Modulating the in vitro digestibility of chemically modified starch ingredient by a non-thermal processing technology of ultrasonic treatment

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ARTICLE INFO

Keywords:
Chemically modified starch
In vitro starch digestibility
Structural changes
Ultrasonic treatment
Non-thermal processing technology

ABSTRACT

Chemically modified starch (RS4) was commercially available as a food ingredient, however, there was a lack of knowledge on how ultrasonic treatment (non-thermal technology) modulated the enzymatic resistance of RS4. In this study, structural change of RS4 during ultrasonic treatment and its resulting digestibility was investigated. Results from scanning electron microscopy, particle size analysis, chemical composition analysis, X-ray diffraction, differential scanning calorimetry, and Fourier transform infrared spectroscopy showed that ultrasonic treatment remained the granule morphology, increased the apparent amylose content, reduced the particle size, destroyed the crystalline structure, decreased the helical orders, but enhanced the short-range molecular orders of ultrasonic-processed RS4. In vitro digestibility analysis showed that the total content of rapidly digestible starch and slowly digestible starch was increased, whereas the content of resistant starch was decreased. Overall, ultrasonic treatment substantially reduced the enzymatic resistance of RS4, indicating that RS4 was not stability against the non-thermal processing technology of ultrasonic treatment.

1. Introduction

Chemically modified starch (RS4) is a type of resistant starch (RS), which plays a very important role in functional food [1]. RS4 has attracted the interest of many research groups over the last decade, mainly because its anti-digestibility property, which can escape digestion from small intestine and be fermented in the colon to improve the colonic health [1]. RS4 can be prepared by a variety of different chemical reactions such as esterification [2], etherification [3], and cross-linking [4]. RS4 is commercially available as a commercial RS ingredient in the food industry [5]. In the newly proposed regulations, which are given by the U.S. Food and Drug Administration, cross-linked phosphorylated starch (RS4) is accepted as a dietary fiber (FDA, 2019) [6]. Compares with traditional fibers, RS4 is a valuable ingredient as a fiber source when includes in food formulations at a relatively high level without noticeably sacrificing the textural property and sensory quality of the final products [5,7]. For instance, Aribas, Kahraman, & Koksel [7] reported that RS4 supplementation in spaghetti caused higher total dietary fiber content and comparable glycemic index value at the same bran supplementation level with lower negative effects on the total organic matter value, color and textural properties. To date, RS4 has been successfully used as a fiber-enriching ingredient in a range of food systems, such as spaghetti [7], extruded snacks [8], and cookies [9].

RS has the disadvantage of easily losing the enzymatic resistance upon heat treatment, such as cooking, baking, fry, and extrusion. There are several studies investigating the effect of heat treatment on the enzymatic resistance of RS, high temperature tends to reduce the RS content because these treatments induce structural changes, by disrupting starch granules, melting crystallites, forcing molecular breakdown, creating new helical structures [10]. In a study investigating the effects of high-temperature processing on the digestibility properties of commercial RS2-4, it was found that high-temperature processing reduced the enzymatic resistance of HA50 (RS2), HA 70 (RS2), and Fibersym (RS4) but did not considerably alter the RS contents of Novolose 330 (RS3) and FiberRite (RS4) [5]. Thermal treatments are traditional methods for food processing, which may cause the damage in functional and sensory characteristics [11]. Nowadays, emerging non-thermal food processing technologies have attracted extensive attention from the food industry and researchers, which can maintain the nutritional and sensory properties for food products [11].

There is a lack of comprehensive understanding of how non-thermal...
food processing technologies influence the enzymatic resistance of RS ingredients and how the changes are related to the alterations in the structure and physicochemical properties of starch. Ultrasound can be considered as an emerging non-thermal technology, which has been widely used in food processing, including microbial inactivation, texture tenderization, emulsification, extraction, freezing and thawing [11]. Ultrasonic treatment has been introduced as an environmental friendly method of starch modification because there is no chemical reagents used or retained in the modified starch, and the effect of ultrasonic treatment can be mainly explained by the mechanical force, micro jets and powerful shock waves and mechanical, thermal and cavitation effects [11,12]. It is shown that ultrasonic treatment can modify the structural arrangement of starch chains within the amorphous and crystalline domains, and thus affects the digestibility, thermal features, and functional properties for different starches such as foxtail millet starch, pea starch, and waxy corn starch [13,14]. For example, ultrasonic treatment increased the content of amylose [15], decreased the mean volume diameter [14], destroyed the crystal structure [16], facilitated the starch-lipid complex formation [14].

In the view of the current literature, there are a lot of publications about the effects of ultrasonic treatment on the digestibility properties of varying kinds of starches, whereas no research has investigated the mechanism of ultrasonic treatment modulating the enzymatic resistance of RS4 ingredient. Hence, this study not only advances the understanding of ultrasonic treatment modulating the enzymatic resistance of RS4 but also reveals the relationships among the enzymatic digestibility of RS4 and their structure. The new knowledge gains from this work will be of great values for development and utilization of RS4 ingredient in food products, especially for those processes under non-thermal food processing technologies – such as high pressure processing, pulsed electric fields, pulsed light, cold plasma and ozone.

