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Synergistic effect of endogenous gluten and oleic acid on wheat starch digestion by forming ordered starch-fatty acid-protein complexes during thermal processing

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ABSTRACT

The aim of this study was to understand the potential of endogenous gluten inhibiting the digestibility *in vitro* of wheat starch (WS) in starch-fatty acid-protein system. Therefore, the influences of gluten and whey protein isolate (WPI) on the properties, multi-scale structure and *in vitro* digestibility of WS in WS-oleic acid (OA)-protein system were compared. The results of digestibility *in vitro* indicated that the ternary system of starch-fatty acid-protein showed higher resistant starch (RS) content as well as lower rapidly digestible starch (RDS) content than the binary system of WS-OA, demonstrating protein decreased WS digestion of WS-OA system. The results of pasting properties showed that gluten and WPI both increased the viscosities of WS-OA system during the cooling period due to the formation of WS-OA-protein ternary complex. The results of swelling power and solubility analysis showed that gluten and WPI both decreased the swelling power and solubility of WS-OA binary system. Laser Confocal Raman and X-ray diffraction (XRD) studies indicated that gluten and WPI both increased the ordered degree of WS-OA binary system by decreasing the full width at half maximum (FWHM) of the peak at 480 cm^{-1} and increasing crystallinity degree. Strikingly, compared with WPI, gluten had greater effects on the digestibility *in vitro*, pasting properties and ordered degree of WS in WS-OA-protein system. Therefore, gluten as an endogenous protein has the potential application in reduction the enzymatic digestibility of WS by regulating the reassembly of starch and fatty acid during thermal processing.

1. Introduction

As the chief source of carbohydrate for human nutrition, starch provides 20–50% of total energy requirements for human body (Feng et al., 2019). However, cooked starch commonly has a limit ascribed to its easy digestion by digestive enzymes inside the human body, which can lead to an increased incidence rate of postprandial hyperglycemia. The risk of chronic diseases and damaged glucose tolerance may be increased due to long-term hyperglycemia, causing the happening of diabetes (Li et al., 2021). The high prevalence of chronic complications is related to the increase in obesity and diabetes, which has become a global public-health problem (Ji, 2018). Therefore, increasing the resistant digestibility and inhibiting postprandial blood glucose of starch have become the critical areas for researchers (Wang et al., 2021). Current reports have revealed that the digestibility of starch is regulated by its multi-scale structures, for instance, crystalline features, short-range ordered structures, lamellar structures and micromorphology and plays a critical role in human nutrition (Chi et al., 2021a; Lin et al., 2020). Hence, how to regulate the multi-scale structure of starch and design starch-based products with low digestibility is one of the key scientific problems to be solved urgently in the development of starch-based products.

Numerous studies have reported regulating the digestibility of starch by assorted kinds of physical or chemical modifications (Li et al., 2018).

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Among these methods, the complexation of starch with non-starchy constituents is a simple method for regulating the reassembly behaviors and multi-scale structures of starch during thermal processing and cooling (Chi et al., 2021b). As one type of RS (resistant starch), starch-lipid complexes and their effects on the digestibility and nutritional properties of starch have been broadly investigated (Gutiérrez and Tovar, 2021; Wang et al., 2020). Interestingly, the formation of amylose-lipid complex was promoted by protein because protein could complex with lipid through hydrophobic and electrostatic interaction with lipophilic/hydrophobic regions of the protein (Wang et al., 2017). Furthermore, several reports have demonstrated that the digestibility of starch-fatty acid-protein ternary complex was lower than that of the corresponding starch-fatty acid binary complex. Therefore, co-complexation of starch with lipid and protein is supposed as an effective method for inhibiting starch digestibility by alteration starch structuration (Chi et al., 2021b).

Recent studies have demonstrated that fatty acids act as bridges between thermally antipathic amylose and protein molecule, which acts as an organizer of starch-protein-fatty acid complex (Zhang et al., 2010). Three structural elements of a starch-fatty acid-protein complex might be mainly starch-fatty acid complex, protein-fatty acid complex and protein polymer linked with disulfide bonds (Parada and Santos, 2016). Additionally, Lin et al. (2020) reported that whey isolate protein (WPI) with an isoelectric point less than 7.0 was more likely to form starch-lipids-protein complex than gelatin with an isoelectric point greater than 7.0. Hence, the properties of protein such as isoelectric point also influenced the formation of starch-fatty acid-protein complexes and the digestibility of starch in starch-fatty acid-protein system. As a unique cereal protein, gluten accounts for 80%–85% of the total wheat protein (Zhou et al., 2020). With the molecular weights ranged from 30 to more than 10,000 KDa and an isoelectric point of 6–8, gluten is a polymeric protein bound by both intramolecular and intermolecular disulfide bonds (Panozzo et al., 2016). Therefore, gluten may be a potential raw material of protein for preparing anti-digestive wheat starch-based foods by forming starch-fatty acid-protein complex. Furthermore, as an endogenous protein with special structure, gluten might interact with wheat starch (WS) and fatty acid differently from other reported animal proteins including whey protein, bovine serum albumin and gelatin. However, there are few studies about the effect of endogenous gluten on the digestibility *in vitro* and properties of WS in the coexistence system of starch and fatty acid commonly found in wheat flour-based products. Therefore, the influences of endogenous gluten on enzymatic digestibility *in vitro* as well as pasting properties, swelling power and solubility of WS in the coexistence system of starch and fatty acid were investigated. Additionally, the influences of endogenous gluten on multi-scale structure of WS in the coexistence system of starch and fatty acid were revealed by multiple analytical methods, including Fourier transform-Raman spectroscopy (FT-Raman) and X-Ray Diffraction (XRD). Furthermore, the effects of gluten and WPI on the starch digestibility, properties and multi-scale structure of WS in starch-fatty acid system were compared. This information from the present study is necessary to the rational design of starch-based products and precise regulation of starch digestibility.

