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A thermal analysis investigation of partially hydrolyzed starch

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Abstract

Porous starch granules are formed by the partial hydrolysis of starch using glucoamylases. These porous granules can be used to reveal structural information about the starch itself. Starches from four different sources were used as substrates in this study. These starches were partially hydrolyzed using glucoamylase. The material left behind after enzyme treatment was then treated to thermal analysis using TG and DSC to characterize the hydrolysis product. A structural analysis was made using SEM and powder X-ray diffraction to investigate whether the partial hydrolysis had any effect on the granule structure and the crystallinity of the different starches. In each case, except for potato starch, combustion in air took place at a lower temperature. On the other hand, the gelatinization temperature increased as the porosity increased. © 1998 Elsevier Science B.V.

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1. Introduction

Raw starch granules are used as substrates for the hydrolysis to glucose. The two most common hydrolysis enzymes used are: glucoamylase and α-amylase. α-Amylase breaks the α-1,4 linkages present in starch but cannot act on the α-1,6 links. Glucoamylase, on the other hand, can act on both, α-1,4 and α-1,6 links. Thus, the end product of glucoamylase action is primarily glucose. Depending on the source or origin of the starch, the susceptibility to amylase may differ.

In recent years, the importance of enzymatic saccharification of raw starch without heating has been well-recognized, mainly from the viewpoints of energy-saving and effective biomass utilization. When starch is treated with glucoamylase, the native starch granules degrade, leaving behind porous starch granules. Many studies describing the action of enzymes on starch have been conducted, but description of the partially degraded starch is limited and it was thought that this porous material may be used to reveal structural information about the starch. Enzymatic hydrolysis can help in studies of the physical and chemical structure of starch granules [1]. Starch degradation does not occur uniformly. SEM can be used to examine the granules produced after amylosis. This study was performed in order to obtain a better understanding of the effect of partial hydrolysis on starch due to the action of enzymes.

Starch provides a high proportion of the worlds food-energy intake. Before consumption, starchy food materials are generally heated to a state where a

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transition known as gelatinization occurs. Thus, gelatinization is an important operation in food preparation and processing. The transition entails a loss of structural order [2]. The starch double helices disappear, though some of the resultant polysaccharide coils may form a different helical structure by interacting with lipids. A long-established trend in food research is to take the principles underlying the methods of studying synthetic polymers, and to apply these to food systems. Thermal-analysis techniques provide powerful methods of probing the transitions that take place when synthetic polymers are heated [3]. Recently, Biliaderis et al. [4] suggested that ungelatinized starch can be considered a partially crystalline glassy polymer and that gelatinization is the nonequilibrium melting process that occurs upon the application of heat. Native starches are insoluble in cold water, and swell only slightly due to diffusion and absorption of water into the amorphous regions. This swelling is reversible on drying. However, when starch granules are heated in water to progressively higher temperatures, a point in reached when granule swelling becomes irreversible and structural order disappears. In recent advances in the study of cereal-based food systems, thermal events such as melting and glass transition, have been of much interest to food scientists in both industry and academia. The structural components, responsible for these products (mostly starch), play a key role in determining the desired textural quality, for example, crunchiness, softness/moistness, hardness, etc. In addition, deterioration of texture is often a common subject of study in terms of molecular mechanisms, so that it can be minimized or inhibited. Thus, characterization of cereal biopolymers by thermal analysis is an important approach to the understanding of the functionality of starch on a structural and molecular level.

In this study, starch from corn, wheat, rice, and potato were treated to enzyme attack. The resultant, partially hydrolyzed porous starch granules were then examined using SEM. This material was then treated to thermal analysis, using thermogravimetry and derivative thermogravimetry (TG-DTG) to examine whether porosity had any effect on the thermal behavior of starch granules. The enzyme-treated and native material was also examined using DSC, and the change in the gelatinization temperature recorded.

