Food Chemistry 199 (2016) 287–295

Contents lists available at ScienceDirect

Food Chemistry

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

Combination of extrusion and cyclodextrin glucanotransferase treatment to modify wheat flours functionality

Laura Román [a], Ángela Dura [b], Mario M. Martínez [a], Cristina M. Rosell [b], Manuel Gómez [a,*]

[a] Food Technology Area, College of Agricultural Engineering, University of Valladolid, 34004 Palencia, Spain
[b] Institute of Agrochemistry and Food Technology (IATA-CSIC), Avda Agustin Escardino, 7, Paterna, 46980, Spain

ARTICLE INFO

Article history:
Received 22 September 2015
Received in revised form 3 December 2015
Accepted 9 December 2015
Available online 10 December 2015

Keywords:
Wheat flour
CGTase
Extrusion
Cyclodextrin
Starch characteristics

ABSTRACT

This research aims to vary functional properties of native and extruded wheat flours combining cyclodextrin glucanotransferase and extrusion treatments. The level of released cyclodextrins (CD) was assessed, besides the microstructure, crystallinity, pasting properties and starch hydrolysis of the flours. Photomicrographs of enzymatically treated flours suggested the production of fragile structures that broke easily. Enzymatic hydrolysis was significantly higher in extruded flours, as confirmed the CD levels, being predominant the γ-CD followed by α-CD, whereas very low β-CD values were obtained probably due to the formation of CD–lipid complexes, as suggested X-ray diffractometry results. Both extruded and native samples showed very low viscosity and flat pasting profile consequence of the enzyme hydrolytic activity on the starch chains. Enzymatically treated flours (native and extruded) showed higher hydrolysis rates at the early hydrolysis stage, and extruded flours exhibited higher fractal exponent *h* in agreement with the extended crystalline structures resulting from enzymatic treatment.

1. Introduction

Starch and starch based products, such as flours, are common raw materials used in food industry because they have unique thermal, structural and functional properties that permit their use in food products and industrial applications. Starch and starch based products can be modified by chemical, physical or enzymatic treatment to improve industrial applications. Physical and enzymatic treatments of these products allow the modification of their nutritional and functional properties. Nevertheless, when enzyme treatment is utilised, native starch is only partially accessible for the enzyme catalysis, thus it is necessary to promote the damage or breakage of the starch granules (Uthumporn, Shariffa, & Karim, 2012). Hydrothermal treatment, such as extrusion, which combines high temperature and pressure, fosters gelatinisation and dextrinization depending on the conditions of the extrusion (Martínez, Calviño, Rosell, & Gómez, 2014). After gelatinisation, starch is more accessible and it is therefore directly available for enzymatic modification (Martínez, Pico, & Gómez, 2015; Patel, Day, Butterworth, & Ellis, 2014).

Cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) is an endoenzyme that catalyses four different reactions (hydrolysis, cyclisation, coupling and disproportionation) by cleaving α-1, 4-glycosidic bonds present in the inner part of a polysaccharide chain (Terada, Yanase, Takata, Takaha, & Okada, 1997). Among these reactions, cyclisation is the specific enzymatic reaction that releases cyclic oligomers, known as cyclodextrins (CDs), from starch or starch derivatives (Li, Chen, Gu, Chen, & Wu, 2014). The most common CDs are α-, β-, and γ-CDs (with six, seven, and eight 1,4-linked D-glucose units, respectively), containing trace amounts of CDs with more than nine D-glucose units (Terada et al., 1997). CDs are extensively used in the food industry for different applications such as food additives, encapsulation of molecules (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gandara, 2009; Astray, Mejuto, Morales, Rial-Otero, & Simal-Gandara, 2010) and as a source of dietary fibre (Artiss, Brogan, Brucal, Moghaddam, & Jen, 2006). The enzyme CGTase has been also proposed to slow down starch retrogradation and staling in starch or flour based products (Gujral, Haros, & Rosell, 2003; van der Maarel & Leemhuis, 2013) and to improve the quality of bakery products (Gujral, Guardiola, Carbonell, & Rosell, 2003). Therefore, the modification of starch with CGTase provides modified starches with the additional functionality that offer the released CDs.

