Inca Peanut Seed Albumin Promotes the Retrogradation of Corn Starch

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ABSTRACT: While starch and protein are two major biopolymer components that usually coexist in foods, how protein influences starch retrogradation, and subsequently the food matrix structure and properties, is complex and dependent on factors such as protein and starch types. Herein, the effect of the addition of Inca peanut seed albumin (IPA, ≤10%) on the retrogradation of native corn starch (NCS) upon aging for ≤7 days was investigated. Dynamic rheological results show that the addition of IPA significantly promoted the short-term (1 day) retrogradation of NCS as reflected by the increased storage modulus and smaller loss tangent. Differential scanning calorimetry results indicate that IPA increased the retrogradation enthalpy, especially with a high content (≥5%) of IPA (P < 0.05). In addition, with a higher level of IPA, the hardness increased after storage for 1 or 7 days. The ratio of the band intensities at 1047 and 1022 cm⁻¹ and the relative crystallinity of the NCS/IPA mixture also increased after storage, especially with a high content (≥5%) of IPA. Scanning electron microscopy results show that the addition of IPA increased the surface roughness and porosity of the starch gel after storage. This work shows that the addition of IPA as a plant protein could be a promising way to modify the properties of starch-based foods by inducing retrogradation while enhancing the nutritional and functional properties of foods.

KEYWORDS: retrogradation properties, starch structure, protein, Inca peanut seed albumin (IPA), rheological properties, thermal properties

1. INTRODUCTION

Starch, a very common biopolymer in cereal products, is widely used in food processing. Upon being heated with water, starch gelatinizes. Gelatinized starch can undergo retrogradation upon storage, and retrogradation is a major factor affecting the quality of starch products. Starch is mainly composed of amylose and amylopectin. Amylopectin forms semicrystalline lattices in the starch structure, and amylose is scattered among the clusters formed by amylopectin to form the amorphous regions of starch. Starch retrogradation is a process by which amylose and amylopectin molecules form ordered structures and can be a short- or long-term process. Short-term retrogradation is due to amylose recrystallization, while long-term retrogradation is the orderly recombination of amylopectin chains. Starch retrogradation is interesting both scientifically and practically because it greatly affects food quality, consumer acceptance, and shelf life. In most cases, starch retrogradation is undesirable because it can make foods such as bread hard or stale. On the contrary, retrogradation can be used to improve the texture and sensory properties of starchy foods, such as Chinese rice vermicelli and breakfast cereals.

It has been demonstrated that the process of starch retrogradation is usually affected by many factors, such as the amylose/amylopectin ratio, moisture content, storage conditions, and additives. Protein, as a typical ingredient in starchy foods, also plays an important role in affecting the mobility of water molecules in starch, the gelatinization and aging of starch, and the viscosity of starch pastes. For instance, Zhang et al. found that the presence of rice protein hydrolysates could inhibit the short- and long-term retrogradation of wheat starch. Hu et al. also indicated that whey protein hydrolysate could be a potential retrogradation inhibitor, as it prevented molecular associations and the formation of hydrogen bonds among starch chains. Moreover, Xijun et al. isolated albumin, globulin, gliadin, and glutenin from wheat flour and found that glutenin delayed the retrogradation of wheat starch while albumin, globulin, and gliadin promoted its retrogradation. Guo et al. also showed that glutathione promoted the degree of retrogradation of wheat flour. These studies have shown that the influence of protein on the gelatinization and retrogradation behavior of starch could depend on the protein and starch types.

Recently, bioactive protein components from plants have attracted a great deal of attention because of their positive effects on human health. Inca peanut seed albumin (IPA) isolated from Inca peanut seed protein is a valuable source of dietary protein. In our previous work, the effect of IPA on the microstructure and rheological and thermal properties of...
native corn starch (NCS) was studied. However, how the addition of IPA affects the retrogradation of gelatinized NCS has not been explored, and that is the basis of this work. Therefore, the objective of this study was to prepare IPA and reveal the effect of IPA on the retrogradation of NCS, through a multianalytical study involving rheology, mechanical testing, differential scanning calorimetry (DSC), Fourier transform infrared (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). This study could be useful for the application of IPA in controlling the properties and quality of starchy foods.

2. MATERIALS AND METHODS

2.1. Materials and Reagents. NCS with 26.00% amylose and 12.78% moisture was obtained from National Starch Pty Ltd. (Lane Cove, Australia). IPA was isolated on the basis of the previous method of Li et al. and Huang et al. Briefly, Inca peanut seed flours were soaked and stirred constantly in a 2 M NaCl solution (1:20, w/v, 30 °C) for 2 h, dialyzed using dialysis bags (molecular mass cutoff of 6–8 kDa), and then collected by centrifugation at 4 °C and 8000g for 30 min. Afterward, the supernatant was freeze-dried to obtain IPA. Other chemical reagents were of analytical grade.

