Physicochemical characteristics of resistant starch prepared from Job’s tears starch using autoclaving–cooling treatment

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ABSTRACT
Job’s tears is an essential cereal with rich nutrients and functional chemical compositions. However, systematic knowledge of its starch content is still lacking. This study sought to investigate the resistant starch (RS) properties prepared from Job’s tears starch by autoclaving–cooling treatment. RS contents for native and autoclave-cooled Job’s tears starches were measured as 8.35%-10.53% and 26.57%-31.65%, respectively. The “Maltese crosses” of RS disappeared, and its morphology exhibited irregular sheet structure with layered strips and gullly shapes on the surface. Additionally, the XRD pattern of RS presented a combination of B and V types, and disrupted short-range ordered structure. Compared with native starch, the water solubility, swelling power, light transmittance, and water-holding capacity of RS were higher, whereas its hardness, chewiness, resilience, and gumminess were significantly lower. These results will provide insights into the future study of starch in Job’s tears and application of RS in the food industry.

Características fisicoquímicas del almidón resistente preparado a partir de almidón de lágrimas de Job (Coix lacryma-jobi) empleando un tratamiento de autoclave y enfriamiento

RESUMEN
Las lágrimas de Job son un cereal esencial que contiene ricos nutrientes y posee composiciones químicas funcionales. Sin embargo, el conocimiento sistematizado de su contenido de almidón aún es escaso. Por este motivo, el presente estudio se propuso investigar las propiedades del almidón resistente (RS) preparado a partir del almidón presente en las lágrimas de Job, mediante un tratamiento de autoclave y su posterior enfriamiento. Los contenidos de RS para almidones de lágrimas de Job nativos y los tratados en autoclave siendo posteriormente enfriados registraron las siguientes mediciones: 8.35%-10.53% y 26.57%-31.65%, respectivamente. Se constató que las “cruces de Malta” existentes en el RS desaparecieron y su morfología exhibió una estructura de lámina irregular con tiras estratificadas y formas de barranco en la superficie. Además, el patrón de DRX del RS presentó una combinación de tipos B y V, así como una estructura ordenada de corto alcance interrumpida. En comparación con el almidón nativo, la solubilidad en agua, el poder de hinchamiento, la transmittancia de luz y la capacidad de retención de agua del RS fueron mayores. En cambio, su dureza, masticabilidad, resiliencia y gomosidad fueron significativamente menores. Estos resultados proporcionan información que aporta al futuro estudio del almidón de las lágrimas de Job y la aplicación del RS en la industria alimentaria.

1. Introduction
Job’s tears (Coix lacryma-jobi L.), a tall grain-bearing tropical plant relative to maize, is originated from Southeast Asia and mostly planted in the tropical and temperate regions of Asian countries (Fu et al., 2019). According to archaeological evidence, its planting history in China is approximately more than 6,000 years. Job’s tears has an inherent high tolerance to low pH and viruses and adapted to waterlogging. Therefore, this plant has been well acknowledged as a vital minor cereal in China with a planting area of 73, 000 ha and abundant germplasm resources (Diao, 2017). Job’s tears grains can be processed into soups, porridges, and some traditional foods due to their richness in starch, protein, unsaturated fatty acid, calcium, phosphorus, iron, and total dietary fiber content (Fu et al., 2019). Besides, it contains some functional chemical compositions and exhibits various grain morphology types, which are often used to prepare medicine (anti-inflammatory agent, cancer drugs, and digests), healthy tea, and crafts (Zhu, 2017). Therefore, owing to the increasing demand, further studies on Job’s tears should be emphasized.

Resistant starch (RS), is a type of dietary fiber that is poorly digested and absorbed in the small intestine. Several studies have focused on RS due to its positive health benefits, such as preventive functions to diabetes, obesity, and colon cancer. RS is classified into four types, namely, RS1 (physically inaccessible starch), RS2 (botanical source starch), RS3 (retrograded starch), and RS4 (chemically modified starch) (Shu et al., 2013). Of them, RS3 has received extensive attention due to its thermal stability during processing. RS3

