Bioresource Technology 149 (2013) 191–199

Contents lists available at ScienceDirect

Bioresource Technology

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

Use of an extruder for pre-mixing enhances xylanase action on wheat bran at low water content

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HIGHLIGHTS

- Xylanase action (AX solubilisation and hydrolysis) on wheat bran was studied.
- Impacts of mixing method, water content and bran particle size were studied.
- With xylanase, MW of WEAX was not significantly affected by these variables.
- Extruder enhanced AX solubilisation as compared to blade mixing at low water content.
- Plasticization in extruder probably enhanced xylanase action via improved diffusion.

ARTICLE INFO

Article history:
Received 14 July 2013
Received in revised form 3 September 2013
Accepted 6 September 2013
Available online 20 September 2013

Keywords:
Wheat bran
Enzymatic hydrolysis
High solids
Mixing
Particle size

ABSTRACT

The aim of the work was to test the hypothesis that at low water content enzyme action on biomass is enhanced when the raw material is in the form of a continuous mass instead of powder/granular form. Effects of two pre-mixing methods, blade-mixing and extrusion, on xylanase action were studied during stationary incubation of wheat bran of different particle sizes, also in comparison with incubation at high water content with continuous stirring. The use of an extruder enhanced arabinoxylan (AX) solubilisation at low water content (<54%), as compared to blade-mixing. AX solubilisation was highest in the high-water stirring treatment, but based on molecular weight, xylanase action on solubilised AX was similar as in the extrusion-aided process. Pre-mixing by extrusion enabled efficient enzyme action at low water content without the requirement for continuous mixing, probably due to the enhanced diffusion by the formation of a continuous mass in the extruder.

1. Introduction

In the extrusion process food ingredients are forced to flow, under mixing, heating and shear, through a die that forms and/or puff-dries the ingredients (Rossen and Miller, 1973). Extrusion technology can be used either to form ready-to-eat foods (snacks, cereals, pasta, confectionery) or to modify food ingredients. A less studied approach is to use extruders as bioreactors for enzymatic processes under elevated temperature, pressure and shear, at moisture levels as high as 70% or more. "Wet extrusion" with feed moisture content above 40% has been possible only since the late 1980s due to developments with twin screw extruders including sophisticated barrel designs, screws and dies (Akdogan, 1999). Compared to traditional stirred-tank reactors, extruders and screw reactors present a cost competitive alternative reactor type for enzymatic modification of biomaterials, especially when targeting dry end products, since the extruders can operate at higher solids content, thus reducing the need for addition and removal of large amounts of water.

The use of an extruder for enzymatic modification has previously been studied mainly for liquefaction of starch by thermostable α-amylase (Linko, 1989; Tomás et al., 1997; de Mesa-Stonestreet et al., 2012). The impact of water content has also been studied, and in most cases the conversion has been highest at the highest water content studied, typically at 55–70% water content, as reviewed by Linko (1989) and Akdogan (1999), although Tomás et al. (1997) reported maximum starch hydrolysis at an intermediate water content of 60% when studying in the range of 55–65%. However, as starch needs to be gelatinized for efficient liquefaction, these extrusion treatments were typically performed above 70 °C or even above 100 °C. Studies concerning the use of extrusion for enzymatic modification of other cereal materials or for other targets are rare. However, two recent papers reported the use of cell wall degrading enzymes for enzymatic hydrolysis of oat bran β-glucan at a water content of 50% (Sibakov et al., 2013a) and for modification of brewer's spent grain at a water content of 65% (Steinmacher et al., 2012).

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http://dx.doi.org/10.1016/j.biortech.2013.09.029

Several biocatalytic and thermomechanical methods and processing conditions have been studied as potential means to modify the properties of wheat bran and its components, since wheat bran is a nutritionally appealing (high in dietary fiber, protein and phytochemicals) and widely available raw material which is currently under-utilised as a food ingredient due to its technological and sensory challenges (Sibakov et al., 2013b). Particle size is an important parameter for the use of bran, affecting both its physiological effects and technological functionality (Hemery et al., 2011; Robin et al., 2012; Zhang and Moore, 1999). Decreasing the particle size by grinding increases the surface area available for reactions. It has been shown that decreasing the particle size of plant materials may enhance their enzymatic hydrolysis (Silva et al., 2012; Niemi et al., 2012; Mahasukhonthachat et al., 2010; Dasari and Berson, 2007). Reduction of particle size can also affect the physicochemical properties of bran, such as water uptake and solubility (Mahasukhonthachat et al., 2010; Zhu et al., 2010), as well as the rheological behaviour of biomass slurries (Dasari and Berson, 2007; Viamajala et al., 2009), which may play an important role in enzymatic processes especially at low water content, when only a limited amount of free water is available.

