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Changes in crystal structure of chickpea starch samples during processing treatments: An X-ray diffraction and starch moisture analysis study

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ABSTRACT

To detect more credibly the changes in the crystal structure of chickpea starch during processing treatments, two methods, a deconvolution method based on X-ray diffraction (XRD) pattern of starch and a moisture analysis method based on starch moisture content, were applied to determine the relative crystallinity (*RC*) of A- and B-type polymorphs (RC_A and RC_B) in the same chickpea starch sample. It was found that the values of RC_A for chickpea starch samples determined by these two methods were close and showed similar trend. The results suggested that these two methods could be used to estimate RC_A in the same chickpea starch sample and provide mutual corroboration. Based on the deconvolution method, it was observed that the crystalline region of chickpea starch was less susceptible to α-amylase hydrolysis than its amorphous region, and B-type polymorph in chickpea starch was more resistant to α-amylase hydrolysis than A-type polymorph.

1. Introduction

Chickpea (*Cicer arietinum* L.) is the third important pulse crop in the world and is cultivated mainly in the semi-arid regions of Asia and Africa (Hiremath et al., 2011; Jukanti, Gaur, Gowda, & Chibbar, 2012). It can be divided into two types according to the seed morphology: Desi variety with dark coated and small seed and Kabuli variety with light color coated and large seed (Chavan, Kadam, Salunkhe, & Beuchat, 1986; Jukanti et al., 2012). The grains of chickpea are rich in starch (37.2–50.8%) which is considered to be as a C-type starch (Chavan et al., 1986; Miao, Zhang, & Jiang, 2009; Xu et al., 2013).

Native starch granules are semi-crystalline with two polymorphic forms of A- and B-types that differ in their conformations and structural water contents (Buléon, Colonna, Planchot, & Ball, 1998; Imberty, Chanzy, Perez, Buleon, & Tran, 1988; Imberty & Perez, 1988; Sarko & Wu, 1978). Based on the types of polymorphs present in the granules, starches can be classified into A-type (A-type polymorph), B-type (B-type polymorph) and C-type (a mixture of A- and B-type polymorphs) starches which are mainly found in cereal, tuber and pulse starches, respectively (Hoover, Hughes, Chung, & Liu, 2010).

The proportions of A- and B-type polymorphs are considered to have impacts on the properties of C-type starch, specifically thermal property (Bogracheva, Wang, Wang, & Hedley, 2002; Tahir, Ellis, Bogracheva, Meares-Taylor, & Butterworth, 2011). At present, the proportions in C-type starch can be determined by X-ray diffraction (XRD) or differential scanning calorimetry (DSC) method. The XRD method is used to determine the proportions of A- and B-type polymorphs in C-type starch by analyzing their contribution in XRD pattern, while the DSC method is based on the difference in thermal properties between A- and B-type polymorphs (Bogracheva et al., 2002; Cairns, Bogracheva, Ring, Hedley, & Morris, 1997; Davydova, Leont'ev, Genin, Sasov, & Bogracheva, 1995; Gernat, Radosta, Anger, & Damaschun, 1993; Gernat, Radosta, Damaschun, & Schierbaum, 1990; Lopez-Rubio, Flanagan, Gilbert, & Gidley, 2008; Mutungi et al., 2011; Mutungi, Passauer, Onyango, Jaros, & Rohm, 2012). However, these two methods are not generally applied to analyze a same starch sample because XRD and DSC methods are only available for dry starches and wet starches, respectively (Bogracheva et al., 2002). In our previous study, we developed a new method for determining the relative crystallinity (*RC*) of chickpea native

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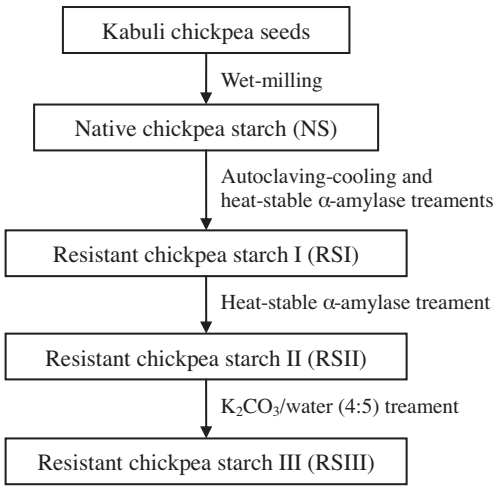

Fig. 1. General scheme for preparation of chickpea starch samples.

and resistant starches by using Fourier-transform infrared (FT-IR) spectroscopy (Sun et al., 2014). In order to detect more credibly the changes in crystal structure of chickpea starch during processing treatments, two methods were applied in the present study to determine the proportions of A- and B-type polymorphs in the same chickpea starch sample. One is a deconvolution method based on the XRD pattern of chickpea starch sample, another is a moisture analysis method based on the moisture content of chickpea starch sample.

