New Proposal for "Crystalline Index" of Starch

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Abstract: In starch science, X-ray diffraction (XRD) has been solely used for the evaluation of starch type and/or the content of crystalline and non-crystalline (amorphous) materials in starch. However, XRD allows us to estimate the crystalline regularity and deficit. In this study, we newly propose a "crystalline index" of starch that was introduced by peak height and the full-width at half maximum (FWHM) by means of XRD to evaluate the destruction or discrimination of amylose and amylopectin in the starch production process of plants. Amylose, amylopectin, corn, potato and sago starch samples were used in this study. The crystalline index of amylose, amylopectin and other starch samples ranged from 609 to 1266, in the order of amylose > amylopectin > corn > potato > sago.

Keywords: crystalline index, sago starch, wave-form separation, X-ray diffraction

Introduction

Starch is a mixture of amorphous and crystalline components. The crystalline components of starch are composed of amylose and amylopectin. Amylose is a linear and slightly branched $(1\rightarrow4)$-α-D-glucan and amylopectin is a highly branched $(1\rightarrow4)$-α-D-glucan and $(1\rightarrow6)$-α-D-glucan with linear or slightly branched molecules (Cheetham and Tao, 1998). X-ray diffraction (XRD) revealed the crystalline structure of starch (Scherrer, 1920; Katz and van Itallie, 1930). Katz and van Itallie (1930) found the crystalline materials in starch grains and divided starch into three types, A, B, and C, based on XRD patterns. Meanwhile, Bear and French (1941) showed that starches are typical A type, B type and the complex of A/B type and insisted that there was no C type starch. Rundle et al. (1941) also developed the starch structure research, especially the relative amount of crystalline materials in starch using XRD, because the relative crystallinity of starch was related to the functions of starch, such as solubility, gelatinization and retrogradation. Nikuni et al. (1948) revealed the results of XRD of different starches; potato, tulip, lily, scilla, red spider lily, lotus, taro, sweet potato, red

bean, rice, wheat, buckwheat, cassava, and corn. In addition Hizukuri and Nikuni (1957a) reported the XRD patterns of potato, corn and rice starches with different moisture contents and concluded that rice and corn starch showed A type while potato starch is B type. Hizukuri and Nikuni (1957b) clearly found that C type was the mixture of A and B type with different ratios, based on the XRD pattern. A type is a close-packed arrangement (monoclinic system) with water molecules between each double helical structure, and B type is a hexagonal system, which has a central cavity surrounded by six double helices (Wu and Sarko, 1978a, b; Imberty and Perez, 1988; Imberty et al., 1988). Moreover, Paris et al. (1999) revealed that both A and B type starchs showed sharper peaks in XRD with increasing moisture content. Meanwhile the addition of amylose from 0 to 84 % of total weight provided the broader peaks of maize starch (Cheetham and Tao, 1998).

The definition of the relative crystallinity of starch in starch science is the percentages of crystal material to total starch. The relative crystallinity of starch, therefore, has been argued for a long time among starch scientists. It is still difficult to discuss the

relative crystallinity of starch, because XRD peaks of starch were not assigned to the specific starch structure and reflection surface completely. Accordingly, determination of the relative crystallinity of starch by the separation of XRD intensity using a smoothing curve method was proposed to estimate the percentages of crystalline materials to total materials of the starch samples (Nara, 1978; Nara and Komiya, 1983; Cheetham and Tao, 1998). This method has been accepted in the field of starch science and used the name "relative crystallinity". In this study, we try to propose the new viewpoint on the concept of crystalline structure in starch. The crystalline index is one of the indexes of the regular structure and the definite direction of crystalline materials, such as amylose and amylopectin. When amylose and amylopectin lost their regular structure or held voids, the crystalline index decreased.