2. Material and methods

The starches used in this investigation were those of corn, wheat, rice and potato. These were obtained from Sigma. Glucoamylase (22 500 IU g^{-1}) was also obtained from Sigma. (One international unit (IU) of glucoamylase is the amount which releases 1 mg of glucose every 3 min.) The starch samples were treated with glucoamylase, i.e 200 IU ml^{-1} at 37°C for 32 h. The reaction was stopped by the addition of 0.2 M HCl and the sample dried at room temperature. SEM micrographs were obtained using a JOEL JSM-6100 scanning electron microscope. Since starch is a nonconductive material, the granules had to be lightly coated with gold. For the DSC studies, a slurry of starch and water at a ratio of 1 : 10 was prepared. This mixture was stirred for 1 h prior to use. Thereafter, 10 mg of this slurry were weighed into an aluminum crucible and hermetically sealed using a sample encapsulation press. The gelatinization temperature was determined using DSC (Dupont, model 990) calorimeter cell. The instrument was calibrated with pure indium. Each sample was analyzed thrice against an empty reference pan over the 20–100°C range. A heating rate of 10°C min^{-1} was used for all the samples. The TG analysis was conducted using a simultaneous TG-DTA unit from TA instruments, model #2960. The experiments were conducted in an atmosphere of flowing dry air, at a steady gas flow rate of 100 ml min^{-1}. Sample sizes varied between 4 and 5 mg. Platinum crucibles were used to hold the samples, with empty crucibles used for reference. A rising-temperature method of thermal analysis was used, with a heating rate of 10°C min^{-1}. X-ray diffraction patterns were obtained using a Scintag XRD 2000 X-ray powder diffractometer. This is mounted on an X-ray generator, operating at the CuK_α wavelength (1.542 Å). Samples were pressed into a sample holder and scanned for the $2<\theta<35$ range.

3. Results and discussion

The action pattern of various amylases on granular starches has been investigated [5–7] earlier, with particular attention being given to the influence of the amylase source [8]. In this study, the action of *Rhizopus* glucoamylase on several types of starches

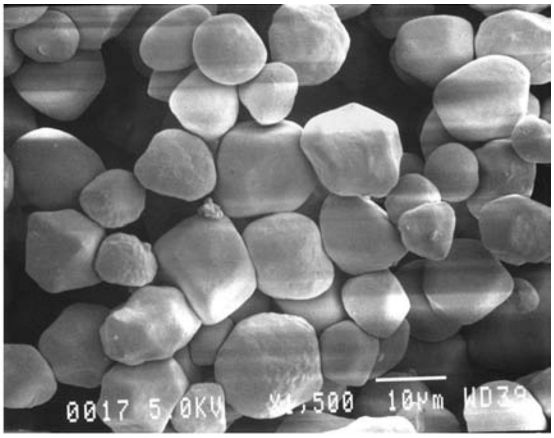

Fig. 1. SEM micrograph of native corn starch granules.

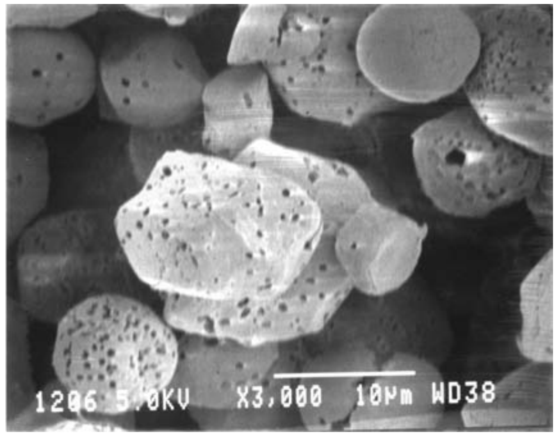

Fig. 2. SEM micrograph of enzyme-treated corn starch granules.

was undertaken to determine the relative susceptibility and extent of the reaction. The starches were carefully selected so as to give a broad spectrum of starch types.

The partially degraded starch granules were than examined using SEM. This was done to study the morphology of the starch granules before, and after, treatment with glucoamylase by SEM. From the SEM photographs, it was evident that hydrolysis does not occur uniformly. Some areas are much more susceptible to attack than others. The SEM photographs of dry granules of commercial native corn, wheat, rice and potato starch were conducted along with the enzyme treated samples. For example, corn starch (Fig. 1) when treated with glucoamylase overnight showed the appearance of large pin holes (Fig. 2). The enzyme acts by first attacking the surface and forming pores on the surface [9]. Rice and wheat starch granules also behave in the same way (Figs. 3 and 4). When potato starch was hydrolyzed, there was heterogeneity in the starch granule degradation, with most granules remaining intact or being only slightly modified. Enzymes cause surface alterations and degrade the external part of the granule by exo-corrosion. When endo-corrosion occurs, the internal part of the granule is corroded through small pores by which enzymes penetrate the granule. In a given starch sample, small granules are more rapidly hydrolyzed than bigger ones, because of a larger available surface area, as previously reported [10]. It appears that the starches which are readily, or rapidly, digested with