2. Materials and methods

2.1. Materials and reagents

Kabuli chickpea seeds were provided by Xinjiang Agricultural University (Urumqi, Xinjiang, China). Amyloglucosidase from *Aspergillus niger* (100 U/mg) was purchased from Shanghai Kayon Biological Technology Co., Ltd (Shanghai, China) and heat-stable amylase from *Bacillus licheniformis* (20,000 U/mL) was purchased from Jiangsu Ruiyang Biotech Co., Ltd (Wuxi, Jiangsu, China). All other reagents were of analytical grade.

2.2. Preparation of chickpea starch

The preparation of chickpea starch samples was performed according to the reported method (Sun et al., 2014). The procedures for preparation of chickpea starch samples are outlined in Fig. 1. Briefly, native chickpea starch (NS) was prepared from Kabuli chickpea seeds by a wet-milling process. Resistant chickpea starch I (RSI) was prepared from NS by autoclaving treatment at 121 °C for 30 min, cooling treatment at 4 °C for 24 h and enzymatic digestion with heat-stable-amylase (pH 6.0, 500 U/g starch, 96 °C) for 30 min and amyloglucosidase (pH 4.5, 500 U/g starch, 59 °C) for 30 min. Resistant chickpea starch II (RSII) was obtained from RSI by extensive hydrolysis with heat-stable-amylase (5000 U/g wet basis, 96 °C, pH 6.0) for 24 h and amyloglucosidase (500 U/g starch, 59 °C, pH 4.5) for 30 min. Resistant chickpea starch III (RSIII) was isolated from RSII by the treatment with high concentration of K_2CO_3 solution (ratio of K_2CO_3/water, 4:5 (w/v)). All chickpea starch samples were vacuum freeze-dried under the same conditions.

2.3. Chemical analysis

The nitrogen content of chickpea starch sample was measured by an Elementar Vario EL II analyzer in duplicate, and the protein content of chickpea starch sample was calculated as 6.25 times of the nitrogen content. The starch content of chickpea starch sample was determined by the phenol-sulfuric acid method (Fox & Robyt, 1991) in triplicate. The moisture content of chickpea starch sample was determined gravimetrically by oven drying at 105 °C to a constant weight in triplicate.

2.4. XRD analysis

XRD analysis was carried out on a Bruker D8 Advance Diffractometer. The radiation used was Cu-Kα (wavelength of 0.15406 nm). The scan was performed at 40 kV and 40 mA for a 2θ range of 5–37° with a step size of 0.02° and a collection time of 6.7 s at each step. All analyses were performed in triplicate.

2.5. Data analysis

SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Significance of difference was tested at $P = 0.05$ by using ANOVA and Post Hoc Test (LSD).

3. Results and discussion

3.1. XRD analysis

As shown in Fig. 2a, NS, RSI, RSII and RSIII all displayed C-type crystallinity with a weak peak at 2θ about 6° (characteristic of B-type polymorphs) and strong peaks at 2θ of 17° and 23° (characteristic of A-type polymorphs) according to their XRD patterns (Hughes et al., 2009; Mutungi, Rost, Onyango, Jaros, & Rohm, 2009; Salman et al., 2009). The results indicated that the polymorphs of these starch samples all consisted of A- and B-type polymorphs. Furthermore, it was observed that the chickpea starch samples showed marked changes in their XRD pattern (Fig. 2a). Firstly, NS displayed a peak at 2θ about 15°, while the corresponding peak was not observed in RSI, RSII and RSIII. Secondly, RSI, RSII and RSIII exhibited a broader and higher peak at 2θ about 23° compared with NS. These results strongly indicated that the changes in the crystal structures of chickpea starch samples occurred due to processing treatments.

