen bonds must satisfy two basic conditions: a hydrogen donor and a hydrogen acceptor (Silverman & Holladay, 2014). Therefore, in mixed starch–protein matrices, hydrogen bonds are the most prominent hydrophilic interactions, due to the presence of abundant ─OH groups in starches (López-Barón et al., 2017). These ─OH groups interact not only with protein side chains containing polar residues (e.g. aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), glutamic acid (Gln), arginine (Arg), and serine (Ser)), but also with protein backbone amine and carbonyl groups (Fernández-Alonso et al., 2012; López-Barón et al., 2017). For example, Yang et al. (2019) have reported, based on a rheological study, that the main interactions between corn starch and whey protein isolate are by hydrogen bonding. Additionally, Fourier-transform infrared (FTIR) analysis of extruded samples has clearly indicated enhanced hydrogen bonding between wheat starch and hydrolyzed pea protein (López-Barón et al., 2018), suggested to be due to hydrolyzed pea protein having more free carboxyl groups, thereby increasing its ability to hydrogen bond with wheat starch (López-Barón et al., 2018).

As illustrated in **Fig. 2I**, in addition to hydrogen bonding, there can also be hydrophobic interactions between processed proteins and starches (Li et al., 2020; López-Barón et al., 2017). Hydrophobic interactions result from the tendency of hydrophobic residues to aggregate with each other, thereby avoiding water (Pace et al., 2011). In proteins, hydrophobic interactions provide a major driving force for their folding, leaving hydrophobic residues inside native protein molecules (Aryee et al., 2018). Ryan and Brewer (2007) found that exogenous soy proteins could bind to wheat starch granules through hydrophobic interactions, as there are proteins inherently on the surface of wheat starch granules. If the surface of starch granules is rendered hydrophobic, hydrophobic interactions between the starch and denatured protein can occur. Studies (Li et al., 2020; Wang et al., 2013) have shown that the hydrophilic groups of wheat gliadin protein remain on its surface, while the hydrophobic groups are located inside the gliadin molecules, which may lead to a more stable double-helical structure of the gliadin–starch system, resulting from hydrophobic interactions and hydrogen bonding. Furthermore, heating causes the swelling of starch granules and the leaching of starch chains (amylose and amylopectin) and the polypeptide chains of protein are partially expanded (e.g. exposing the hydrophobic amino acids buried inside native proteins), and then the starch chains can also bind with polypeptide chains through hydrophobic interactions and/or hydrogen bonding (López-Barón et al., 2017; López-Barón et al., 2018).

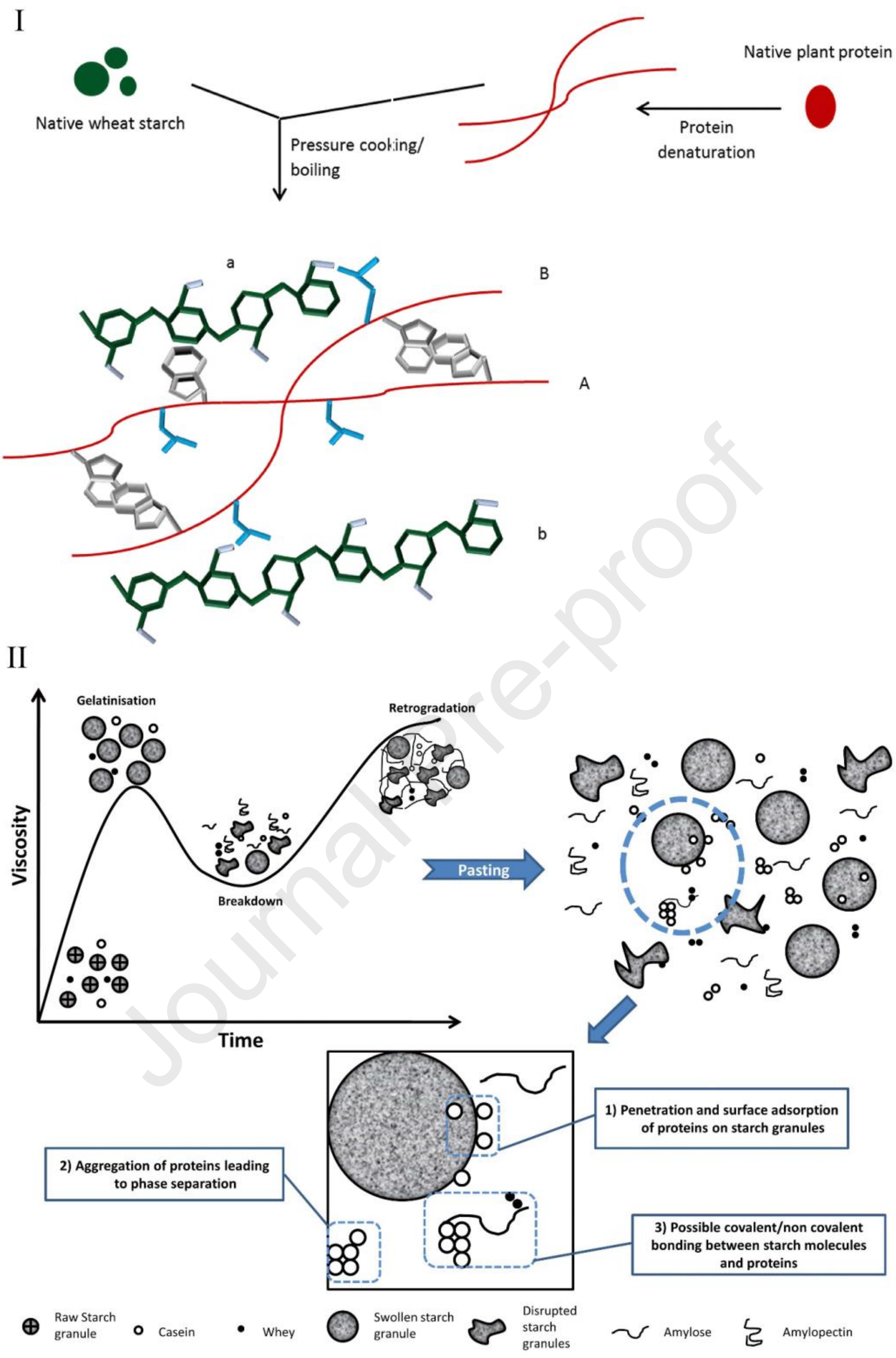

Fig. 2 (I) Polypeptide chains A and B present tryptophan residues (gray) and aspartic acid residues (blue) as binding sites, which create non-polar and polar interactions with starch chains a and b (green). Polypeptide chains A and B interact through tryptophan residues (i.e. hydrophobic interactions), facilitating a coating effect by denatured protein on the surface of the gelatinized starch

matrix. Also, polypeptide chains (B) interact with the starch chains (a and b) through aspartic acid residues (blue), thereby connecting the two starch chains. Reprinted from López-Barón et al. (2017), copyright (2017), with permission from Elsevier. **(II)** Graphical representation of starch–milk proteins interactions during continuous-shear heating. Reprinted from Kumar et al. (2017), copyright (2016), with permission from John Wiley and Sons.

Electrostatic interactions occur mainly between anionic groups on starches and positively charged groups on proteins (Jamilah et al., 2009). Starch molecules are generally considered to be neutral macromolecules, but the amylopectin from potato, root and tuber starches is negatively charged, due to the presence of phosphate groups; and some modified starches (e.g. phosphate starch) are also charged (Quiroga Ledezma, 2018). The charge on a protein surface depends on the protein's p*I* (isoelectric point) and the pH of the system, and the presence of salts can affect the net charge of the protein (Quiroga Ledezma, 2018). Therefore, interaction forces can be affected by the environmental pH, the presence of salts, and the charges on the starch and protein (Warnakulasuriya & Nickerson, 2018). Sun et al. (2016) have reported that the interactions between modified starches (e.g. phosphate starch, hydroxypropyl starch, and octenyl succinic esters of starch) and casein involved electrostatic adhesion and hydrogen bonding, both of which were affected by steric hindrance, and that electrostatic adhesion was the main type of interaction between starch phosphate and casein. Ionic interactions may occur between negatively charged starches and amino acid residues of proteins; however, few studies on such ionic interactions have been reported (Majumdar, Sen, & Ray, 2019).

2.3.2 Covalent interactions

Covalent interactions can be described as strong chemical bonds between specific reactive groups on macromolecules. For example, enzyme conjugation, chemical crosslinking, and the Maillard reaction are common ways of forming covalent bonds between polysaccharides and proteins (de Oliveira, Coimbra, de Oliveira, Zuñiga, & Rojas, 2016; Nicoletti Telis, 2019; Wei & Huang, 2019). The Maillard reaction is safe and convenient as no additional chemicals have been added and the conditions under which it occurs are simple and controllable (de Oliveira et al., 2016). The Maillard reaction conditions for the generation of protein–polysaccharide conjugates, the functional properties of such conjugates, and their applications in food are reviewed by de Oliveira et al. (2016). However, because starch molecules contain terminal reducing groups, the Maillard reaction is considered to be the most common type of covalent binding reaction between starches and proteins in starch-based, processed food systems, which typically produces attractive aromas, colors, and flavors in baked products (Pérez, Matta, Osella, de la Torre, & Sánchez, 2013; Wei & Huang, 2019), but condensation reactions between reducing sugars and lysine amino side chains during Maillard reaction result in a severe reduction of lysine availability (Pérez et al., 2013). Additionally, dry-heating and wet-heating are common processing operations used for creating Maillard reactions (Consoli et al., 2018). Covalent bonding can occur between terminal carbonyl reducing groups on starch molecules and amino groups on proteins, and requires the control of reaction temperature and time, system pH, and humidity conditions (de Oliveira et al., 2016).

2.3.3 Factors influencing interactions

Many factors have been reported to affect the interactions between starches and proteins, especially including processing conditions such as temperature, pH, and ionic strength (Heertje, 2014; Li & Huang, 2015; Warnakulasuriya & Nickerson, 2018; Wei & Huang, 2019).