Sago starch grains are temple-bell-shaped and 10 to 65 µm (mean: 31 µm) in diameter (Takahashi et al., 1981; Ahmad et al., 1999; Karim et al., 2008). Under a polarized microscope the Maltese cross, derived from crystalline materials, was observed in the granule (Kobayashi, 1993; Hibi et al., 1993). Takahashi et al. (1981) showed that the XRD pattern of sago starch was C type, the intermediate type between A and B types with a 3:1 ratio. Kawabata et al. (1984) also revealed that sago starch was the mixture of A and B types based on the X-ray peaks. The XRD pattern of sago starch at various portions of the trunk at different growth stages (9 to 14.5 years after transplanting) and the relative crystallinity of sago starch corrected by the internal standard of CaF_2, ranging from 22 to 40°, were reported (Hamanishi et al., 1999; Hamanishi et al., 2000).

XRD was an important tool for elucidation of the crystalline structure of starches, which deeply related to the functions of starches. We propose a new concept of the crystalline index of starch by the method of determination using the XRD intensity after wave-form separations.

Materials and Methods

1. Starch

Corn, potato and sago starches, as well as amylose and amylopectin, were used in this study. Corn starch was purchased from Kosakai Pharmaceutical Co., Ltd., which was certificated under Japanese Pharmacopoeia. Potato starch was from Miyazawa Pharmaceutical Co., Ltd. Sago starch samples were collected in Leyte, Philippines, in 2006. Amylose and amylopectin were obtained from Sigma-Aldrich, Inc., and MP Biomedicals, Inc., respectively. The moisture contents of amylose and amylopectin and of corn, potato and sago starches were 12.2, 10.5, 3.80, 8.69, and 12.4% (w/w), respectively.

2. XRD and crystalline index

The X-ray identification was carried out by an X-ray diffractometer (Mini Flex, Rigaku) operating at 30 kV and 15 mA. Copper (Cu) Kα radiation was used with a nickel filter. The scanning speed was recorded at 2° min^{-1}. The operation angle was 3 to 45° for 2θ. XRD data were treated with the Lorentz polarization factor correction, the scattering factor correction, and the absorption factor correction (Rigaku, 1962). After those corrections, the smoothing treatment was done using a weighted mean method (smoothing number: 11). The background subtraction was done using the Sonneveld-Visser method (peak width: 0.25, threshold intensity: 0.01), and the wave-form separation was introduced in the diffraction angle ranging from 12 to 28°. Peak fitting was done using Gauss function. The wave-form separation was carried out by PeakFit version 4.12 (SeaSolve Software Inc., Framingham, MA, USA). The peak number was named after Hamanishi et al. (2000).

The crystalline index of starch was determined by height and the full-width at half maximum (FWHM) of the third b (3b) and fourth a (4a) peaks after the correction and wave-form separation.

Crystalline index = peak height $_{3b}$ / FWHM $_{3b}$ + peak height $_{4a}$ / FWHM $_{4a}$ $\qquad$ (1)

Results and Discussion

### 1. XRD pattern of starches and their analysis

XRD patterns of sago starch and other starches are shown in Fig. 1. Sago starch showed diffraction peaks
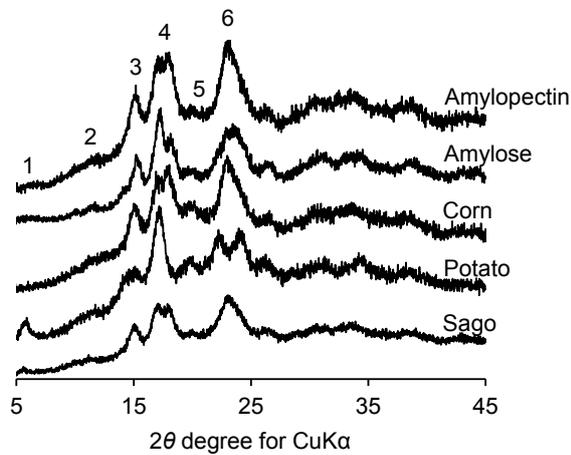
Fig. 1. X-ray diffraction patterns of sago starch
Amylopectin: MP Biomedicals Inc.
Amylose: Sigma-Aldrich Inc.
Corn: Kosakai Pharmaceutical Co.
Potato: Miyazawa Pharmaceutical Co.
Sago: taken from the Philippines
The peak numbers are named after Hamanishi et al. (2000)