To detect the changes in crystal structure, a deconvolution method was performed to quantify *RC* and *RC* of the A- and B-type polymorphs (RC_A and RC_B, respectively) in chickpea starch samples according to the method reported by Lopez-Rubio et al. (2008) with slightly modifications. Taking RSI as an example, the steps of the deconvolution method were as followings: (1) the XRD pattern of RSI was smoothed with a Savitzky–Golay algorithm (8.0%) and baseline-corrected by drawing a tangent line between $2\theta = 5$–37° using PeakFit software version 4.12 (Seasolve Software Inc., CA, USA); (2) the smoothed curve was further baseline-corrected with the amorphous baseline which was acquired following the literature (Nara & Komiya, 1983) using MDI Jade 5.0 software (Materials Data Inc., Livermore, CA) and the resulted pattern represented the crystalline fraction (Fig. 2b); (3) the crystalline fraction pattern of RSI was modeled with 9 Gaussian function peaks at 2θ about 6°, 11°, 15°, 17°, 18°, 23°, 24°, 30° and 33° by using PeakFit software version 4.12 (Fig. 2c) and the model R^2 was at least 0.99; (4) the fitting peaks were determined the contribution from A- or B-type polymorph according to the approaching degree of the actual peak position and *RC* apportioned to peak to the respective standard peak (Table 1); and (5) the *RC* was the ratio of the area of crystalline fraction pattern to the area of the total XRD pattern according to the method described by Nara and Komiya (1983), the RC_A was the ratio of the sum area of peaks contributed from the A-type polymorphs to the area of the total XRD pattern and the RC_B was the ratio of the

Table 1
X-ray diffraction peaks and relative crystallinity apportioned to peak.

Sample	X-ray diffraction peaks at 2θ (°) and relative crystallinity (%) apportioned to peak										
	6	10	11	14	15	17	18	23	24	26	30	33
A-type polymorph[a]	N/A	9.98	11.19	N/A	15.11[b]	17.14[b]	18.14[b]	22.93	23.68	26.27[b]	30.30	33.08
B-type polymorph[a]	5.51	10.01	11.02	13.85	14.60	16.85	N/A	22.30	23.71	26.16	30.61	33.84
NS												
P_d	5.43 ± 0.22	9.63 ± 0.45	11.77 ± 0.09	nd	15.09 ± 0.11	17.46 ± 0.12	nd	nd	23.32 ± 0.20	nd	30.73 ± 0.77	33.23 ± 0.80
P_c	0.3 ± 0.3	0.3 ± 0.1	0.4 ± 0.1	nd	5.4 ± 2.7	13.3 ± 0.7	nd	nd	5.6 ± 2.3	nd	3.0 ± 1.7	2.7 ± 2.2
RSI												
P_d	5.97 ± 0.09	nd	11.07 ± 0.02	nd	15.14 ± 0.13	17.00 ± 0.07	18.80 ± 0.14	22.73 ± 0.11	24.38 ± 0.75	nd	30.81 ± 0.12	33.64 ± 0.55
P_c	1.1 ± 0.1	nd	1.2 ± 0.3	nd	9.4 ± 1.8	17.6 ± 1.7	4.2 ± 1.3	6.4 ± 1.9	2.5 ± 1.5	nd	3.8 ± 1.1	2.0 ± 0.7
RSII												
P_d	6.02 ± 0.16	nd	11.02 ± 0.04	nd	15.13 ± 0.16	17.02 ± 0.05	18.81 ± 0.27	22.69 ± 0.14	23.71 ± 0.39	25.94 ± 0.40	30.63 ± 0.11	33.50 ± 0.33
P_c	1.7 ± 0.8	nd	2.0 ± 0.6	nd	10.9 ± 1.3	18.6 ± 1.4	5.7 ± 2.5	5.0 ± 1.4	4.3 ± 1.7	1.1 ± 0.8	3.8 ± 0.5	3.0 ± 0.5
RSIII												
P_d	5.99 ± 0.45	nd	11.05 ± 0.04	nd	15.09 ± 0.06	17.01 ± 0.07	18.90 ± 0.29	22.66 ± 0.16	23.92 ± 0.23	25.87 ± 0.37	30.55 ± 0.13	33.62 ± 0.43
P_c	0.8 ± 0.4	nd	1.4 ± 0.8	nd	8.5 ± 1.5	19.3 ± 0.6	3.8 ± 1.1	6.7 ± 1.4	3.7 ± 1.4	1.3 ± 0.4	3.7 ± 0.6	3.3 ± 0.6

P_d, diffraction peak position; P_c, relative crystallinity apportioned to peak; N/A, not applicable; nd, not detected; the data with shading is contributed from the A-type polymorphs.
[a] Data adapted from the reference Lopez-Rubio et al. (2008).
[b] Strong diffraction peak.