Thermal treatments can affect starch and protein conformations and, consequently, the occurrence of interactions. For example, cooking can result in starch gelatinization and protein denaturation, thus exposing additional sites for reaction, which may facilitate interactions between the starch and protein, ultimately affecting the structural and digestive properties of various food products (López-Barón et al., 2017; Lu, Donner, Yada, & Liu, 2016; Paliwal, Thakur, & Erkinbaev, 2019; Petitot, Abecassis, & Micard, 2009). Baking is also highly impactful on the interactions between starches and proteins (Paliwal et al., 2019). During baking, protein unfolding and protein–protein interactions, as well as interactions of protein with starch in a paste, may occur, thus affecting structural properties (Quiroga Ledezma, 2018). During extrusion, biopolymers can undergo many physical and chemical transformations. Specifically, starches can be gelatinized and proteins can become unfolded, realigned, hydrolyzed, and can physically crosslink with starch chains, all of which may ultimately lead to improved product texture and digestibility (Cabrera-Chάvez et al., 2012; Heredia‐Olea, Contreras‐Alvarado, Perez‐Carrillo, Rosa‐Millάn, & Serna‐Saldivar, 2019; Moisio, Forssell, Partanen, Damerau, & Hill, 2015; Philipp, Buckow, Silcock, & Oey, 2017; Philipp, Oey, Silcock, Beck, & Buckow, 2017; Yu, Ramaswamy, & Boye, 2012). Kumar et al. (2017) have recently reviewed the application of dairy proteins in starch-based extruded products, reporting that starch–protein complexes may be formed during extrusion, and that the formation of such complexes can prevent starch fragmentation during extrusion and thereby affect the viscosity, hardness, water absorption, and water solubility of the resulting extrudates.

pH can often affect the charges in mixed starch–protein systems. In particular, the surface charge on a protein depends on the protein’s p*I* and the pH of the system, and thus, the behavior of proteins in food processing operations is affected by the pH used (Ghosh & Bandyopadhyay, 2012; Quiroga Ledezma, 2018). For example, Chen et al. (2020) have reported that the elasticity and viscosity of potato starch gel matrices containing added whey protein fibrils were higher at pH 3.5 than at pH 6.8, possibly because electrostatic interactions between the starch (with its anionic components) and protein could be promoted, as the pH decreased below the protein’s p*I* (Chen et al., 2020; Firoozmand, Murray, & Dickinson, 2012).

The addition of salt ions can affect the degree of network formation, through electrostatic shielding of protein molecules, thus affecting interactions with starch (Lambrecht, Rombouts, Nivelle, & Delcour, 2017a, 2017b). At low salt concentrations, salt ions can preferentially interact with protein molecules, and such interactions can interfere with protein–protein and protein–starch interactions. However, higher salt concentrations do not favor protein dissolution, which thus can limit protein interactions with starch (Joshi et al., 2014; Li & Huang, 2015). Therefore, a better understanding of starch–protein interactions, under different pH and salt conditions of processing operations for starch-based foods, can be conducive to improving the quality of many such products.

3. Morphology and structure of starch-based matrices containing protein

3.1 Morphology

The microstructure of starch-based food matrices is highly dependent on their composition, processing, and post-processing storage, and plays a crucial role in determining the textural, sensory, and digestive properties of such foods (Singh, Kaur, & Singh, 2013).

Many studies (Feng, Mu, Zhang, & Ma, 2020; Joshi et al., 2014; Liu et al., 2019; Phongthai et al., 2017; Sun & Xiong, 2014; Wang, Zhang, et al., 2020; Xie et al., 2017; Zhang, Chen, Chen, & Chen, 2019; Zhou, Liu, & Tang, 2018) have used scanning electron microscopy (SEM) to observe the micromorphology of doughs and mixed starch–protein gel systems and found that proteins affect the micromorphology of such mixed systems. Morphological observations of doughs have shown that starch granules are trapped in a protein matrix, and the dough structure can be changed by different dough preparation conditions (i.e. mixing time and pressure) (Gao, Koh, Tay, & Zhou, 2017; Liu et al., 2019; Liu et al., 2015; Sarabhai & Prabhasankar, 2015). Structural properties of starches and proteins, in doughs and close-contact dough fractions, may lead to certain interactions, which consequently change the rheological properties of such doughs and restrict the swelling of starch granules (Feng et al., 2020; Liu et al., 2019). For example, for gluten-free pasta, its structural characteristics depend on the type of exogenous protein added, and the resulting protein network encapsulates starch granules, which can limit the access of water during cooking, thereby affecting the starch’s digestive characteristics (Giuberti, Gallo, Cerioli, Fortunati, & Masoero, 2015; Phongthai et al., 2017). Additionally, when different proteins (gluten and egg white protein) were mixed with sweet potato starch, leached amylose and low-molecular-mass amylopectin interacted with denatured protein during gelatinization and formed different network structures, resulting in altered textural and cooking properties of the final sweet potato vermicelli products (Feng et al., 2020). For mixed starch–protein gel matrices, in general, both starch and protein gels can form three-dimensional network structures (Joshi et al., 2014). Starch gels generally have dense and porous network structures, while protein gel structures can differ, depending on a given protein’s gelation ability (Joshi et al., 2014; Yang, Liu, Ashton, Gorczyca, & Kasapis, 2013; Zhang et al., 2019). Some studies (Joshi et al., 2014; Zhang et al., 2019) have reported that gels with protein have a tighter and more homogeneous structure, compared to gels without protein, while other researchers (Sun & Xiong, 2014; Wang et al., 2021) have reported that mixed starch–protein gels become looser with an increasing proportion of protein in the blend. Such behavior is thought to be related to the ability of proteins to form gels and to their interactions with starches (Sun & Xiong, 2014; Zhang et al., 2019).

The distribution of starch and protein phases in mixed gels can be observed by confocal laser scanning microscopy (CLSM). CLSM observations have shown that proteins tended to adsorb onto the surfaces of starch granules, which resulted in a steric hindrance effect to keep the starch granules separated (López-Barón et al., 2017; Ye et al., 2018; Yu et al., 2018). It has been reported that such mixed gels may be homogeneous or exhibit two-phase separation (such as by protein aggregation) (Noisuwan, Hemar, Wilkinson, & Bronlund, 2009; Vu Dang, Loisel, Desrumaux, & Doublier, 2009). Chanvrier, Colonna, Della Valle, and Lourdin (2005) have reported that, during thermomechanical processing, proteins in a corn starch–zein mixture aggregated, and the blend morphology was affected by the starch/zein ratio, which in turn largely affected the mixture’s mechanical properties.

Moreover, added protein hydrolysates, because of their small molecular mass, are said to be able to easily penetrate starch granules and reach the granule’s center, and thus, form a network structure with starch chains (Kong, Niu, Sun, Han, & Liu, 2016; Niu, Wu, & Xiao, 2017; Xiao & Zhong, 2017).

Atomic force microscopy (AFM) can be used to reveal nanoscale structural features of samples, which are considerably smaller than those observable by CLSM (Niu et al., 2017; Xiao, Niu, Wu, Li, & He, 2019; Xiao & Zhong, 2017). AFM studies have demonstrated that the number and height of protrusions on the surfaces of starch–protein hydrolysate mixtures were significantly altered, compared to those of starch-only samples, suggesting that protein hydrolysates can affect the aggregation of amylose and amylopectin on a nanoscale (Kong et al., 2016; Niu et al., 2017; Xiao et al., 2019; Xiao & Zhong, 2017).

3.2 Long-range molecular ordered structures

The long-range ordered structures of starch granules are generally considered to involve crystalline structures, which are believed to form through the parallel packing of left-handed coaxial double helices in extended regular arrays (Wang, Xue, Yousaf, Hu, & Shen, 2020). Native starches possess either an A-type, B-type, or C-type polymorphic structure (Wang & Copeland, 2013). X-ray scattering has been widely used to characterize the long-range ordered structures of starches, including their relative crystallinity (Wang, Li, et al., 2015). The crystalline structure of native starches is destroyed during gelatinization, leading to the disappearance of the XRD peaks representing crystallites (Wang et al., 2021). With increasing storage time, many cooked starch matrices typically manifest diffraction peaks due to recrystallization or retrogradation (Hu et al., 2020; Xiao & Zhong, 2017). XRD analyses have shown that the addition of proteins or protein hydrolysates can reduce the crystallinity of starch gel matrices after long-term storage by effectively inhibiting starch retrogradation (Hu et al., 2020; Niu et al., 2017; Xiao & Zhong, 2017; Zhang et al., 2019). For example, compared to gelatinized rice starch alone, rice starch samples with an increasing ratio of whey protein hydrolysate displayed gradually reduced relative crystallinity after storage for 7 and 14 days (Hu et al., 2020). The inhibition of starch retrogradation by proteins could be due to interactions between the protein and starch granules, which could hinder the penetration of sufficient water into the granules, resulting in reduced starch gelatinization (Wang, Zhang, et al., 2020). In this way, the protein together with un-gelatinized starch could prevent starch chain rearrangement. It has also been suggested that the hydrogen bonding between starch and water molecules could be hindered, due to hydrophobic interactions between protein chains, thus inhibiting starch retrogradation (Wang, Zhang, et al., 2020). However, Chen, Wang, Fan, Yang, and Chen (2019) have reported that retrograded samples of mixtures of enzyme-modified wheat protein–potato starch exhibited a typical V-type XRD pattern, and the relative crystallinity of the mixture samples increased with increasing concentration of the enzyme-modified wheat proteins, which could have promoted amylopectin recrystallization. It has been found that the removal of endogenous proteins from rice starch granules did not destroy the crystalline morphology of the starch, but decreased the extent of crystallinity, suggesting either that both types of endogenous rice proteins (i.e. starch granule–channel proteins and starch granule–surface proteins) might be involved in the formation of crystalline structures or that the operative extraction methods partially destroyed starch crystallites

(Hu et al., 2017; Zhan et al., 2020). Moreover, processing operations can influence the multi-scale structures of starch–protein systems, possibly by promoting interactions between starches and proteins which lead to specific structures (Chen, Luo, et al., 2019; Chi, Li, Zhang, Chen, & Li, 2018; Li et al., 2018; Qiu et al., 2015; Xiao et al., 2019). For instance, starches (corn and potato) complexed with soybean peptide, subjected to HMT, have been reported to display higher relative crystallinity than their non-HMT counterparts, which was attributed to interactions between starch chains and side-chain groups in the soybean peptide during HMT (Chen, Luo, et al., 2019).

Small-angle X-ray scattering (SAXS) can complement wide-angle X-ray scattering (WAXS), in understanding long-range order at nanometer scales (Li, Senesi, & Lee, 2016). Using a SAXS technique, Chi et al. (2018) have reported that the SAXS intensity of rice starch samples varied with the addition of rice protein or protein hydrolysates, thereby revealing changes in long-range starch order. Thus, the ordered structures and amorphous structures of rice starch can be altered by interactions with rice proteins or protein hydrolysates (Chi et al., 2018). This suggests that such structural features of rice starch can be altered simply by complexing with rice protein or protein hydrolysates before cooking (Chi et al., 2018). Despite this earlier work, to date, there have been few other reports on the use of SAXS to study structural properties of starch–protein mixtures. Thus, more work is warranted on the use of SAXS to explore the effects of proteins on starch structures.

3.3 Short-range molecular ordered structures

Short-range ordered structures in starches are said to mainly involve double-helices and V-type single helices, both of which are involved in starch crystallinity (Wang, Xue, et al., 2020). FTIR spectroscopy can provide valuable information about the presence of ordered and amorphous structures in starches (Chávez-Murillo, Veyna-Torres, Cavazos-Tamez, de la Rosa-Millán, & Serna-Saldívar, 2018). FTIR peaks at 1047, 1022, and 995 cm^{-1} have been assigned to short-range order, amorphous content, and hydrated crystallites, respectively, in starches; two FTIR peak intensity ratios, 1047/1022 cm^{-1} and 995/1022 cm^{-1}, have been used to estimate the short-range order in various starches, and increases in their ratios indicate an increase in structural order. (Chen, Wang, et al., 2019; Li et al., 2020; Liu et al., 2015; Lu et al., 2016; Yang et al., 2019). Moreover, since the characteristic peaks in FTIR spectra correspond to bond stretching, possible interactions in starch-based systems can be inferred from the changes in those characteristic peaks (Lu et al., 2016).