at 5.6, 17, 18 and 23° (2θ) for Cu Kα radiation, which corresponded to 1.6, 0.52, 0.49, and 0.39 nm, respectively, for reflection surface distance at the specific phase. Hatta (2001) ascribed the Miller index on XRD peaks of corn and potato starches according to the atomic coordinates of Imberty et al. (1988) and Imberty and Perez (1988), who suggested that the peak at 5.6° (2θ) for Cu Kα was attributed to 1.6 nm in the reflection surface distance of potato starch. The XRD pattern indicated the complex of A/B type starch. The third and fourth peaks of the XRD of sago starch showed the doublet peak (the third a (3a) and b (3b), and the fourth a (4a) and b (4b)). The doublet peaks will be separated by the following steps. The XRD peaks of sago starch generally showed a mixture of different diffraction surfaces. The main sharp peaks at around 15° (3a and 3b) and 17/18° (4a and 4b) were selected to evaluate the crystalline index of starch, because those peaks commonly belonged to both A and B type starches.
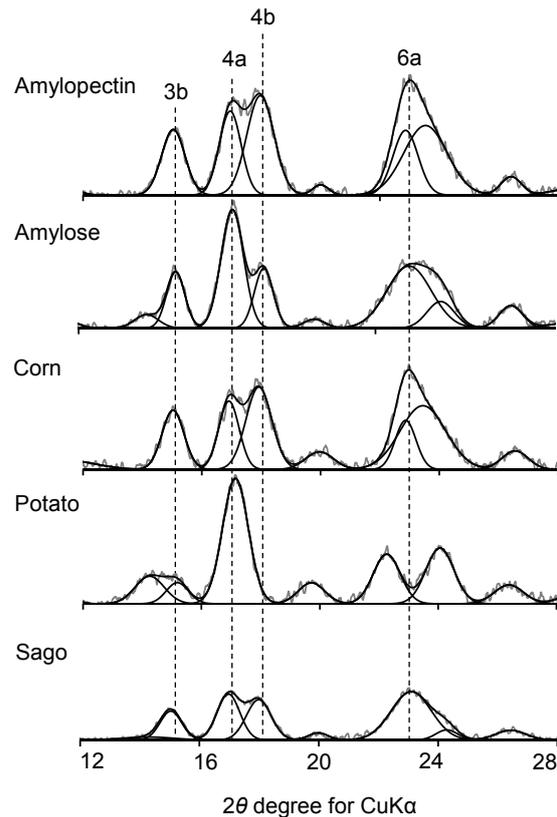
Fig. 2. X-ray diffraction after wave-form separation
Amylopectin: MP Biomedicals Inc.
Amylose: Sigma-Aldrich Inc.
Corn: Kosakai Pharmaceutical Co.
Potato: Miyazawa Pharmaceutical Co.
Sago: taken from the Philippines

Figure 2 shows the results of the XRD intensity of sago starch after wave-form separation. Seven to eight peaks were obtained at 12 to 28° (2θ). The two main peaks at 3b and 4b were clearly separated by the wave-form separation. Hamanishi et al. (2000) used CaF$_2$ as a standard to evaluate the relative crystallinity of sago starch and selected the 3b and 4b peaks to calculate the relative crystallinity based on the relative intensity of XRD. However, the 3b peak and the 4a peak, commonly presented in A and B type starches, were sharp, easily separated and relatively stable.