2. Materials and methods

2.1. Materials

Wheat flour containing 15.64% gluten, 12.0% water, 2.5% fat and 0.5% ash was obtained from Jiangsu Nanshun Food co. Ltd (Jiangsu, China). Whey protein isolate (WPI) with the purity of 80% and WS were purchased from Shanghai yuan-ye Bio-technology Co., Ltd (Shanghai, China). Oleic acid and amyloglucosidase were purchased from Aladdin Industrial Corporation (Shanghai, China). Amylase was obtained from Sigma-Aldrich Co. LLC (Santa Clara, USA). Other chemicals of analytical grade were obtained from China national pharmaceutical group corporation (Beijing, China).

2.2. Preparation of gluten

The gluten separation was performed according to the method of Wang et al. (2014). Specially, 150 g of wheat flour was blended with 0.4 mol/L of NaCl solution (80 mL) for 5 min in a dough mixer (SPI 11, Pingjiang County Hongyu Machinery Manufacturing Co. LTD, China). After resting for 10 min, the wheat flour dough was washed with 0.4 mol/L of NaCl solution until viscoelastic gluten formed. Before lyophilization, the viscoelastic gluten was washed again with distilled water until NaCl was removed. After grinding into powder, the gluten-rich fraction was blended with 150 mL of dichloromethane at 25 °C for 30 min to defat and filtered with filter paper. The above processes were repeated three times and then the gluten-rich fraction was dried in fume cupboard at 25 °C. The obtained gluten was stored at -20 °C for less than two weeks prior to prepare WA-OA-protein sample.

2.3. Preparation of starch samples

WS (2.0 g), WS-OA (2.0 g of WS and 0.1 g of OA), WA-OA-protein (2.0 g of starch, 0.1 g of OA and 0.2 g of gluten or WPI) and an appropriate amount of deionized water (total weight of 28.0 g) were weighed into RVA canisters. After maintaining at 50 °C for 1 min, the above liquid sample suspension was heated to 95 °C and maintained for 2.5 min, and then cooled to 50 °C and held for 3 min. During the above processes, the heating rate and cooling rate were 14 °C/min and the speed of the blending paddle was set at 960 rpm for the first 10 s, then maintained at 160 rpm for the remainder of the test. The obtained paste was frozen for 24 h at −20 °C, and then freeze-dried in a lyophilizer (LGJ-10C, Foring Technology Development (Beijing) Co., Ltd). The freeze-dried sample was pulverized and sieved with a 0.15 mm sieve. The dried sample was deposited in valve bag at −20 °C for determination of the structure and digestion properties.

2.4. Digestibility of starch samples in vitro

On the ground of the Englyst method, the digestion of starch samples was determined with some modifications. Briefly, sample (100 mg) was blended with phosphate buffer solution (pH 5.2, 0.1 mol/L, 5 mL) containing 6.67 mM $CaCl_2$. After incubation at 37 °C for 15 min, 2.5 mL of enzyme solution (122 U/mL of amylase and 16.5 U/mL of amyloglucosidase) was added into the above mixture and then also incubated at 37 °C for a period of time. During hydrolysis, the hydrolysate (0.1 mL) was removed at a certain time (0, 20 and 120 min) and immediately blended with 95% ethanol (0.9 mL). After centrifugation at 10,000 rpm for 5min, the glucose content in the above solution was analyzed by the DNS method. Specially, the enzymatic hydrolysate after enzyme inactivation (0.1 mL), distilled water (0.3 mL) and DNS reagent (0.8 mL) were blended and incubated for 5min in boiling water bath, and then cooled with ice water. After adding distilled water (4.8 mL) and blending, the absorbance of the mixture was determined at 540 nm. The glucose content in enzymatic hydrolysate was quantified with a standard curve of D-glucose. Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) were obtained based on the following equations:

$$\text{RDS}\ (\%) = (\text{G20} - \text{G0}) \times 0.9 \times 100$$

$$\text{SDS}\ (\%) = (\text{G120} - \text{G20}) \times 0.9 \times 100$$

$$\text{RS}\ (\%) = 100\% - \text{RDS} - \text{SDS}$$

where G0, G20 and G120 represent the glucose content after digestion for 0, 20 and 120 min, respectively.