The short-range ordered structures in starches are said to be maintained mainly by hydrogen bonding (Lu et al., 2016; Zhan et al., 2020). During gelatinization, hydrogen bonds between starch chains are thought to be broken, due to the entry of water molecules, leading to weakening or even a disappearance of characteristic absorption peaks, especially at 995 cm^{-1} (Li et al., 2020). Then, starch chains can rearrange during cooling and retrogradation, as reflected by increases in the characteristic FTIR peak intensities (Li et al., 2020). Since proteins can interact with starch molecules, especially by hydrogen bonding, the addition of proteins or protein hydrolysates can affect the structural changes of starches at a molecular level (López-Barón et al., 2018). Several studies have shown that proteins or protein hydrolysates can inhibit the hydrogen-bonded crosslinking of starch chains during aging, and thus, the formation of ordered structures (as reflected by a decreased intensity of the 1047/1022 cm^{-1} ratio) with this inhibitory effect dependent on the protein/protein hydrolysate concentration (Guo, Lian, Kang, Gao, & Li, 2016; Hu et al., 2020; Lian, Zhu, Wen, Li, & Zhao, 2013; Xiao et al., 2019; Zhang et al., 2019). Thus, the presence of proteins or protein hydrolysates can be seen to inhibit starch retrogradation. However, other researchers (Chen, Wang, et al., 2019; Xijun, Junjie, Danli, Lin, & Jiaran, 2014; Yang et al., 2019) have found that proteins can promote starch retrogradation, and that more ordered structures can result from the addition of proteins. Such discrepancies might be explained by differences in protein structure and hydration capacity (Chen, Wang, et al., 2019; Wang, Zhang, et al., 2020; Xijun et al., 2014; Zhang et al., 2019). In any event, the reasons behind the different effects of proteins on starch retrogradation are not entirely understood, and therefore warrant further investigation.

^{13}C cross-polarization/magic-angle-spinning nuclear magnetic resonance (^{13}C CP/MAS NMR) has also been used to analyze the short-range order of starches in starch-based systems (Chi et al., 2018; Xijun et al., 2014). Normally, the C4 resonance peak can be correlated with a starch's amorphous fraction, and the C1 resonance peak can be highly correlated with the contents of amylose V-type single-helices and amylopectin double-helices in various starches (Chi et al., 2018; Flores-Morales, Jiménez-Estrada, & Mora-Escobedo, 2012). As shown in **Fig. 3**, Guo et al. (2016) have suggested, based on ^{13}C NMR results, that tyrosine (Tyr) on wheat protein may interact with the amylose in wheat starch, and have described a mechanism for the inhibition of amylose retrogradation by wheat glutenin. Related studies have also reported that the addition of proteins can result in changes in starch–protein interactions (as reflected by changes of the peak signals in ^{13}C CP/MAS NMR spectra), which can subsequently affect the retrogradation and digestive properties of starches (Flores-Morales et al., 2012; Guo et al., 2016; Renzetti et al., 2012; Xijun et al., 2014). Additionally, Chi et al. (2018) have reported that rice starch with added pepsin–pancreatin protein hydrolysates exhibited more amorphous starch, suggesting that the pepsin–pancreatin protein hydrolysates hindered rice starch aging after gelatinization. In the studies discussed above, the ^{13}C CP-MAS/NMR results consistently supported the results obtained by FTIR.

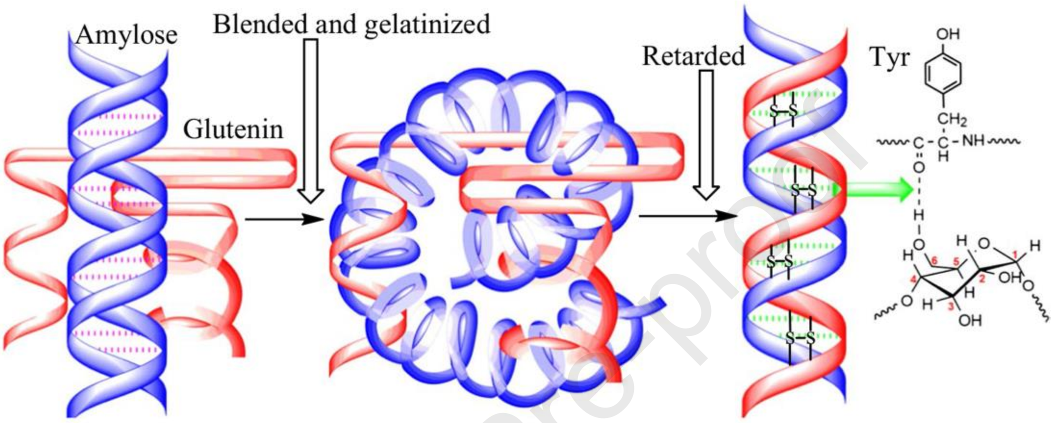

Fig. 3 Mechanism of glutenin retardation of the retrogradation of amylose

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4. Properties of starch-based matrices containing protein

For mixed starch–protein matrix systems, protein structure–property relationships, including the hydration ability, thermal properties, and gelation behavior of the proteins, largely influence the features of the mixed systems. Specific effects reported have included the following: the hydration ability of proteins affects the interactions of starches with water (López-Barón et al., 2017; Wang et al., 2021; Yang et al., 2019); due to the thermal properties of proteins, heat-induced conformational changes of proteins can result in protein–starch interactions (López-Barón et al., 2017; Wang et al., 2021); and the gelling ability of proteins can cause crosslinking or phase separation in starch–protein mixtures during the gelation process (Warnakulasuriya & Nickerson, 2018). Thus, in these ways, the inclusion of proteins can significantly affect the textural, sensory, pasting, thermal, rheological, and digestive properties of starch-based matrices.

4.1 Pasting properties

The pasting behavior of starch is central to many starch-based food matrices and is usually characterized by changes in viscosity on heating, holding, and cooling (Li et al., 2018). Pasting temperature, peak viscosity, final viscosity, breakdown viscosity, and setback viscosity can all be easily determined using a rapid visco-analyzer (RVA) (Gani et al., 2015). Many studies have reported the effects of different proteins on the viscosity of different starch systems, for which viscosity is of great significance to the applications of such biopolymers in various food (Joshi et al., 2014; Kim, Kee, Lee, & Yoo, 2014; Kumar et al., 2017; Li et al., 2018; Sarabhai & Prabhasankar, 2015). For example, **Fig. 2II** shows the viscosity changes for a starch–milk proteins system under continuous-shear and -heating conditions, and the possible interactions between the starch and proteins that can cause such viscosity changes (Kumar et al., 2017). Proteins on the surfaces of starch granules can limit the swelling of the granules, and such interactions between starches and proteins can affect the viscosity parameters of such systems (Chinma, Ariahu, & Abu, 2013; Kett et al., 2013; Qiu et al., 2015; Reddy Surasani, Singh, Gupta, & Sharma, 2019; Ribotta, Colombo, & Rosell, 2012; Ribotta & Rosell, 2010). For instance, Gani et al. (2015) reported that wheat flour supplemented with milk protein hydrolysates exhibited a lower viscosity during cooling than that without supplementation, and the low setback viscosity could be expected to result in softer cookie crumb texture.

Pasting temperature corresponds to the minimum temperature required for starch cooking and the temperature at which viscosity increases during heating (Barak et al., 2014). Many studies (Baxter, Blanchard, & Zhao, 2014; Bravo-Núñez, Garzón, Rosell, & Gómez, 2019; Chen, Luo, et al., 2019; Chinma et al., 2013; Joshi et al., 2014; Kong et al., 2016; Likitwattanasade & Hongsprabhas, 2010; Sarabhai & Prabhasankar, 2015; Shevkani, Kaur, Kumar, & Singh, 2015; Wang, Zhang, et al., 2020; Xiao et al., 2019; Zhang, Sun, Wang, Wang, & Zhou, 2020) have reported that the pasting temperatures of starch–protein mixtures are higher than those of starch-only systems. For example, removal of protein fractions from rice flour has been found to reduce the pasting temperature of such rice-based systems (Baxter, Zhao, & Blanchard, 2010; Likitwattanasade & Hongsprabhas, 2010). Two explanations for how proteins limit the pasting temperature of starches have been suggested: 1) the absorption of excess water by proteins; and/or 2) the formation of complexes between starches and proteins, which restrict starch–water interactions and starch gelatinization (Joshi et al., 2014; Reddy Surasani et al., 2019; Wang, Zhang, et al., 2020).

In contrast, significant decreases in the pasting temperatures for various starch–protein mixtures have also been reported (Qiu et al., 2015; Ribotta et al., 2007; Ribotta et al., 2012; Ribotta & Rosell, 2010). Elsewhere, starch pasting temperatures have been reported to be unchanged by the addition of wheat gliadin and glutenin (Barak et al., 2014; Chen, Zhang, Li, Xie, & Chen, 2018). Thus, proteins can affect starch gelatinization in different ways, depending on their ability to retain water and their interactions with starch granule surfaces and starch molecules (Ribotta et al., 2007; Wang et al., 2021).

Addition of proteins to starch-based systems has been shown to reduce the pasting viscosity (Baxter et al., 2014; Chen, Luo, et al., 2019; Joshi et al., 2014; Kim et al., 2014; Kong et al., 2016; Sarabhai & Prabhasankar, 2015; Shevkani et al., 2015; Sun & Xiong, 2014; Xiao et al., 2019), and the degree of viscosity reduction has been found to be related to protein type (Barak et al., 2014; Shin, Gang, & Song, 2010; Storck et al., 2013). For example, Barak et al. (2014) have reported that, compared to glutenins, gliadins were more effective at decreasing the pasting viscosity of dough. Both types of proteins reduced the system viscosity because they competed for the water along with the starch granules. However, glutenins have a β-sheet structure and tend to form an entangled network upon hydration, and thus glutenins formed a network throughout the flour paste, being more resistant to stirring blades than gliadins (Barak et al., 2014). Additionally, viscosity decreased more when protein hydrolysates rather than proteins were added, which was attributed to the fragmentation of proteins during hydrolysis, resulting in a loss of the water entrapment ability of proteins (Gani et al., 2015; Xiao et al., 2019). From other related studies, the following possible explanations have been suggested for the reduction in starch pasting viscosity due to the addition of proteins:

- Competition between starches and proteins for water decreases the swelling power of starch (Chávez-Murillo et al., 2018; Hu et al., 2020; Li et al., 2020; Shevkani et al., 2015; Sun & Xiong, 2014; Wang, Zhang, et al., 2020; Zhang et al., 2020);

- Interactions between starches and proteins may reduce the amount of amylose leached out during gelatinization (Bravo-Núñez et al., 2019; Hu et al., 2020; Reddy Surasani et al., 2019; Sun & Xiong, 2014);
- Protein addition causes starch dilution (Marti et al., 2014; Sarabhai & Prabhasankar, 2015; Sciarini, Ribotta, León, & Pérez, 2010; Shevkani et al., 2015; Zhang et al., 2020);
- Proteins can act as inert fillers, to hinder hydrogen bonding between starch chains (Kumar, Brennan, Zheng, & Brennan, 2018; Sarabhai & Prabhasankar, 2015; Sciarini et al., 2010; Sopade, Hardin, Fitzpatrick, Desmee, & Halley, 2006).

