Carbohydrate Polymers 148 (2016) 290–299

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Oxidation of amylose and amylopectin by hydroxyl radicals assessed by electrospray ionisation mass spectrometry

Joana Simões [a,*], Ana S.P. Moreira [a], Elisabete da Costa [a], Dmitry Evtyugin [b], Pedro Domingues [a], Fernando M. Nunes [c], Manuel A. Coimbra [a], M. Rosário M. Domingues [a]

[a] QOPNA, Departamento de Química, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal
[b] CICECO, Departamento de Química, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal
[c] Departamento de Química, Universidade de Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal

ARTICLE INFO

Article history:
Received 4 November 2015
Received in revised form 14 March 2016
Accepted 15 March 2016
Available online 18 March 2016

Keywords:
Starch
Fenton reaction
Electrospray ionization mass spectrometry
Maltotriose

ABSTRACT

The hydroxyl radicals (HO•) are one of the most reactive oxygen species (ROS) involved in the oxidative damage of biological molecules, including carbohydrates. During the industrial processing of food, ROS can be formed. In order to identify the structural changes induced in starch by oxidation, amylose, amylopectin, and maltotriose, an oligosaccharide structurally related to these polysaccharides, were subjected to oxidation with HO• generated under Fenton reaction conditions (Fe^{2+}/H_2O_2). The oxidised polysaccharides were hydrolysed by α-amylase and the obtained oligosaccharides were fractionated by ligand-exchange/size-exclusion chromatography. Both acidic and neutral α-amylase resistant oligosaccharides were characterized by mass spectrometry. In oxidised neutral products, new keto, hydroxyl, and hydroperoxy moieties, and oxidative ring scission were observed at the reducing end of the oligosaccharides. The acid sugar residues occurred at the reducing end and included gluconic and glucuronic acid derivatives, and acids formed by oxidative ring scission, namely, arabinonic, erythronic, glyceric and glycolic acids.

1. Introduction

During the industrial food processing, reactive oxygen species (ROS) can be formed and the food components (e.g. starch) can be chemically modified by ROS promoting adverse changes in organoleptic and chemical properties of food. Oxidation is one of the most common modifications of starch during the industrial processing, besides hydrolysis by enzymes or under acid conditions, esterification, and etherification (Xie, Liu, & Cui, 2005). Starch oxidation has been the subject of various studies, using sodium hypochlorite (Kuakpetoon & Wang, 2001), hydrogen peroxide (Tolvanen, Mäki-Arvela, Sorokin, Salmi, & Murzin, 2009), and ammonium persulfate (Harmon, Gupta, & Johnson, 1971) as oxidant reagents. Several processes take advantage of the hydroxyl radicals (HO•) (Gilbert, King, & Thomas, 1984), one of the most reactive oxygen species (ROS) involved in the oxidative damage of biological molecules, including carbohydrates (Duan & Kasper, 2011). Previous works on starch oxidation processes have been mainly focused on the physico-chemical properties changes (Kuakpetoon & Wang, 2008) of the modified starch, pasting and adhesive properties, morphology, thermal and gel texture (Fonseca et al., 2015), carbonyl and carboxyl groups content (Mathew & Adlercreutz, 2009), degree of crystallinity (Kuakpetoon & Wang, 2001), as well as determination of the oxidation degree (Chen et al., 2015). The evaluation of starch oxidation products can be carried out by infrared spectroscopy (Mathew & Adlercreutz, 2009), and carboxyl (—COOH) and carbonyl (—CO) contents determined by titration (Tolvanen et al., 2009). Also by nuclear magnetic resonance (NMR) few structural details have been obtained, as the introduction of carboxyl and carbonyl groups at hydroxyl (—OH) groups of C2, C3 and C6 in the glucose units (Ye et al., 2011), and as the determination of the average degree of substitution and monomer composition (Mischnick & Momcilovic, 2010). In the published data on starch oxidation still clearly lacks detailed information about the modifications occurred on the polysaccharide structure during oxidation.

