Characterization and Retrogradation of Maize Starch by Asymmetrical Flow Field Flow Fractionation

Qi Shen¹, Panpan Guo¹, Xiaoyue Zhang², Zhen Mao², Li Huo¹* and Haiyang Dou¹,²*

¹Key Laboratory of Analytical Science and Technology of Hebei Province, College of Chemistry and Environmental Science, Hebei University, Baoding, China
²College of Medicine, Hebei University, Baoding, China

*Corresponding Author: Haiyang Dou, Key Laboratory of Analytical Science and Technology of Hebei Province, College of Chemistry and Environmental Science, Hebei University, Baoding, China.

Received: March 23, 2018; Published: April 30, 2018

Abstract

In this work, the capacity of AF4 coupled with multiangle light scattering (MALS) and differential refractive index (dRI) detectors to monitor the maize starch retrogradation behavior in situ was investigated. AF4 provides separation of starch molecular based on their hydrodynamic sizes, and MALS-dRI yields the molar mass and the radius of gyration. The two key operation conditions of AF4 analysis (i.e. injection amount and cross flow rate) were optimized. Under optimization operation conditions of AF4 analysis, the effect of storage conditions (i.e. storage time and storage temperature) on maize starch retrogradation was studied by AF4-MALS-dRI. The results reveal that AF4-MALS-dRI seems to be a useful tool for the better understanding of starch retrogradation mechanism.

Keywords: Asymmetrical Flow Field Flow Fractionation; Maize Starch; Characterization; Retrogradation

Introduction

Starch is one the most important source energy in human diet. For nutritional purposes, starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS), based on the rate and extent of digestion [1]. RS has gained importance as a new source of dietary fiber. RS can escape digestion before the colon and be fermented by the colonic bacteria, which plays an important role in digestive physiology. At present, RS is classified into five categories: RS1, RS2, RS3, RS4, and RS5. Among them, RS3, retrograded starch gel, is primarily interest, because of its thermal stability [2]. The retrograded starch (RS3) is safer than those from chemical modification (i.e. SR4), because the starch retrogradation process requires no chemical reagents. The starch retrogradation takes place when amylose and amylopectin chains realign themselves, transitioning from a disordered to an ordered state [3]. Amylose mainly experiences the early stage transition via an irreversible process, while amylopectin spends a longer retrogradation time due to its intrinsic molecular rigidity [4]. Because of retrograded starch's health benefits and industrial significance, many efforts have been made to better understand starch retrogradation. However, there is no consensus on the elusive retrogradation mechanism of starch. The information about the retrogradation behavior of starch is insufficient.

The starch retrogradation is a complex process which is affected by a lot of factors, such as storage conditions (e.g. storage temperature and time), and techniques used [5-7]. Up to now, many methods have been applied for the study of starch retrogradation, such as texture profile analysis (TPA), differential scanning calorimetry (DSC), fourier transform infra-red spectroscopy (FT-IR), X-ray diffraction (XRD), and nuclear magnetic resonance (NMR) [8-11]. Ambigapalalan [12] reported that the different techniques used for studying starch retrogradation may measure different retrogradation. It is reported that the ratio of amylose to amylopectin and their sizes play a criti-

Characterization and Retrogradation of Maize Starch by Asymmetrical Flow Field Flow Fractionation

In recent years, the role in starch retrogradation process [7]. Thus it is important to separate and characterize starch for better understanding of starch retrogradation. Size exclusion chromatography (SEC) is well established as a separation technique for the analysis of polymer; problems associated with its use for very large macromolecules such as amylopectin can include exceeding the exclusion of the column packing, irreversible interaction of the sample with column packing and subsequently low recovery, and shear degradation [13].

Asymmetrical flow field-flow fractionation (AF4), an alternative separation technique, has been widely employed for the separation of colloids and macromolecules [14-17]. AF4 provides for the separation of components based on their hydrodynamic sizes or molar masses [18]. Unlike SEC, AF4 uses an open channel that requires no stationary phase. Thus, the sample degradation or loss in minimized in AF4, and there are fewer problems of sample adsorption than in SEC. In addition to separation, characterization of analytes by direct measurement of physicochemical properties is one of the key features of AF4. Besides hydrodynamic radius (R_h), molar mass can be obtained by coupling AF4 with multi-angle light scattering detector (MALS) [19]. Although there have been a number of studies reported on the application of AF4 to the analysis of polysaccharides, few studies have been focused on starch retrogradation. In this study, the objective was to evaluate the capacity of AF4 coupled with MALS and refractive index (dRI) detectors to monitor the maize starch retrogradation behavior in situ.

Materials and Methods

Materials

Ferritin, bovine serum albumin (BSA) and bromphenol blue (BPB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). BSA was used to validate the performance of AF4 system. BPB was used to determine the focusing time. Sodium nitrate (NaNO₃), sodium azide (NaN₃), sodium chloride, ethanol, and dimethyl sulfoxide (DMSO) were purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Amylose (~90%, from potato) was obtained from Beijing Solarbio Science and Technology Co., Ltd (Beijing, China). Maize starch was purchased from the local market (Baoding, China). Deionized water was obtained from a Milli-Q Advantage A10 Ultra-Pure Water system (Millipore, MA, USA). All chemicals were of analytical reagent grade and used without further purification.

