Review Article

Factors Affecting the Formation, Structure and Digestibility of Starch-Lipid-Protein Complexes – A Mini Review

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ABSTRACT

Starch, lipid and protein have been reported to form a ternary V-type complex that is resistant to digestion. This starch-lipid-protein complex is categorised as type-5 resistant starch with beneficial effects on health. The study of ternary complex is an emerging field with scarce knowledge. This article provides the review of the latest research (2020-2023) on this subject matter focusing on the reported variables affecting the formation of the complexes. Among the factors discussed are the effect of type of proteins, type of fatty acids, type of starch, environmental pH and cooking methods. The structural modification and resultant digestibility are elucidated.

Keywords: starch digestibility; starch-lipid-protein complexes; type-5 resistant starch; V-type complexes

1. Introduction

Starch is one of the most essential carbohydrates in the human diet. It is a complex macromolecule made up of amylose and amylopectin molecules. Upon digestion in the human body, starch will be broken down by enzymes into glucose to provide energy for the normal functions of the human body. The final stage of starch digestion is completed in the small intestine to release glucose that causes significant metabolic problems inherent in obesity, cardiovascular diseases and diabetes (FAO/WHO, 1998). The starch digested as glucose and having effect on postprandial glycaemia is called "digestible", whereas the undigested starch that passes to the large intestine and may be fermented by the gut microbiota to produce short-chain fatty acids with many beneficial health effects (Mortensen & Clausen, 1996; Zhang *et al.,* 2023) is known as "resistant" (Parada & Santos, 2016; Cumings & Englyst, 1995). The digestion of starch depends on its physical form, the nature of the granules and the effects of food processing. According to Cummings and Englyst (1995), starch can be classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) based on the digestion rate. Chi *et al.* (2021) recently pointed out that the digestibility of starch is governed by multi-scale structures of the starch that include the fine structures of amylose and amylopectin; short-range ordered structures; helical, crystalline, lamellar, aggregate structures; and newly formed aggregated structures after food processing. Alteration of these structures allows mitigation of starch digestibility including formation of SDS and RS which enables targeted regulation of starch digestibility.

Starch, particularly the amylose molecules form V-type complex with lipid to become type-5 RS (RS5). These complexes have more compact and stable structures to resist digestion in the gastrointestinal tract (He *et al.,* 2020). Apart from this traditionally classified RS5, there are other emerging V-type complexes resultant from the interaction of starch with other non-starch molecules such as glycerol, amino acids, peptides, proteins, other polysaccharides, etc. These new complexes are proposed to be classified as RS5

due to their non-digestible nature (Gutiérrez & Tovar, 2021). The binary starch-lipid complexes have been extensively investigated but the study of ternary starch-lipid-protein complexes is relatively limited (Kang *et al.,* 2021a). Ternary starch complexes were reported to contain higher RS and lower *in vitro* digestibility when compared to starch-lipid or starch-protein binary complexes (Wang *et al.,* 2020; Zheng *et al.,* 2018), thus attracted more research interest in recent years. The formation of starch-lipid-complexes affects the functional and nutritional properties of finished products in term of flavour, texture, shelf life and digestibility (Wang *et al.,* 2020). The review by Wang *et al.* (2020) revealed the scarcity of knowledge on factors that can affect the formation of starch-lipid-protein complexes. Therefore, this mini review reports and summarises the most current findings related to starch-lipid-protein complexes published between year 2020 until 2023 with the aim to expand the knowledge in this interesting and important field.

2. Formation of Starch-Lipid-Protein Complexes

Naturally, amylose and amylopectin in starch arrange to form densely packed single and double helices, these structures are then orderly arranged into highly crystalline structure which protect the starch from the attack of amylolytic enzymes. Therefore, starch digestibility is reduced as a function of helical structure content (Chi *et al.,* 2021). Perfectly packed helical structures increase the RS content, whilst helical structures with imperfect association will be slowly digested to mainly yield SDS content (Zhang *et al.,* 2008). The formation of single helical starch-lipid-protein complexes is related to non-covalent interactions among the components such as electrostatic interactions, hydrogen bonds and van der Waals forces (Wang *et al.,* 2022). Amylose molecules are mainly responsible for the formation of V-type complexes by including the hydrophobic ligands inside the left-handed helix (Lu *et al.,* 2019; Kang *et al.,* 2022). Amylopectin may also be involved in the formation of V-type complexes but limited to the long side chains due to steric hindrance of the short amylopectin chains (He *et al.,* 2020; Lim *et al.,* 2019).