2. Materials and methods

2.1. Materials

RS4 (Fibersym® RW) was obtained from commercial sources (Atchison, KS, U.S.A.), which consisted 20.83% of amylose, 0.09% of protein, 0.16% of fat, 1.07% of ash, 0.36% of phosphorus as determined following the iodine colorimetric reaction [15] and AOAC protocol [17], respectively. Fibersym was cross-linked phosphorylated normal wheat starch and retained the granular form [5]. Amylose and amylopectin standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Aspergillus niger amylglucosidase (activity: 64 unit/mg) and pancreatic α-amylase (activity: 25 unit/mg) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA).

2.2. Ultrasonic treatment

RS4 (3 g, dry basis) was weighed and 30 mL distilled water was added, followed by the constant agitation (10 min) to acquire a homogeneous suspension. Subsequently, the suspension was subjected to an ultrasonic probe sonicator (950 W nominal power at 20 kHz, JY92-IIN, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) equipped with a circulating water system (SDC-6, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China). Sonication conditions were pulsed on-time 2 s and off-time 2 s with the duration of 30 min and temperature of 25 °C. The temperature of the reactor was maintained at 25 °C throughout the experiment by the circulating water system. Tests were carried out at various ultrasonic powers at 100, 200, 300, 400, 500, and 600 W using a 6-mm titanium diameter ultrasonic probe, and marked as RS4-100, RS4-200, RS4-300, RS4-400, RS4-500, and RS4-600, respectively. After treating, ultrasonic-processed RS4 was washed with distilled water by centrifuging for 20 min at 4000 rpm, and then dried in a hot air oven at 45 °C for 24 h and milled to powder. Ultrasonic-processed RS4 passed through a 100-mesh sieve and stored in sealed plastic bags at room temperature before the analysis.

2.3. Scanning electron microscopy (SEM) analysis

The morphology of samples was observed with a SEM (JSM-6490LV, Japan) at an accelerating potential of 20.0 kV. Samples were attached to SEM stubs using double-side adhesive tape and coated with gold under vacuum. Samples were observed with magnifications of 3000× and 5000×, respectively.

2.4. Light microscopy analysis

Sample was suspended in a mixture of glycerol/water (1,1 V/V) and a drop of the suspension was observed using an optical polarized light microscope (GIPOL, Nikon, Japan). All micrographs were observed at 400-times magnification.

2.5. Particle size distribution (PSD) analysis

Particle size analysis was measured by a laser light scattering particle size analyzer (LS13320, Beckman Coulter, USA). Starch powders were dispersed with continuously mechanical agitation in distilled water (10%, w/v). The obscuration was kept at the range of 40–45%, and 1.33 was used as the refractive index for size measurement.

2.6. Zeta-potential analysis

The zeta-potential of samples was determined using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, U.K.). The sample was suspended in distilled water (0.01%, w/v), and about 1 mL of the suspension was placed in a clear disposable zeta cell. The measurement was performed at 25 °C for 120 s.

2.7. Apparent amylose content analysis

The apparent amylose content of samples was determined using iodine colorimetric reaction based on a previous study with slightly modifications [15]. Briefly, starch samples (50 mg) including amylose and amylopectin standards were dissolved in 9 mL of KOH (2 mol/L) and 1 mL of ethanol. The mixture was incubated in boiling water for 15 min. After cooling to room temperature, the mixture was transferred into a 50 mL volumetric flask. Then, 3.0 mL of the mixture was mixed with 0.5 mL iodine solution (2% KI, 0.2% I2). The mixture was adjusted to a final value of 50 mL with deionized water before the pH was adjusted to 7 using 1 mol/L HCl. The absorbance of this mixture was measured at 620 nm after standing at room temperature for 20 min. The amylose content was then calculated using a standard curve based on mixture solutions of amylose and amylopectin.

2.8. X-ray diffraction (XRD) analysis

Crystalline structures of samples were analyzed using an X-ray diffractometer (X'Pert PRO MPD, D8, Ultima IV) with Cu Kα radiation (λ = 0.154 nm), and the generator voltage was 40 kV and the current was 40 mA. Dried samples were scanned over the range of 4–40° (2θ) at a rate of 5°/min. The relative crystallinity (%) was calculated as the relative ratio of the corresponding crystalline area to the total area by using software (MDI Jade 5) [18].

2.9. Fourier transform infrared (FTIR) spectroscopy analysis

The FTIR analysis was performed using a FTIR (IRTracer-100, Shimadzu, Japan) according to the method reported by Kang et al. [12]. Each sample (2.0 mg) was mixed with dried KBr powder (200.0 mg) and pressed into a pellet before placing in the sample holder. The spectra were acquired in the range of 400–4000 cm⁻¹ at a resolution of
1 cm$^{-1}$. For each spectrum, 64 scans were recorded.

2.10. Differential scanning calorimetry (DSC) analysis

The ordered molecular structure of samples was investigated using a DSC (TA Instruments, Norwalk, USA). Dried samples and distilled water (w/v, 1:2) were accurately weighed and placed into an aluminum pan, and then sealed hermetically. Samples were heated at a rate of 10 °C/min from 30 °C to 180 °C. An empty aluminum pan was used as a reference. The enthalpy change (ΔH), onset (T$_{on}$), peak (T$_{p}$) and conclusion (T$_{c}$) temperatures were recorded [14].

2.11. Swelling power analysis

Starch sample (200 mg, dry basis) was dispersed in distilled water (10 mL) in the 50 mL centrifuge tubes, and heated in a water bath at 65, 80, and 95 °C for 30 min with regular shaking. The samples were cooled to room temperature for 30 min and centrifuged at 4000 rpm for 10 min. The starch swelling power was the ratio of the weight of the sediment swollen starch to the dry weight of starch.

