Journal of Cereal Science 70 (2016) 207–213

Contents lists available at ScienceDirect

Journal of Cereal Science

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

Starch characterization after ozone treatment of wheat grains

Perrine Gozé [a], Larbi Rhazi [b], André Pauss [c], Thierry Aussenac [b, *]

[a] PAULIC Minoteries, Moulin du Gouret, 56920 Saint Gérand, France
[b] Institut Polytechnique LaSalle Beauvais, 19 rue Pierre Waguet, 60026 Beauvais Cedex, France
[c] Sorbonne universités, Université de Technologie de Compiègne, ESCOM, EA4297 TIMR, Centre de recherche Royallieu, CS 60319, 60203 Compiègne Cedex, France

ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form
28 May 2016
Accepted 10 June 2016
Available online 11 June 2016

Keywords:
Ozone treatment
Starch oxidation
Wheat grain matrix
Semi-industrial reactor

ABSTRACT

Soft wheat grains were subjected to oxidation by ozone treatment. A Box Benhken design with three variables (humidification rate, ozone inlet concentration and reaction time) was used for the experiment. Two wheat cultivars differing on their technological properties were used. The products were characterized by determining rheological, thermal and other physicochemical properties such as RVA (Rapid Visco Analyzer), DSC (Differential Scanning Calorimetry), molecular weight distribution and amylopectin branched chain length distribution. Contrary to previous works, results clearly show no significant effect of ozone treatment on wheat starch in our experimental conditions (i.e. starch extracted from ozonated whole wheat grain) whatever the cultivar studied. Only slight increases of carboxyl groups have been noticed with increasing ozonation. Modifications observed on rheological properties in flour samples (i.e. alveographic and RVA measurements) in previous works (Gozé et al., 2015) could mainly be explained by the oxidation of others molecules such as proteins and non-starch polysaccharides.

1. Introduction

Starch is used in large quantities in many industries especially in food. However, natural starches do not often have the properties desired for particular uses. The main required effects of oxidized starches are better cooking characteristics (lower viscosity, improved stability of starch dispersions, binding properties) and resistance to degradation to avoid retrogradation and gelling tendencies. It was found that oxidized starches do not exhibit structural differences from the native starches: X-ray diffraction diagrams in particular remain the same. This, seems to confirm the hypothesis that oxidation affects mostly amorphous phases of the granule (Scallet and Sowell, 1967). Among oxidizing agents, sodium hypochlorite is the most commonly used (Kuakpetoon and Wang, 2008). Hydrogen peroxide (Guerra Dias et al., 2011; Zhang et al., 2012), acidic bromate (Komulainen et al., 2013); potassium permanganate and sodium chlorite have been used too. Ozone, recognized by U.S. Food and Drug Administration (FDA) as a GRAS (i.e. Generally Recognized as Safe) in 1997, is also a powerful oxidizing agent highly reactive. One advantage of ozone, compared to oxidizing chemicals cited above, is that it finally breaks down into atmospheric oxygen, eliminating the potential residue of hazardous chemicals (McDonough et al., 2011). It can therefore be safely used in both gaseous and aqueous forms. Due to its strong oxidizing properties, ozone has been reported to modify significantly the technological properties (i.e. rheological properties in particular) of treated cereal flours (Sandhu et al., 2011). Our previous works on wheat storage proteins demonstrated significant modifications on molecular properties; especially on protein SDS-solubility, molecular mass distribution and disulfide-sulfhydryl interchange reactions which strongly affected bread making quality. However, to our knowledge, few published works have investigated the modifications of extracted starch from ozonated whole wheat grains. Most of them have focused their studies on the oxidative effects of ozone on powdered starches (cassava, corn, yam, potato, wheat) and flours (An and King, 2009; Chan et al., 2009, 2011; Oladebeye et al., 2013; Sandhu et al., 2012). Sandhu et al. (2012) studied starch isolated from ozonated flour and ozonated starch after isolation from control flour. Their results show modifications on molecular properties (i.e. partial depolymerization of high molecular weight amylopectin producing low molecular weight starch polymers; higher amount of carboxylic groups in amylopectin fractions) and on rheological properties (i.e. increase of starch granules swelling and increase in paste

* Corresponding author.
E-mail address: thierry.aussenac@lasalle-beauvais.fr (T. Aussenac).

http://dx.doi.org/10.1016/j.jcs.2016.06.007
0733-5210/

breakdown). It has well demonstrated that under identical ozone treatment conditions, the extent of starch oxidation (on the molecular structure and rheological properties) strongly varied among the different starch types (Chan et al., 2011).

In this study, we investigated the major effects of ozone treatment on starch modifications, particularly ozone applied on grain matrix. This work aimed to evaluate the carboxyl contents, the X-ray diffraction and DSC patterns, the pasting properties and the molecular weight distribution of wheat starch from ozonated wheat grains compared to native extracted wheat starch. The results obtained from this work will be helpful in the comprehension of starch implication on technological variations (i.e. alveographic and rheological modifications) induced by whole wheat grains ozonation observed in previous works (Gozé et al., 2015).

2. Materials and methods

2.1. Plant material

The two bread wheat cultivars used in this study were Rubisko and Diamento differing on their HMW-GS composition (7-8+2-12 and 7-9+5-10 respectively) (Certified seeds, harvest 2013, RAGT France). Wheat flour was obtained after ozone treatment of wheat grains with a laboratory mill (CD1 model, CHOPIN, France).