2.2. Preparation of Starch/Protein Pastes. Starch/protein mixtures were prepared according to the previous procedure described by Chen et al. with some modification. NCS powder (5%, w/w) in the presence of different levels of IPA (0%, 1%, 5%, and 10%, based on the starch weight, w/w) was stirred constantly at 100 °C for 30 min to obtain a gelatinized paste. The starch/protein pastes were cooled in an ice/water bath to 4 °C for 30 min and then stored at 4 °C for 0, 1, and 7 days.

2.3. Dynamic Rheological Measurement. The rheological properties of the NCS pastes containing different levels of IPA (0%, 1%, 5%, and 10%) were determined using a DHR-3 rheometer (TA Instruments) configured with a parallel-plate geometry (25 °C, 40 mm diameter, 0.5 mm gap). To prevent the evaporation of water during the measurement process, silicon oil was applied to the edge of the samples. Changes in the storage moduli (G') and the loss factor (tan δ) during an isothermal time sweep of 6000 s at 25 °C were recorded at a constant frequency of 1 Hz and a constant strain of 1% within the linear viscoelastic region.

2.4. Differential Scanning Calorimetry (DSC). The effects of the addition of IPA on the thermal properties of the NCS samples were analyzed by a model 4000 differential scanning calorimeter (PerkinElmer, Inc., Waltham, MA). Both pure NCS and the starch samples containing different levels of IPA (1%, 5%, and 10%, w/w, based on the starch weight) were measured. Approximately 3 mg of the samples was weighed into a DSC pan, and deionized water (~6 μL) was added using a microsyringe to give a water/mixture ratio of 2:1 (w/w). Immediately thereafter, the mixtures were hermetically sealed in the DSC pans and equilibrated at 25 °C for 12 h. The samples were heated from 30 to 100 °C at a rate of 5 °C/min to determine their gelatinization enthalpy (ΔHg). After the first-run heating, the gelatinized samples were cooled and preserved at 4 °C for 1 and 7 days. The stored samples were reheated under the same conditions to determine the retrogradation enthalpy (ΔHr).

2.5. Textural Analysis. The back extrusion measurement of the gelatinized NCS-IPA pastes was carried out using a texture analyzer (Shimadzu, EZ-SX 500N) equipped with a probe (45 mm diameter) and a cylinder. The pretest speed, test speed, post-test speed, and trigger force were 2 mm s⁻¹, 2 mm s⁻¹, 10.0 mm s⁻¹, and 5.0 g, respectively. The pastes were compressed at a 30% deformation level. Hardness data were recorded by the Texture Exponent Lite software.

2.6. FTIR Analysis. The infrared spectra of the NCS samples containing different levels of IPA were recorded with a spectrometer (FTIR, Nicolet 6700) over the range of 400–4000 cm⁻¹ with a resolution of 4 cm⁻¹. The FTIR curves within a wavenumber range of 1200–900 cm⁻¹ were baseline-corrected by the OMNIC software, and the ratios of absorbance at 1047 cm⁻¹ to that at 1022 cm⁻¹ (R_{1047/1022}) were calculated.

2.7. XRD Analysis. The XRD curves of the freshly gelatinized samples and retrograde samples (4 °C, 0, 1, and 7 days) were obtained by scanning from 4 °C to 40 °C (2θ) at a rate of 2°/min. The relative crystallinity was calculated and analyzed by using Jade version 6.5. The ratio of the crystallization area to the total diffraction area was taken as the relative crystallinity.

2.8. SEM. The micromorphology of all of the freeze-dried samples was imaged using a scanning electron microscope at a magnification of 5000×. Before observation, the samples were covered with gold.

2.9. Statistical Analysis. The experimental results were statistically analyzed with SPSS 17.0. Duncan’s test was performed to evaluate the significant difference (P < 0.05).

3. RESULTS AND DISCUSSION

3.1. Dynamic Viscoelastic Analysis. The storage modulus (G’) is closely related to the aggregation of amylose in the early retrogradation stage. Specifically, G’ is controlled by the aggregation state of amylose during the cooling process, reflecting the initial development of a three-dimensional gel network of NCS pastes. Figure 1A shows the G’ curves for the NCS pastes containing different levels of IPA. For all of the NCS pastes, G’ continued to increase, indicating that amylose...
Table 1. DSC Results for NCS Samples Containing Different Levels (0%, 1%, 5%, and 10%) of IPA

| level of IPA (%) | ΔHr (J/g) | ΔHi (J/g) | R (°C) | ΔHr′ (J/g) | Rr (%) |
|-----------------|----------|----------|--------|-----------|--------|
| 0               | 13.175 ± 0.393 a | 3.784 ± 0.002 b | 28.7 | 4.521 ± 0.117 c | 34.3 |
| 1               | 13.241 ± 0.286 a | 3.861 ± 0.254 b | 29.1 | 4.739 ± 0.109 bc | 35.8 |
| 5               | 13.442 ± 0.063 a | 4.456 ± 0.341 ab | 33.2 | 5.017 ± 0.060 b | 37.3 |
| 10              | 14.039 ± 0.323 a | 4.977 ± 0.009 a | 35.5 | 5.427 ± 0.037 a | 38.7 |

