

DEVELOPMENT OF A GEL PERMEATION CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF STARCHY PRODUCTS

Monica Mironescu

Department of Food Biotechnology, “Lucian Blaga” University of Sibiu, Romania

Abstract

A new gel permeation chromatographic (GPC) method using dimethyl sulfoxide (DMSO) for the analysis of starch components amylose and amylopectin and intermediate products was developed. The paper presents a new method for the sample preparation and the influence of flow rate, column temperature and injection volume on the accuracy of GPC analysis, by using two methods. The optimal conditions for the chromatographic analysis were: eluent flow rate 0.4 ml/min., injection volume 100 μ l, column temperature 60°C.

Keywords: *gel permeation chromatography GPC, starch, DMSO*

Introduction

Starch is the cheapest and most abundant food biopolymer worldwide. There are many challenges in the study of starch processing as a result of the compositional and structural complexities of starchy systems. From a molecular perspective, starch consists of two types of molecules, amylose (AMY) and amylopectin (AMP). Both consist of polymers of α -D-glucose units in the 4C_1 conformations bound through glycosidic bonds. In amylose these are linked α -(1 \rightarrow 4), whereas in amylopectin about one residue in every twenty or so is also linked α -(1 \rightarrow 6) forming branch-points (Anderson, 2001; van den Einde, 2004). The relative proportions of AMY to AMP and α -(1 \rightarrow 6) branch-points both depend on the source of the starch, e.g. amylomaizes contain over 50 % amylose whereas “waxy” maize has almost none (Singh, 2005).

For certain applications in the food and packaging industries, starch is extruded to achieve a desired product texture and quality (Ilo, 1996). The main processes, shearing and melting, that occur during starch extrusion modifies starch at all structural levels. At molecular level, the

covalent bonds break down and macromolecules depolymerise under shear action; especially AMP is affected (Meuser, et al., 1982). At granular level the mechanical treatment determines granular fragmentation (van den Einde, 2004).

An understanding of the mechanisms involved in the degradation of starch during extrusion is therefore required. Chromatography is a modern tool used to predict and/or manipulate the process parameters as desired via calculated structural changes (Cheyne et al., 2005) or to optimise processing characteristics through structural studies (van den Einde, 2004).

The major aim of this research is to develop and optimise a chromatographic method for the characterization and analysis of structural changes of extruded starch.

Experimental

Chemicals: In order to study the molecular mass distribution of starch before and after the extrusion, corn starch C*Gel from Cerestar with about 27 % AMY was used. For the starch solubilisation and as mobile phase, pure dimethyl sulfoxide (DMSO) for spectroscopy “Uvasol” (Merck, Darmstadt, Germany) was used. Solutions of 90 % DMSO in water were used for the sample preparation and solutions of 100 % DMSO for the chromatographic analysis.

HPLC device: A HPLC installation from Shimadzu Corporation, Japan consisting on LC-20AD micro-volume double plunger pump (flow rate 0.0001–5 ml/min, pressure 1–40 MPa), SIL-20A autosampler, CTO-20AC column oven with forced air circulation (temperature range (-10) - 85°C), RID-10A differential refractometric detector (measurement range 0.01-500 ·10⁻⁶ RIU), (cell temperature settings 30-60°C) was used. A Phenogel Linear(2) mixed-bed column, filled with SDVB (Styrene-divinylbenzene) with particle size 5 µm was used. Column selection is M_w in the domain 10² - 10⁷ Da.

Sample and eluent preparation: In order to develop a short and sensitive analytical method, a new method for sample preparation was tested. The method has four steps: mixing of a mixture of starch sample and DMSO 90% (rapport starch:DMSO=10:1 w/w) at 200 rpm for 15 min., incubating at 90°C for 30 min., mixing at 200 rpm for 2 h

and centrifuged at 12000 rpm for 30 min. The eluent was always degassed for minimal 2 hours before running, using ultrasounds.

GPC analysis: Two methods, named **GPC1** and **GPC2**, were tested. The differences between the experimental methods used are listed in Table 1. Sample concentration was 10 mg/ml. Two injection volumes (50 and 100 μ l) were used in both methods. Because the column packed with SDVB is not adequate for water, DMSO 100 % was used as mobile phase.

Table 1. Variations between the two GPC methods

PARAMETER	CHARACTERISTICS	
	Method GPC1	Method GPC2
Temperature	40 °C	60 °C
Flow rate	0.5 ml/min	0.4 ml/min

Results and Discussions

Preliminary tests showed that flow rates higher as 0.5 ml/min give too high pressure in the column, due to the increased viscosity of the eluent DMSO, even at temperatures of 60°C. Literature recommends also a flow rate of 0.5 ml/min DMSO (Chuang, 1987) used. Thus, the flow rate of 0.5 was considered as the maximal flow rate to be tested.

