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Amyloglucosidase Hydrolysis of High-Pressure and Thermally Gelatinized Corn and Wheat Starches

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The study of glucose production using amyloglucosidase as a biocatalyst was carried out using high-pressure and thermally gelatinized corn and wheat starches. For corn starch, the measured initial rate of glucose production obtained from thermal gelatinization is faster than that obtained from the two high-pressure treatments, but the equilibrium yield of glucose was found to be similar for the three treatments. High-pressure treatments of wheat starch significantly improve the equilibrium yield of glucose compared with those obtained from the thermally gelatinized wheat starch. This difference has been related to the formation of amylose−lipid complexes during heating and could also explain previous physicochemical differences observed between high-pressure and thermally gelatinized starch.

Keywords: *Starch gelatinization; high pressure; biocatalysis; starch hydrolysis*

INTRODUCTION

Recent progress in research into the use of high-pressure techniques in food science and technology (Hayashi, 1992; Balny et al., 1992) has led to the manufacture of products at high pressure. As well as the well-established effects of pressure on microorganisms and proteins (Hawley, 1971; Kunigi, 1993; Dumay et al., 1994; Ledward, 1995), previous work has shown that high-pressure treatment alone will bring about gelatinization of starch (Douzals et al., 1996, 1998). Starch consists of two main macromolecules, amylose and amylopectin. Amylose is an essentially linear polymer consisting of α-1,4-linked D-anhydroglucose units, while amylopectin is highly α-1,6-branched (Cui and Oates, 1999). It is generally agreed that these macromolecules are assembled in a cluster structure, in which the starch granule is composed of crystalline and amorphous regions (Buléon et al., 1998; Blanchard, 1987). Starch gelatinization corresponds to a combined mechanism of semicrystalline polymer melting and hydration (Eliasson, 1980) since gelatinization occurs in an aqueous medium.

The hydrolysis of starch to low-molecular weight products is widely applied in the sugar, spirits, and textile industries, as well as in brewing. Enzymatic hydrolysis exhibits some substantial advantages over acid hydrolysis (Yankov et al., 1986), since there is no need for high temperature and pressure. The use of thermostable enzymes leads to additional advantages such as an increase in reaction rate, operation without contamination by microorganisms, a higher stability of the enzyme to the denaturing action of solvent, detergents, and proteolytic enzymes, etc. The increase in reagent stability and the decrease in medium viscosity at higher temperatures are additional advantages of using thermostable enzymes.

Since 1980, several papers have indicated that high-

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pressure gelatinization of starch is possible by adding heat treatment to compression (Muhr and Blanchard, 1982; Hayashi, 1992; Kudla and Tomasik, 1992; Hibi et al., 1993). Recent work (Douzals et al., 1999) has shown the effects of combined treatments on starch gelatinization that occurred under different conditions of temperature and pressure, and the isogelatinization curve for 100% gelatinization was drawn. The total gelatinization of wheat starch was found to occur after high-pressure treatment at 600 MPa and 25 °C for 15 min (Douzals et al., 1996) and after thermal treatment at 80 °C for 15 min (Douzals et al., 1998). Complete gelatinization was also obtained on cooling at −20 °C and 400 MPa for 15 min (Douzals et al., 1999).

In recent studies, microscopic and macroscopic measurements allowed a more precise understanding of starch gelatinization under pressure (Douzals et al., 1996, 1998). A comparison between thermal and pressure gelatinization processes was based on the achievement of wheat starch gelatinization given by differential scanning calorimetry (DSC). Wheat starch suspensions of 5% dry matter (DM) were treated at 86 °C for 15 min or at 600 MPa and 25 °C for 15 min. Under the conditions described above, starch suspensions subjected to pressure gave original products in terms of swelling index (water binding), amylose release, specific gravity, and preservation of granule structure. Peculiar properties were expected for pressure-induced gels of 30% DM obtained at 600 MPa and 25 °C for 15 min. High pressure generated denser gels than a thermal treatment at 86 °C for 15 min (Douzals et al., 1996). By using Young modulus measurements, calorimetry, X-ray diffraction, and drying kinetics experiments (Douzals et al., 1996), the results showed a limited retrogradation for wheat gels obtained under high pressure.