* Corresponding author.
E-mail address: joana.simoes@ua.pt (J. Simões).

http://dx.doi.org/10.1016/j.carbpol.2016.03.034
0144-8617/

The main goal of this work was the study of the starch oxidation pattern induced by HO• generated under conditions of Fenton reaction (Fe^{2+}/H_2O_2). Starches are composed by two main polysaccharides, amylose and amylopectin. Amylose is a linear polyglucan composed by (α1 → 4)-linked-D-glucopyranosyl residues and amylopectin is a highly branched polyglucan composed by (α1 → 4)-linked-D-glucopyranosyl residues branched at the O-6 position of the glucose residues (Sharma, Yadav, & Ritika, 2008). Due to the different structural features of both starch polysaccharides, namely the branching pattern, the amylose and amylopectin were studied separately. The oxidation products formed were enzymatically hydrolysed with an α-amylase, then the resulting products were separated using semi-preparative ligand-exchange/size exclusion chromatography (LEX/SEC) and analysed by electrospray mass spectrometry (ESI–MS) and tandem mass spectrometry (ESI–MS/MS). Mass spectrometry (MS) has been used for the structural characterization of polysaccharides and oligosaccharides (Asam & Glish, 1997; Azenha, Coimbra, Moreira, Domingues, & Domingues, 2013; Simões et al., 2007; Simões, Nunes, Domingues, & Coimbra, 2010; Simões, Nunes, Domingues, Coimbra, & Domingues, 2012; Da Costa et al., 2012; Zhou, Ogden, & Leary, 1990), including the oxidation of oligosaccharides (Moreira et al., 2014; Tudella et al., 2011). In order to complement and better understand starch oxidation, the oxidation induced by HO• generated under conditions of Fenton reaction of a starch oligosaccharide model compound, maltotriose, was also studied. The present study also deals with analyses of oxidative modifications induced by HO• on a gluco-oligosaccharide, which are reported for the first time.

2. Experimental

2.1. Oxidation reaction of polysaccharides

The oxidation of two different polysaccharides, amylose from corn and amylopectin from potato (Sigma-Aldrich, St., Louis, MO), was induced by the hydroxyl radicals generated under Fenton reaction. Stock solutions (4 mg/mL in ultrapure water) of amylose (AL) and amylopectin (AP) were prepared. For each polysaccharide, the mixture for Fenton reaction was prepared by adding of 300 μL of the polysaccharide stock solution, 60 μL of 0.5 M H_2O_2 solution (30 wt.% in H_2O, Sigma-Aldrich), 12 μL of 5 mM $FeCl_2{\cdot}4H_2O$ solution (99.99%, Sigma-Aldrich), and ultrapure water to reach a total volume of 600 μL (pH 5.5). The mixture was vortex mixed, sonicated for 10 min, vortex mixed again, and left to react at 50 °C in the dark for 24 h under stirring. At the end, catalase from bovine liver (Sigma-Aldrich) was added to the mixture to destroy the remaining H_2O_2. Control mixtures of AL and AP were prepared by replacing H_2O_2 by an equal volume of ultrapure water, submitted to the same conditions.

An additional assay of Fenton oxidation of AL and AP was done to further perform a separation of the high and low molecular weight materials by dialysis with a 12 kDa cut-off membrane for 4 days, with changes of distilled water twice a day. After dialysis, the materials were concentrated to dryness.

2.2. Oxidation reaction of the oligosaccharide model compound

The oxidation of maltotriose (Glc_3, Sigma-Aldrich, St., Louis, MO) was induced by the hydroxyl radicals generated under Fenton reaction conditions as described in Section 2.1 for the polysaccharide samples, with the exception of the oxidation reaction time of 6 h, since it is an oligosaccharide (Moreira et al., 2014).