However, Shin et al. (2010) have reported that added whey protein inhibited the pasting viscosity of rice flour, whereas added TGase enzyme increased the pasting viscosity by crosslinking between the added and rice proteins. Additionally, other studies (Chinma et al., 2013; Qiu et al., 2015; Ribotta et al., 2007; Ribotta et al., 2012; Ribotta & Rosell, 2010) have suggested that certain proteins (e.g. soy protein and pea protein) can increase the overall viscosity of protein–starch pastes, due to enhanced protein–starch interactions during heat treatment. Those interactions might involve crosslinks formed between hydrophilic groups on the protein and starch molecules during heat treatment (Chinma et al., 2013; Qiu et al., 2015; Ribotta et al., 2012; Ribotta & Rosell, 2010).

4.2 Thermal properties

The effect of proteins on the thermal properties of starch–protein blended matrices has been widely reported, as related to the characteristics and quality of various starch-based foods (Chen, Wang, et al., 2019; Jamilah et al., 2009; Li, Wei, Fang, Zhang, & Zhang, 2014; Liu et al., 2019; Lu et al., 2016; Zhu et al., 2020). DSC is most often used to study structural changes in proteins and starches as a function of temperature, and onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and ΔH values have been obtained (Jamilah et al., 2009; Marín, Alemán, Montero, & Gómez-Guillén, 2018; Wan, Liu, & Guo, 2018).

DSC heating curves for acetylated potato starch–whey protein blended systems have shown two obvious peaks, reported to represent acetylated potato starch gelatinization (about 61 °C) and whey protein denaturation (about 73 °C) (Ren et al., 2017). However, single endothermic peaks for other starch–protein blends have also been reported in other studies (Lu et al., 2016; Yang et al., 2019). The following two explanations for these latter observations have been suggested: 1) the protein concentration in the mixed system was low (≤20%), so that the thermal events for the protein were overwhelmingly overlapped by those for the starch; and/or 2) the protein in the mixed system had already been denatured and thus thermally inactivated (Lu et al., 2016; Sciarini et al., 2010; Yang et al., 2019). Moreover, no new endothermic peaks have been seen to appear in DSC thermal curves for such mixed systems, suggesting that no new substances were generated during thermal treatment (Wang, Zheng, Yu, Wang, & Copeland, 2017). Various studies e.g. (Villanueva, Mauro, Collar, & Ronda, 2015) have reported significant effects of the presence of proteins on starch gelatinization. Often, proteins are found to have an inhibitory effect on the gelatinization of starches, consistent with RVA results (Lu et al., 2016). The extent of the DSC peak shift to higher gelatinization temperature is considered to be closely related to the type and proportion of protein present. For example, Li et al. (2020) have suggested that the higher T_p value for wheat starch gelatinization, obtained for a glutenin–wheat starch mixture as compared to a gliadin–wheat starch mixture, was probably due to the higher hydrophobicity of glutenin than gliadin (Mittal & Best, 2008). López-Barón et al. (2017) have reported that the addition of denatured and hydrolyzed plant proteins to wheat starch increased the T_p value for wheat starch gelatinization more obviously than did the corresponding addition of native plant proteins, possibly due to changes in surface hydrophobicity and WHC of such plant proteins after their heat-induced denaturation or hydrolysis. It has been speculated that the effects of proteins on starch gelatinization may result from a competition for available water between these two types of biopolymer, leading to a water re-distribution between them (Li et al., 2020; Lu et al., 2016; Ren & Wang, 2019; Yang, Luan, Ashton, Gorczyca, & Kasapis, 2014; Zhu et al., 2020), or from interactions between starch granules and proteins and/or between material leached out of starch granules and proteins (Chen, Zhou, Yang, & Cui, 2015; Chen, Luo, et al., 2019; Li & Zhu, 2017; Ribotta et al., 2007; Wang et al., 2021; Zhu et al., 2020). In these ways, the starch gelatinization temperature could be significantly affected by the specific type and dosage of protein and/or by various starch–protein interactions.

Starch retrogradation is a process in which certain chains of gelatinized starch re-align and re-associate to form crystallites during cooling, which results in significantly influenced textural and sensory characteristics of many starch-based food systems (Chen, Wang, et al., 2019; Lu et al., 2016; Wu, Chen, Li, & Wang, 2010). Thermal analysis can provide information on starch aging in starch–protein mixtures; from DSC analysis, the effects of proteins on the retrogradation properties of starch gels can be better understood (Wang, Li, et al., 2015). It has been reported that the ΔH value measured by DSC mainly reflects the energy required to melt the potato amylopectin crystallites recrystallized during retrogradation (Chen, Wang, et al., 2019). Thus, measured changes in ΔH can reflect whether the aging process of starch matrices is inhibited or promoted. Various studies have found that the measured enthalpies for retrograded starch–protein systems were higher than those for starch-only systems, but could be decreased with even higher amounts of added proteins (Chen, Wang, et al., 2019; Lu et al., 2016; Yang et al., 2019). Moreover, other studies have reported that the addition of rice (or other) protein hydrolysates can significantly reduce the measured ΔH for retrograded rice or wheat starch, indicating that such protein hydrolysates can inhibit the retrogradation of such starches (Niu et al., 2017; Xiao & Zhong, 2017; Zhang et al., 2020). This phenomenon may be due to the active polyhydroxyl groups in protein hydrolysates, which may block or insert into the hydrogen bonds among starch molecules (Niu et al., 2017). For a food product such as Chinese rice vermicelli, starch retrogradation is desired, as it provides preferred food characteristics, including improved textural, sensory, and digestive properties (Karim, Norziah, & Seow, 2000). However, in many other cases, inhibiting starch retrogradation is advantageous for prolonging the shelf-life of various starch-based food products (Chen, Wang, et al., 2019; Niu et al., 2017; Xiao & Zhong, 2017; Zhang et al., 2020), most familiarly exemplified by bread staling. Thus, understanding the effects of proteins on starch retrogradation characteristics can be highly beneficial to the informed development of improved starch-based food products with desired properties.

4.3 Rheological properties

The rheology of various food products plays a significant role in quality control, sensory evaluation, process assessment, and product development (Jamilah et al., 2009). Many studies (Amjid et al., 2013; Brandner, Becker, & Jekle, 2019; Considine et al., 2011; Jamilah et al., 2009;

Kumar et al., 2017) have reported the effects of various proteins on the rheological properties of starches and highlighted component interactions. Understanding how proteins affect the rheological characteristics of starch-based food systems can be of great significance for food processing.

A temperature sweep technique can be used to study rheological changes during heating and cooling of starch-based systems and to identify their starch gelatinization temperatures (Yang et al., 2013). For example, temperature sweep studies of mixed lentil starch–lentil protein gel matrices have shown that the gel development process in lentil protein-dominated composites was much slower than that in lentil starch-dominated composites, and lentil starch-rich composite gels exhibited more solid-like properties (Joshi et al., 2014). During heating, the storage modulus (G') values for starch–protein mixtures have been found to increase with temperature until reaching peak values (the swelling of starch granules), then to decrease upon further heating (the breakage of swollen starch granules); with increasing protein content, the temperature where G' reached a peak increased, while the G' value at this point decreased, which may be due to the proteins limiting starch swelling (Ghumman, Kaur, & Singh, 2016; Hu et al., 2020; Lu et al., 2016; Xiao & Zhong, 2017). Furthermore, Zhou et al. (2018) have reported that there were obvious differences in the G' and loss modulus (G'') behaviors, as affected by protein addition, between wheat flour–soy protein doughs and wheat flour–whey protein doughs. The difference in modulus changing may be due to the higher water absorption and weaker gelation abilities of soy proteins than whey proteins (Comfort & Howell, 2002). For wheat flour–whey protein doughs, both the G' and G'' peak values first decreased slightly, as the whey protein level was increased from 0 to 10 wt%, and then increased significantly, as the whey protein level was increased further to 30 wt% (Zhou et al., 2018). Regarding this, the crosslinking sites between whey protein and wheat starch increased with increasing protein concentration, subsequently affecting the properties of the dough (Zhou et al., 2018). Thus, differences have been found in the effects of both protein type and protein concentration on the G' and G'' values for different starches (Ghumman et al., 2016). During cooling, gelatinized starch chains can undergo retrogradation, which typically results in increased moduli (Yang et al., 2019). Also, the formation of starch–protein gel networks stabilized by interactions has been reported to be responsible for increasing gel elasticity (Ghumman et al., 2016; Xiao & Zhong, 2017; Yang et al., 2019). From the discussion above, we can conclude that the trend of modulus change determined from temperature sweep studies is consistent with the trend of changing RVA viscosity; that is, the moduli and viscosity of various mixed starch–protein systems first increase and then decrease during heating, while the moduli and viscosity both increase again during subsequent cooling; and the moduli and viscosity of mixed starch–protein systems are lower than those of corresponding starch-only systems.

Frequency sweep testing can be used to provide information about the type of gel formed in mixed starch-protein samples; protein addition to starch-based batters has been reported to have a significant effect on moduli (Patraşcu, Banu, Vasilean, & Aprodu, 2016). Generally, protein gels show greater frequency dependence, indicating that they are weaker gels, while starch gels are stronger and more independent of frequency (Joshi et al., 2014). Several studies (Kim et al., 2014; Sang et al., 2018; Wang, Chen, Yang, & Cui, 2017; Yang et al., 2019; Zhou et al., 2018) have reported that the addition of protein decreased the G' and G'' values of various starch-based systems, whereas other studies have reported increased G' and G'' values in a certain frequency range (Chen et al., 2018; Qiu et al., 2015; Ribotta et al., 2012; Ronda, Oliete, Gómez, Caballero, & Pando, 2011; Wang, Huang, Kim, Liu, & Tilley, 2011). A decrease in moduli indicates a weakened starch gel structure, while increased moduli indicate a stronger gel structure (Yang et al., 2019). Moreover, the *G′* values for mixed corn starch–soy protein pastes have been found to increase significantly after dry-heating (the moisture content was <10%, at 130 °C for 4 h), possibly due to enhanced interactions between the involved starches and proteins (Qiu et al., 2015).

Steady-shear, creep-recovery, strain-sweep, stress-sweep, and time-sweep tests, also used to determine the rheological properties of mixed protein–starch systems, have been helpful in understanding the starch–protein interactions in such systems (Chen, Wang, et al., 2019; Feng et al., 2020; Li et al., 2018; Villanueva, De Lamo, Harasym, & Ronda, 2018). It is of obvious importance to establish a food system with stable rheological properties, which would be favorable to food processing operations and the avoidance of collapse of product structure (Larrosa, Lorenzo, Zaritzky, & Califano, 2016; McCann, Le Gall, & Day, 2016; Zhang, Mu, & Sun, 2018).