On the basis of these differences in physicochemical properties (Douzals et al., 1996, 1998) of thermally and high-pressure treated starches, it could be suggested that the molecular structure of starch gelatinized thermally is relatively or completely different from that obtained under high pressure. To test this hypothesis, amyloglucosidase was used as a molecular marker in the enzymatic hydrolysis of both thermally and high-pressure gelatinized starch.

MATERIALS AND METHODS

Materials. All samples were prepared with commercial wheat starch (Prolabo, France) and corn starch (Prolabo, France). Liquid amyloglucosidase (EC 3.2.1.3) from *Aspergillus niger* purchased from Sigma was used with an activity of 6000 units/mL. Amyloglucosidase is capable of hydrolyzing α-1,6-linkages as well as α-1,4-linkages, and the speed of cleavage of α-1,4-linkages is faster than that of α-1,6-linkages (Kusunoki et al., 1982). A diagnostic kit for glucose analysis was purchased from Boehringer (Mannheim, Germany).

Starch Treatment Conditions. Corn or wheat starch suspensions were prepared with 4% DM (on a dry matter basis) with distilled water and sealed in rubber bags of about 15 mL (60 mm length and 18 mm diameter). Thermal treatments were applied in a water bath at 80 °C for 15 min. The temperature was measured with a precision of 1%. High-pressure treatments were realized at 600 (25 °C, 15 min) or 400 MPa (−20 °C, 15 min), using a 5 or 150 mL high-pressure cell connected to a high-pressure circuit previously described by Douzals et al. (1996). Pressure was generated with a hydropneumatic pump (NOVA SWISS). Compress and release rates were about 20 MPa s^{-1}. The high-pressure circuit included high-pressure tubing, valves, and pressure sensor (NOVA SWISS). The pressure was measured with a precision of 7% of the total scale.

For the high-pressure treatment at 400 MPa (−20 °C, 15 min), the 5 mL high-pressure cell was placed in a controlled temperature bath with a range of −25 to 100 °C.

Optical Analysis of Granule Gelatinization. Previous treatments were chosen so a complete starch gelatinization could be involved. Complete starch gelatinization (100%) was assessed after each treatment by the loss of optical birefringence of the starch granules. The birefringence of corn and wheat granules was checked using an inverted light microscope provided with a polarization analyzer. The melted fraction was determined by the number of granules that lost their birefringence, divided by the total number of starch granules. One hundred starch granules were counted three times. The precision in the melted fraction determination was about 5%.

Hydration Measurements. Hydration of 100% gelatinized starch was assessed after thermal and/or pressure treatment using the swelling index method described by Doublier (1981) and Douzals et al. (1998). The swelling index was determined by the amount of water bound to insoluble dry matter. Starch products were centrifuged at 2000 rpm for 5 min at 25 °C. Supernatants were isolated, and DM was quantitated from dried pellets (after 48 h at 105 °C). The swelling index is given in grams of water per gram of DM with a precision of ±0.5 g of water per gram of DM.

Assay of Amyloglucosidase Activity. Experiments were carried out in a 100 mL glass flask whose contents were being stirred. The gelatinized starch solution (4% DM) was adjusted to pH 5.35 with 0.012 M acetate buffer to obtain s 0.2% gelatinized starch solution; 50 mL, corresponding to 0.1 g of gelatinized starch, was immersed in a water bath at 50 °C. The solution was stirred by a magnetic stirrer. After attainment of the desired temperature, the enzymatic reaction was initiated by adding 4.16 μL of amyloglucosidase. The reactions were carried out at 50 °C, and at given time intervals, the glucose concentration in the reaction medium was determined spectrophotometrically according to the glucose diagnostic kit.

Kinetic Parameters Calculation. The values of the measured initial rates of glucose production were determined from the time course curves of the corresponding treatments and were expressed in micromolar per minute per unit of amyloglucosidase. Each experiment was independently performed three times. Blanks were made in duplicate with buffer replacing the enzyme. Bars represent the confidence interval of the mean (95% level).