Typically, starch-lipid-protein complexes are manufactured *via* self-assembly of starch and lipid (fatty acid) into binary complexes followed by self-assembly again with a protein (Gutiérrez & Tovar, 2021) to form ternary starch-lipid-protein complexes. The formation of self-assembled starch-lipid-protein nanoparticles was first reported by Zhang and Hamaker (2003) as an uncharacteristic peak in the pasting curve of a gelatinized flour. Later the formation of this starch ternary complexes was confirmed with High Performance Size Exclusion Chromatography (HPSEC) by Zhang *et al.* (2003). In the ternary complex, the hydrophobic tail of fatty acids interacts with starch to form starch-fatty acids inclusion complexes, whilst the negatively charged carboxyl group of the fatty acids interacts with protein (Bhopatkar *et al.,* 2015; Chao *et al.,* 2018). The stacking of these ternary complexes produces crystalline structures that were reported to have a greater degree of crystallinity than the corresponding binary starch-lipid complexes with lower enzymatic digestion rate (Chao *et al.,* 2018; Zheng *et al.,* 2018). Lately, Wang *et al. (*2022) used Molecular Dynamics (MD) simulation to show that the formation of amylose-lauric acid (LA)- β-lactoglobulin (βLG) complexes was ascribed to the hydrophobic interactions, van der Waal forces between amylose and LA, and van der Waals forces and hydrogen bonds between amylose and βLG. The hydrophobic interactions and hydrogen bonds contributed more than electrostatic forces to the formation of the ternary complexes.

3. Factors Affecting Formation of Starch-Lipid-Protein Complexes

Most of the starchy food that we consume daily is a complex system containing proteins, lipids and other types of components/ingredients that are either naturally present or added into the food matrix. Starch, protein and lipids are macromolecules containing their own characteristic set of functional groups that contribute greatly to their unique chemical properties and functionalities. These diverse hydrophobic and

hydrophilic functional groups between the macromolecules may react with each other to form new conformation and structures. When these macromolecules undergo different processing conditions (presence of water or ions, shearing, pH change, etc.), interaction between the components may take place causing structural transformation and formation of new processing-derived microstructure (Parada & Santos, 2016).

3.1 Type of Protein

As proteins contain charged amino acids, their conformation and structural stability is highly dependent on the environmental pH. At the pH corresponding to the protein's isoelectric point (IEP), the protein carried no net charge; at pH values far away from IEP, the protein will be highly charged and the repulsion between the similarly charged amino acid residues of the protein increases, subsequently alter the secondary structure of the proteins (Guckeisen *et al.,* 2021). As IEP is specific to a protein, this inherent property critically determines the interactions with starch and lipids. The interactions between proteins and lipids lies in the electrostatic interactions with the negatively charged carboxyl group of the fatty acid, therefore positively charged proteins have higher ability to interact with fatty acid as opposed to negatively charged proteins (Lin *et al.,* 2020). *Table 1* shows the summary of the research work investigating the effects of protein type on the structure and digestibility of rice and wheat starch.

Lin *et al.* (2020) compared the formation of starch-lipid-protein complexes between type-A gelatin (IEP *ca.* 8.0-9.0) and whey protein isolate (IEP *ca.* 7) with linoleic acid (LA) and rice starch (RiS). Gelatin (GE) with IEP higher than 7.0 (more positively charged) had higher affinity towards LA and compete with RiS to retard the formation of starch complexes. Comparatively, WPI was more effective in promoting short-range ordered structure, helical structure, crystallites hence lowered the starch digestibility. Ris-LA-WPI was found to contain the highest amount of RS. Irrespective of the protein type, all the ternary starch complexes exhibited higher ordered structure and lower digestibility than the corresponding binary complexes, indicating the synergistic effect of proteins on starch reassociation during retrogradation to resist starch digestibility in a higher magnitude. Protein may emulsify the lipid in starch slurry by increasing its water solubility, subsequently facilitate the interaction between lipid and starch to form inclusion complexes (Chao *et al.,* 2018; Wang *et al.,* 2017). The ratio of FTIR (Fourier Transform Infrared Spectroscopy) absorption band of 1041 and 1020 cm^{-1} (IR1041/1020) which indicated the short-range ordered structure of the starch samples was found to positively correlated to the SDS (0.933**) and RS (0.974**) content of the samples. The absorption band around 1047 cm^{-1} corresponds to starch crystalline regions, whereas around 1022 cm^{-1} represents the amorphous regions (Chi *et al.,* 2017).

