Dry heat treatment induced the gelatinization, rheology and gel properties changes of chestnut starch

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\section*{ABSTRACT}

The effects of continuous dry heat treatment (CT) and repeated dry heat treatment (RT) on gel and structural properties of chestnut starch (CS) were investigated. CT and RT both reduced the swelling degree of starch and showed significant variations in pasting viscosity, viscoelasticity, gel strength and hardness varying from high to low after dry heat treatment, and CT was lower than that of RT. Neither dry heat treatment nor gelatinization produced new functional groups, and both reduced short-range ordered degree. There were significant decrease in spin-spin relaxation time ($T_2$) with dry heat treatment (CT and RT), which made the starch in the samples closely combine with water. These results are helpful to better understand the changes of physicochemical properties of starch gel products during dry heat treatment and provide some theoretical references for the application of CS in food industry.

1. Introduction

Chestnut is rich in nutrition and unique in flavor which has attracted wide attention of many researchers (Zhu, 2016). As the highest content component in chestnut, chestnut starch (CS) is gluten-free and can be used as energy material for celiac disease (Mir et al., 2019). Starch is the main energy source of human diet, and its rapid digestion can induce many diseases, such as obesity, diabetes and cardiovascular diseases, so new slow digestion starch resources are being gradually developed to meet market demand. CS might serve as a novel starch source with high resistance content and applied in both food and non-food industries, which were beneficial to prevent the incidence of type-II diabetes (Cruz et al., 2013).

Native starch with the thermal instability and easy retrogradation and other undesirable characteristics during processing, starch modification has been widely concerned. During the traditional processing methods, such as baking or boiling, starch would undergo irreversible changes, and its crystal structure would be lost (Silva et al., 2016). Therefore, other starch modification methods to improve the gelatinization and structural characteristics of CS were warranted.

Compared with other modification methods, dry heat treatment has the advantages of simplicity, safety and no chemical residue (Sun et al., 2014). Dry heat treatment is continuously treat samples (moisture content less than 10\%) at 140 $^\circ$C for a certain time (Gou et al., 2019). Dry heat treatment usually include two types, continuous dry heat treatment (CT) and repeated dry heat treatment (RT). Sample treated by CT was that the sample was continuously heated for a period of time, while the RT meant heating for a period of time, then cooling for a period of time, which was regarded as a cycle, and then the operation of this cycle was repeated. Zhou et al. (2021) also found CT and RT had a significant effect on the pasting properties of quinoa starch, but they only focused on the properties of native starch and the effect of dry heat treatment on CS gel and structure properties has not been studied previously.

The study of starch gel properties after dry heat treatment, such as rheological properties, gel strength and hardness, can provide some theoretical reference for whether dry heat treatment starch is beneficial to the formation of gel products. And it is also interesting to understand the migration changes of internal water molecules when starch is modified. Therefore, the purpose of the present study are 1) to explore the effects of CT and RT on the pasting, gel and structural properties of CS and 2) to compare the differences between CT and RT, which will also
help us to understand the characteristics of CS, thus providing some theoretical basis for the development of CS and expand the application range of CS.

2. Materials and methods

2.1. Materials and reagents

Chestnuts were obtained from Tianhong supermarket (Jiangxi, China). The starch content kit (A148-1-1) was bought from Jiancheng Bioengineering Co. (Nanjing, China). Other chemicals were all analytical grade.

2.2. Sample preparation

CS was extracted by water extraction method, and was slightly modified according to the method of Liu et al. (2021). The total starch content was 98.09% by the means of content kit. The apparent amylase content in CS was 16.23%.

Dry heat treated samples were prepared using the method of Gou et al. (2019). Native CS (water content less than 10%) was continuously hot treated in an oven at 140 °C for 3, 6, 9 and 12 h and then cooled at room temperature to obtain the CT-3, CT-6, CT-9 and CT-12 starch respectively.