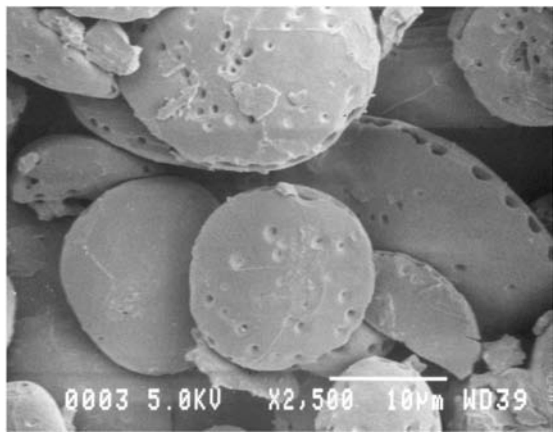

Fig. 3. SEM micrograph of enzyme-treated wheat starch granules.

Fig. 4. SEM micrograph of enzyme-treated rice starch granules.

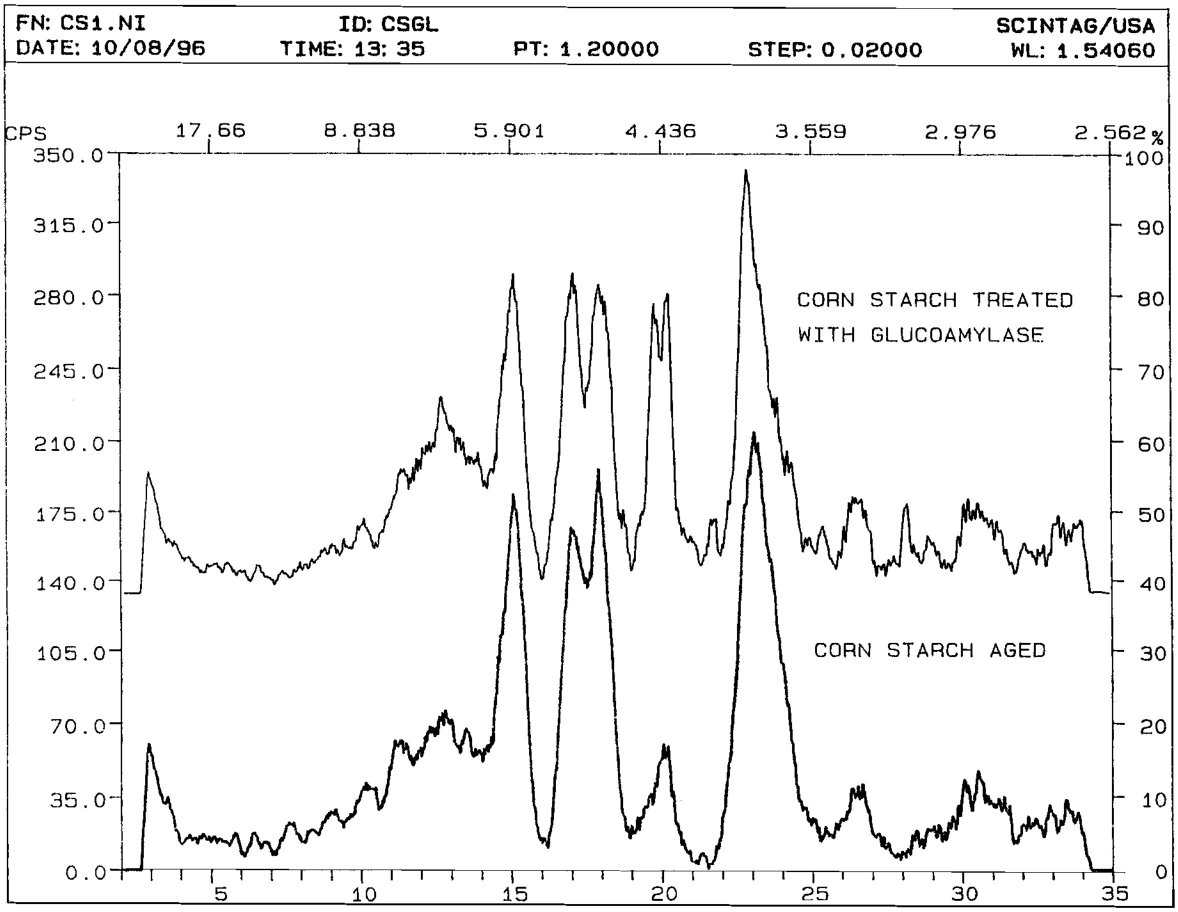

Fig. 5. XRD plot of native and enzyme-treated corn starch granules.