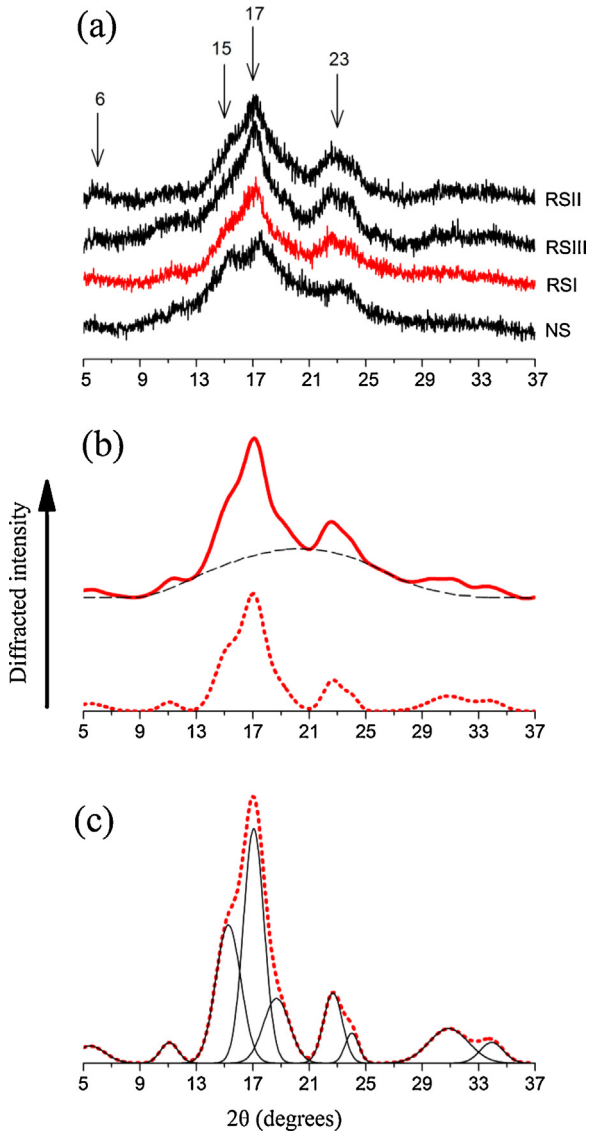

Fig. 2. The XRD patterns of chickpea starch samples. (a) the XRD patterns of NS, RSI, RSII and RSIII, (b) the curve-smoothed XRD pattern of RSI (solid line) fitted with the amorphous baseline (dash line) and the crystalline fraction pattern of RSI (dot line) which is the resulting XRD pattern baseline-corrected with the amorphous baseline, and (c) the crystalline fraction pattern of RSI (dot line) fitted with Gaussian function peaks (solid line).

sum area of peaks contributed from the B-type polymorphs to the area of the total XRD pattern (Table 2).

In order to compare the relative changes of B-type polymorphs in chickpea starch samples during processing treatments, the percentage of B-type polymorphs in total polymorphs (B_P) of the chickpea starch sample was calculated as the RC_B divided by the RC of the chickpea starch sample and listed in Table 2.

3.2. Moisture analysis

Water is distributed in different regions of starch granules and plays a crucial role in determination of the structure of starch granules such as structural water of the starch polymorphs and a plasticizer of the granule amorphous regions. However, the moisture content is not distributed uniformly in starch granules and affected by the components of starch granules (starch and non-starch solid components) and the regions of starch (amorphous, A-type crystalline and B-type crystalline

Table 2
The relative crystallinity of chickpea starch samples.

	Chickpea starch sample			
	NS	RSI	RSII	RSIII
RC (%)	31.0 ± 1.1a	48.2 ± 0.9b	56.2 ± 3.4d	52.5 ± 0.9c
RC_A (%)[A]	25.0 ± 1.1a	37.6 ± 1.1b	40.3 ± 4.3b	38.3 ± 0.8b
RC_B (%)[A]	6.0 ± 0.4a	10.6 ± 1.0b	15.9 ± 1.6c	14.2 ± 1.3c
B_P (%)[A]	19.23 ± 1.16a	22.03 ± 1.97a	28.43 ± 3.86b	27.03 ± 2.04b
RC_A (%)[B]	26.0–31.0	31.4–36.4	34.1–39.9	32.1–36.7

RC, the relative crystallinity; RC_A, the relative crystallinity of A-type polymorphs; RC_B, the relative crystallinity of B-type polymorphs; B_P, the percentage of B-type polymorphs in total polymorphs. Different small letters in the same line indicate significant difference at the 0.05 level.
[A] Acquired from the deconvolution method.
[B] Acquired from the moisture analysis method.

regions) (Bogracheva, Wang, & Hedley, 2001; Hartley, Chevance, Hill, Mitchell, & Blanshard, 1995; Imberty et al., 1988; Imberty & Perez, 1988; Sarko & Wu, 1978). If we can determine the partitioning of water in different components and regions of starch in starch granules, the relative amounts of A- and B-type polymorphs of starch granules can be calculated from the moisture content of starch granules.