The chromatograms obtained at the GPC analysis of corn starch at two flow rates are presented in figure 1. Four regions can be distinguished in the chromatogram 1a. It is known that for non-waxy starch, AMP has a greater molecular mass and elutes first (Brümmer, 2002). Amylose, which has a smaller molecular mass, is eluted after longer time. So, the region *Ia* corresponds to amylopectin, which seems to elute not as singularly peak, the existence of a shoulder being registered in the chromatogram. The region *IVa* could be attributed to AMY and the compounds eluted in the regions *Ila* and *IIla* could be intermediate products, which are found in the starch composition (White, 2000). The result obtained is similar to other chromatograms (Jackson, 1990) or much better as with other SEC-methods (You, 2000). Other methods are more adequate for AMY, which is better evidenced as with this method (Ao, 2007).

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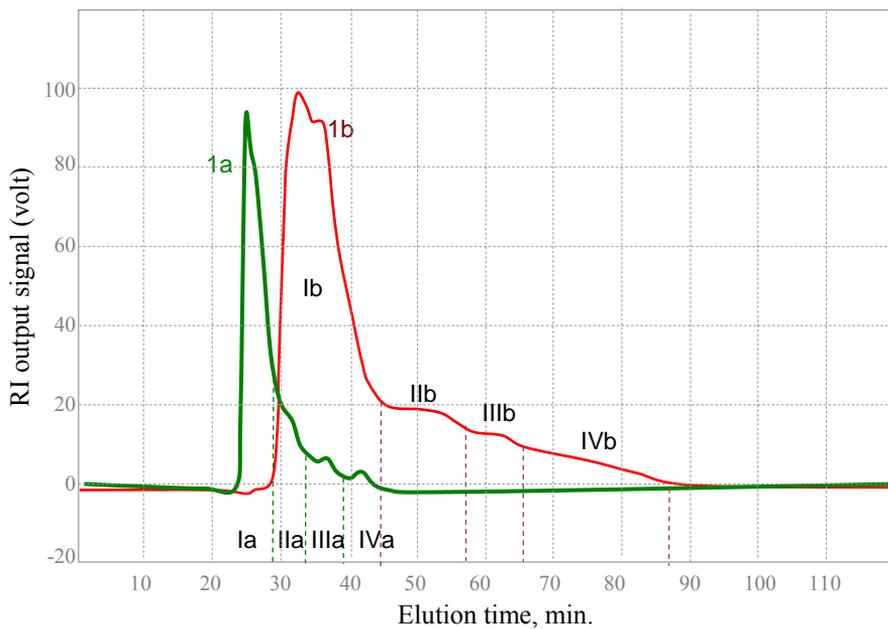


Figure 1. GPC chromatograms of corn starch analysed with the method GPC1 (chromatogram *1a*) and GPC2 (chromatogram *1b*). Injection volume 50 μ l

The result obtained with lower flow rate is presented in the chromatogram *1b*. Four regions could be defined; first, a double peak, which could be attributed to AMP (region *Ib*) appears. The regions *IIb* and *IIIb* seem to correspond to the intermediates identified in the zones *IIa* and *IIIa*. The fourth peak is also present, most probably indicating the presence of AMY.

For a rigorous identification of peaks, further analyses with standards of pure AMP and pure AMY are necessary.

The two chromatograms in figure 1 show that the use of faster flow rates (0.5 ml/min) allows the samples to elute faster compared with the analyses at lower flow rate. In the first case, the peak separation begins after 23 minutes of running and finish after approximately 45 minutes, whereas in the case of the flow rate 0.4 ml/min, the peak separation begins after 28 minutes of running and finish after approximately 90 minutes. But flow rates of 0.4 ml/min offer more information on the

molecular mass distribution, this flow rate being considered more adequate for the purpose of this research.

Practically, it was observed that the pump pressure increased continuously during the analyses performed at 40°C. So, the working temperature was increased from 40°C (method GPC1) to 60°C (method GPC2), the maximal temperature allowed for the RID. The increase of the temperature in the method GPC2 decreases the solvent viscosity and so the pressure at the pump and in the column. In the same time, the increase of the temperature allows a better solubilisation of starch in DMSO and improves the separation. No great influences in the elution profiles due to the temperature increase were observed.

