Carbohydrate Polymers 158 (2017) 29–36

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

An improved approach for evaluating the semicrystalline lamellae of starch granules by synchrotron SAXS

Binjia Zhang[a,e], Fengwei Xie[c], David K. Wang[c], Siming Zhao[a], Meng Niu[a], Dongling Qiao[b,*], Shanbai Xiong[a], Fatang Jiang[b], Jie Zhu[d], Long Yu[e]

[a] Laboratory of Environment Correlative Dietology (Ministry of Education), College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
[b] Glyn O. Philips Hydrocolloid Research Centre at HUT, Hubei University of Technology, Wuhan 430068, China
[c] School of Chemical Engineering, The University of Queensland, Brisbane, Qld 4072, Australia
[d] College of Chemistry and Environmental Engineering, Dongguan University of Technology, Dongguan 523808, China
[e] Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, South China University of Technology, Guangzhou 510640, China

ARTICLE INFO

Article history:
Received 29 August 2016
Received in revised form
24 November 2016
Accepted 1 December 2016
Available online 2 December 2016

Chemical compounds studied in this article:
Starch (PubChem CID: 24836924)
Water (PubChem CID: 962)

Keywords:
Starch
Granule
Semicrystalline lamellae
X-ray scattering
Methodology

ABSTRACT

A fitting method combined with a linear correlation function was developed as an improved approach for the SAXS analysis of the semicrystalline lamellae of starch granules. Using a power-law function with two Gaussian plus Lorentz functions, the SAXS pattern was resolved into sub-patterns of the net lamellar peak and the power-law scattering plus scattering background ($PL+B$). The ratio of the net lamellar peak area (A_{peak}) to the total scattering area (A_{total}) was proposed equal to the proportion of the lamellae within the starch granule (P_{SL}). Along with this fitting method, we obtained a better profile of linear correlation function, with the elimination of the interference of non-lamellar amorphous starch (*i.e.*, amorphous growth rings). Then, we could accurately calculate the lamellar parameters, *e.g.*, P_{SL}, the thicknesses of semicrystalline (d), crystalline (d_c) and amorphous (d_a) lamellae, and the volume fraction (φ_c) of crystalline lamellae within semicrystalline lamellae. Quantitative analysis revealed that P_{SL} was positively correlated with the crystallinity (X_c) of starch. It was confirmed that the distribution of lamellar thickness was more important than the starch botanical origin in affecting the validity of the developed fitting method. We also proposed a criterion to test the validity of the proposed method. Specifically, the total SAXS pattern should be mostly tangent to the profile of $PL+B$ at a high q tail (close to 0.2 $Å^{-1}$).

1. Introduction

As the main storage carbohydrate in higher plants, starch is normally used as a food ingredient providing energy for humans (Juansang, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2012). Also, starch has attracted huge interest in the development of functional foods (Fuentes-Zaragoza et al., 2011), bioactive carriers (Pu et al., 2011) and biomaterials (Situ, Li, Liu, & Chen, 2015). There are two kinds of starch polymers, *i.e.*, amylose and amylopectin (Jiang, Gao, Li, & Zhang, 2011; Liu, Halley, & Gilbert, 2010). These two biopolymers are organized on multiple scales in the starch granule to form its semicrystalline structure, including the whole granule, the growth rings, the semicrystalline lamellae and the crystallites (Buleon, Colonna, Planchot, & Ball, 1998; Luengwilai and Beckles, 2009; Perez & Bertoft, 2010; Pikus, 2005; Zhang, Chen, Xie et al., 2015). The semicrystalline structural features of starch such as crystallinity and lamellar ordering are crucial in the determination of the physicochemical properties, *e.g.*, digestibility (Blazek & Gilbert, 2010; Lopez-Rubio, Flanagan, Shrestha, Gidley, & Gilbert, 2008) and thermal behaviors (Liu, Xie, Yu, Chen, & Li, 2009; Xie, Halley, & Avérous, 2012). Thus, to understand a specific functionality of starch, analytical techniques should be used to accurately evaluate the semicrystalline structure of starch.

Small-angle X-ray scattering (SAXS) is a powerful technique for the characterization of starch lamellae on the nanoscale (Doutch & Gilbert, 2013; Lopez-Rubio, Flanagan, Gilbert, & Gidley, 2008; Zhang, Chen, Li, Li, & Zhang, 2015). Particularly, the average thickness (d) of the semicrystalline lamellae is normally calculated with Woolf-Bragg's equation (Zhang, Chen, Li et al., 2015; Zhang, Zhao, Li, Zhang et al., 2014). Additional lamellar parameters can be obtained

* Corresponding author.
E-mail address: qdttkl@163.com (D. Qiao).

http://dx.doi.org/10.1016/j.carbpol.2016.12.002
0144-8617/

Nomenclature

WMS	Waxy maize starch
RMS	Regular maize starch
GMS	Gelose 50 high-amylose maize starch
PS	Potato starch
SAXS	Small-angle X-ray scattering
WAXS	Wide-angle X-ray scattering
φ_c	The volume fraction of crystalline lamellae within semicrystalline lamellae
d	The thickness of semicrystalline lamellae for starch
d_c	The thickness of crystalline lamellae for starch
d_a	The thickness of amorphous lamellae for starch
Δr	The electron density difference between the crystalline and amorphous lamellae of starch
P_{SL}	The proportion of the semicrystalline lamellae within the starch granule
$PL+B$	Power-law scattering (PL) plus scattering background (B)
X_c	The relative crystallinity of starch
$L(r)$	Linear correlation function
α	Power-law exponent
A_{peak}	Area under the net lamellar peak of the SAXS pattern for starch
A_{total}	Total scattering area of the SAXS pattern for starch
A_{PL+B}	Area under the $PL+B$ profile of the SAXS pattern for starch
$R_{X/\varphi}$	The ratio of X_c to φ_c

using the paracrystalline model (Cameron & Donald, 1993a, 1993b), the liquid-crystalline model (Daniels & Donald, 2004) and the linear correlation function (Zhang, Chen, Li et al., 2015; Zhang, Chen, Xie et al., 2015). Those parameters include the thicknesses of crystalline (d_c) and amorphous (d_a) lamellae, the electron density difference ($\Delta\rho$) between the crystalline and amorphous lamellae, and the volume fraction (φ_c) of the crystalline lamellae within the semicrystalline lamellae. The distribution of lamellar thickness has also been studied using the interface distribution function (Cardoso & Westfahl, 2010).

