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Towards an optimal process for gelatinisation and hydrolysis of highly concentrated starch–water mixtures with *alpha*-amylase from *B. licheniformis*

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Abstract

The enzymatic hydrolysis of starch is usually carried out with 30–35 w/w% starch in water. Higher substrate concentrations (50–70 w/w%) were reached by using a twin-screw extruder for gelatinisation and for mixing enzyme with gelatinised starch prior to enzymatic hydrolysis in a batch reactor. The aim of this study was to determine which parameters are important for gelatinisation of wheat starch and to investigate the effects of different extrusion conditions on the enzymatic hydrolysis. After extrusion, the degree of gelatinisation was measured. During hydrolysis, the carbohydrate composition, the dextrose equivalent (DE) and the *alpha*-amylase activity were measured. Gelatinisation measurements showed that mechanical forces lowered the temperature required for complete gelatinisation. During hydrolysis experiments, high DEs were observed even if starch was not completely gelatinised during extrusion. Due to high substrate concentrations, the residual *alpha*-amylase activity remained high throughout enzymatic hydrolysis, although high temperatures were used. Increased substrate concentrations did not affect the carbohydrate composition of the product. Furthermore, the time required for the batch hydrolysis step could be varied by choosing a different enzyme-to-substrate ratio. This article provides a basis for detailed optimisation of this process to develop an industrial-scale process at high substrate concentrations.

Keywords: Starch gelatinisation; Enzymatic hydrolysis; Extrusion; Process design; Ethanol production

1. Introduction

The enzymatic hydrolysis of starch is an important industrial process that consists of three steps: gelatinisation, liquefaction and saccharification. In industry, a jet cooker is used to gelatinise starch by mixing the starch slurry with steam under pressure at 100–175 °C (Van der Maarel et al., 2002). The residence time in such a jet cooker is in the order of seconds. After gelatinisation, liquefaction and saccharification can take place in a hydrolysis reactor. Usually, a thermostable *alpha*-amylase is used that is mixed with the starch slurry before passing through the jet cooker. The dextrose equivalent (DE) of the product depends 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 (Kennedy et al., 1988).

The industrial gelatinisation process described above is usually carried out with a 30–35% dry solids starch slurry.

Abbreviations: DE, dextrose equivalent; DG, degree of gelatinisation; DP, degree of polymerisation; DSC, differential scanning calorimetry; HPLC, high-performance liquid chromatography; SME, specific mechanical energy; TSE, twin-screw extruder

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Increasing the substrate concentration during the enzymatic hydrolysis can yield a higher productivity, and a higher enzyme stability (De Cordt et al., 1994; Klibanov, 1983). When the starch concentration increases, the temperature required to reach complete gelatinisation increases rapidly (Donovan, 1979). Moreover, the viscosity of the starch slurry increases with increasing starch content and this complicates further processing. Conventional jet cookers cannot be used anymore at high substrate concentrations due to the increased viscosity. Since the gelatinisation temperature increases, addition of the enzyme during the gelatinisation process is unfavourable, because it can lead to enzyme inactivation. A different process is therefore needed to handle more concentrated starch slurries. Extruders appear to be suitable for this purpose.

Several authors have used extruders for non-enzymatic starch processing (Blanche and Sun, 2004; Cai and Diosady, 1993; Cai et al., 1995; Colonna et al., 1984; Davidson et al., 1984; Gomez and Aguilera, 1984; Jackson et al., 1990; Zheng and Wang, 1994). Other researchers used an extruder to gelatinise native starch and hydrolyse it enzymatically (Ćurić et al., 1998; Govindasamy et al., 1997a, b; Lee and Kim, 1990; Roussel et al., 1991; Vasanthan et al., 2001) or used it only to hydrolyse pre-gelatinised starch (Komolprasert and Ofoli, 1991a). Because the residence time in an extruder is limited, it is not possible to produce a hydrolysate with a high DE (more than 25). If a higher DE is desired, a batch reactor is often linked to an extruder to increase the hydrolysis time (Chouvel et al., 1983; Linko et al., 1980, 1983; Reinikainen et al., 1986; Van Zuilichem et al., 1990). Komolprasert and Ofoli (1991b) also used the combination of an extruder and a batch reactor, but they started with pre-gelatinised starch. The extruder was used to mix pre-gelatinised starch with the enzyme and perform the liquefaction before saccharification in a batch reactor. Note that the use of separate process steps for gelatinisation and enzymatic hydrolysis makes it possible to optimise both processes independently.