Specifically, from steady-shear tests, it has been observed that addition of proteins can influence the flow behavior of starch-based samples to different extents, depending on the protein concentration (Chen et al., 2018; Kumar et al., 2018; Qiu et al., 2015; Ronda et al., 2011; Vu Dang et al., 2009; Wang, Chen, et al., 2017). For example, an increase in added wheat protein (glutenin treated by protein-glutaminase) concentration from 0.5 wt% to 1.0 wt% has been reported to lead to increased shear stress for a potato starch-based system, while a protein concentration further increased to 1.5 wt% led to reduced shear stress for the same system (Chen et al., 2018). Higher shear stress is said to indicate that the network structure of a sample is more resistant to shear (Chen et al., 2018). Furthermore, replacing starches with proteins has been found to reduce the viscosity of various mixed systems, compared to corresponding starch-alone systems, but to support the same shear-thinning behavior (Kumar et al., 2018; Wang, Chen, et al., 2017). For example, the addition of soy proteins has been found not to change the non-Newtonian shear-thinning behavior of corn starch systems (Qiu et al., 2015).

Creep and recovery measurements can provide insights into dough macrostructure (Zhang et al., 2018). It is said to be important to use large-deformation and/or -stress measurements, because the resulting rheological behavior of doughs can be determined under conditions similar to those for actual dough processing (Federici, Jones, Selling, Tagliasco, & Campanella, 2020). Sometimes, the addition of certain proteins can decrease the strength of specific gluten-free doughs and their recovery ability (Feng et al., 2020; Hernández-Estrada, Rayas-Duarte, Figueroa, & Morales-Sánchez, 2014; Sarabhai & Prabhasankar, 2015), but the opposite behavior can also result, due to the addition of different proteins (Ronda et al., 2014). Besides, strain-sweep testing has been used to determine the linear viscoelastic range for various starch-based samples, from which the effect of protein addition on the moduli of such starch-based systems in this range can be observed (Feng et al., 2020; Villanueva, Ronda, et al., 2018; Yu et al., 2020).

The following possible explanations for changes in the rheological properties of starch–protein mixed gel matrices during dynamic sweeping have been proposed:

- Different WHC characteristics of proteins and starches can result in proteins competing with starches for available water, which, in turn, can affect the rheological properties of various mixed systems (Feng et al., 2020; Joshi et al., 2014; Ronda et al., 2011);
- Different interactions in mixed starch–protein gels can result in different rheological properties. Interactions can occur between proteins and starch chains (Chen et al., 2018; Joshi et al., 2014; Qiu et al., 2015; Ribotta et al., 2012; Ronda et al., 2011; Ronda et al., 2014; Sang et al., 2018; Yang et al., 2019; Zhang et al., 2018), or between starch chains alone (Chen et al., 2018; Ribotta et al., 2012; Zhang et al., 2018), and can lead to crosslinking in proteins (Phongthai, D'Amico, Schoenlechner, & Rawdkuen, 2016; Singh & Singh, 2013; Zhang et al., 2018), or to self-aggregation of proteins (Ribotta et al., 2012; Ronda et al., 2014; Zhou et al., 2018), while phase separation between starches and proteins may also occur (Chen et al., 2018).

In summary, compared to starch-only gel systems, protein addition can strongly affect starch gel moduli, but such an effect can also be linked to many other factors, such as starch or protein type, protein/starch ratio, and treatment and environmental conditions.

4.4 Textural properties

Textural information is important in food product development, quality control, and in determining product shelf-life and evaluating characteristics associated with product sensory analysis (Levine & Finley, 2018; Rodriguez Furlán, Pérez Padilla, & Campderrós, 2015). Levine and Finley (2018) provide a comprehensive introduction to the food texture, including basic definitions of texture, measurement of texture, texture profile, and applications in food. A Texture Analyzer device is often used to determine the textural properties of food product samples and measure textural parameters such as hardness, cohesiveness, resilience, springiness, and chewiness (Joshi et al., 2014; Lu et al., 2016; Yang et al., 2013). Proteins often affect the properties of starch-based gels and doughs, and thus, the resulting textural characteristics of starch-based products (e.g. breads, cookies, and pasta). As illustrated in **Table 2**, protein addition can affect the textural properties of many different types of starch-based food systems, the extent of the effects being dependent on the type and dosage of protein added, as well as on acid addition (Villanueva et al., 2015).

Table 2 Textural properties of starch-based food systems

Test sample	Added protein and content	Textural results	Reference
Rice-based bread	Pea protein isolate (5 wt%)	Increased crumb firmness significantly	(Villanueva et al., 2015)
	Egg albumin (5 and 10 wt%)	Firmness increased at 10% level	
	Calcium caseinate (5 and 10 wt%)	Did not promote any crumb hardening	
Rice-based bread	Soy flour (10 wt%)	Diminished crumb hardness to half the value of rice-alone bread	(Sciarini et al., 2010)
Wheat-based bread	Whey protein and soy protein (0–30 wt%)	Both increased bread hardness, but the addition of whey protein	(Zhou et al., 2018)

		increased it more; gumminess and chewiness showed similarly increasing trends.	
Chinese noodles	Wheat gluten protein (7.0–19.6 wt%)	Noodle hardness increased sharply with gluten protein content >14.3%. Noodle springiness increased remarkably at 19.6% protein content.	(Zhang, Lu, Yang, & Dan Meng, 2011)
Gluten-free pasta	Rice bran protein concentrate (RBPC) and soy protein concentrate (SPC) (6 and 9 wt%, respectively)	Addition of RBPC or SPC significantly reduced the firmness of these rice flour-based gluten-free pasta.	(Phongthai et al., 2017)
Vermicelli	Defatted soy flour (DSF), whey protein concentrate (WPC) (0–15 wt%)	Addition of DSF and WPC improved hardness, springiness, and cohesiveness, but there were differences in the degrees of improvement.	(Lakshminarayan, Rajeswari, & Rao, 2010)
Cookies	Whey and casein protein concentrates and hydrolysates (0, 5, 10, and 15 wt%)	The fracture force of cookies increased significantly with increasing protein content up to 15%. The fracture force was higher with added hydrolysates than with added concentrates.	(Gani et al., 2015)

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For various starch gel matrices, the textural profile can be significantly changed by the addition of proteins. Compared to a lentil starch gel, a lentil protein gel has been reported to have a lower hardness (Joshi et al., 2014). The hardness of various starch-based gels tends to decrease with the addition of proteins, and the resulting mixed starch–protein gels are reported to be softer (Bravo-Núñez et al., 2019; Hu et al., 2020; Joshi et al., 2014; Lu et al., 2016; Ribotta et al., 2007; Sun & Xiong, 2014; Wang, Zhang, et al., 2020; Xie et al., 2017). However, in contrast, Baxter et al. (2014) have found that increasing glutelin and globulin protein levels in rice starch and flour resulted in linear increases in gel hardness, in agreement with their earlier findings on albumin addition to rice flour (Baxter et al., 2010). The influence of added proteins on the cohesiveness, springiness, and elasticity of starch-based gels is often limited by specific protein type and concentration, resulting in large differences in these parameters for different mixed starch–protein gels (Baxter et al., 2014; Lu et al., 2016; Sun & Xiong, 2014). For example, it has been suggested that interactions between rice starch granules and water largely determine the textural properties of such starch slurries, so that added proteins with strong WHCs may cause incomplete gelatinization of certain starches, thereby impacting mixed gel texture (Baxter et al., 2010). Additionally, the addition of proteins can change the structure of starch-based gels and thus, their texture (Bravo-Núñez et al., 2019; Joshi et al., 2014; Xie et al., 2017). For example, changes in gel structure caused by interactions of proteins with starch chains may affect the formation of hydrogen bonds between amylose and amylopectin or the “inert filler” effect of proteins (i.e. certain proteins can act as inert filler, to hinder hydrogen bonding between starch chains) (Bravo-Núñez et al., 2019; Hu et al., 2020; Xiao & Zhong, 2017).

An increased understanding of the texture of doughs can help to optimize dough processing and predict the quality of freshly baked products (Kweon, Slade, Levine, & Gannon, 2014). The effect of protein addition on dough hardness has been shown to be influenced by protein type (Barak et al., 2014; Campbell, Euston, & Ahmed, 2016; Larrosa et al., 2016; Manoj Kumar et al., 2019; Storck et al., 2013). For example, the addition of wheat glutenin protein has been reported to increase the hardness of a control wheat flour dough, while the addition of wheat gliadin protein decreases the control dough’s hardness (Barak et al., 2014). Campbell et al. (2016) have reported that a wheat flour-based dough containing added cowpea protein isolate was significantly harder than the corresponding wheat flour-only control dough, while dough hardness was decreased by the addition of thermally denatured cowpea protein isolate and was the softest with added glycated cowpea protein isolate, possibly due to the enhanced WHC of such protein isolates after denaturation and glycation (Campbell et al., 2016). In this way, the magnitude of the effect of protein addition on dough hardness can be influenced by the WHC of such proteins, such that a protein with strong WHC can significantly reduce dough hardness. Additionally, the cohesiveness and adhesiveness of doughs can also be significantly affected by added proteins, with protein type being one of the key factors. For instance, the stickiness of wheat flour-based bread dough has been reported to increase with increasing whey protein content but decrease with the addition of soy protein (Zhou et al., 2018). Elsewhere, Barak et al. (2014) have determined that added wheat gliadins were responsible for the cohesiveness and extensibility of wheat flour-based doughs, while added wheat glutenins made those doughs more rubbery and elastic. It has been suggested that the effect of different proteins on dough texture could likely be due to corresponding differences in the hydration capacities of such proteins, with higher hydration ability leading to doughs being softer and stickier, or else to the structural changes in doughs caused by protein crosslinking (Campbell et al., 2016; Manoj Kumar et al., 2019; Storck et al., 2013; Zhou et al., 2018).

Obviously, texture is an important sensory attribute of many food products, in the context of assessing product acceptability (Levine & Finley, 2018; Yu et al., 2020). The textural characteristics of various starch-based food systems, including firmness, springiness, and cohesiveness, are of prime importance, as they can decide consumer acceptance (Shevkani & Singh, 2014). Therefore, a better understanding of the effects of the type and dose of added proteins on the textural properties of starch-based matrices can be highly beneficial to the production of high-quality food products.

4.5 Sensory properties

Sensory evaluations of many different types of starch-based food products are most commonly carried out by human sensory panels (Philipp, Buckow, et al., 2017; Rosa-Sibakov et al., 2016; Sarabhai & Prabhasankar, 2015). Initially, training sessions, attended by groups of sensory panelists, are typically conducted to identify and agree on each relevant descriptive attribute (Philipp, Buckow, et al., 2017). Participants then analyze the different sensory attributes specified, according to the different sensory characteristics of the particular type of product being paneled, and finally evaluate that food product’s overall acceptability. Starch-based food products such as breads, cookies, and noodles often contain proteins added for the purpose of adjusting sensory properties (Campbell et al., 2016; Mancebo, Rodriguez, & Gómez, 2016; Manoj Kumar et al., 2019; Phongthai et al., 2016; Wani, Sogi, Singh, Sharma, & Pangal, 2012). Frequently, the type and amount of added protein critically determine the sensory properties of such starch-based food matrix systems(Campbell et al., 2016; Gani et al., 2015; Shin et al., 2010).