enzyme are those whose surfaces are readily attacked with the formation of canals (wheat, rice, and corn starches). In the case of potato starch, the surface of the granule shows a much greater resistance to enzymatic attack than that of cereal starches. The rate of hydrolysis of nonsolution starch is dependent on the accessible surface area.

Starch-granule crystallinity can be studied with X-ray diffraction technique. Normal starches contain two types of crystallinities, an A-pattern, obtained for cereal starches (except high amylose variety), and B-pattern obtained for root and tuber starches [11]. In native starch, i.e. starch with both amylose and amylopectin, it is the branched molecule (amylopectin) that constitutes the crystallites. The branches of the amylopectin molecules form double helices that are arranged in crystalline domains. The A and B patterns are, thus, different polymorphic forms of starch that differ in the packing of the amylopectin double helices [12]. In order to study the effect of enzymatic degradation on starch, the resulting porous starch was examined by powder X-ray diffraction. Fig. 5 shows both, the native corn starch and the partially degraded starch granules after enzyme action. The native corn-starch granules show a pattern typical for that found for other cereal starches which show the A-pattern, called the cereal type. The enzyme-degraded, partially porous starch also showed the same basic A-type pattern; however, it showed a more crystalline character. The peaks appeared sharper and well defined as compared to those for the native material. This observation is in keeping with the