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has been prepared through different methods, including ultrahigh pressure treatment, extrusion treatment, ultrasonic treatment, microwave radiation, and crystal induction (Shah et al., 2016). However, starch source plays a vital role in RS production as the RS prepared from different starch sources exhibit different properties. Accumulating studies have reported the physicochemical properties of RSs prepared from rice (Ashwar et al., 2016), pea (Zhou et al., 2019), and chestnut (Gu et al., 2018), but only a few have been reported on the starch of Job’s tears. Although several studies have reported genetic diversity (Fu et al., 2019) and chemical composition (Zhu, 2017) of Job’s tears, systematic knowledge of its starch content is still lacking. Additionally, autoclaving–cooling treatment is an economical, safe, and suitable method that does not apply any residual chemical reagent to partly alter the physicochemical characteristics and properties of starch. This method efficiently changed the structural, physicochemical and digestive properties of Job’s tear starches and can provide a better insight into the effect of physical modifications on Job’s tear starches in order to find new food applications (Blaszczyk et al., 2015; Zeng et al., 2019).

This study elucidated the effect of autoclaving–cooling treatment on the physicochemical properties of starch prepared from Job’s tears. Furthermore, a comparative analysis between the amylose content, granule morphology, starch granule distribution, structural characteristics, pasting properties, thermal properties, physicochemical properties, and texture properties of native and RSs was performed. The results will provide a theoretical basis to obtain new starch materials and treatment methods for RS in the food industry.

2. Materials and methods

2.1. Materials

Four Job’s tear grain varieties, namely, Xingrenbaike (X), Ribenyiyi (R), Qianyi 2 (Q), and CL63 (C) were supplied by the Guizhou Academy of Agricultural Sciences, Guizhou, China.

2.2. Preparation of RS

Starch was extracted by the alkaline steeping method described by Chao et al., (2014). 100 g of Job’s tears flour and 700 mL of NaOH (3 g/L) were mixed in a water bath at 35°C for 28 h and then passed through a 100- and 200-μm mesh screen. The mixture was centrifuged at 4000 r/min for 10 min, and the supernatant and impurities were removed. This step was repeated three times. Later, 50 mL of H2O was added, stirred, and neutralized with HCl (0.1 mol/L), followed by centrifugation at 4000 r/min for 10 min. Afterward, the supernatant was removed, and the precipitate was washed thrice with water and dried at 45°C for 48 h. Finally, it was ground to powder and sieved with a 100 mesh sieve. The native starches were named as N-X, N-R, N-Q, and N-C.

Starch suspensions were obtained by mixing 160 mL of distilled water with 40 g (dry) of Job’s tears native starch in a 250 mL conical flask. The starch suspension was then sealed and heated at 115°C for 15 min in an autoclave. Afterward, it was cooled to room temperature and stored at 4°C for 24 h. The suspension was subsequently dried at 60°C in an oven, ground, and passed through a 150-μm mesh screen. The resulting RS products were named as R-X, R-R, R-Q, and R-Q.

2.3. Determination of total starch, amylose content, and resistant starch

Amylose and total starch contents were determined using an amylose test kit (Megazyme Co., Ltd., Bray, Ireland), a total starch test kit (Megazyme Co., Ltd., Bray, Ireland), and a resistant starch test kit (Megazyme Co., Ltd., Bray, Ireland), respectively.

2.4. Starch granule distribution

The samples were suspended in H2O and stirred at 2000 r/min. The obscuration was 10%–15% in all measurements. Later, the starch granules distribution was determined by Mastersizer 2000 (Malvern, England).

2.5. Scanning electron microscopy (SEM)

The starch samples were glued to the double-sided tape and sprayed with gold. Afterward, the starch granules morphology was examined under SEM (S4800, Hitachi, Tokyo, Japan). An acceleration potential of 10 kV was used during micrography.

2.6. Polarized light microscopy (PLM)

A 10% (w/v) starch suspension was observed in 50% glycerol using an Olympus BX53 polarized light microscope (Tokyo, Japan) under polarized light.

2.7. X-ray diffraction (XRD)

The XRD patterns of the starches were analyzed by an XRD (Bruker, Falkenried, Germany) at a target voltage of 40 kW, a target current of 100 mA, a scan range of 5°–50° (2θ), and a scanning speed of 10.0°/min.

2.8. Fourier transform infrared (FTIR) spectroscopy

The sample was mixed with dried KBr power (starch/KBr 1:100). The FTIR spectra of starches were obtained according to the methods described by Khawas and Deka (2016). The samples were scanned from 900−1 to 1200 cm−1 at a resolution of 4 cm−1 (Nicolet i550, Thermo Fisher Scientific, USA).