For various starch-based food products prepared from wheat flour, wheat gluten can have a greater influence than wheat starch on the sensory properties of the system, because gluten proteins can form three-dimensional protein networks that impart unique viscoelasticity to such wheat flour-based doughs (Kweon et al., 2014), and can limit the gelatinization of starch during baking or cooking (Ortolan & Steel, 2017). Ortolan and Steel (2017) have reported that increasing the wheat gluten protein content in bread dough can increase resulting bread volume, improve bread texture (i.e. softness) and uniformity, and give the product a better color (i.e. golden brown). Additionally, adding certain formula amounts of exogenous proteins (e.g. milk proteins, cowpea protein isolates, or watermelon seed protein isolates) can improve the sensory properties of products such as pasta and baked goods such as wheat bread, sponge cake, and cookies (Campbell et al., 2016; Gani et al., 2015; Giménez et al., 2012; Wani et al., 2012). Campbell et al. (2016) have reported that there was a trend toward higher sensory acceptability scores for wheat breads prepared with added glycated cowpea protein isolate, compared to those prepared with added thermally denatured cowpea protein isolate. They also found that, for sponge cakes, replacing 20% of whole egg with glycated cowpea protein isolate did not affect product sensory acceptability (Campbell et al., 2016). Such findings have confirmed that different types of added protein can have different effects on the sensory properties of various starch-based food matrices.

Many recent studies have been devoted to the development of various wheat gluten-free food products with desirable nutritional and acceptable sensory properties (Mancebo et al., 2016; Manoj

Kumar et al., 2019; Miñarro, Normahomed, Guamis, & Capellas, 2010; Phongthai et al., 2016; Sarabhai & Prabhasankar, 2015; Shin et al., 2010; Villanueva et al., 2015). Most gluten-free food products are based on the use of different gluten-free flours, such as from rice, buckwheat, and chestnut (Mancebo et al., 2016). Because the proteins endogenous to such non-wheat flours lack the ability to form viscoelastic networks unique to wheat gluten (Kweon et al., 2014), the addition of exogenous proteins is often needed to improve the sensory properties of such gluten-free products. For example, Sarabhai and Prabhasankar (2015) have reported that the addition of an optimal amount of whey protein concentrate to cookie dough improved the color, appearance, taste, and overall acceptability of the resulting gluten-free cookies, whereas the overall acceptability of those cookies decreased, when the level of added protein differed from the optimized amount. Mancebo et al. (2016) have found that gluten-free cookies made from mixtures of rice flour and pea protein had lower hardness, darker baked color (similar to that for control cookies made from wheat flour), and higher acceptability. Phongthai et al. (2016) have reported that substituting rice bran protein concentrate for added egg albumin can improve the quality of gluten-free bread. However, while added proteins can often improve the appearance, color, and texture of various starch-based food matrices, the unpleasant odors of certain proteins can adversely affect the overall acceptability of such products (Villanueva et al., 2015). Nevertheless, the use of added proteins along with TGase has been reported to be able to improve the quality of some gluten-free products, such as pasta and bread (Manoj Kumar et al., 2019; Shin et al., 2010). For example, Manoj Kumar et al. (2019) have found that the hardness, integrity, and overall acceptability of gluten-free pasta were increased by mixing pearl millet flour with milk proteins and then treating the mixtures with TGase.

Extrusion is one of the most widely used snack production processes, for which ingredient materials that provide adequate product expansion and texture need to be selected (Witczak et al., 2016). Studies have demonstrated that starch and protein types and their ratios play an important role in expansion, and that the interactions between starches and proteins during extrusion can affect the sensory properties of final products (Philipp, Buckow, et al., 2017; Witczak et al., 2016). For example, extrudates with higher contents of added pea protein isolate have been reported to exhibit higher pea flavor intensity, harder and more brittle texture, darker color, and less uniform shape and surface appearance than those with higher proportions of rice flour to pea protein (Philipp, Buckow, et al., 2017). In summary, it can once again be seen to be important to identify the best type and proportion of added protein for use in the development of starch-based food products with satisfactory sensory properties.

4.6 Starch digestibility

Starch digestion properties can be shown by the amount of glucose released and the contents of rapidly digestible starch (RDS), slowly digestible starch (SDS), and RS (López-Barón et al., 2017). Meanwhile, a first-order kinetic model and an associated logarithm of the slope (LOS) plot have been applied to characterize the reaction rate of starch amylolysis (Zou, Sissons, Gidley, Gilbert, & Warren, 2015; Zou, Sissons, Warren, Gidley, & Gilbert, 2016). With increasing demand for low-glycemic-index foods, starch-based foods with reduced digestibility have attracted wide interest (Singh et al., 2010). Starch digestibility has been determined to be significantly influenced by the presence of proteins in cereal-based food systems; typically, proteins have been found to inhibit starch digestion (e.g. increasing RS content and reducing digestion rate) (Bhattarai et al., 2016; Chen, Wang, et al., 2019; Petitot et al., 2009; Singh et al., 2010; Toutounji et al., 2019). For example, *in vitro* digestion experiments on pasta samples — i.e. spaghetti and powdered pasta were prepared from different varieties of durum wheat semolina, and starch was purified from each variety — have shown that the embedding of gluten and the compact microstructure of the pasta reduced starch digestion rates (Zou et al., 2015). Oñate Narciso and Brennan (2018) have found that the amount of glucose released from glutinous rice decreased with increasing content of added proteins, and that both pea protein isolate and whey protein concentrate affect the amount of glucose released in a similar manner. Lu et al. (2016) have reported that the RS content of processed potato starch–protein mixtures was significantly affected by processing, in the order cooled after cooking > just cooked ≈ reheated after cooking and cooling. Furthermore, HMT can promote interactions between starches and proteins, which can restrict starch hydrolysis (Chen, He, Fu, & Huang, 2015; Chen, Luo, et al., 2019; Vu, Bean, Hsieh, & Shi, 2017). Thus, the digestibility of starches in starch–protein mixtures can be influenced by many different factors, such as the types of starch and protein, the starch–protein mixing ratio, and processing conditions (e.g. heat treatment methods).

To date, the following four possible mechanisms, by which proteins can affect starch digestion properties, have been proposed:

- Proteins as physical barriers impact the digestion of starches (López-Barón et al., 2018; Lu et al., 2016; Oñate Narciso & Brennan, 2018; Rosa-Sibakov et al., 2016; Ye et al., 2018);

- Proteins interact with starches, thereby blocking the binding sites of amylase enzyme, so that starch molecules are not easily bound by amylase (Chen, He, et al., 2015; Hu et al., 2020; López-Barón et al., 2017; Yang et al., 2013);
- The binding of proteins to amylase results in the inhibition of amylase activity or prevents the otherwise typical binding of amylase to starch molecules (Bhattarai et al., 2016; Chen, He, et al., 2019; Oñate Narciso & Brennan, 2018; Yu et al., 2018; Zou et al., 2016);
- Gelatinized starches and proteins rapidly form compact protein–amylose aggregates (Chen, Wang, et al., 2019; Chi et al., 2018; Xiao et al., 2019).

With regard to the first mechanism, proteins can interact with starch granules and thus encapsulate them, thereby acting as physical barriers that effectively hinder digestion of the starch. It can be seen from CLSM images (**Fig. 4I**) that proteins can actually enwrap starch granules to form a physical barrier (López-Barón et al., 2017). Along the same lines, it has been reported that the inhibition of starch digestion by endogenous proteins is often due to the latter’s physical barrier effect that limits the contact of starch with amylase (Hu et al., 2017; Ye et al., 2018). Moreover, protein networks can encapsulate starches, thereby limiting starch swelling and gelatinization and starch’s contact with digestive enzymes (Chen, Wang, et al., 2019; Chen, He, et al., 2015; Feng et al., 2020; Yang et al., 2019).

With regard to the second mechanism, Yang et al. (2013) have suggested that combinings between whey protein and wheat starch chains may reduce the number of available action sites for enzymes on the starch. Starch–protein interactions can play an important role in this mechanism, wherein such interactions depend on the molecular configurations of proteins (López-Barón et al., 2017).

With regard to the third mechanism, CLSM images of FITC (fluorescein isothiocyanate) -amylase conjugates in the presence of cooked protein (**Fig. 4II**) suggest that the interactions between α-amylase and proteins were sufficiently strong to cause the starch to compete with the protein to bind digestive enzymes, thereby affecting the digestive properties of the starch (Chen, He, et al., 2019).

With regard to the fourth mechanism, Chi et al. (2018) have presented a schematic diagram illustrating the effect of rice protein and its hydrolysates on starch digestibility, indicating that some starch–rice protein samples had higher double-helix content and more compact, aggregated structures with less amylose leaching during short-term cooling, thus inhibiting the otherwise usual attack by digestive enzymes.