3.2.1. Partitioning of water in starch and non-starch solid components

In order to estimate the moisture contents in starch and non-starch solid components of chickpea starch samples, the following considerations were taken into account: chemical composition and the partitioning of water in solid components. As shown in Table 3, the chickpea starch samples mainly consisted of starch, protein and moisture. According to the reports, the samples could be ranked by decreasing the equilibrium moisture contents in the same dry environment: starch ≥ the natural or artificial mixtures (mainly consisted of starch and protein) > protein (Hartley et al., 1995; Riganakos & Kontominas, 1997; Roman-Gutierrez, Guilbert, & Cuq, 2002). As freeze-dried solid protein is an amorphous material (Hill, Shalaev, & Zografi, 2005; Wang, Tchessalov, Warne, & Pikal, 2009), the protein in chickpea starch sample could be considered to be in amorphous form and it would not affect the crystal structure analysis of chickpea starch sample. Therefore, the moisture content associated with starch in the chickpea starch sample (M_{SC}) could be estimated by the following inequality: $M_{SC} \geq M_C \times S_C/(100 - M_C)$, where M_C and S_C are the moisture content and starch content of the chickpea starch sample, respectively. As a result, the M_{SC} of chickpea starch samples are presented in Table 3.

Table 3
Chemical compositions and M_{SC} of chickpea starch samples.

Composition (%)	Starch sample			
	NS	RSI	RSII	RSIII
Protein	1.00 ± 0.07	2.26 ± 0.46	7.81 ± 0.16	3.61 ± 0.08
Starch	92.65 ± 0.13	90.16 ± 0.80	85.16 ± 1.58	89.87 ± 0.28
Moisture	2.56 ± 0.18	5.76 ± 0.39	7.23 ± 0.30	6.71 ± 0.22
M_{SC}	2.43–2.56	5.51–5.76	6.63–7.23	6.47–6.71

M_{SC}, the moisture content associated with starch in the chickpea starch sample.

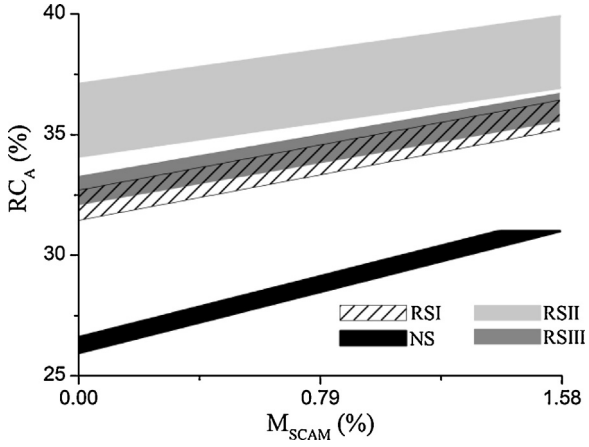

Fig. 3. The RC_A of the chickpea starch samples at different M_{SCAM}.

3.2.2. Partitioning of water in amorphous and crystalline regions of starch

It has been reported that the structural water content is fixed in A-type polymorphs (about 5%) and B-type polymorphs (about 25%) (Imberty et al., 1988; Imberty & Perez, 1988; Sarko & Wu, 1978). Therefore, we assumed that the moisture content is 5% in A-type polymorphs and 25% in B-type polymorphs, and the moisture contents are the same in amorphous regions of starch samples dried under the same condition. Then the RC_A of chickpea starch samples could be obtained by the following linear equations (1):

