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Physicochemical modification of native and extruded wheat flours by enzymatic amylolysis

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ABSTRACT

Enzymatic hydrolysis could be an alternative way to modify flour functionality. The effect of two different enzymes, α-amylase and amyloglucosidase, and their combination on microstructure, oligosaccharide content, crystalline order, pasting, gel hydration, and colour properties of native and extruded wheat flours was investigated. Micrographs showed different mechanisms of actuation of the different enzymes on native and extruded flours, achieving greater than 300% and 500% increases of glucose and maltose contents, respectively, in extruded flours compared with their native counterparts. Native flours displayed higher values of water absorption capacity and swelling power than extruded flours. Flours treated by a combination of amylase and amyloglucosidase showed low swelling power. Regarding colour, native flours were darker and more reddish than extruded flours, whereas flours treated by amyloglucosidase, and therefore had a higher glucose content, were darker and more reddish.

1. Introduction

Native starches and flours are widely used as raw materials, due to their particular polymeric characteristics, which make them suitable for numerous food applications. However, the new demands of the food industry are forcing manufacturers of starchy ingredients to find new functionalities. Starch modification by enzymatic hydrolysis could be an alternative way to control the functionality making the label cleaner unlike the chemically modified starches or syrups. Starch hydrolysis generates products with different dextrose equivalents (DE), depending on the time of incubation and the amount and type of enzyme being used. Two major hydrolysis products are maltodextrins that consist of partly hydrolysed starch chains with a DE below 30, and glucose and maltose syrups with a DE above 40 that contain mono-, di-, and some higher saccharides (Baks, Kappen, Janssen, & Boom, 2008). Maltodextrins are non-sweet, cold water soluble, and have water-holding characteristics. They can be used as carrier or bulk agents, texture providers, spray-drying aids for the production of flavour enhancers, fat replacers, film formers, freeze-control agents to prevent crystallisation, or to supply nutritional value (Ba et al., 2013). Meanwhile, glucose and maltose syrups are employed in a variety of foods like soda water, sweets, baked products, ice-creams, sauces, baby food, conserves, and tined food.

Amylases, together with amyloglucosidases, are the enzymes most commonly used in starch hydrolysis. Alpha-amylase is an endoamylase that cleaves the α-1,4 glycosidic bonds of the amylose or amylopectin chain at internal positions (endo) to yield products (oligosaccharides with varying lengths and branched oligosaccharides called limit dextrins) with an α-configuration. Meanwhile, amyloglucosidase catalyses the hydrolysis of both α-1,4 and α-1,6 glycosidic bonds at the branching point to release β-D-glucose residues of the polymer substrate (van der Maarel, van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002). Because of these different mechanisms of amylolysis, selection of the type and amount of enzyme is important, since it will determine the physicochemical properties of the final flour or starch.

Native starch granules are semi-crystalline and resistant to enzyme hydrolysis. Native granular starch is hydrolysed very slowly by both amylases and amyloglucosidase, but disruption of the starch granular structure (gelatinisation) could enhance its chemical reactivity towards hydrolytic enzymes (Uthumporn, Shariffa, & Karim, 2012). Extrusion cooking is a hydrothermal treatment of high temperature and short duration, during which flours or starches are subjected to high temperatures and mechanical shearing at relatively low levels of moisture content (Camire, Camire, & Krumhar, 1990). By means of extrusion, it is possible to gelatinise the starch present in cereal flour (Martínez, Calviño, Rosell, & Gómez, 2014). Several authors have used extruders to gelatinise native starch and hydrolyse it enzymatically (Govindasamy, Campanella, & Oates, 1997a, 1997b; Lee & Kim, 1990; Vasanthan, Yeung, & Hoover, 2001).

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The vast majority of the studies about hydrolysis of cereal-based products focus on starch modification whereas flour modification has been scarcely investigated. Vasanthan et al. (2001) studied the dextrinisation of barley flours with alpha-amylase by extrusion. Flours are fine, powdery materials obtained by grinding and sifting the starch-containing plant organelles. Components often found in flours include starch, non-starch polysaccharide, sugar, protein, lipid, and inorganic materials. Thereby, the interactions between starch and non-starch components of flour during hydrothermal and enzymatic treatments are possibly different from that of starch. Commercial wheat flour is produced by milling of wheat kernels, whereas wheat starch is generally obtained by gluten agglomeration. Such a treatment involves four major issues to consider: raw materials, products, cost and operability (Maningat, Seib, Bassi, Woo, & Lasater, 2009). Moreover, water consumption and effluent disposal demand careful operation of the plant (Maningat et al., 2009). Therefore, the lower cost and environmental impact of subjecting wheat flour instead wheat starch to enzymatic hydrolysis could made flour modifications a better alternative for industrial processes.

Despite the particular physicochemical characteristics of extruded flours and their high susceptibility to enzymatic hydrolysis, the properties of their hydrolysed products have never been studied, nor have they been compared with hydrolysed products of native flours. The objective of the present study was to investigate the effect of a potential feasible industrial enzymatic hydrolysis (by alpha-amylase, amyloglucosidase, or a blend of both) on microstructure, oligosaccharide composition, crystallinity, pasting, colour, and hydration properties of native and extruded wheat flours.