2.12. In vitro digestibility of starch

RDS, SDS, and RS contents were determined using the method of Ning et al. [19] with a slight modification. Starch (100 mg, dry basis) was dispersed in 5 mL of sodium acetate buffer (0.2 mol/L, pH 5.2) and 1 mL of enzyme mixture containing porcine pancreatic α-amylase and amylglucosidase. The mixture was incubated at 37 °C with stirring at 160 rpm in a water bath. Aliquots (0.5 mL) of the hydrolysate were withdrawn at specific time points (20 and 120 min), and transferred to a boiling water bath for 10 min to inactivate the enzymes. After centrifuging at 4000 rpm for 15 min, the supernatant was collected and used for determination of the hydrolyzed glucose content by a 3, 5-dinitrosalicylic acid (DNS) method. The glucose content at intervals of 20 and 120 min was labeled as G$_{20}$ and G$_{120}$ respectively.

The kinetics of starch digestion could be fitted to the first-order equation.

$$C_t = C_0e^{-kt}$$

where $C_0$ showed the degree of starch digested at t time, $C_0$ was the estimated percentage of hydrolyzed starch at the end of reaction, $k$ was the kinetic constant and $t$ was the hydrolysis time [19].

2.13. Statistical analysis

All results were measured at least in triplicate, and the results were reported as means ± standard deviations. Data were analyzed by one way analysis of variance (ANOVA) using IBM SPSS Statistics 19.0. A statistical difference of P < 0.05 was considered to be significant.

3. Results and discussion

3.1. Granule morphology

As shown in Fig. 1, the morphology of RS4 and ultrasonic-processed RS4 was analyzed using SEM and polarized light microscopy (normal and polarized light). As seen in Fig. 1A, the distinct starch granules could be seen in RS4, varying in shape from spherical to irregular with rough surface, although some smooth granules could also be observed. Moreover, RS4 with this morphology showed high resistance to starch hydrolysis by amylase. As shown in Fig. 1B–G, ultrasonic-processed RS4 presented similar granule surfaces with RS4, in which the majority features of RS4 were remained after ultrasonic treatment. This phenomenon was also observed by Cao and Gao [16], who reported that there was no major differences in the microstructures among the native and ultrasound-treated potato starch. However, ultrasonic treatment likely to cause pores, cracks and depressions on the surface of waxy corn starch [14], sweet potato starch [20], and corn starch [21]. Overall, the SEM observations revealed that RS4 was able to preserve their granular structure after ultrasonic treatment, with no apparent leaching of starch molecules.

Under the normal light microscope (Fig. 1A1–G1), most of ultrasonic-processed RS4 retained the intact granule structure, which was consistent with the SEM observation (Fig. 1A–G). As shown in Fig. 1A2–G2, ultrasonic-processed RS4 exhibited obvious birefringent patterns with the characteristic Maltese cross, which was similar with RS4. These results suggested that ultrasonic-processed RS4 still remained the concentric arrangement in crystalline regions, which could give rise to birefringence.

3.2. Particle size distribution

To quantify the granule size features, the particle size distributions were collected in Fig. 1A2–D2, and the results were shown in Table 1. As shown in Fig. 1A2–D2, the particle size distributions of all samples were distributed in three fractions. For RS4, dominant fraction of the particles was followed in the range from 3.0 to 30.0 μm, while two small fractions of particles were observed in the range from 0.5 to 2.5 μm and 160.0 to 450.0 μm, respectively. Compared with RS4, the granule size distributions of all ultrasonic-processed RS4 slightly shifted to the smaller particle size. The third peak volumes of ultrasonic-processed RS4 became weaker and migrated to a smaller region, which meant that the proportion of the small starch fragments increased. The weakened volume fractions might have been related to the presence of dissociated RS4 granules.

Results in Table 1 were expressed as d (4.3), d (0.1), d (0.5), and d (0.9) values, which represented the volume mean diameter, particles 10% undersize diameter, mean size diameter, and 90% undersize diameter, respectively [22]. It could be observed from Table 1, the d (4.3), d (0.1), d (0.5), and d (0.9) values of RS4 were 42.04 ± 2.61, 2.70 ± 0.24, 14.05 ± 0.06, and 84.11 ± 2.50, respectively. However, the d (4.3), d (0.1), d (0.5), and d (0.9) values for ultrasonic-processed RS4 were significantly reduced, which demonstrated that ultrasonic treatment could break the RS4 particles. The particle size distribution could play a role in starch digestibility that the smaller particle size. The third peak volumes of ultrasonic-processed RS4 became weaker and migrated to a smaller region, which meant that the proportion of the small starch fragments increased. The weakened volume fractions might have been related to the presence of dissociated RS4 granules.

Results obtained from zeta-potential measurement were presented in Table 1. As shown in Table 1, zeta-potential of RS4 was determined to be −25.0 mV, which increased significantly (P < 0.05) after ultrasonic treatment. The increases in zeta-potential of ultrasonic-processed RS4 might be due to the escalation in anionic charge (hydroxyl groups) on the surface, which was related to the degradation of polymer matrix during the ultrasonic treatment [24]. Results form Table 1 indicated that ultrasonic treatment provided energy inputs for stabilization of attractive Van der Waal forces among the ultrasonic-processed RS4 particle [24].