$$\begin{cases} M_{SC}^{NS} = 0.05 \times RC_A^{NS} + 0.25 \times (RC^{NS} - RC_A^{NS}) + (S_C^{NS} + M_{SC}^{NS} - RC^{NS}) \times M_{SCAM} \\ M_{SC}^{RSI} = 0.05 \times RC_A^{RSI} + 0.25 \times (RC^{RSI} - RC_A^{RSI}) + (S_C^{RSI} + M_{SC}^{RSI} - RC^{RSI}) \times M_{SCAM} \\ M_{SC}^{RSII} = 0.05 \times RC_A^{RSII} + 0.25 \times (RC^{RSII} - RC_A^{RSII}) + (S_C^{RSII} + M_{SC}^{RSII} - RC^{RSII}) \times M_{SCAM} \\ M_{SC}^{RSIII} = 0.05 \times RC_A^{RSIII} + 0.25 \times (RC^{RSIII} - RC_A^{RSIII}) + (S_C^{RSIII} + M_{SC}^{RSIII} - RC^{RSIII}) \times M_{SCAM} \end{cases} \quad (1)$$

where M_{SC}^{NS}, RC^{NS}, RC_A^{NS} and S_C^{NS} are M_{SC}, RC, RC_A and starch content of NS, respectively, and the rest may be deduced by analogy, M_{SCAM} is the moisture content of amorphous region in starch.

When the M_{SC}, RC and starch content of chickpea starch samples were applied to the linear equations (1), we obtained the value ranges of M_{SCAM} (0.00–1.57%) and RC_A of chickpea starch samples which are shown in Table 2 and Fig. 3, respectively.

3.3. Comparison of the two methods

It was found that the RC_A of chickpea starch samples acquired from the deconvolution method were close to those obtained from the moisture analysis method (Table 2). Moreover, the RC_A of chickpea starch samples determined by these two methods showed the same trend which followed the order: RSII > RSIII ≈ RSI > NS (Table 2 and Fig. 3). The results suggested that the two methods could be used to estimate the RC_A in the same chickpea starch sample and provide mutual corroboration.

The RC_A acquired from the deconvolution method was not located within the value range of RC_A obtained from the moisture analysis method, the reason was possible due to that the moisture contents of amorphous, A- and B-type polymorphs were slightly overestimated or underestimated in different chickpea starch samples.

3.4. Changes in crystal structure of chickpea starch samples during processing treatments

RSI was prepared from NS by enzymatic digestion after autoclaving-cooling. It was found that the RC, RC_A and RC_B of RSI

significantly increased and the B_P showed a small increase compared with NS. These changes might be related with the preparation process of RSI which was produced by three main steps: gelatinization, retrogradation and enzymatic digestion. After gelatinization, the starch gel was stored at 4 °C for retrogradation which might be favored to the formation of B-type polymorphs in starch (Shamai, Bianco-Peled, & Shimoni, 2003; Shamai, Shimoni, & Bianco-Peled, 2004). Then the starch gel was digested by amylase which occurred mainly in the amorphous region and had a lower digestion rate for B-type polymorphs than A-type polymorphs (Gerard, Colonna, Buleon, & Planchot, 2001; Kim, Park & Lim, 2008; Naguleswaran, Vasanthan, Hoover & Bressler, 2013; Planchot, Colonna & Buleon, 1997; Planchot, Colonna, Gallant & Bouchet, 1995).

RSII was obtained from RSI by extensive hydrolysis with α-amylase. It was found that the *RC*, RC_B and B_P of RSII significantly increased while the RC_A increased only slightly compared with RSI. The results showed that the crystalline region of RSI was less susceptible to α-amylase hydrolysis than its amorphous region, and moreover, B-type polymorph in RSI was more resistant to α-amylase hydrolysis than A-type polymorph. This conclusion is consistent with the literatures (Gerard et al., 2001; Kim et al., 2008; Naguleswaran et al., 2013; Planchot et al., 1995, 1997).

RSIII was prepared from RSII by the treatment with high concentration of K_2CO_3 solution. It was observed that the *RC* value of RSIII showed a significant decrease whereas the RC_A, RC_B and B_P did not show significant difference compared those of RSII. The results indicated that A-type polymorph was not as stable as B-type polymorph in the high concentration of K_2CO_3 solution.

4. Conclusions

In the present study, a deconvolution method based on starch XRD pattern and a moisture analysis method based on starch moisture content were applied to determine RC_A and RC_B in the same chickpea starch sample. The results indicated that both methods could be used to estimate the RC_A in the same chickpea starch sample and provide mutual corroboration. Furthermore, the present results demonstrated that (1) the crystalline region of chickpea starch was less susceptible to α-amylase hydrolysis than its amorphous region, and B-type polymorph in chickpea starch was more resistant to α-amylase hydrolysis than A-type polymorph; (2) A-type polymorph of chickpea starch was not as stable as B-type polymorph in the high concentration of K_2CO_3 solution.