Even though several studies have been focused on the production of CDs from different tuber and cereal starches (Calsavara, Dias da Cunha, Balbino, Zanin, & de Moraes, 2011; Gujral & Rosell, 2004; Yamamoto, Zhang, & Kobayashi, 2000), CDs production from flour has never been attempted. Flour in comparison with starch, contains proteins, lipids, sugars and other

* Corresponding author.
E-mail address: pallares@iaf.uva.es (M. Gómez).

http://dx.doi.org/10.1016/j.foodchem.2015.12.040
0308-8146/

non-starchy components. Therefore, enzymatic treatment of flours can possibly be influenced by the interactions between starch and those non-starch components, giving rise to different properties than those of starch. Moreover, flour modification can be a good alternative to starch modification for their use in industrial processes, being economically a more viable process and with lower environmental impact (Eckhoff & Watson, 2009).

The objective of this research was to provide wheat flours with diverse functional properties by enzymatic treatments. In pursuing the aim, CGTase was applied to native and extruded wheat flours and the level of released CDs was assessed. In addition, enzymatic treatment was carried out at two ratios of liquid volume to starch mass, given the impact of that ratio on the absorption of the enzyme to the starch surface and also considering the economic impact of drying when industrial application of the process. To determine the functionality of enzymatically treated flours, the microstructure, crystallinity, pasting properties, hydration properties and digestibility, were also investigated.

2. Materials and methods

2.1. Materials

Native and extruded wheat flours were supplied by Molendum Ingredients (Zamora, Spain). Extrusion of native wheat flour (11.73% of moisture, 11.78% of protein and 4.97% of damage starch contents) was carried out by Molendum Ingredients in a single screw extruder Bühler Basf (Bühler S.A., Uzwil, Switzerland). The length to diameter (L/D) ratio for the extruder was 20:1. The extrusion conditions were carried out based on preliminary experiments in order to ensure starch gelatinization. Wheat flour was extruded at a maximum barrel temperature of 160 °C and a feed moisture content of 50 L/h with a feed rate of 500 kg/h and a screw speed of 340 rpm. Extruded product was dried by convection air up to 10.40% of moisture content and then ground with a compression roller till particle size was lower than 200 microns.

Cyclodextrin glucanotransferase (CGTase) from *Bacillus licheniformis* Toruzyme® 3.0 L (declared activity: 3.0 KNU/g) was kindly provided by Novozymes (Bagsvaerd, Denmark).

2.2. Methods

2.2.1. Flour measurements

Native wheat flour composition was analysed following AACC Methods (AACC, 2012) for moisture, method 44-16.01; damaged starch 76-30A; and protein content, method 46-30.01.

2.2.2. Flour modification by CGTase

First, the enzyme solution was prepared by dissolving 41.65 μL ± 0.001 μL (0.15 KNU) of CGTase in the appropriate volume of distilled water (40 mL or 80 mL). Then, a pre-weighed amount of starch (10 g) were suspended in 40 mL or 80 mL of enzyme solution to obtain ratios of flour mass to liquid content of 1:4 or 1:8, respectively. Slurries of native and extruded flours were also prepared in 40 mL or 80 mL distilled water without CGTase addition, as control. Flour slurries were well mixed with a glass rod, covered by plastic film to avoid drying of the sample and then incubated at 60 °C for 60 min. During incubation, flour slurries were vigorously stirred each 15 min so as to avoid the flour particles to settle down. To stop the enzymatic reaction and to dry the flour slurries, the pastes were heated at 105 °C for 5 h. Afterwards, samples were rested in a desiccator at room temperature for 3 min, before milling in a Moulinex super juniors (Groupe Seb Iberica, S.A, Barcelona, Spain) for 20 s. Flours were stored in airtight plastic containers perfectly sealed at 4 °C until analysis. Thereby, the whole process of flour hydrolysis was performed considering the feasibility of scaling up the process in the food industry.