2.9. Rapid visco analyzer (RVA)

The pasting properties of samples were determined using RVA (RVA 4500, Perten, Sweden) according to the method described by Yang et al. (2019). Three grams sample was mixed with 25 mL of H2O to determine the pasting properties of starches. The mixture was held at 50°C for 1 min, heated at 12°C/min to 95°C, maintained at 95°C for 2 min, then cooled at 12°C/min to 50°C and finally held for 1 min.
2.10. Differential scanning calorimetry (DSC)

The thermal properties of the samples were studied using DSC (Q 2000, TA Instruments, USA). 6 μL of deionized water was added to 3 mg starch (dry basis) in the DSC pan and then placed at room temperature for 2 h. The samples were then heated at 10°C/min from 30°C to 150°C.

2.11. Water solubility and swelling power

The water solubility, swelling power, and light transmittance of starches were determined according to the method described by Yang et al. (2019). Approximately 0.5 g of sample and 20 mL of H₂O were placed in a 30 mL centrifuge tube and then heated at 50°C, 55°C, 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, and 90°C for 30 min, respectively. Afterward, the mixture was centrifuged under 3000 r/min for 10 min, and the supernatant was dried at 80°C until constant weight. A (g) is the weight of the supernatant after drying; P (g) is the weight of the sediment; W (g) is the dry basis weight of the sample.

Water solubility (%) = A/W × 100

Swelling power (%) = P/(W(100 – S)) × 100

2.12. Light transmittance and water hold capacity

Firstly, 0.5 g of sample and 50 mL H₂O were heated in boiling water for 30 min, and then the light transmittance of the samples was compared with distilled water and obtained at 620 nm using a spectrophotometer.

Approximately 0.5 g of starch and 10 mL of H₂O were placed in a centrifuge tube and then in boiling water for 30 min. The mixture was centrifuged again under 5000 r/min for 15 min and the supernatant was discarded.

Water – hold capacity (%) = Weight of gelatinized starch – 0.5 / 0.5 × 100

2.13. Texture profile analysis (TPA)

The 20% starch suspension was heated in boiling water for 30 min, transferred to an aluminum box (55 mm diameter, 30 mm depth) and stored in a refrigerator at 4°C for 12 h. The texture properties of the starch pastes were analyzed using TPA (Stable Micro Systems Ltd., Surrey, UK). A two-cycle compression test was performed using a PS cylindrical probe to compress the paste to obtain 70.0% strain at a speed of 1 mm/s.

2.14. Statistical analysis

Statistical analyses were conducted using principal component analysis (PCA) and Duncan’s multiple test (p < .05) (SPSS 16.0, IBM Corp., Armonk, NY, USA).

3. Result and discussion

3.1. Resistant content

The contents of starch, amylose, and resistant starch of native and resistant Job’s tear starches were summarized in Table 1. The total starch of native and resistant Job’s tear starches contained 79.71%–80.57% and 77.67%–81.80%, respectively. The amylose content of N-X, N-R, N-Q, and N-C was 2.11%, 3.35%, 10.33%, and 3.58%, respectively. The amylose content of R-X, R-R, R-Q, and R-C was 2.28%, 2.86%, 10.78%, and 4.45%, respectively. The results indicated that the autoclaving–cooling treatment had no significant effect on amylose content. RS contents for native and resistant Job’s tears starches were measured as 8.35%–10.53% and 26.57%–31.65%, respectively. The RS content of the sample prepared using autoclaving–cooling treatment was significantly (p < .05) higher than the native starches.

3.2. Morphology and distribution of starch granules

The morphological characteristics of Job’s tears native starch and RS were examined under SEM (Figure 1). All the RSs underwent autoclaving–cooling. High pressure and high temperature caused the swelling and destruction of starch granules, as well as leaching of starch components. Cooling caused the rearrangement of the gelatinized amylose molecular chains (F. Zhang et al., 2019). The morphology of RS granules significantly changed compared with the native starch granules. The size of resistant starch was bigger than that of native starch (See the red scale bar). The four native starches exhibited spherical and subspheroidal smooth surfaces, while the four RS granules exhibited irregular sheet structure with layered strips and gully shapes on the surface (See the blue arrows). This observation was consistent with the previous studies on rice (Kim et al., 2015)

| Table 1: Total starch content, amylose content, D (0.5), and IR ratio of native and resistance starches.