RESULTS AND DISCUSSION

Time Course of Gelatinized Corn Starch Hydrolysis. The experimental results of glucose production by amyloglucosidase hydrolysis of corn starch gelatinized by thermal and the two high-pressure treatments are presented in Figure 1. It can be observed that the glucose production from thermally gelatinized corn starch is more rapid than that obtained from the two high-pressure treatments, which are relatively similar. The rate of glucose production from high-pressure and low-temperature (400 MPa and −20 °C for 15 min) treated corn starch seems to increase a little slower than that treated at 600 MPa and 25 °C for 15 min.

A higher conversion yield after 5 min is obtained with thermally gelatinized corn starch (78%) as compared with those obtained with the two high-pressure treatments at 600 MPa and 25 °C and 400 MPa and −20 °C, which are 58 and 43%, respectively. At equilibrium, 90% of the initial treated corn starch is in the form of glucose for the three cases.

The initial rates of glucose formation have been determined graphically from the time course curves (Figure 1). The measured initial rate of glucose production obtained from thermally (80 °C for 15 min) gelatinized corn starch (38.88 μM min^{-1} $unit^{-1}$) is higher than

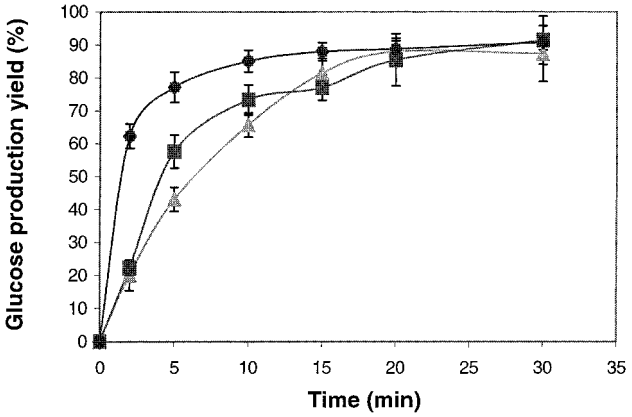

Figure 1. Progress curve of glucose production during gelatinized corn starch hydrolysis carried out at 50 °C with 4.16 μL of amyloglucosidase (see the conditions described in detail in the text): thermal treatment (◆), high-pressure and ambient-temperature treatment (■), and high-pressure and low-temperature treatment (▲). Bars represent the confidence interval of the mean (95% level).

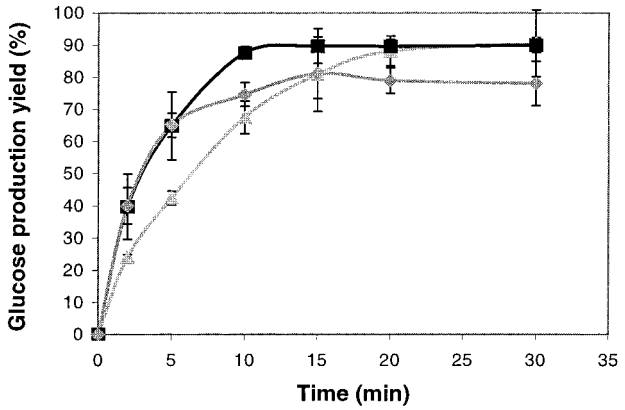

Figure 2. Progress curve of glucose production during gelatinized wheat starch hydrolysis carried out at 50 °C with 4.16 μL of amyloglucosidase (see the conditions described in detail in the text): thermal treatment (◆), high-pressure and ambient-temperature treatment (■), and high-pressure and low-temperature treatment (▲). Bars represent the confidence interval of the mean (95% level).

that obtained from high-pressure and ambient-temperature (600 MPa and 25 °C for 15 min) gelatinized corn starch (15.48 μM min^{-1} unit^{-1}). The lowest is measured for high-pressure and low-temperature (400 MPa and −20 °C for 15 min) gelatinized corn starch (11.52 μM min^{-1} unit^{-1}).

A comparison of the swelling indexes of corn starch gelatinized by thermal and the two high-pressure treatments is presented in Table 1. It can be observed that the swelling index of high-pressure and low-temperature (400 MPa and −20 °C for 15 min) gelatinized corn starch is lower than that obtained under high pressure and ambient temperature (600 MPa and 25 °C for 15 min), which is itself lower than that obtained by thermal treatment (80 °C for 15 min).