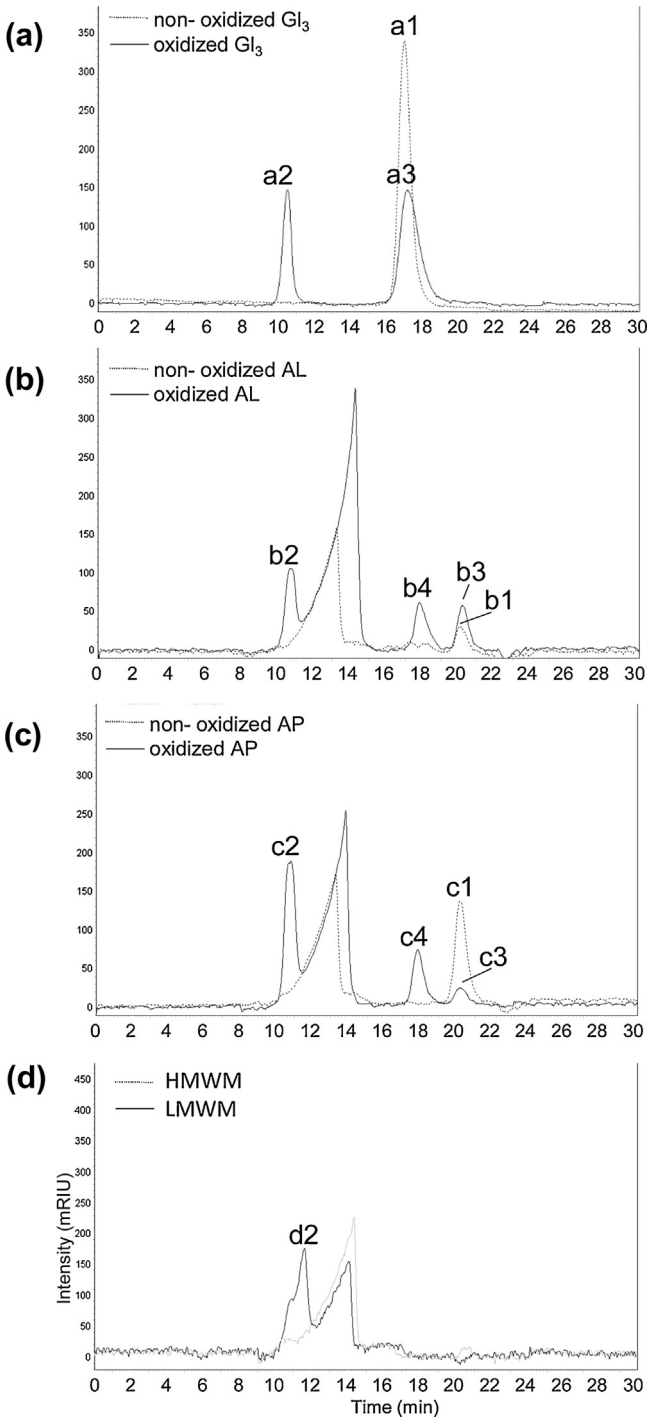

Fig. 1. LEX/SEC chromatograms of oxidised and non-oxidised **(a)** maltotriose, **(b)** amylose, **(c)** amylopectin, and **(d)** high (HMWM) and low (LMWM) molecular weight materials recovered after oxidation of amylose. Different peaks were identified as corresponding to acidic (a2, b2, c2, d2) and neutral (a1, a3, b1, b3, b4, c1, c3, c4) compounds.

2.3. Enzymatic hydrolysis of polysaccharides

The oxidised polysaccharides and the control mixtures (non-oxidised polysaccharides) were dried in a Vacuum Concentrator Centrifuge and cold Trap (Uniequip, Planegg, Denmark) and hydrolysed with 1 U of an α-amylase from *Bacillus subtilis* preparation (Fluka, EC 3.2.1.1) during 24 h at 37 °C with continuous stirring in 50 mM ammonium hydrogen carbonate buffer solution at pH 7.4.