| Total starch | Amylose | Resistant starch content | Granule size | IR ratio |
|--------------|---------|--------------------------|--------------|---------|
|              |         |                          | D (0.5)      | 1047/1022 (cm⁻¹) | 1022/995 (cm⁻¹) |
| N-X          | 80.27 ± 0.72a | 2.11 ± 0.22d | 8.35 ± 0.31 | 12.5 ± 0.2e | 0.419 ± 0.012a | 0.781 ± 0.008b |
| N-R          | 80.57 ± 0.04a | 3.35 ± 0.43c | 8.40 ± 0.27 | 11.5 ± 0.7e | 0.358 ± 0.008b | 0.724 ± 0.015c |
| N-Q          | 79.72 ± 2.35a | 10.53 ± 0.02a | 8.96 ± 0.23 | 11.8 ± 0.0e | 0.419 ± 0.005a | 0.721 ± 0.006c |
| N-C          | 79.76 ± 0.62a | 3.58 ± 0.30bc | 27.99 ± 0.18 | 200.6 ± 3.0a | 0.250 ± 0.007d | 0.778 ± 0.009b |
| R-X          | 81.80 ± 1.35a | 2.28 ± 0.36d | 26.57 ± 0.46 | 178.8 ± 1.1c | 0.236 ± 0.007d | 0.849 ± 0.006a |
| R-R          | 79.45 ± 0.33a | 2.86 ± 0.17d | 31.65 ± 0.45 | 190.9 ± 4.9b | 0.240 ± 0.015d | 0.704 ± 0.011c |
| R-Q          | 78.44 ± 1.39a | 10.78 ± 0.79a | 28.54 ± 0.28 | 155.8 ± 2.6d | 0.210 ± 0.010e | 0.774 ± 0.011b |
| R-C          | 77.67 ± 2.46a | 4.45 ± 0.49b | 31.65 ± 0.45 | 190.9 ± 4.9b | 0.240 ± 0.015d | 0.704 ± 0.011c |

For each column, values not displaying the same letter are significantly different (p < .05).

D (0.5) is the granule sizes wherein 50% of all the granules are smaller by volume.

En cada columna, los valores con letras distintas son significativamente diferentes (p < .05).

D (0.5) es el tamaño de los gráuules en que 50% de todos los gránulos son más pequeños en volumen.
and arrowroot (Astuti et al., 2018) RS3 starches. “Maltese crosses” were typically found in the native starches of Job’s tears but disappeared in the RSs (Figure 1) as the ordered crystal structure in the native starch was destroyed during RS preparation. Similar results were observed in starches with heat–moisture treatment (Chung et al., 2009) and microwave heating (Palav & Seetharaman, 2006).

The four native starches and RSs showed a unimodal distribution. The distribution of N-X starch granules was concentrated, whereas the distribution of N-C starch granules was dispersed (Figure 2). However, the distribution of R-C starch granules was concentrated, whereas of the distribution of R-X starch granules was dispersed (Figure 2). The granule size of RS was larger than the native starch. D (0.5) value of these four native starches ranged between 11.5–12.5 μm with no significant difference. D (0.5) values of these four RSs ranged between 155.8–200.6 μm. R-C exhibited the highest D (0.5), while R-X had the lowest D (0.5).

3.3. X-ray diffraction (XRD)

The XRD patterns of native starches (N-X, N-R, N-Q, and N-C) and RSs (R-X, R-R, R-Q, and R-C) are displayed in Figure 3. The native starches showed a typical A-type (double peak: 17° and 18° 2θ; single peaks: 15° and 23° 2θ) (Yang et al., 2019). The XRD patterns of RSs changed significantly than the native starches. Additionally, the RSs showed lower peak intensities than the native starches. The results indicated that the internal crystalline structure of native Job’s tear starch was extensively damaged by autoclave treatment, which was supported by the morphology of native and resistant starch in SEM. The XRD patterns of RSs were transformed to a combination of B (single peaks: 17° and 22° 2θ) and V (a weak peak approximately at 20° 2θ) types. This finding was consistent with the previous studies (Jing et al., 2019). Starch is likely to form B-type crystal-line structure during retrogradation at low temperatures. The V-type crystal structure is a single, left-handed helix often with a complexing agent included in the helical channel and it is formed by crystallites typical of an amylose helix stabilized by the inclusion of lipophilic compounds (Ding et al., 2019; Wang et al., 2009). During the autoclaving–cooling treatment, the increase in fluidity of starch caused the crystallinity increased, which led to structural changes in the starch crystallization area. The crystal structure of resistant starch was more stable than that of native (Gao et al., 2020). Therefore, the onset, peak, and completion temperatures of resistant starch were higher than those of native starch.