3.3. Apparent amylose content

The apparent amylose content of RS4 and ultrasonic-processed RS4 was presented in Table 1. It has been pointed out that even small differences in amylose content could have large effects in the digestibility
Fig. 1. Representative scanning electron microscopy (A–G), light microscopy images under normal and particle size distribution (A1–G1), and polarized light modes (A2–G2) of RS4 (A), RS4-100 (B), RS4-200 (C), RS4-300 (D), RS4-300 (E), RS4-500 (F), and RS4-600 (G).
properties of starch [25]. As shown in Table 1, ultrasonic treatment increased the apparent amylose content of RS4, with the highest increase for RS4-600 (28.87%). This was because RS4 granules could disintegrate by the mechanical shear force, cavitation effect and powerful shock waves of ultrasonic treatment, resulting in the release of the linear amylose chains [12]. Furthermore, ultrasonic treatment could fractionate the branches of the external layers of amylopectin and therefore the amylose apparently increased [26]. This phenomenon was also observed in the melting of seeds where the starch was fractionated in the branches of amylopectin [26]. Babu, Mohan, and Parimalavalli [13] postulated that the observed changes in apparent amylose content could be attributed to partial depolymerisation of amylose and amylopectin to some extent by ultrasonic treatment. A similar observation was reported by Li et al. [15], who found that the amylose content of Radix Puerariae starch was increased using high-intensity low-frequency ultrasonic treatment applied on RS4 induced lower ΔH values as compared to RS4 (P < 0.05), which indicated that ultrasonic treatment increased the short-range order of RS4. In addition, the increased short-range order indicated that ultrasonic treatment could result in a compact packing density of double helices in the amorphous region, resulting in the enhancement of the slow digestibility properties of ultrasonic-processed RS4. Similar results were also reported on the effects of ultrasonic treatment on short-range molecular order of feld pea starch [28]. Compared with RS4, ultrasonic-processed RS4 exhibited higher R1047/1022 but lower relative crystallinity (Fig. 2A), which indicated that ultrasonic treatment disrupted some long-range molecular order but enhanced the short-range molecular order. The above results suggested that the short-range order was inconsistent with the changes of relative crystallinity. This was consistent with previous reports by Li et al. [31] and Liu et al. [20], who found that local molecular order observed form FTIR did not always correlate with the relative crystallinity observed from X-ray diffraction.

Table 1

| Sample          | RS4-100 | RS4-200 | RS4-300 | RS4-400 | RS4-500 | RS4-600 |
|-----------------|---------|---------|---------|---------|---------|---------|
| Particle size analysis (μm) |         |         |         |         |         |         |
| d (4.3)         | 42.04 ± 2.61 | 36.74 ± 1.67 | 36.00 ± 0.83 | 34.78 ± 0.22 | 34.91 ± 1.31 | 35.49 ± 0.68 |
| d (0.1)         | 2.70 ± 0.24 | 1.54 ± 0.08 | 1.57 ± 0.20 | 1.66 ± 0.06 | 1.99 ± 0.23 | 1.85 ± 0.10 |
| d (0.5)         | 14.05 ± 0.06 | 13.80 ± 0.11 | 13.83 ± 0.04 | 13.71 ± 0.15 | 13.72 ± 0.13 | 13.83 ± 0.07 |
| d (0.9)         | 84.11 ± 2.50 | 50.63 ± 4.70 | 57.51 ± 0.80 | 29.07 ± 1.50 | 36.53 ± 2.74 | 39.11 ± 1.09 |
| Zeta-potential (mV) |         |         |         |         |         |         |
| Apparent amylose content (%) |         |         |         |         |         |         |
| Thermal properties |         |         |         |         |         |         |
| T0 (°C)         | 141.66 ± 4.69 | 142.77 ± 1.97 | 134.18 ± 1.55 | 136.37 ± 3.95 | 132.19 ± 1.36 | 134.41 ± 4.00 |
| Tp (°C)         | 147.52 ± 3.14 | 146.12 ± 1.38 | 135.50 ± 2.33 | 141.03 ± 4.68 | 138.29 ± 4.07 | 138.13 ± 4.93 |
| Tc (°C)         | 159.81 ± 4.69 | 157.91 ± 1.87 | 152.11 ± 2.43 | 155.01 ± 4.36 | 152.64 ± 4.51 | 145.01 ± 3.83 |
| ΔH (J/g)        | 133.75 ± 6.48 | 121.65 ± 7.93 | 90.29 ± 5.81 | 81.64 ± 6.23 | 108.05 ± 6.37 | 86.39 ± 6.39 |
| Hydrolysis parameters |         |         |         |         |         |         |
| k               | 0.047 ± 0.003 | 0.055 ± 0.001 | 0.075 ± 0.005 | 0.055 ± 0.007 | 0.057 ± 0.002 | 0.061 ± 0.002 |
| C∞              | 50.30 ± 0.28 | 54.45 ± 1.66 | 56.41 ± 2.42 | 57.47 ± 1.48 | 55.03 ± 2.46 | 55.34 ± 0.93 |
| R²              | 0.95 ± 0.01 | 0.97 ± 0.00 | 0.98 ± 0.00 | 0.98 ± 0.00 | 0.97 ± 0.01 | 0.96 ± 0.00 |

Means followed by different letters in the same row are significantly different at P < 0.05.