2.5. The determination of swelling power and solubility

Swelling power and solubility were determined according to the method reported by Cui et al. (2014) with slight modifications. The suspension of WS (2% (w/v)), WS-OA (2% (w/v) of WS and 0.1% of OA (w/v)), WA-OA-protein (2% (w/v) of WS, 0.1% of OA (w/v) and 0.2% of protein (w/v)) was successively heated at 55, 65, 75, 85 and 95 °C for 30 min in a magnetic stirring apparatus then cooled to room temperature. After centrifugation at 4000 r/min for 20 min, the supernatant was dried at 105 °C until constant weight and wet sediment was weighed. Swelling power and solubility was calculated as the proportion of wet sediment to dry basis of starch and solid weight to dry basis of starch, respectively.

2.6. Pasting viscosity analysis

Pasting viscosity curves of samples were obtained using a rapid viscosity analyzer (RVA-Super 4) (Perten, Sweden) based on the report of Zhang and Hamaker (2003). The aforementioned protocol, which was explained in methods 2.3, was used to analyze pasting properties.

2.7. The determination of X-ray diffraction (XRD)

The crystalline structure and relatively crystallinity of samples were determined according to the method of Chao et al. (2018) using a D8 Advance 25 X-ray diffractometer (Bruker, Germany) equipped with an X-ray source of Cu Kα. After equilibrium at 25 °C for 5 d with saturated NaCl solution, the equilibrated samples were scanned from 4 to 40° (2θ) at the rate of 4°/min and step size of 0.02°. The relatively crystallinity degree of samples was obtained using a Jade 6 software.

2.8. The determination of fourier transform-Raman spectroscopy (FT-Raman)

The short-range ordered structure of samples was measured according to the report of Wang et al. (2022) with some modifications. Raman spectra ranged from 3000 to 100 cm^{-1} were obtained using a Nicolet iS50 FT-Raman spectrometer (Thermo Fisher Scientific Co., Ltd, USA) equipped with a laser source of 785 nm. The scans times and resolution were 256 and 4 cm^{-1}, respectively. The short-range ordered structure was expressed as the full width at half maximum (FWHM) of the peak at 480 cm^{-1} and obtained by Origin 21 software.

2.9. Statistical analysis

All experiments were performed in triplicate and these results were analyzed with the software of SPSS 21.0 (IBM, Armonk, NY, USA). To compare the significance, one way analysis of variance (ANOVA) with Duncan tests were performed and the significance level was $P < 0.05$.

3. Results and discussion

3.1. Effect of protein on the digestion in vitro of WS in WS-OA system

The digestion of starch is a complicated process and involves some successive events including the diffusion of enzymes into starch, the formation of chain-enzyme complexes and the hydrolysis of glycosidic bonds (Zhen et al., 2022). It could be influenced by numerous factors, such as supramolecular structures of starch, the interactions between food components and starch/digestive enzymes (Li et al., 2021). Evidence from the composition of RDS, SDS and RS content (Fig. 1) showed that gelatinized WS with high content of RDS (85.3%), low content of SDS (9.2%) and RS (5.4%) was easy to be digested. The incorporation of OA significantly decreased RDS content and increased RS content of gelatinized WS, which might be ascribed to the entanglement of starch with hydrophobic guest of OA to form WS-OA inclusion complex (Lin et al., 2020). Compared with the binary system of WS-OA, the ternary system of WS-OA-protein had lower digestibility, lower content of RDS as well as higher content of RS. Zheng et al. (2018) also found that the binary system of maize starch-fatty acid showed slightly lower digestibility than maize starch, while it presented higher digestibility than the ternary system of maize starch-fatty acid-protein. Therefore, protein decreased WS digestion of WS-OA system, which might be ascribed to the increase in the structural order and steric hindrance caused by the formation of WS-OA-protein complex (Zheng et al., 2018). Meanwhile, the existence of protein might decrease enzyme activity and the availability of enzyme to starch, which also conduced to the decrease in WS digestion of WS-OA binary system (Zhen et al., 2022). Furthermore, compared with WPI, gluten had greater effect on RDS content, demonstrating protein types had greater effect on the digestibility of WS in the ternary system of starch-fatty acid-protein. Therefore, endogenous gluten was more effective in preparing starch-based foods with lower digestibility than WPI.