Du *et al.* (2023) found that gluten (endogenous protein from wheat flour) caused formation of more wheat starch (WS) ternary complexes with lower digestibility with oleic acid (OA) than whey protein isolate (WPI) as observed by higher degree of relative crystallinity (24.7% vs. 22.8%). However, the short-range ordered structure of these two samples were statistically insignificant, but higher than the corresponding binary counterparts. The IEP of gluten is between 6-8, higher than that of WPI, between 4.7-5.3 (Ye & Chen, 2019). The variance in the formation of starch ternary complexes may be related to the IEP of the proteins, however, the media pH of the experiment was not reported in the study.

Table 1 Effect of type of protein on the structure, digestibility and formation of V-type complexes in starch ternary system.

Type of starch	Type of lipid (%)	Type of protein (%)	Setback viscosity peak	ATR-FTIR/FTIR	LCM Raman Spectroscopy	X-ray diffraction	DSC	Digestibility/Content of starch fraction	Source
rice starch (RiS)	6% linoleic acid (LA)	6% protein: whey protein isolate (WPI) type-A gelatin (GE)	Setback viscosity: RiS-LA-WPI > RiS-LA-GE Starch ternary system > binary system	IR1047/1022 values: RiS-LA-WPI = RiS-LA-GE starch ternary complex > starch binary complex	NA	% V-type crystallinity: RiS-LA-WPI > RiS-LA-GE > RiS-LA > RiS-WPI = RiS-GE > RiS	ΔH value: RiS-LA-WPI > Ris-LA-GE Ternary complexes > binary complexes	RS content: RiS-LA-WPI > RiS-LA-GE > RiS-LA > RiS-WPI = RiS-GE > RiS SDS content was the same for all samples.	Lin *et al.*, 2020
wheat starch (WS)	5% oliec acid (OA)	10% protein: gluten whey protein isolate (WPI)	WS-OA-gluten > WS-OA-WPI	NA	Short-range order (inverse of FWHM at 480 cm-1): WS-OA-gluten ≥ WS-OA-WPI ≥ WS-OA ≥ WS	The relative crystallinity of WS-OA-gluten > WS-OA-WPI	NA	The starch ternary complexes for both proteins were less than the binary counterparts. Gluten had greater effect on reduction of RDS.	Du *et al.*, 2023
wheat starch (WS)	5% palmatic acid (PA)	10% protein: whey protein isolate (WPI) egg white protein (EWP) soy protein isolate (SPI) pea protein isolate (PPI)	Viscosity peak was seen for all systems, but the cooling viscosity started to increase earlier in the WPI, SPI and PPI, but slower in EWP.	NA	Short-range order (inverse of FWHM at 480 cm-1): WS-PA-WPI = WS-PA-SPI = WS-PA-PPI > WS-PA-EWP = WS-PA	% V-type crystallinity: WS-PA-WPI > WS-PA-PPI > WS-PA-SPI > WS-PA-EWP > WS-PA	ΔH values: WS-PA-WPI > WS-PA-SPI > WS-PA-PPI > WS-PA-EWP	NA	Duan *et al.*, 2023

Table 1 Continue.