“The results are based on three measurements and are represented as means ± the standard deviation. Values in the same column with different letters are significantly different (Duncan’s method; P < 0.05). Abbreviations: ΔHi, enthalpy of gelatinization; ΔHr, enthalpy of retrogradation on the ith day; Rr, percentage of retrogradation on the ith day, i.e., (ΔHr/ΔHr0) × 100.

With an increase in storage time, the hardness of all of the gelatinized samples increased, indicating their rapid retrogradation. The hardness of the NCS paste without IPA increased obviously from 125 to 229 gf (Figure 2) with the storage time increased from 1 to 7 days. This is mainly because, during retrogradation, starch chains rearrange into ordered structures through hydrogen bonds. Similarly, it was previously found that the hardness of wheat starch gels increased with storage period. We observe here that the presence of IPA increased the hardness of the NCS paste significantly, indicating that a strong network could be formed by interaction between NCS and IPA molecules. A similar result was previously reported by Baxter et al., who proved that hardness was increased significantly when albumin was added to rice starch. The enhanced hardness in the presence of IPA agrees with the thermal property results and could be attributed to the formation of hydrogen bonds between IPA and NCS and thus a strengthened gel structure. These results suggest that the
addition of IPA could promote the retrogradation of NCS, and the effect depended on the amount of albumin.

Table 2 shows the relative increases [hardness (day 1)/hardness (day 7)] of the different samples. When a larger amount of albumin was added, the hardness increased (see Figure 2) and the relative increase decreased (Table 2). This indicates that with a higher level of albumin, a greater portion of the whole retrogradation occurred from day 1 to 7. These results show that IPA could be a potential retrogradation additive for corn starch-based foods.

3.4. FTIR Analysis. To further study the effect of IPA on the recrystallization of NCS, FTIR curves in the range of 900–1200 cm\(^{-1}\) were obtained. Generally, \(R_{1047/1022}\) is used to represent the ratio of the ordered structure to the amorphous content in starch granules\(^{13,25}\) and thus can be used to reflect the degree of retrogradation of starch. Figure 3 presents the spectra for the freeze-dried retrograde samples, and Table 2 lists the \(R_{1047/1022}\) values for these samples. One can see that the \(R_{1047/1022}\) for the NCS samples increased with storage time, which can be attributed to the formation of ordered starch structures (single and double helices).\(^{25}\) For example, when the storage time was increased from 1 to 7 days, the \(R_{1047/1022}\) for the NCS sample (without IPA) increased from 0.112 to 0.161 (Table 3). Moreover, compared to that of NCS, the addition of IPA increased the \(R_{1047/1022}\) value. The addition of 1%, 5%, and 10% IPA increased the \(R_{1047/1022}\) for NCS after storage for 7 days to 0.182, 0.198, and 0.229, respectively (Table 3), indicating that the addition of IPA could lead to a more ordered gel structure. A previous study displayed an analogous trend that showed that the addition of modified glutenin and gliadin led to an increase in \(R_{1047/1022}\) for potato starch.\(^{5}\) Moreover, this result was consistent with the thermal and textural results presented in Table 1 and Figure 3, respectively.

3.5. XRD Analysis. XRD is a useful tool for exploring the crystallization characteristics of starch.\(^{22}\) Figure 4 displays the XRD diffractograms for native NCS and the gelatinized NCS/IPA mixtures after storage at 4 °C for 0, 1, and 7 days. As shown in Figure 4A, the XRD diffractogram for native NCS exhibited characteristic peaks at 2\(\theta\) angles of \(\sim15.0^\circ\), \(\sim17.0^\circ\), \(\sim18^\circ\), \(\sim20^\circ\), and \(\sim23^\circ\), indicating the A-type crystalline structure.\(^{27}\) After storage at 4 °C, the XRD diffractograms for the retrograde NCS showed a single peak at 17\(^\circ\) and a peak near 20\(^\circ\), characteristic of the B-type crystalline structure.