Repeated treatment of starch was obtained following the steps: firstly, native CS were treated at 140 °C for 3 h, and then cooled for 1 h, which was recorded as one cycle, then obtain a sample namely RT-1, which was CT-3. Then, this cycle was repeated twice, three and four times, and then marked them as RT-2, RT-3 and RT-4, respectively. Native CS without dry heat treatment was recorded as control.

2.3. Determination of solubility and swelling power

The result of solubility and swelling power were determined with modified method (Gou et al., 2019; Li et al., 2021). 30 mL of starch suspension (2%, w/v) were shaken in a water-bath at 50 °C, 60 °C, 70 °C, 80 °C, 90 °C and heated at 120 rpm for 30 min with continuous stirring. Then cooled to room temperature and centrifuged at 4000 g for 15 min. The supernatant was then dried at 105 °C for 2 h and weighed. The value of solubility (S) and swelling power (SP) were calculated according to the following formula:

\[ S(\%) = \frac{\text{weight of dried supernatant}}{\text{weight of dry starch}} \times 100 \]

\[ SP(g/g) = \frac{\text{weight of sediment}}{\text{weight of dry starch} \times (100-S)} \]

2.4. Pasting properties

The pasting properties of starch were determined with a Rapid-Visco Analyzer (RVA, Newport Scientific, NSW, Australia) following the method of Chen et al. (2018). Dry starch (7%, w/v) and 25 mL distilled water were mixed evenly in an aluminum tank with continuous stirring at 960 rpm, the slurry was balanced at 50 °C for 1 min and heated to 95 °C at a speed of 13 °C min\(^{-1}\) for 3 min. Then cooled to 50 °C at the same speed, stirred at 160 rpm for 4 min, and the starch pastes were collected for later use.

2.5. Rheological properties

The rheometer (DHR-2, American Technical Consulting Company) equipped with a plate (diameter 40 mm, gap 0.5 mm), and dynamic rheological scanning was carried out in the angular frequency range of 1–10 rad s\(^{-1}\) based on the method of Li et al. (2020).

2.6. Gel strength and hardness

A texture analyzer (TA-XTplus, Stable Co., UK; 0.5R probe) was used for measurement. Test speed including before, during and after testing was 2 mm s\(^{-1}\), test distance is 10 mm, trigger force was 5 gf, and trigger type was set as automatic.

2.7. Fourier-transform infrared spectra (FT-IR)

The samples obtained from RVA were frozen in a freeze dryer at −80 °C for 3 d to obtain freeze-dried samples. The freeze-dried samples of control, CT-3, CT-6, CT-9, CT-12, RT-2, RT-3 and RT-4 were respectively designated as D-Control, DCT-3, DCT-6, DCT-9, DCT-12, DRT-2, DRT-3 and DRT-4.

Samples were scanned based on the following procedures: freeze-dried samples and KBr powder in a 1:30 ratio in the mortar full grinding, pressing machine would grind the powder into a transparent sheet and be used to determine. FT-IR (Nicolet 5700, Thermo, USA) had a scanning wavelength of 4000–400 cm\(^{-1}\), with a scanning resolution of 8 cm\(^{-1}\) over 64 scans. The spectra of CT and RT samples (freeze-dried and ungelatinized starch) were obtained, and following previously described procedures (Correia, Cruz-Lopes, & Beirão-da-Costa, 2012; Li et al., 2021; Lyu et al., 2021).

2.8. Low-field nuclear magnetic resonance (LF-NMR)

A 23 MHz NMR analyzer (EDUUMR20-015V-I, Niumag Co., Ltd., Suzhou, China) was used to obtain the T2 according to the previous method (Ma et al., 2019). Transfer 1 mL of gelatinized sample prepared by RVA to NMR glass tube, and shake thoroughly to eliminate bubbles. Set CPMG sequence for determination.

2.9. Statistical analysis

SPSS 21.0 software (IBM software, Chicago, Illinois, USA) and Origin 8.0 software (Stat-Ease Company, Minnesota, USA) were used to evaluate the significance and obtain the graph.