Type of starch	Type of lipid (%)	Type of protein (%)	Setback viscosity peak	ATR-FTIR/FTIR	LCM Raman Spectroscopy	X-ray diffraction	DSC	Digestibility/Content of starch fraction	Source
wheat starch (WS)	3% lauric acid (LA)	5% protein: gliadin (in alcohol) glutenin (in water) glutenin (in buffer with pH 5.2)	NA	IR1047/1022 values: a. glutenin: WS-LA (pH 5.2) > WS-LA-glutenin (water) > WS-LA (water) > WS-LA-glutenin (pH 5.2) > WS (pH 5.2) = WS (water) b. gliadin in ethanol WS-LA > WS-LA-gliadin > WS	Short-range order (inverse of FWHM at 480 cm^{-1}) a. glutenin (water) WS = WS-LA = WS-LA-glutenin b. glutenin (pH 5.2) WS-LA = WS-LA-glutenin ≥ WS c. gliadin WS-LA > WS-LA-gladin > WS	% V-type crystallinity: a. glutenin: WS-LA (pH 5.2) > WS-LA (water) > WS-LA-glutenin (water) > WS-LA-glutenin (pH 5.2) > WS (pH 5.2) > WS (water) b. gliadin in ethanol WS-LA > WS-LA-gliadin > WS	Total ΔH values: a. glutenin: WS-LA (water) > WS-LA-glutenin (pH 5.2) > WS-LA (pH 5.2) > WS-LA-glutenin (water) b. gliadin in ethanol WS-LA > WS-LA-gliadin > WS Only WS-LA glutenin (pH5.2) and WS-LA-gliadin showed single type II complexes endotherm.	The digestibility of WS-LA-glutenin (pH5.2) decreased significantly compared to the water system. Samples prepared in ethanol showed higher RS content due to WS-alcohol complexes.	Kang *et al.,* 2022

NA – not available

Protein polymer linked by disulfide bonds is one of the structural elements in the starch ternary complexes besides starch-fatty acid complex and protein-fatty acid complex (Parada & Santos, 2005), therefore the high enzymatic resistance of WS-OA-gluten was attributed to the higher disulfide bond contents in gluten. Moreover, gluten is known to affect starch digestion by embedding starch particles within the continuous gluten network to restrict starch swelling, and hence the accessibility to α-amylase (Yao *et al.,* 2020).

The effects of gladin and glutenin on the formation of ternary starch complexes with wheat starch (WS) and lauric acid (LA) was further investigated by Kang *et al.* (2022). Since gladin is not water soluble (Kasarda *et al.,* 1967), 65% ethanol was used to prepare the WS-LA-gladin sample, whereas starch ternary system with glutenin was prepared using water and acetic acid buffer (pH 5.2). Neither gliadin and glutenin promoted starch-lipid complexes as the % crystallinity of the binary complexes was higher than the corresponding ternary complexes, in agreement with the results of Raman spectroscopy and FTIR. Instead, the addition of protein improved the perfection of V-type crystallites as evident by the presence of Type II starch-lipid complexes endotherms (melting point above 105 °C) (Putsey *et al.,* 2010) in WS-LA-glutenin (pH 5.2) and WS-LA-gliadin; nonetheless the total complex content was less than WS-LA samples (lower total enthalpy). Among the glutenin samples, RS was found highest in WS-LA-glutenin (pH 5.2), but the RS content in WS-LA-gliadin was less than the WS-LA counterpart. Higher RS in WS-LA-gliadin than WS-LA-glutenin was ascribed to the formation of WS-alcohol complexes. Comparatively, glutenin was less effective than gliadin to promote the ordered structure in WS-LA complexes.

The above findings suggest that proteins may or may not participate in the formation of starch-lipid-protein complexes. Proteins modulate the digestibility of starch by altering the degree of ordered structures in the starch binary and ternary complexes. The extent of structural alteration is highly dependent on the inherent properties of protein such as chemical composition, IEP, emulsifying ability, and molecular mass. The reaction conditions that determine the protein solubility and configuration (related to IEP) are equally critical.

3.2 Type of Lipids

Effects of lipids on the formation of binary amylose-lipid complexes have been extensively reported. The alkyl chain length, degree of unsaturation, solubility, polar head group of lipids are known to affect the properties of amylose-lipid complexes (Wang *et al.,* 2020). Lipids with a longer chain length form stronger hydrophobic interactions within the interior of amylose helix (Kawai *et al.,* 2012, Putseys *et al.,* 2010) to produce more stable complexes. However, if the chain length is too long, the solubility of the lipids will decrease and higher activation energy is required for complex formation (Cui & Oates, 1999; Siswoyo & Morita 2002; Wang *et al.,* 2020). Increasing double bonds in fatty acids impairs the formation of V-amylose complexes by reducing the crystallinity, thermal stability, and formation of particles will less uniform size (Wang *et al.,* 2020). The decreased degree of fatty acid unsaturation induced the formation of more organized and well-defined structures (Zabar *et al.,* 2009).