Solubilisation of the arabinoxylan (AX) of cereal bran by endoxylanases has been shown to modify the technological properties of the bran (Katina et al., 2012; Lebesi and Tzia, 2012). We have previously shown that enzymatic solubilisation of bran AX can be efficiently performed even at low water content (40%) using continuous mixing (Santala et al., 2011, 2012). However, when processing at low water content, i.e. at high consistency, continuous mixing requires a high amount of energy, which may not be feasible in industrial processes. In the previous study (Santala et al., 2011), the enhanced AX solubilisation was related to the formation of a compact plastic mass during the treatment at the water content of 40%, because it resulted in the reduction of bran particle size due to the high shear during the treatment with continuous mixing (Santala et al., 2012). However, it is also possible that the formation of a continuous mass from the granular/powdery material can be used as a means to facilitate enzyme action even without continuous mixing. In the current study the aim was to test this hypothesis by studying the effects of two different pre-mixing and forming methods, blade-mixing and extrusion, on xylanase action during stationary (i.e. without stirring) incubation of wheat bran. The impact of treatment water content and bran particle size on the solubilisation and hydrolysis of AX during the different processes was investigated. Further, the aim was to compare the stationary enzyme incubation at low water content to incubation with continuous stirring at high water content.

2. Methods

2.1. Bran

Commercial wheat bran (Fazer Mill & Mixes, Lahti, Finland) was used as raw material and ground by TurboRotor technology (Mahltechnik Görgens GmbH, Dormagen, Germany) to three different levels of fineness. The particle size distributions of the bran raw materials (Table 1) were determined in triplicate from dry bran dispersions by laser light diffraction (Mastersizer 3000, Malvern, Worcestershire, UK) and calculated from the volumetric distribution of the particles using the Fraunhofer optical model. The heat damage possibly associated with intensive milling treatments could be avoided, since in the grinding technology used the high air throughput and short residence times ensured that the product temperature remained below 45 °C. The dietary fibre (DF) content of the brans (Table 1), was analysed by AOAC method No. 991.43 (Prosky et al., 1988). For the quantification of total AX (Table 1), 0.1 g of bran was mixed with 5 ml of 0.5 M H_2SO_4 and boiled for 30 min and centrifuged, followed by a colorimetric determination (Douglas, 1981).

2.2. Hydration properties

Water holding capacity was determined by the Baumann apparatus as described previously (Santala et al., 2012) using a sample size of 50 mg and measurement time of 30 min.

2.3. Xylanase enzyme preparation

A commercial *Bacillus subtilis* xylanase preparation, Depol 761P (Biocatalysts Ltd., Cardiff, UK), was used for the bran treatments. The activity profile (xylanase 28,660 nkat/g, polygalacturonase 1317 nkat/g, β-glucanase 1625 nkat/g, α-amylase 44 nkat/g, and β-xylosidase 2 nkat/g) of the preparation was previously reported by Santala et al. (2012). According to the manufacturer, the optimum temperature range of the enzyme preparation is 45–55 °C.

2.4. Extrusion-aided and blade-mixed treatments at water contents of 37–60%

The process scheme of the treatments is presented in Fig. 1. For the enzymatic treatments at water contents of 37–60%, the xylanase preparation (in powder form, dosed according to its xylanase activity at 200 nkat/g bran dm) was first mixed carefully with 450 g of dry bran, after which the mixture was pre-conditioned to a moisture content of 20% by adding water slowly while mixing (speed setting 2) with a Kenwood KM300 mixer (Kenwood Ltd., Havant, United Kingdom) with a K-shaped blade for 2 min. Pre-conditioning was also performed for the blank extruder treatments (i.e. without enzyme addition). For the extrusion-aided treatments, the pre-conditioned bran mixture was transferred to the feeding bowl of a co-rotating twin screw extruder (APV MPF 19/25, Baker Perkins Group Ltd, Peterborough, UK) within 20 min and fed to the extruder at a rate of 26 g/min. The barrel temperature was set at 50 °C and it was monitored that the temperature in the barrel remained at 50 °C during all the treatments. The screw speed was 65 rpm. Water was pumped to the barrel at an appropriate rate in order to obtain moisture contents of 37 ± 0.5%, 42 ± 1%, 48 ± 1%, 54 ± 1%, or 60 ± 1% in the

Table 1
Properties of the bran raw materials.

	Unground	Coarse	Fine	Ultrafine
Median particle size (μm)	1001 ± 9	702 ± 59	327 ± 9	81 ± 2
90% of particles < (μm)	2257 ± 16	1873 ± 163	895 ± 57	401 ± 28
10% of particles < (μm)	283 ± 5	127 ± 13	29 ± 1	10 ± 1
Total DF (% bran dm)	48.0	48.9	47.9	48.4
Soluble DF (% bran dm)	3.1	3.5	4.1	4.6
Total AX (% bran dm)	20.6 ± 0.4	20.5 ± 0.3	20.3 ± 0.6	20.6 ± 0.2
WEAX (% bran dm)	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
Water holding capacity(g/g bran dm)	3.7 ± 0.2	3.7 ± 0.2	3.7 ± 0.1	3.3 ± 0.1

The results are expressed as means ($n = 4$) ± standard deviation.