3. Results and discussion

3.1. Solubility and swelling power

Solubility and swelling power were used to reflect the amylose leaching degree and water absorption capacity during starch swelling. The solubility of native starch was increased by CT and RT (Table 1A), which indicated that dry heat treatment promoted the leaching of amylose. And with the increasing of temperature, the solubility of starch also increased, which was consistent with the previous research result that dry heat treatment increased the solubility of starch from Dioscorea (Vashisht et al., 2017). It was also found that the solubility was usually related to amylose, while the swelling power was related to amylopectin and also reported that this increase in solubility caused by the leaching of amylose during dry heat treatment (Gou et al., 2019). In addition, the solubility of CT was greater than that of RT, which might be related to the differences in water absorption capacities and swelling power.

Both CT and RT decreased the swelling power of CS (Table 1B), which indicated that dry heat treatment could inhibit the swelling power of starch granules. Especially, CT-12 (50 °C) reached the lowest swelling power value of all samples. This might also be due to the agglomeration of starch granules during the heat treatment of starch. However, with the increasing of temperature, the swelling power increased slightly, which was attributed to the starch reaching gelatinization temperature during heat treatment, which caused partial gelatinization.
showed as mean values ± SD in triplicate. Different letters (a-g) in the same column show a significant difference (p < 0.05).

### Table 1B
Swelling power of native chestnut starch and dry heat treated starch.

| Samples | Swelling power (g/100g) | 50 °C | 60 °C | 70 °C | 80 °C | 90 °C |
|---------|--------------------------|-------|-------|-------|-------|-------|
| Control | 1.95 ± 0.04^a | 2.22 ± 0.04 | 3.85 ± 0.09 | 12.84 ± 0.12 | 22.59 ± 0.13 |
| CT-3    | 1.68 ± 0.05^b | 2.08 ± 0.05 | 3.55 ± 0.09 | 10.96 ± 0.12 | 18.63 ± 0.13 |
| CT-6    | 1.55 ± 0.02^c | 1.84 ± 0.02 | 3.15 ± 0.03 | 8.81 ± 0.13 | 15.80 ± 0.14 |
| CT-9    | 1.42 ± 0.02^d | 1.62 ± 0.02 | 2.94 ± 0.03 | 7.93 ± 0.13 | 12.69 ± 0.14 |
| RT-2    | 1.27 ± 0.02^e | 1.46 ± 0.02 | 2.84 ± 0.04 | 6.67 ± 0.15 | 10.76 ± 0.20 |
| RT-3    | 1.19 ± 0.01| 1.93 ± 0.01 | 3.22 ± 0.04 | 9.11 ± 0.15 | 15.60 ± 0.20 |
| RT-4    | 1.10 ± 0.01| 1.83 ± 0.01 | 3.05 ± 0.04 | 8.35 ± 0.15 | 13.57 ± 0.20 |
| Control | 1.34 ± 0.03 | 1.61 ± 0.03 | 2.96 ± 0.03 | 7.61 ± 0.15 | 11.28 ± 0.20 |

Note: CS, native chestnut starch; CT-3, CT-6, CT-9, CT-12, preparation of chestnut starch by continuous dry heat treatment for 3, 6, 9, 12h, respectively; RT-2, RT-3, RT-4, preparation of chestnut starch by repeated dry heat treatment for two cycles, three cycles, four cycles, respectively. Results are showed as mean values ± SD in triplicate. Different letters (a-g) in the same column show a significant difference (p < 0.05).