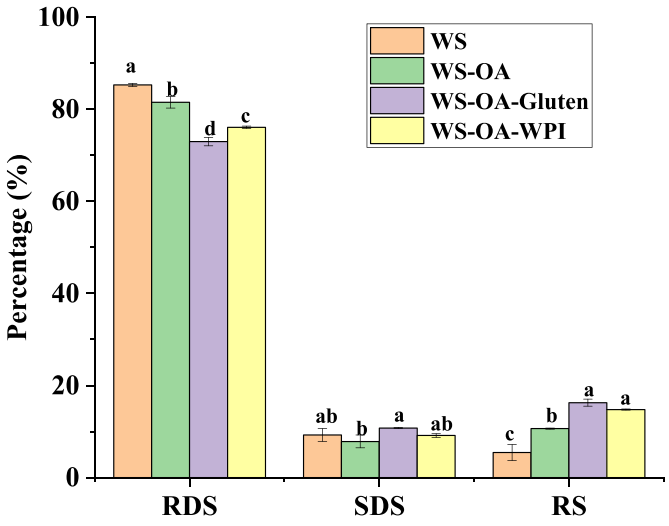

Fig. 1. The digestibility *in vitro* of WS in WS-OA and WS-OA-protein system.

3.2. Effect of protein on the swelling power and solubility of WS in WS-OA system

After heating in superfluous water, the crystalline structure of starch was destroyed and the hydrogen bonds between starch and water molecules were formed, which induced an increase in starch volume (An et al., 2022). As shown in Table 1, the swelling power of WS was slightly decreased by OA, while it was significantly decreased by the coexistence of protein and OA. This result might be ascribed to the interactions including amylose-fatty acid, amylose-fatty acid-protein, and/or the increase in crystallinity of starch (Colussi et al., 2020). The swelling power of WS in WS-OA-Gluten system was slightly lowered than that in WS-OA-WPI system, which might be attributed to more crystallinity structure formation in WS-OA-Gluten system.

Starch solubility defined as the percentage of dissolved solid in starch mass after heating. It results from the leaching of amylose, which

Table 1
The swelling power, solubility and FWHM at 480 cm^{-1} of WS in gelatinized WS, WS-OA, WS-OA-protein system.

Sample	Swelling power (g/g)	Solubility (%)	FWHM
WS	8.58 ± 0.504^{a}	15.0 ± 0.52^{b}	23.5 ± 0.12^{a}
WS-OA	7.95 ± 0.080^{a}	10.9 ± 0.66^{a}	22.9 ± 0.87^{ab}
WS-OA-WPI	6.74 ± 0.139^{b}	10.0 ± 2.06^{a}	21.0 ± 0.38^{bc}
WS-OA-Gluten	6.50 ± 0.404^{b}	9.2 ± 1.10^{a}	19.8 ± 0.56^{c}

Values with different letters in a column represents significantly different ($P < 0.05$).

diffuses and dissociates from the particles during expansion. This leaching is characterized by an alteration from ordered to disordered for starch particles when starch is heated with water (Colussi et al., 2020). As shown in Table 1, the solubility of WS was decreased by OA with/-without protein, which might be due to the reduction of the leaching of amylose caused by the interaction of amylose-OA and/or amylose-OA-protein. This result was consistent with a previous study, which also found that fatty acids with/without whey protein both decreased the solubility of starch at 85 °C and 100 °C (Zhang and Hamaker., 2003). Compared with WPI, gluten had slightly greater effect on starch solubility in starch-OA-protein system, which also could mirror the difference of multi-scale structure in some extent. Therefore, it was necessary to study the effect of protein on the formation of multiple complexes and their multi-scale structure in WS-OA-protein system to discover the underlying mechanism of anti-digestion and lower swelling power and solubility of WS in WS-OA-protein system.

3.3. Effect of protein on the pasting properties of WS in WS-OA system

During thermal processing, starch gradually absorbed moisture and thereby swelled and disintegrated with increasing temperature, which led to the increase of viscosity (Lin et al., 2020). The alterations in the viscosity of starch during heating or cooling could be used to uncover the disassembly and reassembly behaviors of starch. As depicted in Fig. 2, the addition of OA had no remarkable effects on the pasting curves of WS, indicating that OA had slight effects on the disassembly and reassembly behaviors of WS. While the coexistence of protein and OA obviously altered the pasting curves of WS, demonstrating that the coexistence of protein and OA remarkably altered the disassembly and reassembly behaviors of WS. Specifically, in cooling stage, the ternary system of WS-OA-protein showed a distinct viscosity peak, while WS and the binary system of WS-OA both had no viscosity peaks. The presence of a high viscosity peak during cooling stage in ternary system might be attributed to the reassociation and entanglement of gelatinized WS with OA and protein and starch-fatty acid-protein complex formation. Additionally, compared with WS and WS-OA binary system, the first viscosity peak of ternary system caused by gelatinized starch granule was postponed, revealing that the coexistence of protein and OA altered the pasting properties of WS. Comparatively speaking, the viscosity peak of WS in WS-OA-Gluten system was higher than that in WS-OA-WPI system. This suggested that the disassembly and reassembly behavior of WS in WS-OA-protein system was influenced by protein types. Additionally, in WS-OA-protein system, OA was more likely to interact with WS in the presence of gluten than WPI. Lin et al. (2020) found that WPI with an isoelectric point of less than 7.0 had a greater effect on the viscosity parameters of rice starch than gelatin with an isoelectric point of greater than 7.0 in rice starch-lauric acid-protein system. While gluten with an isoelectric point of 6–8 had greater effect on the disassembly and reassembly behaviors of WS in WS-OA-protein system than WPI in the present study. This might be related to the high molecular weight and high content of disulfide bonds of gluten.