Previously, Zhang *et al.* (2010) concluded that the carboxyl group of fatty acids acts as an essential bond between amylose and protein molecules to form a stable ternary complex. The electrostatic interactions among negatively charged carboxyl groups and the poly-ionic protein are the basis for the self-assembly of the complexes. However, latest insight by Wang *et al.* (2022) indicated that hydrophobic interactions and hydrogen bonds contributed more than electrostatic forces to form the amylose-lauric acid-β-lactoglobulin ternary complex. The influence of rice protein (RP) (10%, w/w) and six types of fatty acids (FAs) (lauric acid [LA], C12:0, myristic acid [MA], C14:0, palmitic acid [PA], C16:0, stearic acid [SA], C18:0), oleic acid [OA], C18:1, and linoleic acid [LOA], C18:2) on the formation of starch ternary complexes in indica rice starch (IRS) were compared using model system (Chen *et al.,* 2023). The setback viscosity of IRS-FAs-RP was significantly higher than their corresponding binary systems, especially for LOA. *Table 2* shows that RP facilitated the perfection of V-type crystallites (IR1047/1022 values) in IRS-lipid complexes (especially PA and LOA). RP also increased the RS and SDS contents of IRS-FAs complexes, consistent well with the results of XRD (X-ray Diffraction Analysis) and DSC (Differential Scanning Calorimetry).

LOA with the longest alkyl chain length and highest degree of unsaturation formed complexes with higher crystallinity, lower digestibility, and higher thermal stability; in contrast to the findings of Zheng *et*

al. (2018) who found that fatty acids with shorter alkyl chains and lower degree of unsaturation favour the formation of more ternary complexes but lower in thermal stability.

The influence of RP on the rice starch-lipid complexes formed in instant rice noodle (IRN), a real food system, was also investigated in the same study (Table 2). FAs also exerted significant enhancement on the long-range order of IRN. The high melting enthalpy showed that higher ordered crystalline structures were generated in the noodle contained FAs with higher chain lengths and degrees of unsaturation. IRN fortified with FAs (especially for LOA) displayed significantly higher RS and lower digestibility than natural IRN sample. The *in vitro* hydrolysis rate of IRN was reported as: LOA < PA < OA < SA < LA < MA < control. However, the V-type complexes formed in the noodle was lower than that of IRS-lipid and IRS-lipid-RP. Authors stressed that RP did not involve in the formation of ternary complexes, rather facilitating the formation of IRS-FAs complexes, in agreement with the findings of Chao *et al.* (2018) and Kang *et al.* (2022).

Another group of researchers also explored the effect of lipid on both model and real food systems (Kang *et al.,* 2021a). The effects of lauric acid (LA), glycerol monolaurate (GML), stearic acid (SA) and glycerol monostearate (GMS) on the digestibility of wheat starch (WS) (in the presence of wheat protein) and wheat flour noodle (WFN) were investigated (Table 2). From the RVA (Rapid Visco Analyser) pasting curve, only WS-LA, WS-GML, WS-LA-WP and WS-GML-WP exhibited characteristic setback viscosity for starch-lipid-protein complexes. Since no 1543 cm^{-1} band (Thygesen *et al.,* 2003) was observed in FTIR spectra, the authors concluded that no new ternary starch-lipid-protein complexes were formed; this is supported by the results of TGA (thermogravimetric analysis), XRD and FTIR. The coexistence of protein only promoted the formation of amylose-lipid complexes. LA and GML were more favourable for the formation of starch-lipid complexes than SA and GMS in WS and noodle samples. These observations are in opposite to the findings on rice starch and rice noodle mentioned above (Chen *et al.,* 2023). The digestibility of WS-lipids-WP samples were lower than the corresponding binary samples. Besides enhancing formation of ternary complexes, the added protein may combine with the digestive enzyme which in turn reduce the enzyme digestibility (Geng *et al.,* 2022).