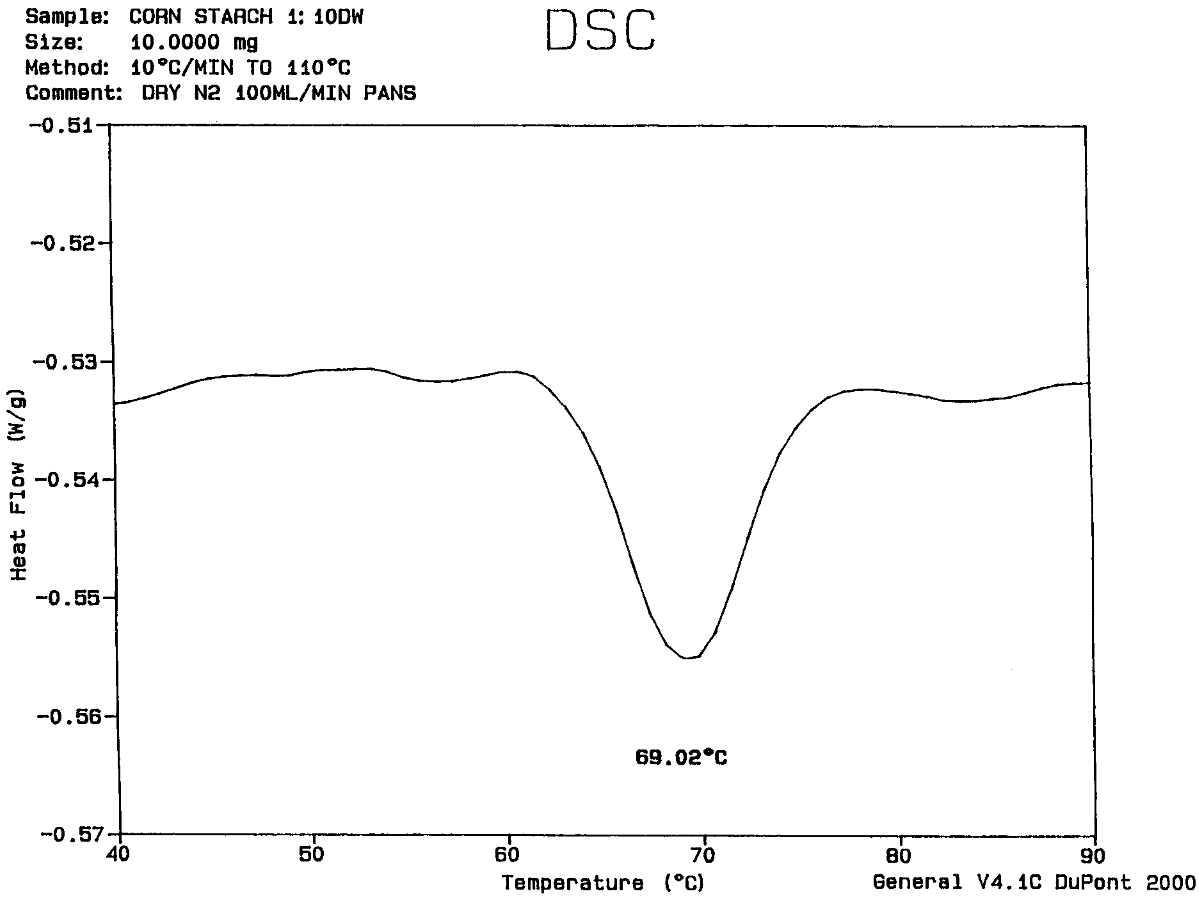

Fig. 6. DSC plot of native corn starch granules mixed with water in the 1 : 10 ratio, showing gelation.

proposal by Gallant et al. [13], mentioned earlier, that the amylosis primarily occurs in the amorphous regions of the starch granules. Similar results were also obtained for wheat and rice starches. On the other hand, there was barely any difference observed between the X-ray pattern of potato starch treated to enzymatic degradation, as compared to the native material. Potato starch is a tuber starch which reveals a B pattern. This shows higher crystalline regions as compared to the cereal starches and has a higher amylose content. On account of this higher crystallinity, the enzyme attack may be restricted as the amorphous regions are not accessible.

When heated, an aqueous suspension of starch in excess water undergoes a cooperative endothermic transition known as gelatinization [14]. A comparison was attempted between starches from four different origins, using the same level of water each time. The onset, T_i, and the peak, T_p, temperatures were determined from the intercepts with the baseline. Untreated wheat starch showed a characteristic melting curve, with T_p=68.6°C. A small shoulder prior to that was also seen (see, e.g. Fig. 6). This is in agreement with the literature values. Partially hydrolyzed corn starch treated with glucoamylase showed an increase in the initial gelatinization temperature (Fig. 7). It was earlier reported [15] that the swelling of larger starch granules occurs at a lower temperature than the swelling of smaller starch granules; therefore it is possible that, for the starches treated with glucoamylase, there is an increase in the relative number of smaller granules after hydrolysis which causes an increase