3.4. Effect of protein on the crystalline structure of WS in WS-OA system

XRD techniques are commonly applied to obtain the information on the long-range structural order of starch. Native starch has three diffraction patterns, which represents A-, B- and C- type, respectively, while there is a different XRD pattern referring to V-type for starch-lipid and starch-lipid-protein complexes (Wang et al., 2020). As shown in Fig. 3, the gelatinized WS was chiefly amorphous-structured with a low relative crystallinity of 14.7% but also had V-type crystallites which exhibited two broad peaks at 17.10° and 20.15° (2θ) due to retrogradation. After complexation with OA, the gelatinized WS with a relative crystallinity of 17.2% showed a new peak at 13.04°, which was ascribed to the formation of V-type crystals between WS and OA. While the gelatinized WS showed two obvious peaks at 13.00° and 19.98° (2θ) after complexation with OA and protein, which were ascribed to V-type crystals of WS-OA-protein complex (Zheng et al., 2018). This result was in agreement with Cai et al. (2021), who also found that the peak intensities caused by V-type crystals of starch-lauric acid-β-lactoglobulin complex were higher than that of the homologous starch-lauric acid complex. Additionally, the peaks intensity of V-type crystals and relative crystallinity of WS in WS-OA-WPI system were lower than that in WS-OA-Gluten system. Specially, the relative crystallinity of WS in WS-OA-WPI and WS-OA-Gluten system was 22.8% and 24.7%, respectively. This suggested that there was a greater amount of crystalline ternary complex of WS-OA-Gluten formed than that of WS-OA-WPI in WS-OA-protein system, which was in agreement with the results of RVA determination.

3.5. Effect of protein on the short-range ordered structure of WS in WS-OA system

Raman spectroscopy as a non-destructive analysis technique has been commonly applied in the characterization of the short-range ordered structure of starch (Wang et al., 2020). According to a previous study, the full width at half maximum (FWHM) of the peak at 480 cm^{-1} could be used to deduce the short-range ordered structure of starch and to identify starch–lipid and starch–lipid-protein complexes formation (Zheng et al., 2018). Generally, starch with small FWHM value and

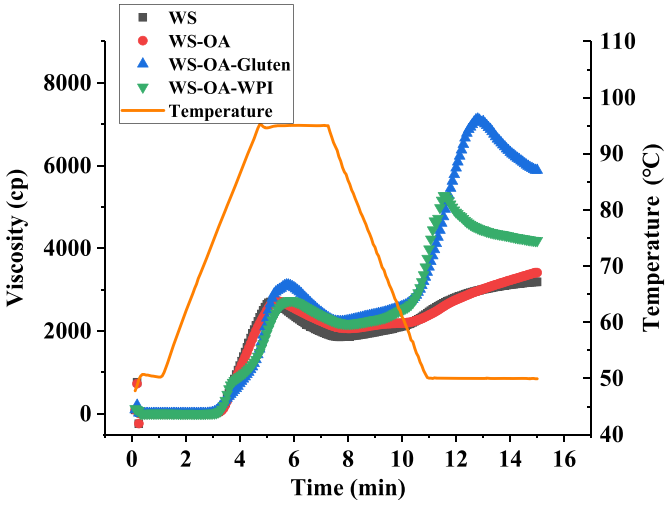

Fig. 2. Pasting viscosity curves of WS in WS-OA and WS-OA-protein system.

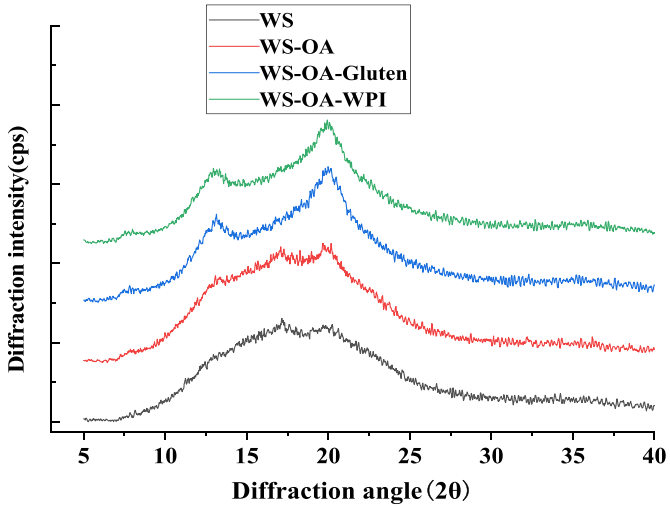

Fig. 3. XRD patterns of WS in WS-OA and WS-OA-protein system.