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References

Bogracheva, T. Y., Wang, Y. L., & Hedley, C. L. (2001). The effect of water content on the ordered/disordered structures in starches. *Biopolymers*, *58*(3), 247–259.
Bogracheva, T. Y., Wang, Y. L., Wang, T. L., & Hedley, C. L. (2002). Structural studies of starches with different water contents. *Biopolymers*, *64*(5), 268–281.
Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules*, *23*(2), 85–112.
Cairns, P., Bogracheva, T. Y., Ring, S. G., Hedley, C. L., & Morris, V. J. (1997). Determination of the polymorphic composition of smooth pea starch. *Carbohydrate Polymers*, *32*(3), 275–282.
Chavan, J. K., Kadam, S. S., Salunkhe, D. K., & Beuchat, L. R. (1986). Biochemistry and technology of chickpea (*Cicer arietinum* L.) seeds. *Critical Reviews in Food Science and Nutrition*, *25*(2), 107–158.
Davydova, N. I., Leont'ev, S. P., Genin, Y. V., Sasov, A. Y., & Bogracheva, T. Y. (1995). Some physico-chemical properties of smooth pea starches. *Carbohydrate Polymers*, *27*(2), 109–115.
Fox, J. D., & Robyt, J. F. (1991). Miniaturization of three carbohydrate analyses using a microsample plate reader. *Analytical Biochemistry*, *195*(1), 93–96.
Gerard, C., Colonna, P., Buleon, A., & Planchot, V. (2001). Amylolysis of maize mutant starches. *Journal of the Science of Food and Agriculture*, *81*(13), 1281–1287.
Gernat, C., Radosta, S., Anger, H., & Damaschun, G. (1993). Crystalline parts of three different conformations detected in native and enzymatically degraded starches. *Starch-Starke*, *45*(9), 309–314.
Gernat, C., Radosta, S., Damaschun, G., & Schierbaum, F. (1990). Supramolecular structure of legume starches revealed by X-ray scattering. *Starch-Starke*, *42*(5), 175–178.
Hartley, L., Chevance, F., Hill, S. E., Mitchell, J. R., & Blanshard, J. M. V. (1995). Partitioning of water in binary biopolymer mixtures at low water content. *Carbohydrate Polymers*, *28*(2), 83–89.
Hill, J. J., Shalaev, E. Y., & Zografi, G. (2005). Thermodynamic and dynamic factors involved in the stability of native protein structure in amorphous solids in relation to levels of hydration. *Journal of Pharmaceutical Sciences*, *94*(8), 1636–1667.
Hiremath, P. J., Farmer, A., Cannon, S. B., Woodward, J., Kudapa, H., Tuteja, R., Kumar, A., BhanuPrakash, A., Mulaosmanovic, B., & Gujaria, N. (2011). Large-scale transcriptome analysis in chickpea (*Cicer arietinum* L.), an orphan legume crop of the semi-arid tropics of Asia and Africa. *Plant Biotechnology Journal*, *9*(8), 922–931.
Hoover, R., Hughes, T., Chung, H. J., & Liu, Q. (2010). Composition, molecular structure, properties, and modification of pulse starches: A review. *Food Research International*, *43*(2), 399–413.
Hughes, T., Hoover, R., Liu, Q., Donner, E., Chibbar, R., & Jaiswal, S. (2009). Composition, morphology, molecular structure, and physicochemical properties of starches from newly released chickpea (*Cicer arietinum* L.) cultivars grown in Canada. *Food Research International*, *42*(5), 627–635.
Imberty, A., Chanzy, H., Perez, S., Buleon, A., & Tran, V. (1988). The double-helical nature of the crystalline part of A-starch. *Journal of Molecular Biology*, *201*(2), 365–378.
Imberty, A., & Perez, S. (1988). A revisit to the three-dimensional structure of B-type starch. *Biopolymers*, *27*(8), 1205–1221.
Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. *British Journal of Nutrition*, *108*(S1), S11–S26.
Kim, J. Y., Park, D. J., & Lim, S. T. (2008). Fragmentation of waxy rice starch granules by enzymatic hydrolysis. *Cereal Chemistry*, *85*(2), 182–187.
Lopez-Rubio, A., Flanagan, B. M., Gilbert, E. P., & Gidley, M. J. (2008). A novel approach for calculating starch crystallinity and its correlation with double helix content: A combined XRD and NMR study. *Biopolymers*, *89*(9), 761–768.