In many cases, the enzyme was added at the beginning of the extruder together with the starch–water mixture (Chouvel et al., 1983; Ćurić et al., 1998; Govindasamy et al., 1997a, b; Komolprasert and Ofoli, 1991a; Lee and Kim, 1990; Linko et al., 1980, 1981, 1983; Reinikainen et al., 1986; Vasanthan et al., 2001). Chouvel et al. (1983) also carried out experiments where the enzyme was added to the extruder approximately half-way along its length. Ćurić et al. (1998) and Linko et al. (1980, 1981) found that the residual *alpha*-amylase activity after extrusion decreased under several extrusion conditions. To reduce the amount of enzyme deactivation during extrusion, the enzyme should be added at the end of the gelatinisation section and not at the beginning of the extruder.

For optimisation of a process suitable for gelatinisation and enzymatic hydrolysis at high starch concentrations, it is important to know whether complete gelatinisation is achieved (to reach a high degree of hydrolysis in the following steps); whether the enzyme is still active during the hydrolysis reaction (to maintain a high hydrolysis rate); and whether the desired product is formed (to decide how much time is needed for the hydrolysis reaction). It is therefore essential to measure the degree of gelatinisation (DG), the enzyme activity and the carbohydrate composition. The residual *alpha*-amylase activity during the enzymatic hydrolysis of starch in a batch reactor at high starch concentrations was not measured before. When the enzyme is added at the end of the extruder, the residence time of the enzyme is small in comparison with the time it spends in the batch reactor. As a result, a much larger decrease in *alpha*-amylase activity can be expected during the enzymatic hydrolysis in a batch reactor in comparison with the decrease in *alpha*-amylase activity during extrusion. Furthermore, in the literature the DE was often determined instead of the carbohydrate composition. DE measurements alone are not suitable for the purpose of defining the product, because the same DE can be found for products with a different carbohydrate composition.

Besides the required changes in the process, an increased starch content can also affect measurements of the DG (Baks et al., 2007), the enzyme activity and stability (Baks et al., 2006a, b; Linko et al., 1983) and the carbohydrate composition.

The objective of this article is to determine which process parameters are important for gelatinisation of starch in a twin-screw extruder (TSE) and how extrusion conditions can affect the subsequent enzymatic hydrolysis of starch in a batch reactor at high starch concentrations. We will focus on several output parameters: the DG, the enzyme activity, the DE and the carbohydrate composition. The enzyme activity and carbohydrate concentration will be measured at different times during the hydrolysis of the gelatinised starch slurry. The knowledge gathered during this study can be used for more detailed process optimisation.

2. Experimental

2.1. Materials

Native wheat starch (Excelsior) was obtained from Latenstein (Nijmegen, the Netherlands) and contained 10.0 ± 0.1 w/w% water (95% confidence interval, based on three measurements). The moisture content was determined by drying wheat starch in a hot air oven at 105 °C until the mass of the samples was constant in time. The water content of wheat starch was taken into account during all experiments. Thermostable *alpha*-amylase from *Bacillus licheniformis* (Termamyl 120 L) was donated by Novozymes (Bagsværd, Denmark). The enzyme concentration used during the experiments is expressed in mass percent of this enzyme stock solution per equivalent mass of substrate (w/w%). Maltose monohydrate, fuming hydrochloric acid, sodium hydroxide, sodium chloride,