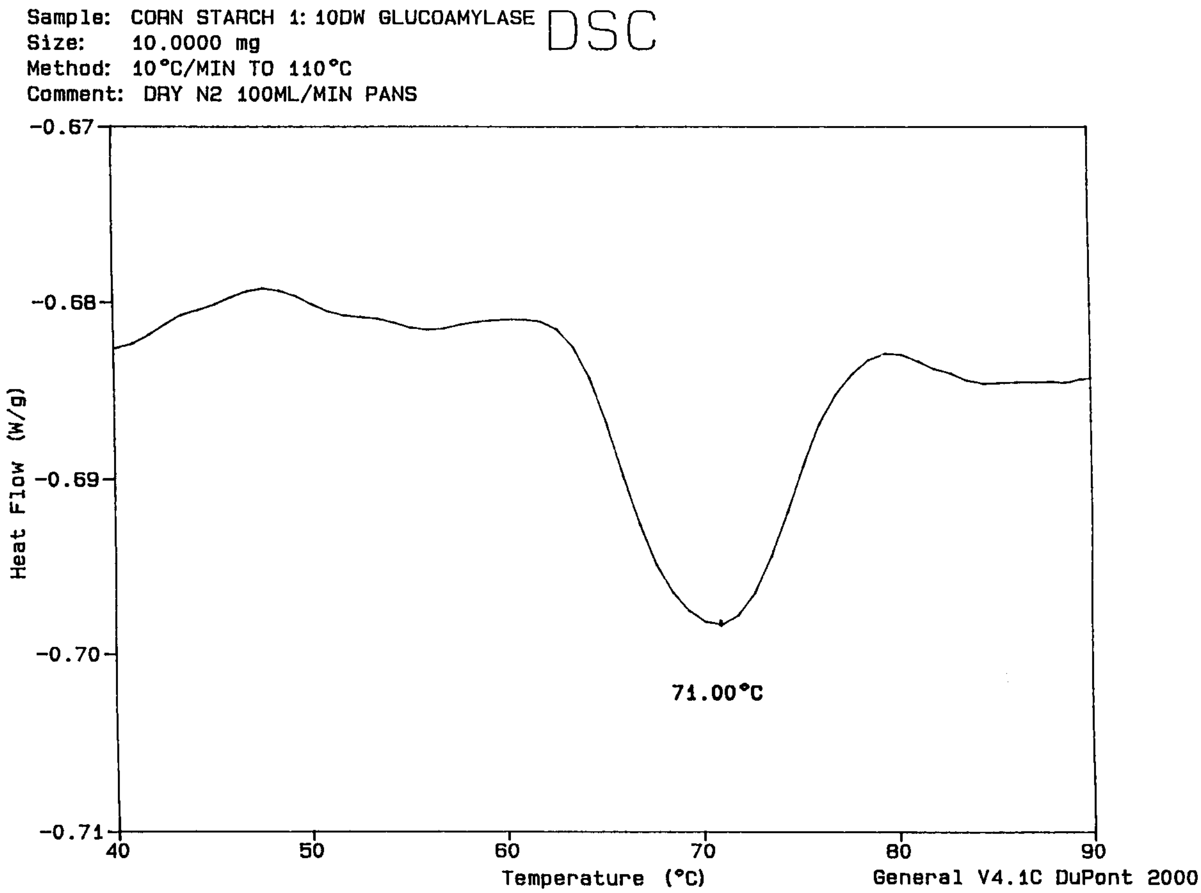

Fig. 7. DSC plot of enzyme treated corn starch granules mixed with water in the 1 : 10 ratio, showing gelation.

Table 1
DSC results, obtained for gelatinization of the native and porous starch granules on account of glucoamylase action (average of three repeats)

1 : 10 Starch in DW*	T_i (°C)	T_p (°C)
Corn starch (CS)	65.2	69.0
CS after hydrolysis	65.0	71.2
Wheat starch (WS)	59.9	62.3
WS after hydrolysis	61.4	65.3
Rice starch (RS)	62.5	63.0
RS after hydrolysis	64.0	64.5
Potato starch (PS)	59.9	64.7
PS after hydrolysis	60.0	64.6

*DW Deionised water.

in the initial gelatinization temperature. As can be seen from Table 1, in each case studied, the partially hydrolyzed starch granules showed a higher peak temperature as compared to the undegraded starch granule. On the other hand, in the case of potato starch, the enzyme-treated and the untreated material showed the same transition temperatures, suggesting that the potato starch granules remained intact even after enzyme treatment. This result is consistent with observations made on the SEM pictures and power X-ray diffraction results on native and enzyme-treated starch granules.

In order to investigate the effect of enzyme treatment on the thermal degradation of the starch granules, they were examined using TG-DTG. Profound modifications and degradation of the molecular structure occur when starch granules are heated. The extent of the changes induced depends on the temperature and time involved and, under extreme conditions, may result in a complete loss of carbohydrate character.