### 3.3. Dynamic rheological measurements

The dynamic rheological test showed the same increasing trend of storage modulus ($G'$) and loss modulus ($G''$) with angular frequency (See Fig. 2), and the $G'$ of all gels was larger than $G''$, which signified that all gels were typical weak gels (Lin et al., 2021). Meanwhile, CT and RT reduced the $G'$ and $G''$ of CS gel, and which was negatively correlated with the treatment time and treatment cycle. The decrease of $G'$ might be due to the following reasons: first, dry heat treatment would reduce the effective water content of starch, and reduced the effective concentration of starch in unit volume, thereby affecting the formation of gel network structure; second, the dry heat treatment would affect the swelling and water absorption performance of starch granules, thus resulting in the difference of gel viscoelasticity of starch granules (Liu et al., 2020). Additionally, $G''$ was related to the extra connection of starch molecular chains and the enhancement of connection tightness, which showed that dry heat treatment would weaken the entanglement points between starch gel molecules, and led to the decrease of $G''$ (Tabarsa et al., 2017).

### 3.4. Gel properties

Dry heat treatment decreased the gel strength and hardness of CS gel, and the effect of CT was more obvious (Table 2). Compared with RT, the gel strength and gel hardness of CT treated samples were relatively small, the main difference was the existence of cooling cycle would influence the thermal degradation of starch hence influence the gel strength and hardness (Colussi et al., 2014). Especially, when the dry heat treatment with 12 h, the gel strength decreased from 0.37 N to 0.06 N and the gel hardness decreased significantly from 0.47 N to 0.10 N. This might be attributed that dry heat treatment would prevent starch retrogradation, which could be explained by the decrease of SB. The changes in gel strength and hardness after CT and RT could be due to dry
viscosity; SB is setback viscosity. It also be noticed that there was a broad peak at 22\(^{-1}\) cm, which was attributed to the O–H stretching vibration of starch amorphous region (Wang et al., 2018). The absorption peak at 1643 cm\(^{-1}\) was associated with angular arrangement and recrystallization of starch granule after dry heat treatment, thus influenced on the short-range order of starch (Luo et al., 2020). However, there was no significant difference among CT samples, RT samples and gelatinized samples, which indicated that the time and cycle of dry heat treatment had no significant effect on short-range order.

### 3.5. FT-IR

Compared with the control group, both CT and RT showed the similar FT-IR patterns, and there were no new absorption peaks appeared (Fig. 3A and B), which indicated that CT and RT did not cause changes in functional groups and did not destroy the original functional groups of native CS. Similar results had been found that dry heat treatment and gelatinization did not cause the generation of new chemical bonds (Zhou et al., 2021). The absorption peak at 1643 cm\(^{-1}\) was attributed to the O–H stretching vibration of starch amorphous region (Wang et al., 2018). It also be noticed that there was a broad peak at 3250-3500 cm\(^{-1}\) and at 3432 cm\(^{-1}\), which was mainly related to the stretching vibration of free, intramolecular and intermolecular free hydroxyl groups. The hydrophilicity of starch was the reason to explain the movement of water molecules. The T\(_2\) value of CT-12 sample was the smallest, which indicated that starch and water were most closely combined in the system. The T\(_2\) value of CT was slightly lower than RT, which was due to the existence of the cooling cycle stage of RT.

### 3.6. LF-NMR

LF-NMR was used to detect the water distribution in gel samples, and T\(_2\), as an important index, mainly reflected the tightness between starch and water (Din et al., 2018; Ma et al., 2019). In T\(_2\) spectra, there are three kinds of water components in starch gel, which were tightly bound water (T\(_2\)), immobile water (T\(_{22}\)) and free water (T\(_{22}\)), respectively. T\(_{21}\), T\(_{22}\) and T\(_{23}\) of CS gel treated with dry heat treatment were lower than control group, which was consistent with the result of T\(_2\) (Table 4). Moreover, there were significant differences between starch with different treatment time and cycle samples and dry heat treatment (CT and RT) decreased the T\(_2\) value of CS gel. The reduction in T\(_2\) might be due to the leached amyllose aggregated or rearranged, thus limiting the movement of water molecules. The T\(_2\) value of CT-12 sample was the smallest, which indicated that starch and water were most closely combined in the system. The T\(_2\) value of CT was slightly lower than RT, which was due to the existence of the cooling cycle stage of RT.