However, though numerous studies have evaluated the lamellar structure of starch, the relative proportion (named as P_{SL}) of the semicrystalline lamellae within the starch granule has never been calculated. Also, among above mentioned methods, the linear correlation function is fairly straightforward, as no predefined assumptions of the starch structure are needed. Nonetheless, the non-lamellar amorphous starch (*i.e.*, amorphous growth rings) exists as a third-phase fraction in the starch granule. The third-phase starch reduces the accuracy of the linear correlation function to calculate the parameters of the two-phase semicrystalline lamellae. This interference prevents us from establishing accurate links between the functionalities and the semicrystalline features of starch, which is undesired for the rational design of starch products with tailored performance. Thus, if the scattering arising from the semicrystalline lamellae could be properly resolved from the total SAXS pattern of starch, it would be possible to calculate P_{SL}. Also, using the net scattering of the lamellae, the lamellar parameters of starch would be accurately obtained from the linear correlation function, due to the elimination of the interference of the third-phase starch.

To this end, a fitting equation based on a power-law function with two Gaussian plus Lorentz functions was developed to aid the decomposition of starch SAXS pattern into sub-patterns of the net lamellar peak and a profile of power-law scattering plus scattering background ($PL+B$). Then, the profile of linear correlation function was largely improved using the fitted net scattering for starch lamellae. Based on this, we obtained not only P_{SL} but also other lamellar parameters (*e.g.*, d, d_c, d_a and φ_c) with increased accuracy.

2. Materials and methods

2.1. Materials

Waxy maize starch (WMS), regular maize starch (RMS) and Gelose 50 high-amylose maize starch (GMS) were purchased from Penford Australia Ltd. (Lane Cove, NSW Australia). WMS, RMS and GMS had amylose contents of *ca.* 3%, 24% and 56%, respectively, as measured using an iodine colorimetric method (Tan, Flanagan, Halley, Whittaker, & Gidley, 2007). Potato starch (PS) (amylose content, *ca.* 36%) was supplied by Avebe (Netherlands). The moisture content of starch was determined using a moisture analyzer (MA35, Sartorius Stedim Biotech GmbH, Germany).

2.2. Small/wide angle X-ray scattering (SAXS/WAXS)

SAXS/WAXS measurements with 1 s acquisition were performed on the SAXS/WAXS beam-line (flux, 10^{13} photons/s) installed at the Australian Synchrotron (Clayton, Australia) at a wavelength $\lambda = 1.54$ Å. A slight overlap in q was established, and the configuration covered $0.015 < q < 2.9$ Å^{-1} simultaneously. The scattering vector, q, was defined as $q = 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle and λ is the wavelength of the X-ray source. The 2D scattering patterns were collected using a Pilatus 1 M camera (active area 169 × 179 mm and pixel size 172 × 172 μm) and a Pilatus 200 K camera (active area 169 × 33 mm and pixel size 172 × 172 μm). The Scatterbrain software was used to acquire the 1D data from the 2D scattering patterns. The starch slurries with a starch concentration of 40 wt% were used as the samples, which were prepared by adding a desired amount of water to the starch. The scattering of pure water with a Kapton tape (5413 AMBER 3/4IN X 36YD, 3 M, USA) on the stage window was used as the background data. All data were background subtracted and normalized using the Scatterbrain software. In particular, the background subtraction was conducted with care through a subtraction between the scattering of the starch slurry with Kapton tape and the scattering of pure water with Kapton tape. Each test was carried out in triplicate to acquire reliable SAXS/WAXS data.

The data in the range of $0.28 < q < 2.8$ Å^{-1} (*ca.* $4^\circ < 2\theta$ for Cu Kα $< 40^\circ$) were used as the WAXS patterns. The relative crystallinity (X_c, %) of starch was calculated using the PeakFit software (Ver. 4.12), according to Eq. (1).

$$X_c = \frac{\sum_{i=1}^{n} A_{ci}}{A_t} \quad (1)$$

In which, A_{ci} is the area under each crystalline peak with index i, and A_t is the total area of the WAXS pattern.

The data in the range of $0.015 < q < 0.20$ Å^{-1} were used as the SAXS patterns. The linear correlation function $L(r)$, as given in Eq. (2) (below) and Fig. S1 (see the Supplementary data), was used to calculate the parameters of semicrystalline lamellae, with Eq. (3).

$$L(r) = \frac{\int_0^\infty I(q)\, q^2 \cos(qr)\, dq}{\int_0^\infty I(q)\, q^2\, dq} \quad (2)$$

$$T = d\varphi_c(1-\varphi_c) \quad (3)$$

Here, r (nm) is the distance in real space. T is the insection of the linear region on $L(r)$ with the abscissa ($L(r) = 0$) (*cf.* Fig. S1 in Supplementary data); d is the second maximum of $L(r)$ (*i.e.*, the average thickness of the semicrystalline lamellae); φ_c is the volume fraction