The relative crystallinity (%RC) of the noodle samples that were reported was in the order of: LA > GML > SA > GMS > control, in similar trend with the model system. Authors attributed the findings to the fact that short chain fatty acids can be easily inserted in the helical structure of amylose; the good dispersion of LA and GML also enhanced the interplay with starch in the noodles. Noodles containing starch-lipid complexes (particularly LA) showed typical V-type diffraction peaks with higher crystallinity and RS. As noodles did not contain 100% starch, the %RC and the amount of starch-lipid complexes in noodles supplemented with fatty acids were lower than the samples of model system. The limited time used in cooking noodle hindered the starch granules in the compact structure of the noodles to absorb sufficient water and release amylose for complexation with lipids. Results of TGA showed that the formation of starch-lipid complexes enhanced the WS samples' resistance to thermal decomposition, but no significant difference was observed between types of fatty acid. However, marked differences was noted in the temperature of maximum decomposition of noodles incorporated with FAs. The noodles supplemented with FAs was more thermal stable than control noodles. The digestion data for the model system and real food system fit well into the first-order kinetic equation (Goñi *et al.,* 1997). The order of starch hydrolysis rate in noodles containing lipids was as follows: GML < LA < SA < GMS. Overall, the digestibility of noodles was lower than that of model systems.

Discrepancies in the findings on how the chain length and degree of unsaturation of FAs affect the formation of V-type complexes in a starch ternary system suggests that the behaviour and impact of FAs is dependent on the type of starch and protein coexistent in the system. The contribution of each macromolecule towards the complex ternary interaction ought to be considered. The positive influence of FAs addition on the digestibility of wheat and rice noodle indicates the potential of modifying noodle formulations to mitigate desirable starch digestion.

Table 2 Effect of type of lipids on the structure, digestibility and formation of V-type complexes in starch ternary system.

Type of starch/food system	Type of lipid (%)	Type of protein (%)	Setback viscosity peak	ATR-FTIR/FTIR	X-ray diffraction	DSC/TGA	Digestibility/Content of starch fraction	Source
Indica rice starch (IRS) / instant rice noodle (IRN)	3% fatty acid: lauric acid (LA), myristic acid (MA), palmitic acid (PA), stearic acid (SA), oleic acid (OA), linoleic acid (LOA)	10% rice protein	All ternary systems exhibited higher setback viscosity than their binary systems. IRS-LOA-RP showed the highest peak.	IR1047/1022 values: Increased in ternary systems except MA & OA No absorption at 1543 cm^{-1} was observed for all ternary systems.	V-type diffraction peaks intensities in IRS-Fas-RP were sharper and stronger than their corresponding binary complexes particularly for PA and LOA. The intensity of the diffraction peak and relative crystallinity of noodle systems: control < SA < PA < OA < MA < LOA < LA	Transition temperatures & ΔH: starch ternary systems > starch binary systems Noodle added with fatty acids > noodle without fatty acids	Natural noodle contained RDS, SDS and RS like IRS. RS: starch ternary complexes > starch binary complexes SDS: starch ternary complexes > starch binary complexes In noodle system: All fatty acids increased the RS content The noodle digestibility: control > MA > LA > PA = SA = OA = LOA	Chen *et al.,* 2023
wheat starch (WS) / wheat flour noodle (WFN)	3% lauric acid (LA), glycerol monolaurate (GML), stearic acid (SA), glycerol monostearate (GMS)	10% wheat flour protein (WP)	Only WS-LA, WS-GML, WS-LA-WP & WS-GML-WP showed setback viscocity peak.	The absorption band of 1710 cm^{-1} was found in all binary and ternary starch systems.	The intensity of V-type diffraction peaks was increased in WS-lipid-Pr samples as compared to WS-lipid samples. % relative crystallinity in noodles: LA > GML > SA > GMS > control	TGS was used. No difference in the decomposition temperature of WS-lipids and WS-lipids-WP samples. Difference was found in the noodle samples: GML > LA > GMS. > SA > control.	The digestibility of WS-lipid-WP < WS-lipid-samples. Starch hydrolysis rate in noodles added with lipids: GML < LA < SA < GMS	Kang *et al.,* 2021a

3.3 Type of Starch

Since amylose is the main complexing component, the amylose content and amylose chain length are among the important determinants of the complexing ability (Wang *et al.*, 2020). Previous studies indicated optimal size (degree of polymerization, DP) of amylose chains may be required to form crystals with greater ordered structures (Putseys *et al.*, 2010; Wang *et al.*, 2020). Recently, Sun *et al.* (2023) reported that the average molecular weight of amylose, average chain length of amylopectin side chains, and the proportion of chains with DP of 6-12 and 25-36 greatly affected their interactions with myristic acid. The complexing state and structural order of starch substantially influenced the digestibility of the binary complexes formed.