3.4. Fourier transform infrared (FTIR)

FTIR is potential method to analyze the starch structure but sensitive to the short-order range. The FTIR spectrum between 900–1200 cm⁻¹ reflected the variations in helicity, chain conformation, and double helix structure. The bands at 995 and 1047 cm⁻¹ were linked to the ordered structure and crystalline region, whereas 1022 cm⁻¹ reflected the disordered phase (Ma et al., 2018). Therefore, the 1047/1022 and 995/1022 cm⁻¹ could be used to express the degree of order structure and double helix, respectively (Zhou et al., 2019). Figure 3(c,d) illustrate that the FTIR spectrum of RSs was similar to the native starches. However, the 1047/1022 cm⁻¹ of RSs (0.210–0.250) was significantly lower than
the native starches (0.315–0.419). This result indicated that autoclaving destroyed the short-range ordered structure of starch (Table 1). These findings were consistent with the reports of Zhou et al. (2019) and Chung et al. (2009). The 995/1022 cm⁻¹ values of N-X, N-R, N-Q, and N-C were 0.781, 0.724, 0.721, and 0.773, respectively. The 995/1022 cm⁻¹ values of R-X, R-R, R-Q, and R-C were 0.778, 0.849, 0.704, and 0.774, respectively. Therefore, the double helix content of N-R increased after autoclaving–cooling. This happened because autoclaving destroyed the hydrogen bonds connecting the adjacent helices rather than dissociating the double helix structure. However, the double helix contents
of the other three starches were not significantly changed due to the difference in sample varieties. This finding was consistent with the results of XRD. The changes in the ordered structure of starch leads to the differences in the physicochemical (pasting properties, thermal properties, and starch paste properties) of starches.

### 3.5. Pasting properties

The pasting properties of Job’s tears native starch and RS are summarized in Table 2. Compared with the N-X and N-R, the peak viscosity (PV) and through viscosity (TV) of R-X and R-R were decreased. Moreover, the final viscosity (FV) of RSs significantly decreased than the native starches. This happened because the high temperature and pressure caused the dissolution and disruption of starch granules (Acevedo et al., 2019). Although RSs had lower pasting viscosities than the native starches, the PV and TV of R-Q and R-C were increased compared to N-Q and N-C. The pasting properties of RS are related to the starch source, treatment process, and amylose (Khawas & Deka, 2016). Ozturk et al. (2009) also reported the same phenomenon. High BD indicated low heat resistance of starch (L. Zhang et al., 2018). Although the damaged starch was retrograded by cooling, it might have a looser structure than the original starch. Compared with the native starches, R-Q and R-C showed a higher breakdown (BD), while no significant change was observed between R-X and R-R (Table 2). Compared with the native starches, the setback (SB) of R-X, R-R, and R-C showed no clear trend of increase or decrease. This outcome was consistent with the result of heat moisture treatment in rice starch (Silva et al., 2017). However, the SB of R-Q was significantly lower than N-Q due to its high amylose content (formed several short chains of amylose) (Khan et al., 2014; Khawas & Deka, 2016). The PT and PTM of Job’s tears RSs were lower than the native starches. This finding indicated that the RSs could be gelatinized easily due to the destruction of starch granules when subjected to high pressure and temperature (Reddy et al., 2014).