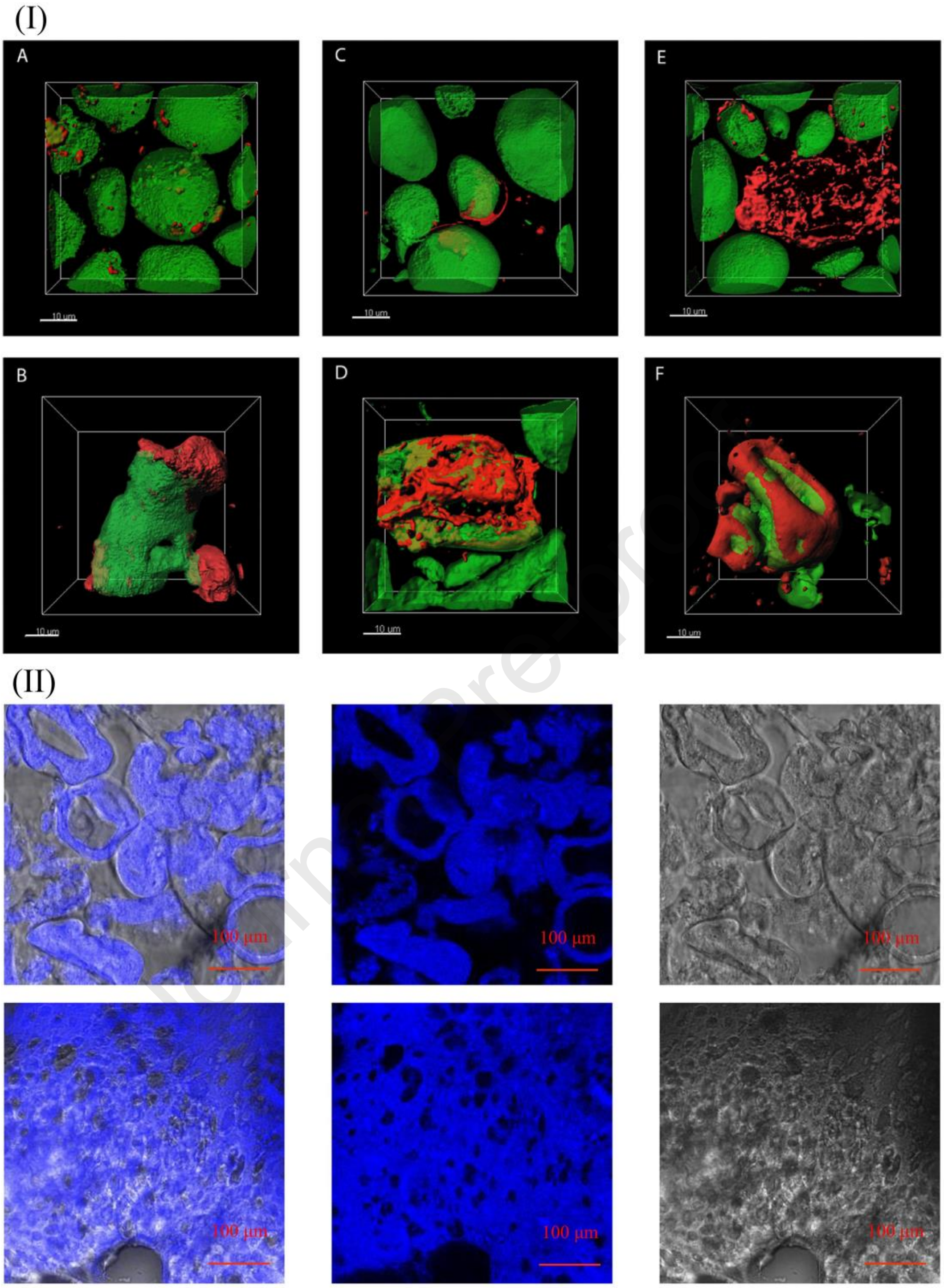

Fig. 4 (I) Confocal laser scanning microscopy (CLSM) images of wheat starch in the presence of purified plant proteins. Wheat starch and cellulose were mixed with (A) pea protein, no cooking, (B) denatured pea protein, pressure-cooked, (C) rice protein, no cooking (D) hydrolyzed rice protein, pressure-cooked, (E) soybean protein, no cooking, (F) hydrolyzed soybean protein, pressure-cooked.

Reprinted from López-Barón et al. (2017), copyright (2017), with permission from Elsevier. **(II)** Representative CLSM images of FITC (fluorescein isothiocyanate)-amylase conjugates bound onto cooked protein surfaces after two successive washings: soy protein isolate (upper) and wheat gluten protein (bottom). Reprinted from Chen, He, et al. (2019), copyright (2019), with permission from Elsevier.

4.7 Morphology/structure–property relationships

Although the structure and physicochemical properties of many starch–protein mixtures have been extensively studied, the structural reasons for changes in physicochemical properties are still not fully understood. To date, structure–property relationships for various starch–protein systems have been established to only a limited extent, and these generalized relationships are described in **Fig. 5**. Microscopic observations have revealed that proteins can be adsorbed on the surfaces of starch granules, thus hindering water molecules from entering the granules and limiting contacts between the starch and amylase, thereby ultimately inhibiting starch gelatinization and digestion (López-Barón et al., 2017; Phongthai et al., 2017; Yang et al., 2019). Protein networks formed in certain starch-based doughs can encapsulate starch granules, ultimately with a similar effect, especially for product applications involving pastas with reduced digestibility (Feng et al., 2020; Giuberti et al., 2015). Porous starch–protein gel networks can be responsible for endowing such gels with elastic/spongy properties and promoting water retention, so the network structures of such gels can provide them with particular textural properties (Joshi et al., 2014). For instance, Sun and Xiong (2014) have reported that the network structure formed by a pea starch–peanut protein mixed gel was significantly different from the three-dimensional network structure of a pea starch-alone gel matrix, and the network structure of the former was affected by the type and proportion of added protein, which ultimately influenced the textural properties of the mixed gel. In another example, Joshi et al. (2014) have reported that the hardness of lentil starch–lentil protein composite gels was reduced, due to the physical separation of protein-rich domains, which may have been related to the failure of such weak lentil protein networks to penetrate such strong lentil starch gel networks. Wang, Zhang, et al. (2020) have suggested that the presence of protein can make the network structure of a mixed rice flour-based gel somewhat more compact, because filling by the protein can make the resulting pore size of the gel smaller, which would be conducive to greater softness of the gel.

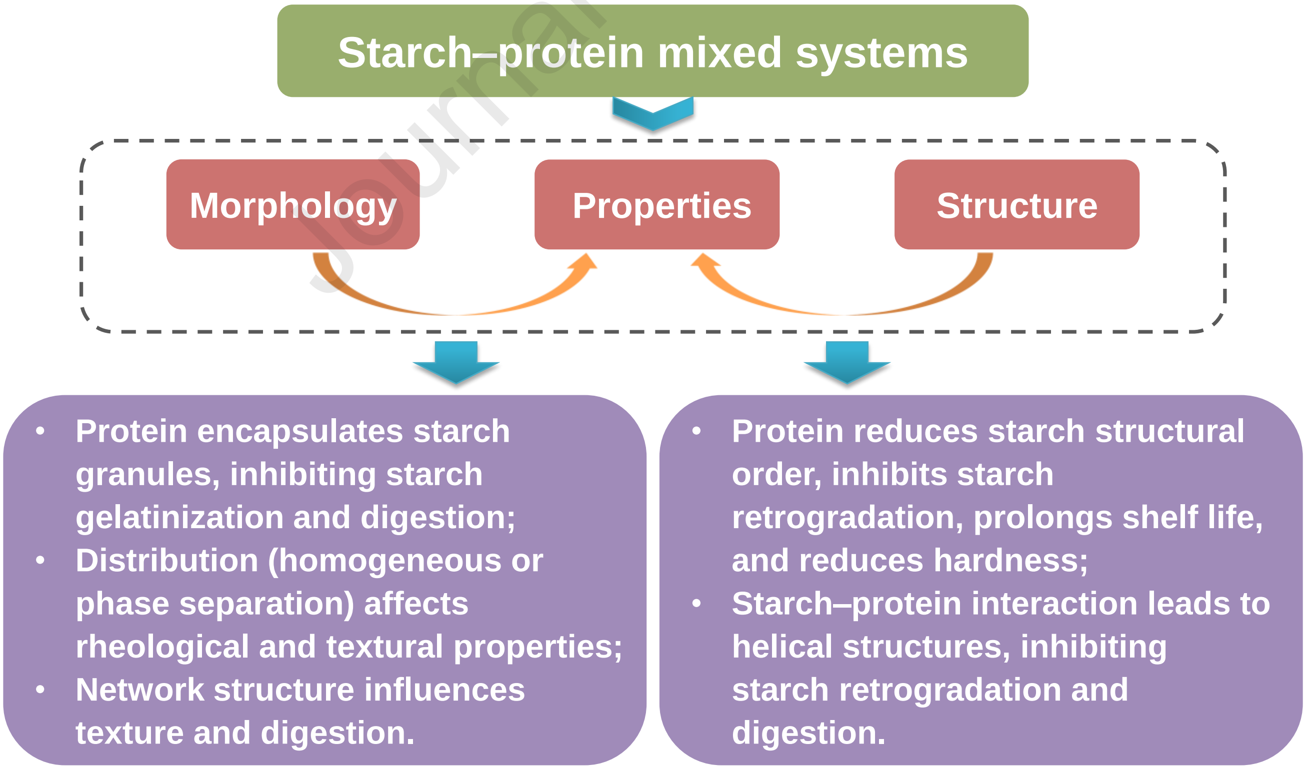

Fig. 5 Overview of the relationships between the morphology/structure and properties of starch–protein mixed systems

The consequences of long-range and short-range ordered structures have been suggested to include the fact that the presence of added protein can generally reduce the extent of starch structural order during retrogradation, thus indicating that protein can inhibit starch retrogradation and thereby help to prolong the shelf-life of various starch-based foods (Xiao & Zhong, 2017; Zhang et al., 2019). Hu et al. (2020) have observed that the significant decreases in rice starch gel hardness and water mobility after the addition of whey protein hydrolysate were related to the protein's retarding effect on starch retrogradation. However, in other instances, the addition of rice proteins has been found to enhance the ordered structure of rice starch, possibly due to interactions between the starch and protein to form more stable helical structures, which significantly increased the hardness of the resulting starch–protein gels and thereby decreased starch digestibility (Chi et al., 2018; Wang, Zhang, et al., 2020). The RS content of various processed starch–protein mixtures has been positively correlated with starch short-range order; in other words, the short-range ordered structures of such mixtures were able to be correlated with starch digestive characteristics (Chen, Wang, et al., 2019; Lu et al., 2016; Xiao et al., 2019). Thus, in the ways just described, the gelatinization, retrogradation, textural, and digestion characteristics of various starch–protein mixtures are closely related to their structural changes. Further investigations need to be conducted to more firmly strengthen the morphology/structure–property links for such starch-based food matrices, and thereby to facilitate the production of starch-based food products with desirable qualities and functions.

5. Applications in food processing and production

Pastas, noodles, breads, steamed breads, biscuits, cakes and many other starch-based food products are popular with consumers worldwide. Their textural, sensory, and nutritional properties have attracted considerable research attention. Such starch-based food products are most commonly prepared from wheat flours, of which the gluten proteins can often form continuous three-dimensional network structures that can encapsulate wheat starch granules, and which are vital to the formation of elastic doughs, from which, through extrusion or baking processes, specific products with certain desirable textural and sensory properties can be produced (Kim et al., 2008; Kweon et al., 2014; Levine & Finley, 2018; Slade, Kweon, & Levine, 2021; Wang, Guo, & Zhu, 2016). Studies (Mohammed, Ahmed, & Senge, 2012; Zhou et al., 2018) have revealed that the addition of certain exogenous proteins can disrupt the continuity of wheat gluten networks, leading to poor texture and other quality defects of baked starch-based products such as breads, while the color of such starch-based matrix products has been found to be closely related to the color of the added proteins. The effects of added proteins on various types of starch-based food products are summarized in **Table 3**.