Preparation of retrograded starch

Starch was dissolved with the method described by Nilsson [20], but with a little modification. 20 mg maize starch was weighed directly into a 5 mL glass vial. Then the sample was dispersed in ethanol (60 μL, 80% ethanol in water v/v) with magnetic stirring at 160 rpm for 5 minutes, after which 0.6 mL DMSO was added with continue stirring for 30 minutes. The glass vial was capped and then heated in a boiling water bath with stirring at 160 rpm for 1h. Then, the sample solutions were quantitatively transferred and diluted with deionized water (55 ~ 60°C) to 10 mL in a volumetric flask. The resulting concentration of sample was 2 mg/mL. And then the sample solutions were stored at 4°C and 25°C, respectively.

AF4-MALS-dRI analysis of maize starch

In this work, Eclipse DUALTEC system (Wyatt Technology, Dernbach, Germany) was employed for AF4 analysis of starch. It was connected to a DAWN EOS multiangle light scattering (MALS) detector (Wyatt technology, Santa Barbara, CA, USA) operating at the wavelength of 690 nm and a 1260 differential refractive index (dRI) detector (Agilent Technologies, Waldbronn, Germany). The dRI detector was calibrated with sodium chloride and temperature was set at 35°C. An Agilent 1260 pump (Agilent Technologies, Waldbronn, Germany) with an in-line vacuum degasser delivered the carrier liquid into the AF4 channel. The channel was assembled with a 350 μm-thick Mylar spacer and a regenerated ultrafiltration cellulose membrane with the cut-off of 10 kDa. The actual channel thickness was measured to be 284 μm from the elution time of ferritin. The channel geometry was trapezoidal with the tip-to-tip length of 26.5 cm and breadths at the inlet and the outlet of 2.2 and 0.5 cm, respectively. The sample injection volume was 50 μL. Injection of the sample into the channel was performed at the flow rate of 0.2 mL/min for 2 minutes. The detector flow rate was constant at 1 mL/min. Carrier liquid was deionized water containing 50 mM NaNO₃ and 3 mM NaN₃.

Characterization and Retrogradation of Maize Starch by Asymmetrical Flow Field Flow Fractionation

Data treatment

In this work, the weight average molecular weight ($M_w$) and the radius of gyration ($R_g$) of starch were determined by online AF4-MALS-dRI using the Berry method [21,22]:

$$\frac{Kc}{R_g} = \sqrt{\frac{1}{M_w} + \frac{16\pi^2}{3\lambda^2} \times R_g^2 \times \sin^2 \left(\frac{\theta}{2}\right)}$$  (1)

Where $K$ is the optical constant, $c$ is the sample concentration, $R_0$ is the Rayleigh ratio, $\lambda$ is the wavelength. MALS data processing was performed using the Astra software (Version 6.1.7, Wyatt Technology). A specific refractive index increment, $dn/dc$, value of starch in water of 0.146 mL/g was used, and the second virial coefficient was assumed to be negligible [20].

Results and Discussion

Optimization of AF4 for analysis starch

A series of preliminary experiments was performed for the optimization of AF4 analysis of starch. To evaluate the effect of injection amount on AF4 analysis of starch, various concentrations of maize amylose solution were injected into AF4 channel. Figure 1 shows AF4-MALS-dRI fractograms of maize amylose obtained with different injection amounts. In order to avoid excessive retention and long elution time, the cross flow rate was started at 1.2 mL/min and decreased exponentially with a half-life of 2.0 minutes. No sample overloading was observed at the injection amount of 50 - 150 μg studied in this work as indicated by the independence of retention times on injection amount (Figure 1a). However, the dRI signal intensity of the void peak significantly increased as the injection amount of sample increased to 150 μg, indicating part of sample was eluted with the void peak. It was also found that the elution peak area of 150 μg sample did not proportionally increase, indicating a low sample recovery which is mainly due to the interaction between the sample and the surface of ultrafiltration membrane. During sample focusing process, a high concentration of sample in the focusing zone can enhance the repulsive force among sample molecules, which could result in a co-elution with the void peak and an interaction between the sample and the surface of ultrafiltration membrane. When AF4 coupled with MASL and dRI detectors, the weight average molecular weight ($M_w$) and the radius of gyration ($R_g$) of starch can be gained. It can be seen from Figure 1(a) that the $M_w$ distribution of main population peaking at 3 minutes ranges from 5 x 10^4 g/mol to 3 x 10^5 g/mol, which corresponds to amylose. The population eluted after 6 minutes with the $M_w$ distribution ranging from 3 x 10^5 g/mol to 10^7 g/mol which corresponds to amylopectin. The sample amylose includes approximately 10% amylopectin as described by the manufacturer. It was found that at the same retention time, the $M_w$ of amylopectin determined with injection amount of 50 μg was lower than that determined with injection amount of 100 μg, which is probably due to a lower intensity of dRI signal. Moreover, two distinct populations were observed from AF4-MALS fractograms shown in figure 1b. This can be explained by the fact that the MALS signal is dependent not only on the sample mass, but also on the sample size. Although, the concentration of amylopectin was low, the intensity of MALS signal of amylopectin was higher due to a larger size of amylopectin as shown in figure 1b. Taking into account the signal intensity of sample and the sample recovery, 100 μg was selected as injection amount for the further study.