### 4. Conclusion

Dry heat treatment, as an important physical modification method, had an influence on gelatinization, rheology and structure of starch. Dry
heat treatment increased the solubility of native CS, which would expand the application of CS. The viscosity (PV, TV, FV, BD), dynamic viscoelasticity, gel strength and hardness of CS after CT and RT was lower than that of native starch, which showed that the treated starch by dry heat treatment was not easy to form gel. Dry heat treatment also decreased the degree of short ordered structure of CS. LF-NMR result showed that dry heat treatment increased the solubility of native CS, which would expand the application of CS. The viscosity (PV, TV, FV, SB, BD), dynamic viscoelasticity, gel strength and hardness of CS after CT and RT were different for the two methods of dry heat treatment, CT and RT. 

Table 3
The absorbance value of 1047/1022 results of samples.

| Samples   | 1047/1022 cm⁻¹ |
|-----------|----------------|
| Control   | 1.277 ± 0.228b |
| CT-3      | 0.886 ± 0.003a |
| CT-6      | 0.949 ± 0.038a |
| CT-9      | 0.929 ± 0.034a |
| CT-12     | 0.915 ± 0.014a |
| RT-2      | 0.946 ± 0.030a |
| RT-3      | 0.889 ± 0.004a |
| RT-4      | 0.893 ± 0.000a |
| D-Control | 0.948 ± 0.003a |
| DCT-3     | 0.940 ± 0.003a |
| DCT-6     | 0.934 ± 0.001a |
| DCT-9     | 0.934 ± 0.005a |
| DCT-12    | 0.905 ± 0.001a |
| DRT-2     | 0.944 ± 0.004a |
| DRT-3     | 0.945 ± 0.008a |
| DRT-4     | 0.931 ± 0.000a |

Note: Control, native chestnut starch; CT-3, CT-6, CT-9, CT-12, preparation of chestnut starch by continuous dry heat treatment for 3 h, 6 h, 9 h, 12 h, respectively; RT-2, RT-3, RT-4, preparation of chestnut starch by repeated dry heat treatment for two cycles, three cycles, four cycles, respectively. D-Control, DCT-3, DCT-6, DCT-9, DCT-12, DRT-2, DRT-3, DRT-4 represented the obtained freeze-dried starch samples, respectively. Results are showed as mean values ± SD in triplicate. Different letters (a-g) in the same column show a significant difference at p < 0.05.

Table 4
Water mobility results of native chestnut starch and dry heat treated starch.

| Samples      | T₂ (ms) | T₂τ (ms) | T₂α (ms) | T₂β (ms) |
|--------------|---------|----------|----------|----------|
| Control      | 272.48 ± 1.96d | 299.94 ± 17.45d | 853.06 ± 18.41d | 8343.11 ± 278.80d |
| CT-3         | 250.12 ± 1.16c | 280.78 ± 21.69bc | 754.05 ± 50.74bc | 7941.76 ± 707.53bc |
| CT-6         | 244.91 ± 0.90g | 204.01 ± 21.54bd | 722.00 ± 28.06bc | 7572.18 ± 136.40bc |
| CT-9         | 241.68 ± 2.35bc | 214.19 ± 18.09a | 704.71 ± 21.75ac | 7141.34 ± 139.71ac |
| CT-12        | 238.94 ± 0.50c | 216.53 ± 36.67bc | 678.24 ± 11.32bc | 6985.39 ± 477.83bc |
| RT-2         | 245.65 ± 2.26d | 250.47 ± 13.39bd | 776.64 ± 82.01bd | 7586.55 ± 706.59bd |
| RT-3         | 243.24 ± 0.30md | 280.35 ± 13.84bd | 712.66 ± 1.65bc | 7208.77 ± 213.13bc |
| RT-4         | 241.24 ± 1.84b | 290.67 ± 70.14bd | 647.84 ± 20.05 | 7019.88 ± 156.07 |