### 3.6. Thermal properties

The thermal properties of Job’s tears native starch and RS are summarized in Table 2. The onset (To), peak (Tp), completion temperatures (Tc), and gelatinization temperature range (Tc-To) of native starches were 68.3°C–70.0°C, 72.3°C–74.2°C, 79.6°C–82.1°C, and 11.2°C–12.1°C, respectively. The To, Tp, Tc, and Tc-To of RSs were 139.1°C–144.0°C, 139.7°C–144.1°C, 143.0°C–147.1°C, and 3.0°C–3.2°C, respectively. These results indicated that the autoclaving cooling increased the To, Tp and Tc and decreased the Tc-To of starches. In contrast, the unstable starch crystallites melted first during the autoclaving, while the remaining stable starch crystallites required high temperatures to melt. Besides, the disrupt starch became stable after rearranging during cooling (Shah et al., 2016). The decrease in Tc-To of RSs showed that the temperature difference between Tc and To is smaller, indicating a more homogeneous and perfect crystallites. This could be to the destroyed crystalline regions containing flaws that further increased the perfection of helical order (Ashwar et al., 2016; Chung et al., 2009; Shah et al., 2016). The ΔH values of N-X, N-R, N-Q, and N-C were 10.7, 11.1, 10.6, and 8.0 J/g, and the ΔH values of R-X, R-R, R-Q, and R-C were 1.2, 1.4, 1.5, and 3.6 J/g, respectively. The native starches showed lower ΔH than RSs, suggesting that the latter had less stable crystals (Chiotelli & Le Meste, 2002). This might have happened due to the retrogradation and the formation of a weak matrix or network requiring less energy to melt by RSs (Ovando-Martínez et al., 2013; Shah et al., 2016).

### 3.7. Water solubility and swelling power

Figure 4 depicts the water solubility and swelling power of the native and RSs. With the increasing temperature, the water solubility and swelling power of starches also increased due to the breakdown of starch granules and exposure of the hydrophilic groups to water (Khawas & Deka, 2016). The water solubility of RSs was initially lower than the native starches. At 90°C, the water solubility of RSs was higher than the native starches. However, the swelling power of RSs was higher than the native starches at 50°C–90°C. Besides, the N-Q and R-Q showed the lowest swelling power in the native starches and RSs, respectively. This result was consistent with the previous studies reporting that amylose inhibits starch swelling (Li et al., 2018).

### 3.8. Light transmittance and water hold capacity

Except for R-C, the RSs showed significantly higher light transmittance than the native starches due to the fragility of starch granules during autoclaving–cooling (Shah et al., 2016). Water holding capacity refers to the ability of starch to retain water and is an indicator for determining the degree of damage of starch granules (Astuti et al., 2018). The water absorption capacity of Job’s tears native starches ranged between 97.5%–114.6% and significantly increased with the autoclaving–cooling treatment (323.1%–369.9%). This result was associated with the destruction of RS granules. Research fronts have reported that the physical modification increased the water holding capacity of starches (Zhou et al., 2019).

### 3.9. Texture profile analysis (TPA)

The textural properties of the native and RSs are summarized in Table 3. The hardness, chewiness, resilience, gumminess, and cohesiveness of RSs were significantly lower than the native starches. This result indicated that autoclaving–cooling decreased the hardness and viscosity of starches as the granules became weak during chain fragmentation and the starch formed a gel network allowing the permeation of a great amount of water (Astuti et al., 2018; Chung et al., 2010). Springiness is an indication of food elasticity (Hedayati & Nia Kousari, 2018). The springiness of RSs was almost similar to the native starches. This finding was consistent with the report of Kim et al. (2015).