Miao, M., Zhang, T., & Jiang, B. (2009). Characterisations of kabuli and desi chickpea starches cultivated in China. *Food Chemistry*, *113*(4), 1025–1032.
Mutungi, C., Onyango, C., Doert, T., Paasch, S., Thiele, S., Machill, S., Jaros, D., & Rohm, H. (2011). Long- and short-range structural changes of recrystallised cassava starch subjected to *in vitro* digestion. *Food Hydrocolloids*, *25*(3), 477–485.
Mutungi, C., Passauer, L., Onyango, C., Jaros, D., & Rohm, H. (2012). Debranched cassava starch crystallinity determination by Raman spectroscopy: Correlation of features in Raman spectra with X-ray diffraction and ^{13}C CP/MAS NMR spectroscopy. *Carbohydrate Polymers*, *87*(1), 598–606.
Mutungi, C., Rost, F., Onyango, C., Jaros, D., & Rohm, H. (2009). Crystallinity, thermal and morphological characteristics of resistant starch type III produced by hydrothermal treatment of debranched cassava starch. *Starch-Starke*, *61*(11), 634–645.
Naguleswaran, S., Vasanthan, T., Hoover, R., & Bressler, D. (2013). The susceptibility of large and small granules of waxy, normal and high-amylose genotypes of barley and corn starches towards amylolysis at sub-gelatinization temperatures. *Food Research International*, *51*(2), 771–782.
Nara, S., & Komiya, T. (1983). Studies on the relationship between water-satured state and crystallinity by the diffraction method for moistened potato starch. *Starch-Starke*, *35*(12), 407–410.
Planchot, V., Colonna, P., & Buleon, A. (1997). Enzymatic hydrolysis of α-glucan crystallites. *Carbohydrate Research*, *298*(4), 319–326.
Planchot, V., Colonna, P., Gallant, D. J., & Bouchet, B. (1995). Extensive degradation of native starch granules by alpha-amylase from *Aspergillus fumigatus*. *Journal of Cereal Science*, *21*(2), 163–171.
Riganakos, K. A., & Kontominas, M. G. (1997). Study of water sorption of flours (wheat and soy) using a hygrometric method: effect of relative humidity during heat treatment. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, *204*(5), 369–373.
Roman-Gutierrez, A. D., Guilbert, S., & Cuq, B. (2002). Distribution of water between wheat flour components: A dynamic water vapour adsorption study. *Journal of Cereal Science*, *36*(3), 347–355.
Salman, H., Blazek, J., Lopez-Rubio, A., Gilbert, E. P., Hanley, T., & Copeland, L. (2009). Structure-function relationships in A and B granules from wheat starches of similar amylose content. *Carbohydrate Polymers*, *75*(3), 420–427.
Sarko, A., & Wu, H. C. H. (1978). The crystal structures of A-, B- and C-polymorphs of amylose and starch. *Starch-Starke*, *30*(3), 73–78.
Shamai, K., Bianco-Peled, H., & Shimoni, E. (2003). Polymorphism of resistant starch type III. *Carbohydrate Polymers*, *54*(3), 363–369.
Shamai, K., Shimoni, E., & Bianco-Peled, H. (2004). Small angle X-ray scattering of resistant starch type III. *Biomacromolecules*, *5*(1), 219–223.
Sun, Y., Wu, Z., Hu, B., Wang, W., Ye, H., Sun, Y., et al. (2014). A new method for determining the relative crystallinity of chickpea starch by Fourier-transform infrared spectroscopy. *Carbohydrate Polymers*, *108*, 153–158.

Tahir, R., Ellis, P. R., Bogracheva, T. Y., Meares-Taylor, C., & Butterworth, P. J. (2011). Study of the structure and properties of native and hydrothermally processed wild-type, lam and r variant pea starches that affect amylolysis of these starches. *Biomacromolecules*, *12*(1), 123–133.

Wang, B., Tchessalov, S., Warne, N. W., & Pikal, M. J. (2009). Impact of sucrose level on storage stability of proteins in freeze-dried solids: I. correlation of protein-sugar interaction with native structure preservation. *Journal of Pharmaceutical Sciences*, *98*(9), 3131–3144.

Xu, Y., Sismour, E. N., Narina, S. S., Dean, D., Bhardwaj, H. L., & Li, Z. (2013). Composition and properties of starches from Virginia-grown kabuli chickpea (*Cicer arietinum* L.) cultivars. *International Journal of Food Science and Technology*, *48*(3), 539–547.