In AF4 analysis, the cross flow rate (i.e., external force) is one of important factors affecting the resolution of sample. For the samples having broad size distribution, a programmed AF4 run may also improve detectability and allows lower sample consumption [23]. Figure 2 shows the AF4-MALS-dRI fractograms, $M_w$ and $R_g$ distributions of maize amylose obtained by an exponentially programmed cross flow rate with a half-life of 2.0 minutes. It was found that the intensity of dRI signal of void peak gradually decreased as the initial cross flow rate.

Figure 1: AF4-MALS-dRI fractograms, $M_w$ and $R_g$ distributions of maize amylose obtained with various injection amounts. (a): AF4-dRI and $M_w$ distribution; (b): AF4-MALS and $R_g$ distribution. The cross flow rate was started at 1.2 mL/min and decreased exponentially with a half-life of 2.0 minutes.
rate increased. It was demonstrated that a high initial cross flow rate improved the resolution of sample from the void peak. However, the sample with size larger than 80 nm was observed as the initial cross flow rate increased to 1.6 mL/min (Figure 2b). The result reveals that the stronger external force acting the sample can lead to intermolecular aggregation. Furthermore, the interaction between the sample and the surface of ultrafiltration membrane occurred as indicated by a decrease in the area of elution peak obtained with an initial cross flow rate of 1.6 mL/min. Thus, initial cross flow rate of 1.4 mL/min was selected for the further study.

**Figure 2:** AF4-MALS-dRI fractograms, Mw and Rg distributions of maize amylose obtained with a programmed exponentially cross flow rate with various initial cross flow rates. (a): AF4-dRI and Mw distribution; (b): AF4-MALS and Rg distribution. Injection amount of sample was 100 μg.
Effect of storage conditions on retrogradation behavior of maize starch

AF4-MALS-dRI fractograms of gelatinized maize starch obtained under optimized operation conditions. In order to investigate the effect of storage temperature on maize starch retrogradation, the gelatinized maize starch was stored at 4°C or 25°C. After that the sample was balanced to room temperature and injected into AF4 immediately. The AF4-MALS-dRI results are shown in figure 3. Two distinct populations were observed from AF4-dRI fractograms (Figure 3a), which corresponds to amylose and amylopectin, respectively. In this case, the $M_w$ and $R_g$ distributions of maize starch range from $5 \times 10^5$ to $5 \times 10^8$ g/mol and from 10 to 240 nm, respectively. It can be seen from figure 3(a) that the area of the first population (i.e. amylose) decreased while for the second population, the area of elution peak increased as gelatinized maize starch was stored at either 4°C or 25°C for 24h. The results indicated that the amylose retrogradation occurred and the amylose aggregates formed during retrogradation process were co-eluted with amylopectin population. On the other hand, no aggregate with size larger than that of amylopectin was observed in figure 3(b), indicating that amylopectin stored at studied conditions in this work did not retrograde. According to the classical polymer crystallization theory, crystallization processed via a three step mechanism: nucleation-propagation-maturation. The low temperature, 4°C, favors to form the nucleus promoting the retrogradation process of gelatinized maize starch.

Figure 3: AF4-MALS-dRI fractograms, $M_w$ and $R_g$ distributions of maize starch obtained with various storage conditions. (a) and (c): AF4-dRI and $M_w$ distribution; (b) and (d): AF4-MALS and $R_g$ distribution. Cross flow rate was started at 1.4 mL/min and decreased exponentially with a half-life of 2.0 minutes. Injection amount of sample was 100 μg.
Characterization and Retrogradation of Maize Starch by Asymmetrical Flow Field Flow Fractionation

Conclusions

For AF4 analysis of the sample with a broad size distribution such as starch, a great care should be taken as an injection amount and/or cross flow rate can result in an undesired sample resolution and/or the sample recovery. The results from AF4-MALS-dRI obtained at optimized operation conditions show that the rate of maize starch retrogradation stored at 4°C is fast compared with 25°C storage. It is demonstrated that in maize starch retrogradation process, the rate-limiting step is nucleation, which is enhanced at lower temperature. In this work, AF4-MALS-dRI was proved to be a useful tool for the separation and characterization of starch. The information obtained by AF4-MALS-dRI is valuable for understanding of maize starch retrogradation mechanism, and thus for developing functional starch-based foods.

Acknowledgements

The authors acknowledge support from the Nature Science Foundation of Hebei Province (B20162010002) and the Hebei Introduction Foundation for the Returned Overseas Scholars (CL201603).

Conflict of Interest

The authors declare no conflict of interest.

Bibliography


Characterization and Retrogradation of Maize Starch by Asymmetrical Flow Field Flow Fractionation


Volume 13 Issue 5 May 2018
©All rights reserved by Haiyang Dou., et al.