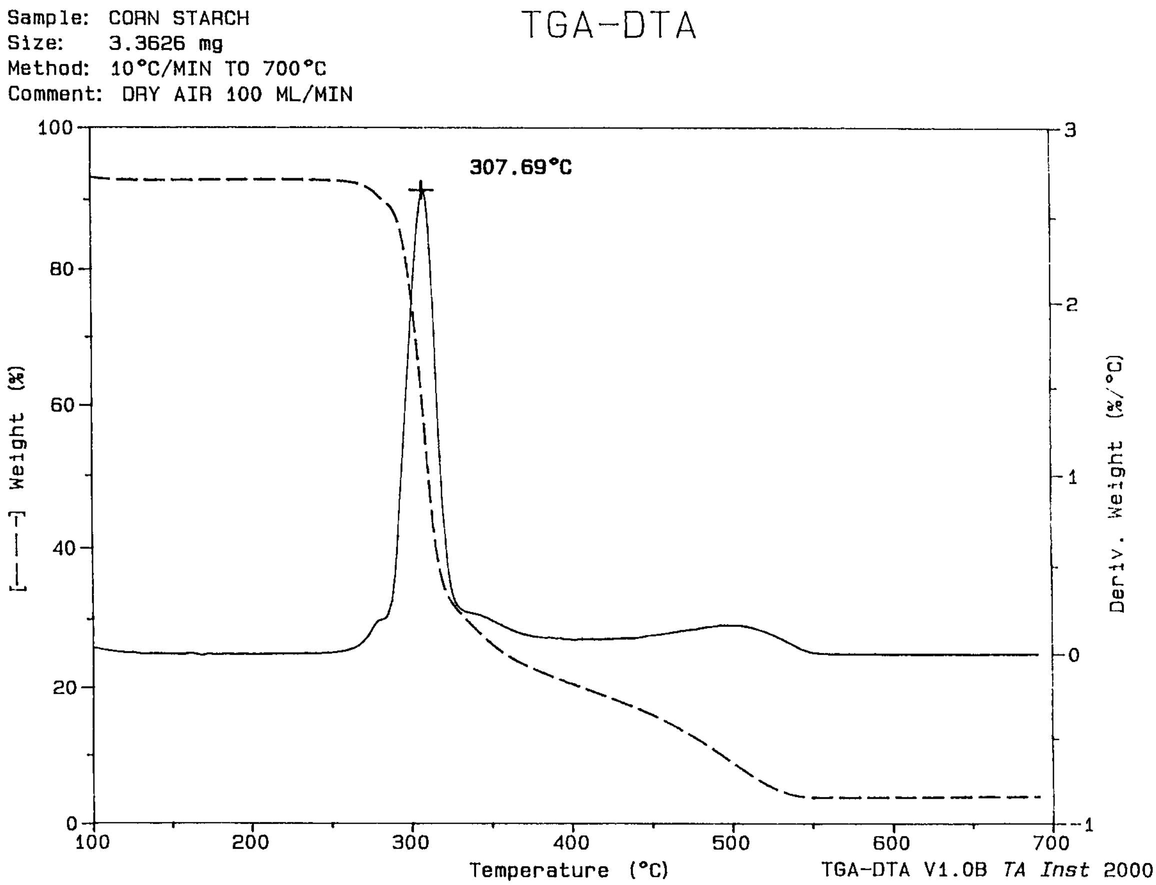

Fig. 8. TG-DTG plot of native corn starch granules.

Thermal treatment of dry starch usually leads exclusively to its depolymerization, unless the temperature applied exceeds 300°C. The starch undergoes a series of irreversible changes. The initial phase causes modification of the polymer structure to form products soluble in cold water, termed pyrodextrins. It has been shown earlier [16], that at higher temperatures, depolymerization of the macromolecules takes place with the formation of β-(1,6) anhydro D-glucopyranose (levoglucosan), 2-furaldehyde (furfural) and a range of lower molecular-weight volatile and gaseous fragmentation products. A carbonaceous residue remains after all the volatile products have been driven off. The degradation pattern of all four enzyme-treated and native starches was followed using TG. The thermograms of all four starches examined were characterized by a distinct endothermic peak, at ca. 310°C in each case. A typical TG thermogram for the degradation of corn starch in an atmosphere of dry air is illustrated in Fig. 8. Earlier reports [17] of qualitative analysis of the gaseous products showed that water was obtained at 310°C, accompanied by straw-colored gases. It has been postulated that the endothermic transition seen is due to selective dehydration, possibly accompanied by transglucosidation. The large peak at 310°C appears to involve further elimination of the polyhyroxyl groups, accompanied by depolymerization and decomposition. This was the general pattern of events observed for all the four starches examined. The T_p for the DTG plot in air showed a lower degradation temperature for the porous granules than the native ones (Fig. 9). This may occur due to the higher surface area seen in porous starch granules, thus indicating that temperatures are lowered due to