strong intensity of the peak at 480 cm^{-1} indicates that the order degree of this starch is high. Raman spectra of WS, WS-OA and WS-OA-protein were depicted in Fig. 4, and the FWHMs of the peak at 480 cm^{-1} were shown in Table 1. OA slightly decreased the FWHM value of gelatinized WS, and slightly increased the peak intensity at 480 cm^{-1} of gelatinized WS. While the coexistence of OA and protein significantly lowered the FWHM values of gelatinized WS, and increased the peak intensities at 480 cm^{-1} of gelatinized WS in WS-OA-protein system. This suggested that OA alone slightly improved the structural order of gelatinized WS, while the simultaneous presence of OA and protein significantly enhanced the structural order of gelatinized WS due to the formation of WS-OA-protein complex. This result was consistent with a previous study, which reported that lauric acid lowered FWHM value of gelatinized WS and the FWHM value of gelatinized WS in lauric acid-WS system was furtherly lowered by β-lactoglobulin (Cai et al., 2021). The FWHM value of WS in WS-OA-WPI system was slightly lower than that in WS-OA-Gluten system, while there was on significant difference between them. This indicated that the short-range ordered structure of WS in ternary system was slightly influenced by protein types. Lin et al. (2020) also found ternary system of starch-LA-protein had more short-range ordered structure of starch than the corresponding binary system of starch-LA. Additionally, ternary system of starch-LA-β-lactoglobulin had no significant difference with starch-LA-gelatin ternary system in short-range ordered structures (Lin et al., 2020).

Protein types had a significant effect on *in vitro* digestibility of WS in starch-fatty acid-protein ternary system due to the ordered starch-fatty acid-protein complexes formation. According to a previous study, WPI was more favorable for the formation of starch-fatty acid-protein complexes with a lower starch digestibility than gelatin (Lin et al., 2020). In the present study, gluten was more favorable for the formation of starch-fatty acid-protein complexes with more ordered multi-scale structure than widely studied WPI. Therefore, gluten was a potential vegetable protein, which could be used to lower WS digestion by interacting with WS and fatty acid and forming starch-fatty acid-protein complex. Lin et al. (2020) found that protein with an isoelectric point less than 7.0 more easily formed starch-lipids-protein complex than protein with an isoelectric point greater than 7.0. The isoelectric point of gluten (6–8) is higher than that of WPI, while gluten is more conducive to the formation of starch-lipids-protein complex than WPI. Might describe the high molecular weight and high disulfide bonds content of gluten. According to a previous study, protein polymer linked by disulfide bonds was one of the three structural elements formed in the starch-fatty acid-protein complex (Parada and Santos, 2016). Additionally, protein linked by electrostatic interaction was also necessary for ternary complex formation, and the formation of disulfide bonds by free cysteine of protein during thermal processing might also probably conduce to ternary complex formation (Zhang and Hamaker, 2005; Zhang et al., 2010). Gluten with molecular weight ranging from 30 to 10,000 KDa consists of gliadin with intramolecular disulfide bonds and glutenin with intramolecular and intermolecular disulfide bonds and has cysteine residues (Abedi et al., 2018). Furthermore, large gliadin-glutenin aggregations are formed by disulfide bonds cross-linking during heating process. Thus, gluten showed more powerful influences on starch-fatty acid-protein complexes formation than other proteins, such as WPI.

4. Conclusion

This study confirmed that protein could further inhibit the digestibility as well as decrease the swelling power and solubility of WS in WS-OA system by forming ordered WS-OA-protein complexes. Additionally, gluten had a stronger ability to form WS-OA-protein complexes than WPI, which led to higher ordered structure and lower digestibility of WS in WS-OA-Gluten system than that in WS-OA-WPI system. This might be ascribed to the high molecular weight of gluten, which might also be one of the critical factors influencing the multi-scale structure and digestibility of WS-OA-protein complexes. However, how the molecular weight of gluten and how the key subfractions of gluten (glutenin and gliadin) influenced the formation of WS-OA-protein complexes were unknown and should be further studied. This study provided a direction for the creation and design of functional wheat flour-based products.