3.5. Short-range ordered structure

FTIR reported to be sensitive to the changes in the molecular structure of short-range order of starch at the spectral region of 1200–800 cm⁻¹ [29]. The ratio between 1047 cm⁻¹ and 1022 cm⁻¹ absorbance (R1047/1022) was used to investigate the changes of ordered structures of starch sample [30]. Fig. 2B showed the FT-IR spectra and R1047/1022 of RS4 and ultrasonic-processed RS4. It could be clearly seen from Fig. 2B, no new absorption peaks were found for ultrasonic-processed RS4 compared to RS4, indicating that no chemical groups were produced during the ultrasonic treatment. As shown in Fig. 2B, the R1047/1022 of ultrasonic-processed RS4 was significantly higher than RS4 (P < 0.05), which indicated that ultrasonic treatment increased the short-range order of RS4. In addition, the increased short-range order indicated that ultrasonic treatment could result in a compact packing density of double helices in the amorphous region, resulting in the enhancement of the slow digestibility properties of ultrasonic-processed RS4. Similar results were also reported on the effects of ultrasonic treatment on short-range molecular order of feld pea starch [28]. Compared with RS4, ultrasonic-processed RS4 exhibited higher R1047/1022 but lower relative crystallinity (Fig. 2A), which indicated that ultrasonic treatment disrupted some long-range molecular order but enhanced the short-range molecular order. The above results suggested that the short-range order was inconsistent with the changes of relative crystallinity. This was consistent with previous reports by Li et al. [31] and Liu et al. [20], who found that local molecular order observed form FTIR did not always correlate with the relative crystallinity observed from X-ray diffraction.

3.6. Molecular ordered structure

The thermal properties of RS4 and ultrasonic-processed RS4 were summarized in Table 1. RS4 exhibited the only endothermic peak with T0 at 141.66 °C, Tp at 147.52 °C, and Tc at 159.81 °C, and the ΔH was found to be 133.75 J/g, representing the melting of the double-helical structures [5]. As shown in Table 1, differences were found between the T0, Tp, Tc and ΔH of RS4 and ultrasonic-processed RS4. Ultrasonic-processed RS4 still included a single endothermic peak, but the transition temperature was lower than that of RS4. This result was mainly attributed to the disruption of the crystalline structure, which could shorten the dissociation of double helices [32]. In addition, the ultrasonic treatment applied on RS4 induced lower ΔH values as compared with RS4, suggesting that less thermal energy was required to melt the crystalline domains of ultrasonic-processed RS4. The decreased ΔH values could be resulted from the partial loss of the double helical.
structures in crystalline and amorphous regions during the ultrasonic treatment, indicating reduced resistant to enzymatic hydrolysis with decreased RS content (Fig. 3). Similar observations were also reported by Huet al. [33], who found that the reduced ΔH due to the disruption of double helices and it was related to the decreased contents of SDS and RS. Meanwhile, the decrease in relative crystallinity of ultrasonic-processed RS4, as observed from the XRD analysis (Fig. 2A), also signified the decrease in ΔH, meaning less perfection in crystalline regions.

### 3.7. Swelling power

As the temperature rose from 65 to 95 °C, the swelling power of RS4 and ultrasonic-processed RS4 was investigated (Fig. 2C). At the low temperature (65 °C), the swelling power of RS4 and ultrasonic-processed RS4 was not significantly different. Heating in water at low temperature could not disrupt the crystalline structure of RS4 and ultrasonic-processed RS4, which impeded water transport into the starch structure, thereby limited swelling of starch granules. However, the swelling power exhibited a significantly increase when the temperature reached 80 °C due to the occurrence of starch gelatinization [28]. Further increased temperature to 95 °C, the swelling power increased slowly, which could be ascribed to the irreversible rupture of starch granules at high temperature [32]. Compared with RS4, ultrasonic-processed RS4 showed a higher swelling power value over the temperature range of 65 to 95 °C, indicating weak bonding forces of molecular chains within the crystalline and amorphous regions in ultrasonic-processed RS4 granules [28]. This result corresponded well with the relatively lower relative crystallinity for ultrasonic-processed RS4 than RS4 (Fig. 2A).
3.8. RDS, SDS and RS content

The starch fractions (RDS, SDS, and RS) of RS4 and ultrasonic-processed RS4 were presented in Fig. 3A. As shown in Fig. 3A, RS4 possessed a relative high RS content (50.46%) and slightly less RDS content (29.44%) and SDS content (20.10%), indicating that RS4 was resistant to digestion with the nature of resistance. However, the RS content of ultrasonic-processed RS4 was significantly reduced to 40.58% (RS4-200) in comparison with that of RS4 (50.46%) by enhancing SDS ranging from 20.10% to 25.94% (RS4-300) and RDS from 29.44% to 35.29% (RS4-200). In a previous study, it was found that the sum of the RDS and SDS contents was defined as digestible starch [34]. With increasing ultrasonic power from 100 W to 600 W, the contents of RDS and SDS (digestible starch) increased from 49.54% to 58.54%, whereas the content of RS (resistant starch) decreased significantly. It could be learned that the ultrasonic-processed RS4 was easier to digest than RS4. These results indicated that the enzymatic resistance of RS4 could be decreased by ultrasonic treatment, which was not in accordance with the previous reports. For example, it had been reported that ultrasonic treatment could increase the RS content of starch, which reduced the rate and extent of starch digestion [13]. The different observation could be attributed to the different ultrasonic treatment conditions (ultrasonic temperature) and starch type.