Table 3 Influence of proteins on properties of starch-based food products

Application	Major components	Characteristics	Reference
Pasta	Durum wheat semolina (68.6% starch, 11.9%	- Protein network encapsulating starch granules can delay starch digestion; - Mixing or sheeting processes	(Kim et al., 2008)

	protein)	produced elastic doughs; - Increased sheeting might reduce starch–protein interactions, thereby increasing starch accessibility to α-amylase.	
Gluten-free pasta	Rice flour, egg albumen (EB, 74.7% protein), rice bran protein concentrate (RBPC, 68.1% protein)	- EB-rich pasta showed a compact and homogenous structure, reduced cooking loss, and improved hardness; - RBPC-rich pasta showed cracked and non-continuous surfaces, resulting in high cooking loss and low firmness.	(Phongthai et al., 2017)
Chinese steamed bread	Wheat flour (11.2% protein)	- Formation of a continuous and three-dimensional gluten network; - The starch granules were embedded in the protein network; - Gluten polymerization was conducive to retaining gas and restricting starch swelling.	(Wang et al., 2016)
Bread	Wheat flour (63.5% starch, 11.9% protein), chickpea flour (51.2% starch, 25.5% protein)	- Addition of chickpea flour increased dough development time, stability, and tensile properties; - Addition of chickpea flour significantly increased bread crumb hardness; - Bread crust color became darker with increased chickpea flour level.	(Mohammed et al., 2012)
Bread	Wheat flour (13.2% protein), whey protein	- Addition of whey protein improved gas retention ability during baking	(Zhou et

	(76.4% protein), soy protein (86.6% protein)	and produced better specific bread loaf volume; - Weak gel formed by soy protein and its poor heat preservation ability led to a decrease in bread volume and poor bread quality.	al., 2018)
Gluten-free bread	Rice flour (90.6% starch, 8.1% protein), corn flour (86.1% starch, 6.9% protein), soy flour (30.6% starch, 55.0% protein)	- High water affinity of soy protein and starch–protein interactions influenced starch gelatinization; - Soy flour incorporation increased bread volume, possibly due to increased batter consistency.	(Sciarini et al., 2010)
Gluten-free cookies	Rice flour (74.4% starch, 8.0% protein), pea protein (80% protein)	- Protein incorporation improved hydration properties of the mixtures and dough consistency; - Cookies with higher protein content showed higher acceptability.	(Mancebo et al., 2016)

For various gluten-free food products, rice flour is typically used along with added exogenous proteins to obtain products comparable to traditional wheat-based counterparts, but there are still product issues related to sensory properties, nutritional value, and consumer acceptance (Rodriguez Furlán et al., 2015; Shevkani et al., 2015; Storck et al., 2013; Villanueva, Ronda, et al., 2018). Thus, gluten-free formulations have been optimized for greater consumer acceptability, for example, by the selection of appropriate individual proteins or protein combinations or by the addition of other components such as polysaccharides (Gularte, Gómez, & Rosell, 2012; Phongthai et al., 2017;

Rodriguez Furlán et al., 2015; Sarabhai & Prabhasankar, 2015; Sciarini et al., 2010). Since such gluten-free starch-based products are mainly composed of starch and protein, it should be intuitively obvious that the characteristics of the particular starches and proteins and their interactions can affect the sensory and nutritional properties of the final food products. For instance, researchers have found that replacing rice flour with pea protein or maize starch could help to change the gluten free cookie characteristics (Mancebo et al., 2016). The inclusion of protein in the formulation reduced cookie size (thickness and width), resulting in cookies with lower hardness values and darker color, while the addition of starch increased cookie size without affecting texture or color (Mancebo et al., 2016). However, considering that protein addition modifies cookie dough rheology, resulting in a more consistent dough, the problem of cookie lamination and formation can be solved if the dough is too soft (Mancebo et al., 2016).

Many pastas and noodles are typically made by mixing wheat flours or semolinas with water to form unfermented doughs, followed by extrusion (Baik, 2010; Li, Zhu, et al., 2014). The quality of cooked pastas or noodles is known to be influenced by gluten protein network formation during dough mixing and wheat starch gelatinization during cooking (Bonomi et al., 2012; Bruneel, Pareyt, Brijs, & Delcour, 2010). When a given gluten network lacks elasticity and compactness, starch granules tend to swell more during cooking, resulting in a greater loss of soluble solids (Bonomi et al., 2012). However, excessive addition of exogenous wheat gluten can make a dough too strong to be easily and efficiently handled during subsequent rolling or extrusion (Li, Zhu, et al., 2014). In contrast, certain exogenous proteins can positively affect the formation of protein networks in doughs, thereby enhancing the structures of pasta and noodle doughs and improving the sensory properties of the final products (Li, Zhu, et al., 2014; Marti et al., 2014; Susanna & Prabhasankar, 2013). For example, the addition of egg albumin to rice flour-based gluten-free pasta dough can lead to the product having less tendency toward structural disintegration, thereby reducing cooking losses (Phongthai et al., 2017). It has been said that starch chains, especially amylose, in cooked noodles can interact with wheat gluten proteins through hydrogen bonding (Baik, 2010; Liu et al., 2019). In this way, amylose chains exuded during cooking may cause rigidity of cooked noodles by interacting with gluten proteins (Baik, 2010). The compact structure and the interactions between starches and proteins can also lead to reduced starch digestibility of pastas (Kim et al., 2008; Zou et al., 2016).

For bread doughs, high elasticity and extensibility are typically required during fermentation and baking, in order to enable dough expansion and retention of leavening gases (Liu et al., 2019). Wheat gluten is well-known to play a key role in determining the processing and baking quality of wheat flours, by imparting WHC, viscosity, cohesiveness, and elasticity to bread doughs (Hernández-Estrada et al., 2014; Zhou et al., 2018). Hence, the absence of wheat gluten can often result in a paste rather than a dough, which, in turn, may result in low loaf volume, poor texture, and other post-baking quality defects of baked breads (Phongthai et al., 2016; Witczak et al., 2016; Zhou et al., 2018). However, it has been reported that gluten-free bread doughs can be rendered capable of forming protein network structures, similar to those of gluten networks, by the addition of certain exogenous proteins (e.g. soybean, egg and pea proteins) and TGase (Nozawa, Ito, & Arai, 2016; Sciarini et al., 2010; Shin et al., 2010). For example, Shin et al. (2010) have reported that added whey protein and caseinate reduced the paste viscosity of rice flour, whereas TGase increased the viscosity by crosslinking between rice protein and the added proteins, and the resulting crosslinked protein network contributed to gas retention during baking. Exogenous proteins are typically incorporated into gluten-free systems to increase elasticity by crosslinking, and to enhance color and flavor development by Maillard reactions, improve structures by gelation, and support foaming (Campbell et al., 2016; Phongthai et al., 2016). Protein unfolding and protein–protein interactions, as well as protein interactions with starches, can occur during baking, leading to improved textural properties (Phongthai et al., 2016). For instance, the springiness of a model-system bread has been reported to result from a combination of gluten protein aggregation, interactions between cassava starch and gluten, and water retention (Liu et al., 2019). Moreover, starch granules embedded in a gluten-free protein matrix produced by baking can result in hindered starch retrogradation (Phongthai et al., 2016). The high WHC of soybean protein and its established interactions with amylopectin can also hinder the starch retrogradation process (Sciarini et al., 2010).

Other baked products such as cookies, crackers, and cakes have been reviewed by several researchers with a focus on their major ingredients, formulas, processes, and effect on quality characteristics (Kweon et al., 2014; Slade et al., 2021). Kweon et al. (2014) also reported that gluten development was promoted in lower-sugar cracker doughs during mixing and sheeting, which was a key factor affecting the quality of baked-crackers. Besides, further studies reported that for these baked products, the protein structures in pastes or doughs and the distribution and interactions of starches with proteins can affect the textural and sensory properties of the final products (Gularte et al., 2012; Kweon et al., 2014; Mancebo et al., 2016; Sarabhai & Prabhasankar, 2015; Slade et al., 2021). It has been reported that a gelatinized starch gel contributes to initial cake crumb firmness (Slade et al., 2021), while the aggregated gluten proteins in cake crumb are responsible for crumb elasticity (Wilderjans, Luyts, Goesaert, Brijs, & Delcour, 2010). A large collection of literature has reported the effects of various starch–protein mixtures on the properties of many such final baked products (Gularte et al., 2012; Kim et al., 2008; Mancebo et al., 2016; Phongthai et al., 2017; Sciarini et al., 2010; Wang et al., 2016; Zhou et al., 2018). Different starches and proteins have been added to model dough systems, in order to prepare doughs with unique microstructures and textural properties (Liu et al., 2019; Sarabhai & Prabhasankar, 2015; Zhou et al., 2018). In such ways, starch-based products with particular sensory properties have been obtained by extrusion, cooking, or baking processes (Kumar et al., 2017; Reddy Surasani et al., 2019; Sarabhai & Prabhasankar, 2015).

6. Conclusions and future perspectives

Many starch-based foods, consisting mainly of starch matrices containing proteins, are important energy and nutrient sources for people. In such starchy foods, the presence of endogenous and exogenous proteins has been shown to have a great impact on their textural, sensory, and digestive properties. Based on descriptions of the structural characteristics of various starch–protein mixtures on different scales (i.e. micromorphology, and long-range and short-range ordered structures), the effects of different proteins on the structural characteristics of different starches have been demonstrated. In particular, proteins with stronger gelation ability and interaction with starch molecules could lead to starch–protein systems with a more compact network structure and being more homogeneous; however, some proteins interact with starch molecules to hinder the formation of a dense network structure. Usually, the interaction of proteins with starch inhibits starch molecular rearrangement during cooling, leading to decreased amounts of long-range crystallites and short-range helices. But under certain conditions, proteins interact with starch strongly, increasing the extent of crystallinity, and protein adsorption on the surface of starch granules inhibits starch gelatinization, enhancing short-range order. At the same time, the presence of different proteins in starch-based food systems has been shown to significantly affect the viscosity, thermal, rheological, textural, and digestive properties of such mixed systems. Due to the WHC, dilution and hindrance effects of proteins and to starch–protein interactions, starch–protein systems generally showed a decrease in viscosity, an increase in the starch gelatinization temperature, and alterations in the rheological and textural properties. Starch digestion could be inhibited due to the physical barrier effect of proteins, starch–protein interactions, protein binding to amylase, and the formation of starch–protein aggregates. However, the currently available information, in terms of structure–property relationships, is still limited. Additionally, various types of starch–protein interactions have been shown to play key roles in the quality and functional properties of many starch-based foods. Therefore, a thorough understanding of such starch–protein interactions in many different starch-based food systems can be of great significance to the development of products with stable thermal and rheological properties, and can guide the processing of such foods, aimed at creating desirable structures and properties.

Looking ahead, starch and protein source materials can be reconstituted, in order to enable the development of starch-based products with particular textural, sensory, and nutritional properties, through specific processing techniques. There is still a strong need to define more comprehensive relationships among biopolymer interactions, food product structures, and product properties. Innovative techniques for the manipulation of starch–protein interactions could open a new dimension for health and nutrition platforms. Furthermore, while many recent studies have reported that the addition of proteins can have a significant impact on the texture, taste, and other aspects of various starch-based products (e.g. breads, cookies, cakes, and pastas), there is still a need for more systematic studies on how the addition of proteins affects the many characteristics of such final products. It is of paramount importance to better understand how the above knowledge can impact starch-based food products and processes, and thus the food industry as a whole.

Conflict of Interest

Declarations of interest: none

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Highlights

- ✓ Starch–protein interactions are affected by biopolymer type, proportion, and processing.
- ✓ Proteins often inhibit starch gelatinization and restrict starch retrogradation.
- ✓ Proteins affect the textural, sensory, and digestive properties of many starch-based foods.
- ✓ Understandings that are instrumental to the development of high-quality, healthy foods are reviewed.

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