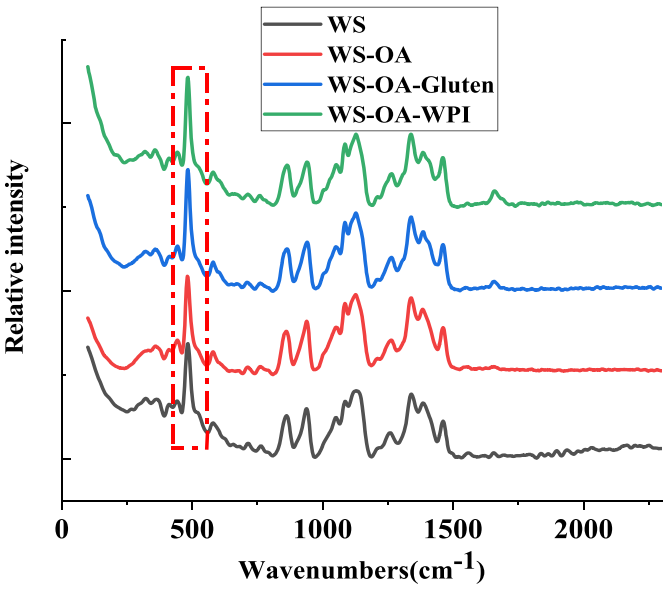

Fig. 4. LCM-Raman spectroscopy of WS in WS-OA and WS-OA-protein system. Red rectangle indicated the band at 480 cm^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

CRediT authorship contribution statement

Jing Du: Investigation, Methodology, Software, Formal analysis, Manuscript revision, Writing – original draft, Writing – review & editing. **Meng-yao Lv:** Resources, Conceptualization, Methodology, Visualization, Data curation. **Hai-long Zhang:** Investigation, Writing – review & editing, Manuscript final version approval. **Shen-sheng Xiao:** Resources, Software, Writing – review & editing, Manuscript final version approval. **Shuang-yi Zheng:** Writing – review & editing, Resources, Investigation. **Xue-dong Wang:** Writing – review & editing, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Abedi, E., Majzoobi, M., Farahnaky, A., Pourmohammadi, K., Mahmoudi, M.R., 2018. Effect of ionic strength (NaCl and $CaCl_2$) on functional, textural and electrophoretic