Therefore, the digestive properties of corn starch (CS) as influenced by ultrasonic temperature (5, 15, 25, 35, 50 °C) were investigated. As shown in Fig. 3B, fractions of SDS and RDS were converted into RS by ultrasonic temperature, except the CS sonicated at 35 °C and 50 °C. Accordingly, the ratio of RS to SDS + RDS of ultrasonic-processed CS increased at low temperature (5 °C, 15 °C and 25 °C). Subsequently, the content of RS decreased while the contents of RDS and SDS increased along with the ultrasonic temperature increased. The RS content after sonication at 5 °C, 15 °C and 25 °C was higher than those of native CS. Moreover, the RS content of CS-5 was about 1.43 times higher than native CS. Ultrasonic temperature increased starch digestibility due to the gelatinization of CS at high temperature (35 °C and 50 °C), but the digestibility decreased due to the recrystallization at low temperature (5 °C, 15 °C and 25 °C). A previous study showed that cooling of gelatinized starch led to retrogradation, thus developed a compact starch structure that was usually more resistant to enzymatic hydrolysis [35].

3.9. Kinetics of starch hydrolysis

Digestion curves of RS4 and ultrasonic-processed RS4 were showed in Fig. 4. The corresponding fit of the hydrolysis parameters (C∞, k, R2) to a first-order equation for starch hydrolysis kinetics were presented in Table 1. As shown in Fig. 4, RS4 and ultrasonic-processed RS4 showed one linear range for the LOS plots, indicating a monophasic digestion manner obeying the first order kinetics. As shown in Table 1, all R2 values were higher than 0.95, illuminating that the hydrolysis kinetic equation model was primly matched for starch hydrolysis in the range of testing time [19]. The k value was related to the enzymatic hydrolysis rate [19]. The k values of ultrasonic-processed RS4 were significantly higher than that of RS4 (P < 0.05), which could attribute to the high RDS content and SDS content in ultrasonic-processed RS4 than in RS4. It was reported that a higher value of C∞ meant less remnant starch [36]. The C∞ values of RS4 were small indicating that most of the RS4 remained undigested. The high C∞ values were exhibited in ultrasonic-processed RS4, which was probably because ultrasonic treatment disrupted the organized structure of crystalline structure that facilitated enzyme access to the starchy matrix. This was consistent with the RDS + SDS content of ultrasonic-processed RS4 being more abundant than in RS4, indicating that ultrasonic treatment contributed a increasing of the digestion degree.

3.10. Explanation for structural and digestibility changes of RS4

These differences among the RS4 and ultrasonic-processed RS4 confirmed the relationship of structural properties with starch digestion. The exact mechanism of digestibility changes of RS4 mitigating by ultrasonic treatment was proposed in Fig. 5. From the morphological observation, ultrasonic-processed RS4 remained the granular structure with birefringence in the center. According to crystalline structure analysis, ultrasonic treatment destroyed the crystalline region of RS4 granules, while the A-type crystalline pattern remained unchanged, indicating that the double helix in the crystalline region might be partly damaged and rearranged into a new amorphous region. The XRD results were complementary to the DSC results. A decrease in the ΔH of ultrasonic-processed RS4 suggested the loss of double helical structures in crystalline and amorphous regions. In addition, ultrasonic-processed RS4 had small particle size and high apparent amylose content. All of these structural changes could affect the digestibility properties of RS4, which was demonstrated in vitro. The decreased particle size, relative crystallinity, and ΔH corresponded to the increase of RDS content and the decrease of RS content of RS4. Accompanying these structural disruptions, the starch chains could reassemble to form more ordered structures at amorphous regions. Based on analyzing results of FTIR, the R1047/1022 of RS4 after ultrasonic treatment increased, which suggested that part of the dispersed starch chains might pack densely in amorphous region. These observations suggested that ultrasonic-processed RS4 with high R1047/1022 might lead to the increase of SDS content. In conclusion, ultrasonic treatment mainly induced RS4 structure disruption rather than reassembly, which led to the decrease of the enzymatic resistance of RS4, and eventually transformed part of RS into SDS and/or RDS. The disrupted long-range molecular order was favorable for the formation of RDS, whereas the enhanced short-range molecular order was favorable for the formation of SDS. Similar observation was also reported by Lee et al. [37], who found that RS could be transformed into SDS due to the disruption of starch granules and crystalline structures through heat-moisture treatment, which resulted in the elevation of enzyme susceptibility. Additionally, the in vitro digestibility conclusions needed to be validated with future in vivo evidences.

On the other hand, previous studies had shown that ultrasonic treatment was relevant to the reduction of starch digestibility [38], which was apparent inconsistent with our results. It was reported that increasing RS content depended on the heating-gelatinization-cooling process [15]. Moreover, Martinez et al. [39] reported that starch susceptibility to enzyme hydrolysis could be reduced by retrogradation through heating and cooling. In our study, we controlled the ultrasonic temperature throughout the ultrasound treatment, which promoted the heating-gelatinization process and restricted the cooling-retrogradation process of ultrasonic-processed RS4. It was important to highlight that RS4 did not retrograde during the ultrasonic treatment with controlled ultrasonic temperature used in our study, which restricted the rearrangement of the dispersed starch chains in an amorphous state to form highly ordered structures (crystalline structure), resulting in decreased RS content of ultrasonic-processed RS4. Our results revealed that ultrasonic temperature, especially at low temperature, played an important role in starch reorganization and consequently in its digestibility. Further experiments were therefore carried out to determine how structural changes of ultrasonic temperature might affect starch digestion.