2.2. Ozone treatment

All experiments were conducted with a specific reactor set up at the Institut Polytechnique LaSalle Beauvais. A 10 kg seed mass freshly homogenized during 15 min with the desired quantity of water was fed in a semi-batch stainless steel reactor equipped with a central endless screw for grain circulation. The temperature in the reactor was controlled at 22 °C by a cooling system. The reactor's pressure was maintained at 0.4 bars by gas vector. Ozone gas was fed at the bottom of the reactor through a micro-porous plate with a flow rate of 33.34 L/min TPN. Ozone was produced from oxygen (99.5%, Air-liquid, France) which was connected with an ozone generator (OZAT® CFS-2G, Ozonia, France). Inlet and outlet ozone concentrations were continuously measured and recorded (Ozone Analyzer BMT 964). The ozone off-gas was destroyed at 350 °C by a thermal destruction unit.

2.3. Experimental design

Box-Behnken experimental design (BBD) was employed in this study. The independent variables chosen were humidification rate during preparation of grain (X_1), the ozone concentration in the ozone inlet (X_2) and the reaction time (X_3). Each factor in the experiment was established and coded at three different levels, low (−1), medium (0) and high (+1) (Table 1). The used X_1 values were 2, 4 and 6% (v/w), those of X_2 were 60, 120 and 180 mg/L and those of X_3 were 15, 37.5 and 60 min, respectively. A total of 15 experimental runs, including 3 replicates at center point, were carried out (Table 1).

Two more treatments encoded −1,−1,−1 and +1,+1,+1 (not included in the Box-Behnken design) were also studied. The results were analyzed using Statistica (version 12.1, Statsoft) with $P < 0.05$.

2.4. Extraction of starch granule

Granule starch extraction has been conducted according to the protocol developed by Bancel et al. (2010) with some modifications. 500 g of wheat grains were milled using a Chopin CD1 Laboratory Mill (Chopin, Villeneuve-la-Garenne, France). In Order to purify starch granules from all other constituents mainly proteins, 3 g of flour were washed twice 30 min and three times 1 h with 15 mL of washing buffer (55 mM Tris-HCl pH 6.8, 2.3% (w/v) SDS, 1% (w/v) dithiothreitol (DTT), 10% (v/v) glycerol) at 20 °C. At the beginning of each washing step, granules were disrupted using sonication for 20 s at a power setting of 20% using a stepped microtip probe (6 mm diameter) (Sonics Materials, Bioblock Scientific, model 75038). The starch granule pellet was then washed three times for 5 min with cold water, once with cold acetone and finally air-dried at room temperature for 12 h. Each washing step was followed by centrifugation at 3500*g* for 5 min.

2.5. Carboxyl group determination

The carboxyl content was determined by the following titrimetric method adapted from ISO 11214 1996: 1996-07(Modified starch-Determination of carboxyl group content of oxidized starch). 1 g of starch granule was stirred for 30 min with 5 mL of 0.1 M HCl. The mixture was washed five times with 20 mL of water. Each washing step was followed by centrifugation at 10,000*g* for 5 min. Pellet was then suspended in 40 mL of distilled water, incubated 30 min at 95 °C and immediately titrated to pH 8.3 with standardized 0.005 M NaOH. A blank experiment with non ozonated wheat starch was performed similarly. Carboxyl content of the sample, expressed as the quantity of carboxyl groups per 100 glucose units (GU), was calculated as follows (Kuakpetoon and Wang, 2006):

$$\text{Percentage of carboxyl content} = \frac{[(\text{sample} - \text{blank})\ \text{mL} \times \text{molarity of NaOH} \times 100 \times 0.045]}{\text{sample weight in grams}}$$

2.6. Asymmetrical flow field flow fractionation (A4F) system and procedure

Sample preparation has been conducted according to the protocol described in detail by Chiaramonte et al., 2012. Briefly, starch granules (10 mg) were dispersed in 95% (v/v) of dimethylsulfoxide (DMSO)/water and incubated at 100 °C for 60 min. Samples were precipitated using 5 vol of ethanol and centrifuged at 20,000*g* for 20 min at room temperature. Supernatants were discarded and pellets were mixed with 4 mL of NaOH (20 mM) and transferred into 10 mL pressurized vessels (CEM, Saclay, France). Solubilization has been conducted with microwave heating (Discover, CEM, Saclay, France) for 8 min at 135 °C. Samples were filtered through 5 μm cellulose nitrate filters (Gelman Sciences, France) before injection (100 μL) into the A4F system. Pullulan (400 kDa) solutions were used for initial operational testing of the AF4 system. A4F analysis was accomplished using an Eclipse3 F System (Wyatt Technology, Santa Barbara, CA, USA) serially connected to a multi-angle light scattering (MALS) detector (Dawn multi-angle Heleos TM, Wyatt Technology, Santa Barbara, CA, USA) and, an interferometric refractometer (Optilab rEX, Wyatt Technology, Santa Barbara, CA, USA). The MALS detector was calibrated with toluene. The RI detector was calibrated with sodium chloride and the temperature was set at 35 °C. The separation channel of 195 mm of length had a trapezoidal geometry. The thickness of the spacer used in this experiment was 0.35 mm. The ultrafiltration membrane forming the accumulation wall was made of regenerated cellulose with a cut-off of 10 kDa (Superon). An Agilent 1200 Series Isocratic HPLC Pump (Agilent Technologies, Germany) with an in-line vacuum degasser was used to deliver the carrier flow to the channel. The mobile phase was de-ionized water with 0.02% NaN_3 (w/v) added