2. Materials and methods

2.1. Materials

Native wheat flour (11.73% and 11.20% w/w of moisture and protein contents, respectively) was supplied by Harinera Castellana (Medina del Campo, Valladolid, Spain). Extruded modified wheat flour was provided by Harinera Los Pisones (Zamora, Spain), which performed the extrusion treatment using a Bühler Basf single screw extruder (Bühler S.A., Uzwil, Switzerland). The extrusion conditions were carried out based on preliminary experiments in order to ensure starch gelatinization. The length-to-diameter (L/D) ratio for the extruder was 20:1. 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 with a screw speed of 340 rpm. The extruded product was dried by convection air till it reached 11.2% of moisture. Then it was ground with a compression roller to a particle size below 200 μm.

The amyloglucosidase from *Aspergillus niger* AMILASE™ AG 300L (300 AGU/mL) and the fungal alpha-amylase Fungamil® 800L from *Aspergullus oryzae* (800 FAU/g) were gently provided by Novozymes (Bagsvaerd, Denmark).

2.2. Methods

2.2.1. Flour hydrolysis

The quantity of enzymes was based on previous experiments, where the minimum amount of enzyme to produce changes in the viscosity of starch slurries was selected. Amylase and amyloglucosidase flour slurries with a 0.2% w/w of enzyme (flour basis) were made by dissolving 0.1 g (±0.001) of amylase or amyloglucosidase solution (20% w/w of enzyme) respectively into 40 mL (±0.01) of distilled water. In the case of using both enzymes simultaneously, 0.05 g (±0.001) of each enzyme was dissolved. The quantity of flour was also selected based on preliminary tests, in order to achieve easily dryable suspensions. Then, 10 g of flour were added to the enzyme solution previously prepared and mixed to achieve a homogenous paste. These pastes were covered by plastic film to avoid drying of the sample and then incubated at 50 °C for 2 h. With the aim of bringing to an end the enzymatic activity, the pastes were heated at 105 °C for 4 h. Afterwards, they were rested in a desiccator at room temperature for 3 min, before being milled in a Moulinex super junior s (Groupe Seb Iberica, S.A, Barcelona) for 20 s. Flours were stored in airtight plastic containers at 4 °C until analysis. Thereby, the whole process of flour hydrolysis was performed considering the feasibility of potential industrial processes in the food industry.

2.2.2. 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).

2.2.3. Oligosaccharide content of flours by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC–PAD)

The aim of the HPAEC–PAD analysis was to determine the content of oligosaccharides in the extruded and non-extruded enzymatically treated flours. D-(+)-Glucose, maltose monohydrate, maltotetraose, and maltopentaose (Neat, Sigma–Aldrich, Steinheim, Germany), isomaltose (98%, Sigma–Aldrich, Steinheim, Germany), and maltotriose hydrate (95%, Sigma–Aldrich, Steinheim, Germany) were the standards employed to analyse these compounds in the flours studied.

Sample treatment consisted of solid–liquid extraction with MilliQ deionised water (Millipore, Molsheim, France) without derivatisation. Then, 0.5 g (±0.09) of the ground sample were weighed in a falcon tube, 15 mL of water were added, and the mixture was shaken for 5 min at 430 rpm in a shaker. Then, 2 mL of Carrez II reagent [potassium hexacyanoferrate (II) trihydrate, Panreac, Barcelona, Spain] were added and the mixture was shaken again for 5 min at 430 rpm. The mixture was centrifuged for 20 min at 12,000 rpm and 20 °C and immediately after, to avoid re-suspension of the precipitate, the supernatant was transferred to a flask. A second extraction was needed; therefore, another 15 mL of water were added to the solid phase obtained from the centrifugation, the mixture was again shaken for 5 min at 430 rpm and centrifuged for 20 min at 12,000 rpm and 20 °C. Afterwards, the supernatant was transferred to the flask containing the first extract. After making up to the volume with water, a suitable dilution was filtered with 0.45 μm nylon filters into the vial and then injected.

HPAEC–PAD analyses were carried out on a Metrohm system (Herisau, Switzerland) consisting of an 850 Professional IC with an isocratic pump, an automatic 858 Professional Sample Processor with ultrafiltration, an 872 extension module to provide another pump and the possibility of making gradients, and an IC Amperometric Detector working as a pulsed amperometric detector (PAD) with a gold electrode as the working electrode and a palladium electrode as the reference electrode. MagICnet software (Metrohm, Herisau, Switzerland) was used to analyse the chromatograms.

Separation was achieved on a Hamilton RCX-30 column and a Metrosep RP2 Guard precolumn from Metrohm (Herisau, Switzerland), with the same stationary phase as the column. Column and precolumn were thermostated at 30 °C and the PAD at 35 °C. The flow rate was 1.0 mL/min constantly and the volume injection was 20 μL. A binary gradient solvent system was used as mobile phase, consisting of (A) 50 mM NaOH (Panreac, Barcelona, Spain) and (B) a mixture of 500 mM NaAcO (Panreac, Barcelona, Spain)