Previous results (Douzals et al., 1996, 1998) have shown that starch gelatinization under high pressure generated a limited granule expansion (water binding), denser gels, and lower rates of amylose release than thermal treatment. These results would mostly explain the specific behavior of corn starch gelatinized under high pressure. The difference between the measured initial rates of gelatinized corn starch hydrolysis obtained from the three treatments could be explained by structural conformation differences and/or by the difference in enzyme diffusion in gaining access to the granule interior.

Time Course of Gelatinized Wheat Starch Hydrolysis. Glucose production by amyloglucosidase hydrolysis of gelatinized wheat starch by thermal and the two high-pressure treatments is presented in Figure 2. As shown by Guerrieri et al. (1997), it can be observed that after the modification in their structure and aggregation, gelatinization considerably improved the availability of wheat starch for enzymatic hydrolysis.

As shown in Figure 2, a higher conversion yield after 5 min is obtained for both thermally (80 °C for 15 min) and high-pressure and ambient-temperature (600 MPa and 25 °C for 15 min) gelatinized wheat starch, which are similar (65%), as compared with that obtained with high-pressure and low-temperature treatment (400 MPa and −20 °C for 15 min), which is only 42%. The equilibrium conversion yield is reached at 10 min for both thermal (80 °C for 15 min) and high pressure and ambient temperature (600 MPa and 25 °C for 15 min),

Table 1. Swelling Indexes of Thermally and High-Pressure Gelatinized Corn and Wheat Starches

	swelling index (g of water/g of starch DM)	
	corn starch	wheat starch
thermal (80 °C for 15 min)	16	10
high pressure (600 MPa and 25 °C for 15 min)	6	6.32
high pressure (400 MPa and −20 °C for 15 min)	4.45	5

which are 75 and 90%, respectively. Nevertheless, the rate of glucose production from high-pressure and low-temperature treated wheat starch (400 MPa and −20 °C for 15 min) seems to increase more slowly than in the previous two cases. The equilibrium yield occurs later (20 min), and its value is similar to that obtained with high-pressure and ambient-temperature (600 MPa and 25 °C for 15 min) treatment (90%).

In the initial stage of the hydrolysis of gelatinized wheat starch (Figure 2), it can be observed that contrary to the lowest measured initial rate of glucose production obtained from high-pressure and low-temperature (400 MPa and −20 °C) treatment (15.96 μM min^{-1} unit^{-1}), there is no significant difference between the measured initial rates obtained from thermal (80 °C for 15 min) and high-pressure and ambient-temperature (600 MPa and 25 °C for 15 min) treatments, which are 27.24 and 26.4 μM min^{-1} unit^{-1}, respectively.

A comparison of the swelling indexes of wheat starch gelatinized by thermal and the two high-pressure treatments is presented in Table 1. It can be observed that the swelling index of high-pressure and low-temperature (400 MPa and −20 °C for 15 min) gelatinized wheat starch is lower than that obtained under high-pressure and ambient-temperature conditions (600 MPa and 25 °C for 15 min), which is itself lower than that obtained by thermal treatment (80 °C for 15 min).

Previous results (Douzals et al., 1996, 1998) have shown that starch gelatinization under high pressure generated a limited granule expansion (water binding), denser gels, and a lower rate of amylose release than thermal treatment at 80 °C for 15 min. These results would mostly explain the specific behavior of wheat starch gelatinized under high pressure. The difference