2. Selection of an XRD peak to evaluate the crystalline index

Starch shows the XRD peaks, which means the presence of crystalline materials; amylose and amylopectin. The main and important peaks, which relate to the characteristics of starch function;

gelatinization and retrogradation, should be selected to evaluate the crystalline index of amylose and amylopectin among the XRD peaks ranging from 12 to 28° (2θ). The first peak of XRD is lost in the A type starch, so that the first peak is not profitable for calculating the crystalline index. The second peak of both A and B type starches is too small to evaluate the crystalline index. The 3b peaks for A and B type starches after peak separation are suitable to evaluate the crystalline index. In addition the 4a peak is clear and sharp after peak separation and suitable for evaluation of the crystalline index. The fifth and seventh peaks are small and thus not suitable for evaluation of the crystalline index. Finally, we chose the 3b and 4a peaks to calculate the crystalline index.

3. Crystalline index

The crystalline index of sago starch is shown in Fig. 3. The values of the crystalline index of starch
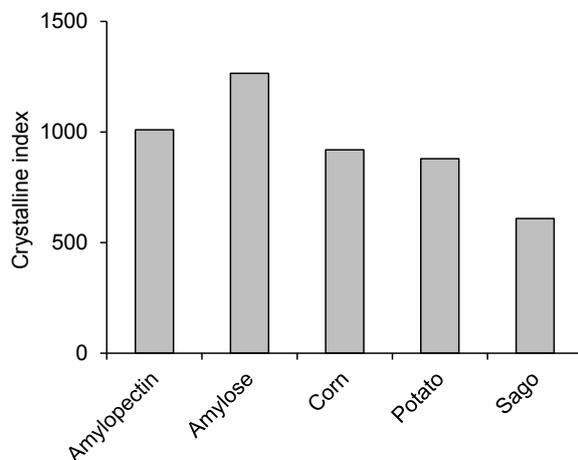
Fig. 3. Crystallinity index of starch

calculated from eq. (1) ranged from 609 to 1266, in the order of amylose (1226) > amylopectin (1010) > corn (919) > potato (879) > sago (609). The crystalline index will be related to the gelatinization and retrogradation of sago starch and other starches.

The FWHM was extremely changed by the moisture content of potato starch (0 to 40 %) and that of corn starch (0 to 47.5 %) (Hizukuri et al., 1964). The increase of moisture content of starch provided

the decrease in FWHM of corn and potato, whilst the decrease in moisture content gave rise to the decrease in FWHM. This means that the presence of water molecules allowed starch to hold the normal crystalline structure.

4. Importance of the crystalline index during the starch deposition process in cytoplasm

The The biosynthetic system of starch granules in the amyloplast falls into the deposition process by the simultaneous activity of the two kinds of starch synthases, a granule-bound starch synthase and a soluble starch synthase, and a branching enzyme (Akazawa, 1965). When the solid starches consisting of the ADP-glucose produced by the starch synthases from the solution of amyloplast provide both A and B type commonly, the starch is considered to be C type, which is not a special case.

Conclusion

The "crystalline index" of starch was calculated by the width and height of 3b and 4a peaks of the XRD pattern. This is the first trial for the crystalline index for sago starch, which is applicable to other starches.

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Appendix

Curve fitting and wave-form separation of X-ray diffraction and crystalline index

1. Corrected XRD data are introduced to PeakFit (Ver. 4.12, SeaSolve Software Inc., Framingham, MA, USA). After that the baseline of XRD should be determined. Although an automatic function of baseline is built into PeakFit, one should manually select appropriate baseline.

2. AutoPeakFit II Second Derivative mode is used for curve fitting and wave-form separation of X-ray diffraction; 4 to 8 % of smoothing range, after determining the baseline. Select Gauss Area in Spectroscopy for Peak type and Vary Widths in Auto Scan.

3. Delete the small peaks that appeared by means of a secondary differentiation. Meanwhile, for example, peak 6a, which shows a non-symmetric and assembled peak, conventionally comprises two peaks for the purpose of better fitting.

4. The curve fitting and wave-form separation of X-ray diffraction continue to repeat until they converge.

5. Finally calculate the crystalline index using the equation of Σ height of peak 3b and peak 4a / the full-width at half maximum of peak 3b and peak 4a.