The nature of starch influences the formation and structure of starch-lipid complexes, but Cai *et al.* (2021) found that starch ternary complexes were affected to a much lesser extent than starch binary complexes (Table 3). In a binary system of starch and lipid (lauric acid, LA), wheat starch (WS) and maize starch (MS) were found to form starch-LA complexes with greater extent than potato starch (PS) which contained lower amylose. The results of DSC suggested PS formed binary complexes with lower degree of crystallinity than MS and WS. But when added with βLG, the setback viscosity of the PS ternary system increased greater than those in MS-LA and WS-LA, the greatest increase of short-range and long-range ordered structures also observed for PS-LA-βLG. This showed that the promoting effect of βLG is more pronounced in PS than MS and WS.

Surprisingly, results obtained also showed that the addition of βLG facilitated the complexation of LA with MWS (maize waxy starch) which is high in amylopectin. The emulsifying effect of βLG (Chao *et al.,* 2018; Zheng, *et al.*, 2018) could be responsible for this observation. This was again evident by the presence of a small endotherm in the thermogram of WMS-LA-βLG but not in WMS-LA. WMS-LA-βLG showed two V-type crystalline peaks at 12.8 and 19.8Θ, further proved that βLG promoted complexation between WMS and LA, consistent with DSC and Raman results. In a nutshell, the influence of βLG on the extent of ternary complexes formation is starch origin dependent.

3.4 Effect of pH

Environmental pH is a critical parameter that influences the structure and properties of protein and provides an effective means of modification (Guo *et al.*, 2023). Zhen *et al.* (2022) investigated the effect of pH on starch-protein-lipid complexes formation in RVA using Rice starch (RiS), steric acid (SA) and whey protein isolate (WPI). When pH was increased from acidic (4 and 6) to alkaline (8), a significant change in the pasting curve of RiS-SA paste was observed. Disruption of hydrogen bonds between starch chains under alkaline condition resulted in higher swelling of starch granules, higher breakdown and setback. However, in starch tertiary system, the molecular structure alteration of WPI by pH changed the molecular interplays in the system. The IEP of WPI is between 4.7 to 5.3 (Ye & Chen, 2019), when close to its IEP, WPI favoured the aggregate structure with lower reactivity. At pH farther than IEP, the protein unfolded to expose more reaction sites to interact with SA and starch molecules. Under this condition, the construction of more perfect V-type crystallites was promoted. The results of SAXS (Small-Angle X-Ray Scattering) revealed that higher pH increased the size of nonperiodic nanoscale structures in ternary starch complexes. The resultant complexes displayed strongest digestion resistibility with higher RS content.

Kang *et al.* (2022) found that glutenin reacted differently to starch and lauric acid depending on the environmental pH. At pH 5.2 (lower than glutenin's IEP), the unfolded protein structure readily interacted with starch and lipids to form more ordered structures, hence increasing the RS content in the ternary complexes formed (Table 1). Whereas with aggregate structure in the water (pH~7), glutenin did not cause remarkable change in the digestion rate due to limited inter-component interactions as proven by the lower diffraction peaks than the WS-LA sample. The starch samples prepared at pH 5.2 showed strongest and higher peak intensity (V-type crystal) than the corresponding counterparts prepared using water.

Table 3 Effect of type of starch, pH and cooking method on the structure, digestibility and formation of V-type complexes in starch ternary system.