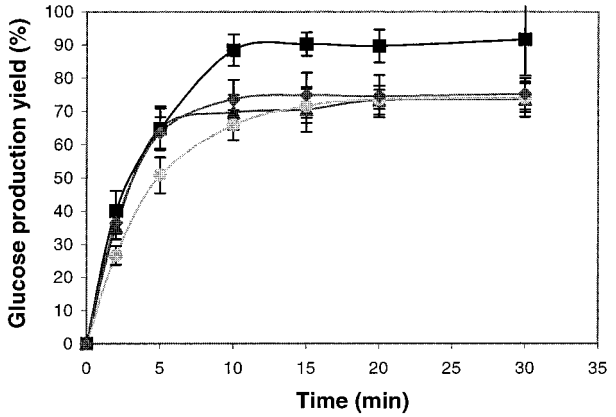

Figure 3. Progress curve of glucose production during gelatinized wheat starch hydrolysis carried out at 50 °C with 4.16 μL of amyloglucosidase (see the conditions described in detail in the text): thermal treatment (◆), high-pressure and ambient-temperature treatment (■), thermal treatment followed by high-pressure treatment (▲), and high-pressure treatment followed by thermal treatment (●). Bars represent the confidence interval of the mean (95% level).

between the measured initial rates of gelatinized wheat starch hydrolysis obtained from the three treatments could be explained by structural conformation differences and/or by the difference in enzyme diffusion in gaining access to the granule interior.

It is important to determine if the lowest equilibrium conversion yield obtained from thermally gelatinized wheat starch is related to structure modifications and if these effects are reversible. Figure 3 presents the time course of glucose production from gelatinized wheat starch obtained by two successive treatments: a high-pressure and ambient-temperature (600 MPa and 25 °C for 15 min) treatment followed by a thermal (80 °C for 15 min) one, and a thermal treatment followed by a high-pressure and ambient-temperature one.

It can be observed (Figure 3) that glucose production increase from thermally gelatinized wheat starch followed by high-pressure treatment is similar to that obtained from a solely thermally treated situation. Compared with the results obtained from only high-pressure and ambient-temperature treatment (600 MPa and 25 °C for 15 min), the thermal treatment (80 °C for 15 min) of high-pressure and ambient-temperature (600 MPa and 25 °C for 15 min) gelatinized wheat starch produces a decrease for both the measured initial rate (17.76 μM min^{-1} unit^{-1} instead of 26.4 μM min^{-1} unit^{-1}) and equilibrium yield (75% instead of 90%) of glucose production.

At equilibrium, the yield of glucose production obtained from the two successive treatments and thermal treatment alone is the same, and is reached approximately in the same time (Figure 3). The reason for such a result may lie in structural changes of the starch granules when complexed with lipids. The formation of amylose−lipid complexes prevents leaching of amylose during sago gelatinization, inhibits swelling of starch granules heated in water, and reduces the water-binding capacity of starch (Cui and Oates, 1999). Similar phenomena for wheat starch have also been reported by Eliasson and Krog (1985) and Holm et al. (1983), and previous studies indicated changes in starch gelatinization behavior that were specifically affected by the presence of lysophospholipids (Eliasson et al., 1988; Huang and White, 1993), which are mainly found in wheat starch (Morisson et al., 1984). From the experimental results, it can be observed that the two high-pressure (low and ambient temperatures) treatments of wheat starch significantly improve the yield of glucose production at equilibrium (90%). The lowest equilibrium yield of glucose production is obtained from thermally treated wheat starch, which could be explained by a complex structural conformation related to the amylose−lipid complexes formed during heating. This assumption is in accordance with the results obtained by Cui and Oates (1999), showing that the amylose−lipid complexes are more resistant to enzyme digestion than free amylose and showing that the digest of the amylose−lysophospholipids complex (found in wheat) is more difficult than the digest of the amylose−monoglycerides complex (found in corn).

CONCLUSIONS

It is clearly shown that a high yield of glucose production by hydrolysis of thermally and high-pressure (400 and 600 MPa) gelatinized corn and wheat starch can be achieved with amyloglucosidase. The difference between the measured initial rates of gelatinized corn and wheat starch hydrolysis obtained from the three treatments could be explained by structural differences and/or by the difference in enzyme diffusion in gaining access to the granule interior. Compared with the yield with thermally gelatinized wheat starch, the two high-pressure treatments of wheat starch significantly improve the yield of glucose production at equilibrium. The lowest equilibrium yield of glucose production is obtained with the hydrolysis of thermally treated wheat starch, which is related to the amylose−lipid complexes formed during heating. This work is now being continued to determine whether an amylose−lipid complex is formed during the two high-pressure treatments.

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