### 3.10. Principal component analysis (PCA)

PCA was employed to determine and compare the characteristics of the native and RSs in Job’s tears (Figure 5). PC1 and PC2 accounted for 55.6% and 20.0% of the total variance (75.6), respectively. The scores plot (Figure 5(a)) demonstrated that the starches could be segregated into two groups. The first group included four native starches, which was located on the positive side of PC1. The second
| PV (cP) | TV (cP) | BD (cP) | FV (cP) | SB (cP) | PT (min) | PTM (°C) | To (°C) | Tp (°C) | Tc (°C) | ΔH (J/g) | Tc-To (°C) |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----------|
| 3339 ± 76d | 1952 ± 25d | 1388 ± 52d | 2270 ± 70bc | 318 ± 45d | 4.1 ± 0.2ab | 769 ± 0.0a | 69.8 ± 0.2d | 73.8 ± 0.1d | 81.0 ± 1.0de | 10.7 ± 0.7a | 11.2 ± 1.2a |
| 3733 ± 2c  | 2404 ± 22a | 1329 ± 24d | 2804 ± 232a | 501 ± 42 c  | 4.3 ± 0.0a  | 75.9 ± 0.1b | 69.5 ± 0.0de | 74.2 ± 0.2d | 81.1 ± 0.8de | 11.1 ± 0.5a | 11.7 ± 0.8a  |
| 3900 ± 25bc | 2259 ± 18b | 1642 ± 8c  | 2963 ± 62a  | 705 ± 45a  | 3.6 ± 0.0de | 75.5 ± 0.5b | 70.0 ± 0.3d | 74.2 ± 0.3d | 82.1 ± 0.4d | 10.6 ± 0.2a | 12.1 ± 0.7a  |
| 3413 ± 22d | 2004 ± 12c | 1409 ± 10d | 2475 ± 48b  | 472 ± 60c  | 4.0 ± 0.0be | 75.9 ± 0.1b | 68.3 ± 1.0e | 72.3 ± 0.5e | 79.6 ± 0.4e | 8.0 ± 0.8b  | 11.3 ± 0.6a  |
| R-X       | 2680 ± 194e | 1304 ± 6g  | 1376 ± 201d | 1646 ± 32e | 342 ± 25d  | 50.0 ± 0.0c | 141.7 ± 0.9b | 142.1 ± 0.7b | 144.9 ± 0.2b | 1.2 ± 0.1d  | 3.2 ± 0.6b   |
| R-R       | 2788 ± 42e | 1514 ± 25f | 1274 ± 16d | 1964 ± 48d | 450 ± 23c  | 5.4 ± 0.0ef | 50.1 ± 0.0c | 140.0 ± 0.3c | 140.7 ± 0.5c | 143.0 ± 0.5c | 1.4 ± 0.0d  | 3.0 ± 0.2b   |
| R-Q       | 4379 ± 18a | 2385 ± 33a | 1994 ± 15b | 2996 ± 23a | 611 ± 9b   | 3.7 ± 0.1cd | 50.1 ± 0.0c | 139.1 ± 1.5c | 139.7 ± 1.4c | 142.1 ± 1.8c | 1.5 ± 0.2d  | 3.1 ± 0.3b   |
| R-C       | 4004 ± 162b | 1705 ± 1e  | 2299 ± 161a | 2168 ± 36cd | 463 ± 35c  | 3.3 ± 0.0f  | 50.1 ± 0.0c | 144.0 ± 0.4a | 144.1 ± 0.1a | 147.1 ± 0.4a | 3.6 ± 0.3c  | 3.1 ± 0.1b   |

For each column, values not displaying the same letter are significantly different (p < .05).

PV, TV, BD, FV, SB, PT, and PTM represent peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, peak time, and pasting temperature, respectively.

To, Tp, Tc, and ΔH represent onset temperature, peak temperature, conclusion temperature, and gelatinization enthalpy, respectively.

En cada columna, los valores con letras distintas son significativamente diferentes (p < .05).

PV, TV, BD, FV, SB, PT y PTM representan la viscosidad máxima, la viscosidad mínima, la viscosidad de ruptura, la viscosidad final, la viscosidad de retroceso, el tiempo máximo y la temperatura de pegado, respectivamente.

To, Tp, Tc y ΔH representan la temperatura de inicio, la temperatura pico, la temperatura de conclusión y la entalpia de gelatinización, respectivamente.
Moreover, were native starches in Xingrenbaike, Ribenyiyi, Qianyi 2, and CL63. N-X, N-R, N-Q, and N-C were native starches in Xingrenbaike, Ribenyiyi, Qianyi 2, and CL63.

Table 3. Texture properties of native and resistance starch pastes.

| Hardness (g) | Springiness | Chewiness (g) | Resilience | Gumminess | Cohesiveness |
|--------------|-------------|---------------|------------|------------|--------------|
| N-X          | 39.7 ± 1.6c | 1.0 ± 0.0a    | 35.1 ± 1.8b | 0.8 ± 0.0a | 35.0 ± 2.0b  |
| N-R          | 47.1 ± 1.1b | 1.0 ± 0.0a    | 39.1 ± 0.9a | 0.7 ± 0.0b | 39.1 ± 0.9a  |
| N-Q          | 26.9 ± 2.6e | 1.0 ± 0.0a    | 23.6 ± 2.8c | 0.8 ± 0.0a | 23.3 ± 2.7c  |
| R-Q          | 57.0 ± 0.0a | 0.9 ± 0.0b    | 34.4 ± 2.8b | 0.4 ± 0.1c | 37.6 ± 2.2ab |
| R-X          | 20.4 ± 2.6g | 1.0 ± 0.0a    | 15.5 ± 1.7e | 0.6 ± 0.0b | 15.3 ± 1.6ef |
| R-R          | 19.4 ± 0.1g | 1.0 ± 0.0a    | 14.1 ± 0.1e | 0.5 ± 0.0c | 14.0 ± 0.1f  |
| R-Q          | 24.6 ± 0.1ef| 1.0 ± 0.0a    | 19.7 ± 0.1cd | 0.7 ± 0.0b | 19.4 ± 0.0d  |
| R-C          | 34.6 ± 4.3d | 0.9 ± 0.0b    | 17.6 ± 1.2de | 0.2 ± 0.0d | 19.1 ± 1.3de |

For each column, values not displaying the same letter are significantly different (p < 0.05).
En cada columna, los valores con letras distintas son significativamente diferentes (p < 0.05).