4. Conclusions

This research provided new insights into the interrelationships between the enzymatic resistance and structural properties of RS4 under the non-thermal processing technology of ultrasonic treatment. Ultrasonic treatment disrupted the long-range ordered structure and molecular ordered structure but rearranged the short-range ordered structure. These structure changes were important reasons responsible...
Fig. 4. The digestion curves and LOS plots of RS4 (A), RS4-100 (B), RS4-200 (C), RS4-300 (D), RS4-400 (E), RS4-500 (F), RS4-600 (G).
for explaining the changes in RDS, SDS, and RS contents, with a decrease in RS content (resistant starch) but an increase in RDS and SDS contents (digestible starch). Overall, results indicated that RS4 was not able to retain its enzymatic resistance processed with the non-thermal processing technology of ultrasonic treatment. For the first time, RS4 had been identified with structural features that resulted in digestible molecular structures after ultrasonic treatment. The findings provided a theoretical foundation for food industry to use RS4 ingredients in food products.

**CRediT authorship contribution statement**

**Yongbo Ding:** Conceptualization, Methodology, Software, Investigation, Data curation, Writing - original draft. **Yiwei Xiao:** Data curation, Writing - original draft. **Qunfu Ouyang:** Visualization, Investigation, Supervision. **Feijun Luo:** Software, Validation. **Qinlu Lin:** Writing - review & editing, Writing - review & editing.

**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.

**Acknowledgment**

The study was carried out with financial support of the Natural Science Foundation of Hunan Province (Grant No. 2020JJ5967).

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2020.105350.

**References**

[1] H. Xia, Y. Li, Q. Gao, Preparation and properties of RS4 citrate sweet potato starch by heat-moisture treatment, Food Hydrocolloid 55 (2016) 172–178.
[2] R. Remya, A.N. Jyothi, J. Sreekumar, Morphological, structural and digestibility properties of RS4 enriched octenyl succinylated sweet potato, banana and lentil starches, Food Hydrocolloid 82 (2018) 219–229.
[3] A. Shah, F.A. Masoodi, A. Gani, B.A. Ashwar, Physicochemical, rheological and structural characterization of acetylated oat starches, LWT - Food. Sci. Technol. 80 (2017) 19–28.
[4] O. Jeong, M. Shin, Preparation and stability of resistant starch nanoparticles, using acid hydrolysis and cross-linking of waxy rice starch, Food Chem. 256 (2018) 77–78.
[5] S. Liu, M. Reimer, Y. Ai, In vitro digestibility of different types of resistant starches under high-temperature cooking conditions, Food Hydrocolloid (2020) 105927.
[6] FDA, Questions and answers on dietary fiber. Accessed, https://www.fda.gov/food/food-labeling-nutrition/questions-and-answers-dietary-fiber, 2019 (Accessed 10 December 2019).
[7] M. Ariban, K. Kahraman, H. Koksel, In vitro glycemc index, bile acid binding capacity and mineral bioavailability of spaghetti supplemented with resistant starch type 4 and wheat bran, J. Funct. Foods 103778 (2020).
[8] A.A. Escobarpuentes, S. Rincon, A. Garciauagrola, A. Zepeda, A.D. Calvolopez, F. Martinezbunutos, Development of a third-generation snack with type 4 resistant sorghum starch: Physicochemical and sensorial properties, Food Biosci. 32 (2019) 100474.
[9] K. Kahraman, E. Aktaaksiylidze, S. Ozturk, H. Koksel, Effect of different resistant starch sources and wheat bran on dietary fibre content and in vitro glycaemic index values of cookies, J. Cereal Sci. 90 (2019) 102581.
[10] B. Zhang, S. Dhillon, B.M. Flanagan, P. Luckman, P.J. Halley, M.J. Gidley, Extrusion induced low-order starch matrices: Enzymic hydrolysis and structure, Carbohydr. Polym. 134 (2015) 485–496.
[11] Y.M. Zhao, M. De Alba, D.W. Sun, B. Tiwari, Principles and recent applications of novel non-thermal processing technologies for the fish industry—a review, Crit. Rev. Food. Sci. 59 (5) (2019) 728–742.
[12] X. Kang, P. Liu, W. Gao, Z. Wu, B. Yu, R. Wang, B. Cui, L. Qiu, C. Sun, Preparation of starch-lipid complex by ultrasoundation and its film forming capacity, Food Hydrocolloid 99 (2020) 105340.
[13] A.S. Babu, R.J. Mohan, R. Parimalavalli, Effect of single and dual-modifications on stability and structural characteristics of foiatx millet starch, Food Chem. 271 (2019) 457–465.
[14] Q.Y. Yang, X.Lu, Y.Z. Chen, Z.G. Luo, Z.G. Xiao, Fine structure, crystalline and physicochemical properties of waxy corn starch treated by ultrasound irradiation, Ultrason. Sonochem. 51 (2019) 350–358.
[15] Y. Li, Z. Wu, N. Wan, X. Wang, M. Yang, Extraction of high-amylase starch from Radix Puerariae using high-intensity low-frequency ultrasound, Ultrason. Sonochem. 59 (2019) 104710.
[16] M. Cao, Q. Gao, Effect of dual modification with ultrasonic and electric field on potato starch, Int. J. Biol. Macromol. 150 (2020) 637–643.
[17] AOAC, Association of Official Analytical Chemists, Official Method of Analysis, 16th ed., Washington, DC, 2000.
[18] S. Gao, H. Liu, L. Sun, N. Liu, J. Wang, Y. Huang, F. Wang, J. Gao, R. Fan, X. Zhang, M. Wang, The effects of dielectric barrier discharge plasma on physicochemical and digestion properties of starch, Int. J. Biol. Macromol. 138 (2019) 819–830.
[19] Y. Ning, B. Cui, C. Yuan, Y. Zou, W. Liu, Y. Pan, Effects of konjac glucomannan on the rheological, microstructure and digestibility properties of debranched corn starch, Food Hydrocolloid 100 (2020) 105342.
[20] H.W. Wang, K. Xu, Y. Ma, Y. Liang, H. Zhang, L. Chen, Impact of ultrasoundization on the aggregation structure and physicochemical characteristics of sweet potato starch, Ultrason. Sonochem. 63 (2020) 104869.
[21] B. Wei, H. Qi, Z. Wang, Y. Bi, J. Zou, B. Xu, X. Ren, H. Ma, The ex-situ and in-situ ultrasonic assisted oxidation of corn starch: a comparative study, Ultrason. Sonochem. 61 (2020) 104854.
[22] F. Zhang, Y.Y. Zhang, K. Thakur, J.G. Zhang, Z.J. Wei, Structural and physicochemical characteristics of lycoris starch treated with different physical methods, Food Chem. 275 (2019) 8–14.
[23] Y. Ji, In vitro digestion and physicochemical characteristics of corn starch mixed with amino acid modified by low pressure treatment, Food Chem. 242 (2018) 421–426.
[24] I. Ullah, T. Yin, S. Xiong, Q. Huang, J. Zhang, A.B. Javaid, Effects of thermal pre-treatment on physicochemical properties of nano-sized okara (soybean residue)
insoluble dietary fiber prepared by wet media milling, J. Food Eng. 237 (2018) 18–26.