Type of starch	Type of lipid (%)	Type of protein (%)	Setback viscosity peak	ATR-FTIR/FTIR	LCM Raman Spectroscopy	Small-angle x-ray scattering	X-ray diffraction	DSC/TGA	Digestibility /Content of starch fraction	Source
Effect of starch										
waxy maize starch (WMS), non-waxy maize starch (MS), potato starch (PS), wheat starch (WS)	lauric acid (LA)	β-lactoglobulin (βLG)	Binary complexes: WS-LA, MS-LA > PS-LA. Increase of peak in ternary complexes of MS, WS and PS were greater than their binary counterparts. No peak for MWS-LA in binary system but was enhanced in ternary system.	NA	βLG decreased the FWHM values (higher short-range order) of all of the starch-LA pastes, with the greatest decrease in PS-LA-βLG. The effect on PS-LA systems were more pronounced.	NA	No diffraction peak for WMS-LA sample. WS-LA & MS-LA had higher % crystallinity than PS-LA. Starch-LA-βLG samples showed higher diffraction intensity and % crystallinity than their corresponding binary counterparts.	Starch-LA-βLG complexes showed higher ΔH than their corresponding starch-LA samples. No formation of WMS-LA could be detected, but a small endothermic transition was observed for WMS-LA-βLG.	NA	Cai *et al.,* 2021
Effect of pH during thermal processing										
rice starch (RiS)	stearic acid (SA)	whey protein isolate (WPI)	Starch binary system - remarkable change in the pasting curve at pH 8. Starch ternary system - significant increase of setback peak viscosity at pH 6 and pH8.	WPI suppressed the alignment of starch chains into short-range orders. The increased pH weakened protein aggregation in the ternary system, which facilitated starch protein interactions and hindered the self-assembly of starch chains.	NA	At high pH, the formation of nanoscale structures with increased sizes was observed in the starch ternary system.	The relative content of V-type crystallites (X_v) increased with pH. Xv for starch ternary samples > binary counterparts at all pH.	NA	The SDS content remained unchanged by pH in both binary and ternary starch systems. At pH 8, RS content in starch-WPI-SA system was significantly increased.	Zhen *et al.,* 2022

Table 3 Continue.

Type of starch	Type of lipid (%)	Type of protein (%)	FTIR	X-ray diffraction	DSC/TGA	Digestibility/Content of starch fraction	Source
wheat starch (WS)	3% lauric acid (LA)	15% gluten (following the composition of native wheat flour)	IR1022/995 value: boiled WS-LA-gluten > WS-LA-gluten > steam WS-LA-gluten > baked WS-LA-gluten	% relative crystallinity: baked WS-LA-gluten > steamed WS-LA-gluten > WS-LA-gluten > boiled WS-LA-gluten	TGA was used. Temperature of decomposition: boiled WS-LA-gluten > baked WS-LA-gluten > steamed WS-LA-gluten > WS-LA-gluten mixture	RS content: baked WS-LA-gluten = steamed WS-LA-gluten > boiled WS-LA-gluten > WS-LA-gluten RDS content: baked WS-LA-gluten < steamed WS-LA-gluten = boiled WS-LA-gluten = WS-LA-gluten	Kang *et al.*, 2021b

NA – not available

3.5 Effect of Cooking Method

Steaming (100 °C, 40 min), boiling (10 min) and baking (200 °C, 30 min) exerted different effects on the structure and physicochemical properties of wheat starch-lauric acid complexes upon addition of gluten (Kang *et al.*, 2021a) as seen in Table 3. The ingredients were mixed into dough for steaming and baking but was made into noodle strips for boiling. Different lamellar structure by gluten network was observed after cooking, the distribution of protein was also found different in the cooked samples. The %RC was reported as: baked WS-LA-gluten > steamed WS-LA-gluten > WS-LA-gluten > boiled WS-LA-gluten. The moisture availability during thermal treatments affected the formation of Type-II starch-lipid complexes. Baking with low moisture heat favour the formation of more perfect Type-II starch-lipid complexes with the highest %RC. Steaming with intermediate moisture content also enhanced the formation of Type-II complexes (Blazek & Gilbert, 2011). Authors attributed the lower crystallinity in the steamed sample to the loss of type-II complexes by boiling at 100 °C. The thermal stability of the complexes was improved in the order: boiling > baking > steaming > WS-LA-gluten. No ternary complexes were formed under these three cooking methods. The RS was highest in baked and steamed samples, follows by boiled sample, and lowest in WS-LA-gluten. Overall, baking and steaming were conducive for the formation of stable Type II starch-lipid complexes. The temperature and moisture content used in cooking could be the controlling factors of the V-type complexes formation. More systematic investigations looking into the effect of temperature and moisture are called for.

4. Conclusion and Future Direction

Overall, the research on starch-lipid-protein complexes is still inadequate to generate generalisation for effective applications. Few studies showed that addition of protein into starch binary system brought about formation of starch-lipid-protein ternary complexes, others found that protein did not participate in the ternary complexes, but only facilitate the formation of starch-lipid binary complexes. Further research is needed to provide in-depth understanding and clarify the reasons behind these observations. Apart from fundamental research, investigation related to applied research such as processing conditions including emerging thermal and non-thermal techniques, food product formulation (to create functional food) are useful to verify the potential of this complex in practical applications.

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