properties of native and acetylated gluten, gliadin and glutenin. Int. J. Biol. Macromol. 120, 2035–2047. https://doi.org/10.1016/j.ijbiomac.2018.09.155.
An, D., Li, H., Li, D., Zhang, D., Huang, Y., Obadi, M., Xu, B., 2022. The relation between wheat starch properties and noodle springiness: from the view of microstructure quantitative analysis of gluten-based network. Food Chem. 393, 133396 https://doi.org/10.1016/j.foodchem.2022.133396.
Cai, J., Chao, C., Niu, B., Copeland, L., Yu, J., Wang, S., Wang, S., 2021. New insight into the interactions among starch, lipid and protein in model systems with different starches. Food Hydrocolloids 112, 106323. https://doi.org/10.1016/j.foodhyd.2020.106323.
Chao, C., Cai, J., Yu, J., Copeland, L., Wang, S., Wang, S., 2018. Toward a better understanding of starch–monoglyceride–protein interactions. J. Agric. Food Chem. 66 (50), 13253–13259. https://doi.org/10.1021/acs.jafc.8b04742.
Chi, C., Li, X., Huang, S., Chen, L., Zhang, Y., Li, L., Miao, S., 2021a. Basic principles in starch multi-scale structuration to mitigate digestibility: a review. Trends Food Sci. Technol. 109, 154–168. https://doi.org/10.1016/j.tifs.2021.01.024.
Chi, C., Li, X., Zhang, Y., Chen, L., Li, L., Miao, S., 2021b. Progress in tailoring starch intrinsic structures to improve its nutritional value. Food Hydrocolloids 113, 106447. https://doi.org/10.1016/j.foodhyd.2020.106447.
Colussi, R., Kringel, D., Kaur, L., da Rosa Zavareze, E., Dias, A.R.G., Singh, J., 2020. Dual modification of potato starch: effects of heat-moisture and high pressure treatments on starch structure and functionalities. Food Chem. 318, 126475 https://doi.org/10.1016/j.foodchem.2020.126475.
Cui, M., Fang, L., Zhou, H., Yang, H., 2014. Effects of amino acids on the physiochemical properties of potato starch. Food Chem. 151, 162–167. https://doi.org/10.1016/j.foodchem.2013.11.033.
Feng, T., Shui, M., Chen, Z., Zhuang, H., Wang, W., Yang, Y., Zhang, J., Ye, R., 2019. Hericium Erinaceus β-glucan modulates *in vitro* wheat starch digestibility. Food Hydrocolloids 96, 424–432. https://doi.org/10.1016/j.foodhyd.2019.05.044.
Gutiérrez, T.J., Tovar, J., 2021. Update of the concept of type 5 resistant starch (RS5): self-assembled starch V-type complexes. Trends Food Sci. Technol. 109, 711–724. https://doi.org/10.1016/j.tifs.2021.01.078.
Ji, Y., 2018. *In vitro* digestion and physicochemical characteristics of corn starch mixed with amino acid modified by low pressure treatment. Food Chem. 242, 421–426. https://doi.org/10.1016/j.foodchem.2017.09.073.
Li, D., Yang, Y., Yang, X., Wang, X., Guo, C., Sun, L., Guo, Y., 2021. Modulation of gelatinized wheat starch digestion and fermentation profiles by young apple polyphenols *in vitro*. Food Funct. 12 (5), 1983–1995. https://doi.org/10.1039/D0FO02752A.
Li, Y., Wang, R., Liang, R., Chen, J., He, X., Chen, R., Liu, W., Liu, C., 2018. Dynamic high-pressure microfluidization assisting octenyl succinic anhydride modification of rice starch. Carbohydr. Polym. 193, 336–342. https://doi.org/10.1016/j.carbpol.2018.03.103.
Lin, L., Yang, H., Chi, C., Ma, X., 2020. Effect of protein types on structure and digestibility of starch-protein-lipids complexes. LWT–Food Sci. Technol. 134, 110175 https://doi.org/10.1016/j.lwt.2020.110175.
Panozzo, A., Manzocco, L., Lippe, G., Nicoli, M.C., 2016. Effect of pulsed light on structure and immunoreactivity of gluten. Food Chem. 194, 366–372. https://doi.org/10.1016/j.foodchem.2015.08.042.
Parada, J., Santos, J.L., 2016. Interactions between starch, lipids, and proteins in foods: microstructure control for glycemic response modulation. Crit. Rev. Food Sci. Nutr. 56 (14), 2362–2369. https://doi.org/10.1080/10408398.2013.840260.
Wang, C., Chao, C., Yu, J., Copeland, L., Huang, Y., Wang, S., 2022. Mechanisms underlying the formation of amylose–lauric acid−β-lactoglobulin complexes: experimental and molecular dynamics studies. J. Agric. Food Chem. 70 (34), 10635–10643. https://doi.org/10.1021/acs.jafc.2c04523.
Wang, N., Wu, L., Huang, S., Zhang, Y., Zhang, F., Zheng, J., 2021. Combination treatment of bamboo shoot dietary fiber and dynamic high-pressure microfluidization on rice starch: influence on physicochemical, structural, and *in vitro* digestion properties. Food Chem. 350, 128724 https://doi.org/10.1016/j.foodchem.2020.128724.
Wang, P., Xu, L., Nikoo, M., Ocen, D., Wu, F., Yang, N., Jin, Z., Xu, X., 2014. Effect of frozen storage on the conformational, thermal and microscopic properties of gluten: comparative studies on gluten-, glutenin- and gliadin-rich fractions. Food Hydrocolloids 35, 238–246. https://doi.org/10.1016/j.foodhyd.2013.05.015.
Wang, S., Chao, C., Cai, J., Niu, B., Copeland, L., Wang, S., 2020. Starch–lipid and starch–lipid–protein complexes: a comprehensive review. Compr. Rev. Food Sci. Food Saf. 19 (3), 1056–1079. https://doi.org/10.1111/1541-4337.12550.
Wang, S., Zheng, M., Yu, J., Wang, S., Copeland, L., 2017. Insights into the formation and structures of starch–protein–lipid complexes. J. Agric. Food Chem. 65 (9), 1960–1966. https://doi.org/10.1021/acs.jafc.6b05772.
Zhang, G., Hamaker, B.R., 2005. Sorghum (Sorghum bicolor L. Moench) flour pasting properties influenced by free fatty acids and protein. Cereal Chem. 82 (5), 534–540. https://doi.org/10.1094/CC-82-0534.
Zhang, G., Hamaker, B.R., 2003. A three component interaction among starch, protein, and free fatty acids revealed by pasting profiles. J. Agric. Food Chem. 51 (9), 2797–2800. https://doi.org/10.1021/jf0300341.
Zhang, G., Maladen, M., Campanella, O.H., Hamaker, B.R., 2010. Free fatty acids electronically bridge the self-assembly of a three-component nanocomplex consisting of amylose, protein, and free fatty acids. J. Agric. Food Chem. 58 (16), 9164–9170. https://doi.org/10.1021/jf1010319.
Zhen, Y., Wang, K., Wang, J., Qiao, D., Zhao, S., Lin, Q., Zhang, B., 2022. Increasing the pH value during thermal processing suppresses the starch digestion of the resulting starch-protein-lipid complexes. Carbohydr. Polym. 278, 118931 https://doi.org/10.1016/j.carbpol.2021.118931.
Zheng, M., Chao, C., Yu, J., Copeland, L., Wang, S., Wang, S., 2018. Effects of chain length and degree of unsaturation of fatty acids on structure and *in vitro* digestibility of starch–protein–fatty acid complexes. J. Agric. Food Chem. 66 (8), 1872–1880. https://doi.org/10.1021/acs.jafc.7b04779.
Zhou, R., Sun, J., Qian, H., Li, Y., Zhang, H., Qi, X., Wang, L., 2020. Effect of the frying process on the properties of gluten protein of you-tiao. Food Chem. 310, 125973 https://doi.org/10.1016/j.foodchem.2019.125973.