[25] M. Lopez-Silva, L.A. Bello-Perez, V.M. Castillo-Rodriguez, E. Agama-Acevedo, J. Alvarez-Ramirez, In vitro digestibility characteristics of octenyl succinic acid (OSA) modified starch with different amylose content, Food Chem. 304 (2020) 125434.

[26] B. Contreras-Jiménez, A. Del Real, B.M. Millan-Malo, M. Gaytán-Martínez, E. Morales-Sánchez, M.E. Rodríguez-García, Physicochemical changes in barley starch during malting, J. I. Brewing 125 (1) (2019) 10–17.

[27] W. Bao, Q. Li, Y. Wu, J. Ouyang, Insights into the crystallinity and in vitro digestibility of chestnut starch during thermal processing, Food Chem. 269 (2018) 244–251.

[28] D. Zhou, Z. Ma, X. Yin, X. Hu, J.I. Boye, Structural characteristics and physicochemical properties of field pea starch modified by physical, enzymatic, and acid treatments, Food Hydrocolloid 93 (2019) 386–394.

[29] H.J. Chung, Q. Liu, R. Hoover, Effect of single and dual hydrothermal treatments on the crystalline structure, thermal properties, and nutritional fractions of pea, lentil, and navy bean starches, Food Res. Int. 43 (2) (2010) 501–508.

[30] K. Liu, C. Chi, X. Huang, X. Li, L. Chen, Synergistic effect of hydrothermal treatment and lauric acid complexation under different pressure on starch assembly and digestion behaviors, Food Chem. 278 (2019) 560–567.

[31] S. Li, C. Wang, X. Fu, C. Li, X. He, B. Zhang, Q. Huang, Encapsulation of lutein into swelled cornstarch granules: structure, stability and in vitro digestion, Food Chem. 268 (2018) 362–368.

[32] B. Chen, S. Zeng, H. Zeng, Z. Guo, Y. Zhang, B. Zheng, Properties of lotus seed starch–glycerin monostearin complexes formed by high pressure homogenization, Food Chem. 226 (2017) 119–127.

[33] X.P. Hu, B. Zhang, Z.Y. Jin, X.M. Xu, H.Q. Chen, Effect of high hydrostatic pressure and retrogradation treatments on structural and physicochemical properties of waxy wheat starch, Food Chem. 232 (2017) 560–565.

[34] H. Hao, Q. Li, W. Bao, Y. Wu, J. Ouyang, Relationship between physicochemical characteristics and in vitro digestibility of chestnut (Castanea mollissima) starch, Food. Hydrocolloid 84 (2018) 193–199.

[35] M. Wang, M. Sun, Y. Zhang, Y. Chen, Y. Wu, J. Ouyang, Effect of microwave irradiation-retrogradation treatment on the digestive and physicochemical properties of starches with different crystallinity, Food Chem. 298 (2019) 125015.

[36] W. Yu, K. Tao, R.G. Gilbert, Improved methodology for analyzing relations between starch digestion kinetics and molecular structure, Food Chem. 264 (2018) 284–292.

[37] C.J. Lee, Y. Kim, S.J. Choi, T.W. Moon, Slowly digestible starch from heat-moisture treated waxy potato starch: preparation, structural characteristics, and glucose response in mice, Food Chem. 153 (4) (2012) 1222–1229.

[38] S.R. Falsafi, Y. Maghsoudlou, M. Aalami, S.M. Jafari, M. Raesi, Physicochemical and morphological properties of resistant starch type 4 prepared under ultrasound and conventional conditions and their in-vitro and in-vivo digestibilities, Ultrason. Sonochemistry 53 (2019) 110–119.

[39] M.M. Martinez, C. Li, M. Okoniewska, I. Mukherjee, D. Vellucci, B. Hamaker, Slowly digestible starch in fully gelatinized material is structurally driven by molecular size and A and B1 chain lengths, Carbohyd. Polym. 197 (2018) 531–539.