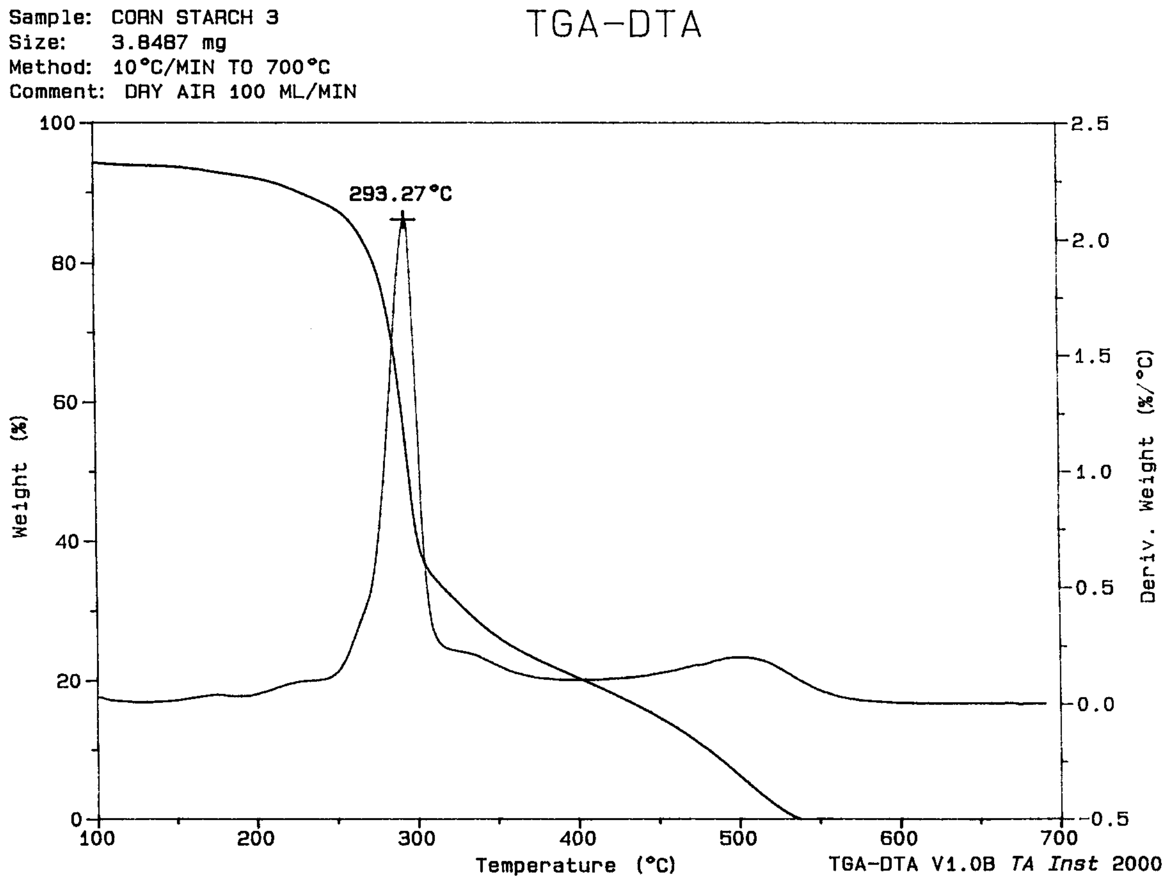

Fig. 9. TG-DTG plot of enzyme-treated corn starch granules.

Table 2
Peak temperature (T_p) obtained for native and enzyme-treated samples subjected to TG-DTG in an atmosphere of flowing dry air (average of tgree repeats)

Starch type	T_p (°C)
Corn starch (CS)	308.9°C
CS after hydrolysis	294.0°C
Wheat starch (WS)	308.1°C
WS after hydrolysis	299.0°C
Rice starch (RS)	311.3°C
RS after hydrolysis	297.6°C
Potato starch (PS)	302.9°C
PS after hydrolysis	294.5°C

increase in surface-area degradation and decomposition. Similar observations were made (Table 2) in the case of all the samples analyzed.

4. Conclusions

The partially degraded product obtained after hydrolysis by glucoamylase formed porous granules, as seen from the SEM micrographs. These porous granules showed a higher gelatinization temperature than their native counterparts when examined using DSC. This indicates that these granules were like lower chain length polymers and, hence, more energy is required to break smaller sized polymers as compared to bigger ones.

The XRD pattern of the porous material appeared more crystalline than the native one suggesting that the enzyme attacked the amorphous regions of the starch; therefore, the material appeared more crystalline.

The TG-DTG data indicates that the combustion and degradation occurs at a lower temperature for the porous granules, probably due to a higher surface area.

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