A group included four RSs, which was located on the negative side of PC1. Figure 5(b) depicts the loading plot of the starch properties. The primary contributors to the native starches were ΔH, Tc-To, IR1047/1022, PT, PTM, hardness, chewiness, and water solubility. The primary contributors to the RSs were To, Tc, Tp, water holding capacity, and D (0.5). Moreover, the N-Q and R-Q (high amylose) were at the top of PC2 (Figure 5(a)). This finding was consistent with the Figure 5(b) result reporting that amylose was at the top of PC2.

4. Conclusion

In the present study, the structural and physicochemical changes of Job's tears starches were determined by autoclaving–cooling treatment. Compared with the native starches, the "Maltese crosses" of RSs disappeared, and the RSs morphology exhibited irregular sheet structure with layered strips and gully shapes on the surface. The RS granule size was larger than the native starch. The native starches presented an A-type XRD pattern and then transformed to a combination of B and V types after autoclaving–cooling. Moreover, the short-range ordered structure of RS was destroyed. Compared with the native starches, the RSs were expected to have lower pasting viscosities. The To, Tp, and Tc of RSs were higher than the native starches, while the Tc-To of RSs was lower than the native starches. The RSs increased the water solubility, swelling power, light transmittance, and water holding capacity but significantly decreased the hardness, chewiness, resilience, gumminess, and cohesiveness than the native starches. The PCA showed that the native starches and RSs were segregated into two groups. This method intensifies the formation of RS by
Figure 5. Scores plot of the samples (a) and the loadings plot of the properties in starches (b). TS, total starch; AM, amylose; IR 1, Infrared 1047/1022 (cm−1); IR 2, Infrared 1022/995 (cm−1); PV, peak viscosity; TV, trough viscosity; BD, breakdown viscosity; FV, final viscosity; SB, setback viscosity; PT, peak time; PTV, pasting temperature; To, onset temperature; Tp, peak temperature; TC, conclusion temperature; ΔH, gelatinization enthalpy; WS, water solubility; SP, swelling power; LT, light transmittance; WH, water-holding capacity; HA, hardness; SP, springiness; CH, chewiness; RE, resilience; GU, gumminess; CO, cohesiveness. N-X, N-R, N-Q, and N-C were native starches in Xingrenbaike, Ribeniyi, Qianyi 2, and CL63. N-X, N-R, N-Q, and N-C were resistant starches in Xingrenbaike, Ribeniyi, Qianyi 2, and CL63.

Figure 5. Diagrama de puntuación de las muestras (a) y diagrama de carga de las propiedades de los almidones (b). TS, almidón total; AM, amílose; IR 1, infrarrojo 1047/1022 (cm−1); IR 2, infrarrojo 1022/995 (cm−1); PV, viscosidad de pico; TV, viscosidad de depresión; BD, viscosidad de ruptura; FV, viscosidad final; SB, viscosidad de retroceso; PT, tiempo de pico; PTV, temperatura de pegado; To, temperatura de inicio; Tp, temperatura de pico; TC, temperatura de conclusión; ΔH, entalpía de gelatinización; WS, solubilidad en agua; SP, poder de hincharse; LT, transmisión de luz; WH, capacidad de retención de agua; HA, dureza; SP, elasticidad; CH, masticabilidad; RE, resistencia; GU, gomosidad; CO, cohesión. N-X, N-R, N-Q y N-C son almidones nativos de Xingrenbaike, Ribeniyi, Qianyi 2 y CL63. N-X, N-R, N-Q y N-C son almidones resistentes de Xingrenbaike, Ribeniyi, Qianyi 2 y CL63.

physical modification (i.e. without using solvents), which is safer for food industry use. In addition, the results would be of great interest for the potential development value of Job’s tear starch and the application of RS in the food industry.

Disclosure statement
The authors declare no conflict of interest.

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