2.2.3. Environmental scanning electron microscopy (ESEM)

Flour photomicrographs were taken with a Quanta 200FEI (Hillsboro, Oregon, USA) ESEM. Photomicrographs were taken in beam deceleration mode (BDM) at 1.5 keV in high vacuum mode with a backscattered electron detector (BSED).

2.2.4. Cyclodextrin content of flour samples

Release of the most common CDs; α-CD, β-CD and γ-CD was followed colorimetrically via the formation of inclusion complexes with different organic compounds. The ability of α-CD to form inclusion complex with methyl orange (MO) was tested following the method reported by Lejeune, Sakaguchi, and Imanaka (1989), slightly modified. The methyl orange (MO) stock solution was prepared at 5 mM in 50 mM sodium phosphate buffer pH 6.0 by agitating at 40 °C. A dilution of 1:50 of MO was prepared, in which final concentration of methyl orange was 0.1 mM. A calibration curve of α-CD was performed in the range 0–1946 μg of α-CD. α-CD in flours were measured by suspending 250 mg in 2.5 mL of 50 mM sodium phosphate buffer, after stirring for 5 min, they were centrifuged at 10,000×*g* for 10 min. Supernatant (2 mL) was mixed with 2 mL MO and two drops of 0.275 N HCl were added. Then, cuvettes were shacked and kept into the fridge for 15 min. Optical density was measured at 505 nm in UVmini-1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

Concentration of β-CD was analysed following the method described by Goel and Nene (1995) based on the decrease in absorbance at 550 nm due to phenolphthalein–CD complex formation, with slight modifications. A calibration curve of β-CD was performed in the range 0–100 μg. The phenolphthalein solution was prepared at 4 mM in 125 mM Na_2CO_3 buffer pH 10.5. Samples (50 mg) were suspended in 500 μL 50 mM Tris–HCl buffer pH 8.0 and stirred for 5 min. After centrifuging, as was described above, 200 μL of supernatant were mixed with 1 mL phenolphthalein solution and absorbance measured immediately at 550 nm in UVmini-1240 spectrophotometer.

γ-CD was determined measuring the colour increase at 630 nm due to the formation of inclusion complexes with bromocresol green (BCG) following the method reported by Kato and Horikoshi (1984) slightly modified. The working BCG solution was prepared by mixing 0.5 mL of 5 mM BCG (in 20% ethanol solution) and 10 mL of 0.2 M citrate buffer pH 4.2. A calibration curve of γ-CD in the range 0–700 μg was performed. Flour sample (150 mg) was extracted with 1500 μL 0.2 M citrate buffer pH 4.2. Clear supernatant (500 μL) obtained after centrifuging were mixed with 1 mL BCG, after shaking the absorbance was read at 630 nm in a UVmini-1240 spectrophotometer.

Experimental results are the average of three replicates.

2.2.5. Flour crystallinity by X-ray diffraction (XRD)

Samples were analysed using a Bruker D8 Discover A25 (Bruker AXS, Rheinfelden, Germany) equipped with a copper tube operating at 40 kV and 40 mA, producing CuKa radiation of 0.154 nm wavelength. Diffractograms were obtained by scanning from 5° to 40° (2theta) at a rate of 1.2°/min, a step size of 0.02°, a divergence slit width variable (DS) of 5 mm, a scatter slit width (SS) of 2.92° and a nickel filter to exclude Kβ radiation.

2.2.6. Pasting properties

Pasting properties of flours were determined following the standard method 61.02.01 (AACC, 2012) by a Rapid Visco Analyser (RVA-4C) controlled by Thermocline software (Perten, Uppsala, Sweden) for Windows. RVA measurements were carried out in duplicate.