Table 3. FTIR Results for NCS Samples with Different Levels (0%, 1%, 5%, and 10%) of IPA\(^a\)

| level of IPA (%) | \(R_{1047/1022}\) on day 1 | \(R_{1047/1022}\) on day 7 |
|-----------------|---------------------------|--------------------------|
| 0               | 0.112 ± 0.002 c           | 0.161 ± 0.003 b          |
| 1               | 0.173 ± 0.001 b           | 0.182 ± 0.002 b          |
| 5               | 0.175 ± 0.002 b           | 0.198 ± 0.001 ab         |
| 10              | 0.198 ± 0.004 a           | 0.229 ± 0.019 a          |

\(^{a}\)The results are based on three measurements and are represented as means ± the standard deviation. Values in the same column with different letters are significantly different (Duncan’s method; \(P < 0.05\)). Abbreviations: \(R_{1047/1022}\), ratio of absorbance at 1047 cm\(^{-1}\) to that at 1022 cm\(^{-1}\).
After gelatinization and retrogradation, the XRD curves for NCS and the NCS/IPA mixtures displayed only the typical B-type crystalline structure ($2\theta = 17^\circ$ and $20^\circ$). Additionally, the XRD curves for the lyophilized retrograde samples were quite different from that of NCS. With an increase in storage time, the crystallinity of the samples increased gradually, reflecting the starch retrogradation process. The addition of IPA did not affect the type of crystalline structure for the NCS samples, though. With an increase in storage time, an increase in relative crystallinity calculated for all of the retrograde samples was observed, implying that more crystals were formed during the retrogradation.

With the same storage time, the crystallinity of the pure NCS was lower than those of the NCS/IPA mixtures, suggesting the latter experience greater retrogradation, as promoted by IPA, which was in line with FTIR and DSC results. Similar to our results, Xijun et al. also found that the addition of albumins isolated from wheat flour promoted starch retrogradation. The effect of IPA on the crystalline structure of NCS may be due to the interactions between IPA molecules and amylose and/or the side chains of amylpectin.

### 3.6. SEM

Figure 5 shows the SEM images of the freeze-dried NCS and NCS/IPA samples after different periods of storage. Freshly prepared starch gel without IPA shows a relatively dense and uniform structure. At the initial stage of storage (1 day), with an increasing level of IPA added, the lyophilized retrograde NCS/IPA mixtures exhibited a clear porous structure, which was mainly generated by the evaporation of water. This result may be due to the presence of IPA, which induced the reaggregation, reordering, and recrystallization of corn starch molecules after cold storage.

With an increase in the storage time, the samples became more porous after cold storage. This phenomenon occurred due to the enhanced interaction between starch and protein chains during low-temperature storage. With the aggregation of starch and protein chains, water gradually flowed out of the gel network, and the gels after freeze-drying exhibited more holes.

For NCS/IPA mixtures, especially with a higher level of IPA, the gel surface roughness increased significantly with prolonged storage duration. The NCS/IPA mixtures containing higher protein fractions (5–10%) showed an inhomogeneous gel network structure (500× micrograph), onto which segregated protein mass was dispersed. Due to the higher concentration and faster continuous gel network forming capacity of starch, the continuous gel network was formed by the corn starch and the segregated mass might be formed by the aggregated IPA fraction. These results demonstrated that IPA could effectively promote the retrogradation of NCS.

In conclusion, while the restricting effect of protein on starch retrogradation has been widely shown, here the addition of IPA as a plant protein to NCS was found to promote the retrogradation of NCS. The results of dynamic viscoelastic analysis show that the presence of IPA facilitated the short-
term retrogradation of NCS paste. Additionally, after storage for 1 or 7 days at 4 °C, the ΔHr and hardness of gelatinized NCS increased with a higher level of IPA. Meanwhile, for all of the samples, $R_{1047/1022}$ increased with an increase in storage time and also increased with a higher level of IPA, indicating a higher degree of short-range ordering. The addition of IPA increased the relative crystallinity of NCS on days 1 and 7 as shown by XRD. SEM images show that the samples with IPA added were more porous, which could also be caused by the retrogradation of starch. With regard to these results, we propose that the interaction of IPA with NCS promoted hydrogen bonding between starch chains and, therefore, the chain aggregation and retrogradation of starch. This work indicates that the addition of IPA could be a promising way to modify the properties of starch-based foods by inducing the retrogradation of starch while enhancing the nutritional properties of these foods.

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Figure 5. SEM images of the freeze-dried NCS samples containing different levels (0%, 1%, 5%, and 10%) of IPA after storage at 4 °C for 0, 1, and 7 days. Magnifications of 500X; scale bars of 100 μm.
Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded by Guangdong Simiao Rice Industrial Clusters (Grant Yue Nong Han [2020] No. 840), the Sail Plan for Talents Development (Grant 2017YT05H077), and the Yunnan Academician (Expert) Workstation Project, Science and Technology Talent and Platform Program (Grant 20190SF150011).

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