Two chromatograms of initial and extruded corn starch obtained with the tested methods are presented in figures 2 and 3. The results offer valuable information on the process, indicating with good accuracy the differences between the extruded and the initial starch sample. So, if the chromatogram 2a is compared with the chromatogram 2b obtained in the same conditions for initial and extruded sample, relevant differences are pointed: two new peaks corresponding to decomposition products of AMP are obtained and the broad area between 60 and 95 minutes indicates the formation of compounds with lower molecular mass as AMY.

In the chromatograms presented in figure 2, two injection volumes were used, the results showing that the increase of the injection volume from 50 µl to 100 µl allows a better observation of peaks.

The use of method GPC2 for the analysis allows the better differentiation of peaks between the untreated sample and the extruded one (figure 3). New peaks appear in the extruded sample (chromatogram 3b) as result of the granule fragmentation caused by extrusion; these peaks are not present in the initial chromatogram (3a), as for example the peaks after 43, 65 or 75 min. of elution.

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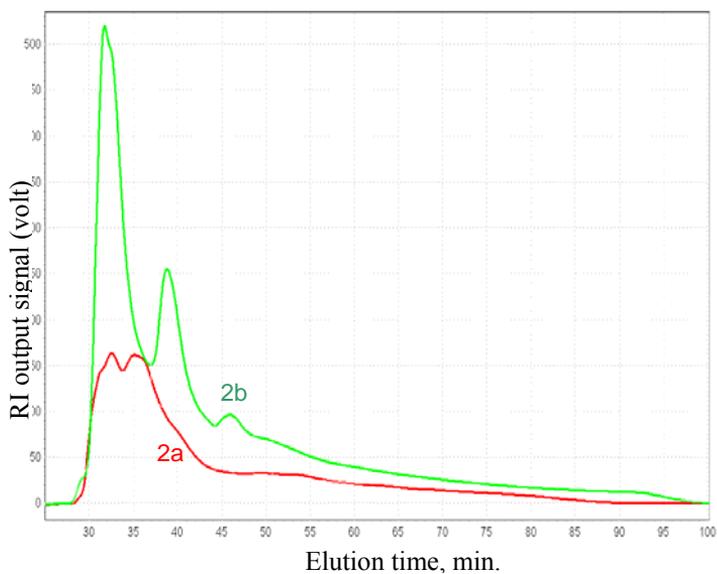


Figure 2. GPC chromatograms of corn starch initial (2a) and after extrusion (2b), analyzed with the method GPC1.

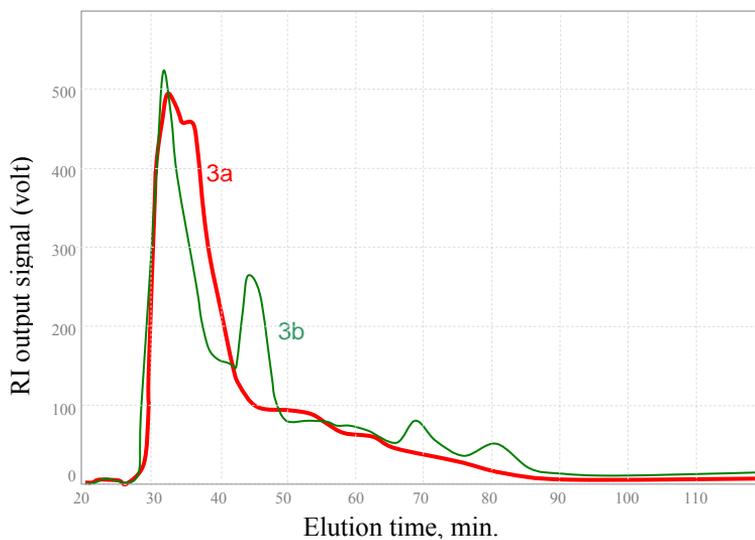


Figure 3. GPC chromatograms of corn starch initial (3a) and after extrusion (3b), analyzed with the method GPC2.

Conclusions

GPC is one of the valuable tools used for the analysis of the molecular decomposition of starch. The use of DMSO for the sample preparation and as mobile phase to flow through a mixed-bed column packed with SDVB give good results at the analysis of starchy products. In this paper, a new shorter and effective method for the sample preparation was developed and used to analyse the effect of extrusion on corn starch. For the chromatographic analysis, two elution volumes and two column temperatures were tested. The method named GPC2 (which use the elution volume of 0.4 ml/min and 60°C – column temperature) is considered to be better as the method named GPC1 (which use the elution volume of 0.5 ml/min and 40°C – column temperature) because allows the separation of more fractions with different molecular masses both for untreated and for extruded corn starch. The sample concentration was established to be 100 µl. Future works will use standards of pure AMP, pure AMY and compounds with intermediate molecular mass for the adequate identification of components.

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