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THERMAL BEHAVIOUR OF CORN STARCH GRANULES UNDER ACTION OF FUNGAL α-AMYLASE

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Corn starch, partially hydrolyzed by fungal α-amylase was investigated by using thermal analysis, microscopy and X-ray diffraction. After enzymatic treatment lower degradation onset temperatures were observed. DSC analysis showed almost similar range of gelatinization temperature, however, the enthalpies of gelatinization increased for the partially hydrolyzed starch granules. According to the X-ray diffraction analysis, stronger cereal pattern peaks were recognized after enzymatic digestion. The results suggested that the hydrolysis was more pronounced in the amorphous part of the starch granules.

Keywords: *enzyme, hydrolysis, starch, thermal analysis*

Introduction

Starch is the most important storage reserve carbohydrate in plants. Many of the starch-storing organs are staple foodstuffs in the human diet [1]. Despite the fact that there are many starchy plants available only a few of them have industrial importance, mainly corn, wheat, potato and tapioca. More than 80% of the world starch market originates from corn [2]. Hydrolytic starch transformations are made by several methods such as chemical, thermal and by enzymatic hydrolysis.

α-amylase catalysed starch hydrolysis is one of the most important large scale enzymatic process [3]. The fact that some enzymes having a particular capacity in transforming starches to shorter polymers composed of glucose units inspired several researches in the recent years [4, 5]. Traditional acid hydrolysis of starch to glucose is being superseded by enzymatic processes. This is due to the fact that acid processes leads to undesirable by-products.

Bakery industry, for instance, is a great consumer of both starches and enzymes. Only in the USA, there is a loss of more than 1 billion U$/year due to bread stalling. Besides, generating fermentable compounds, fungal α-amylase also have an anti-staling effect in baking and they improve the softness retention of baked products [6, 7].

For more than 20 years, differential scanning calorimetry (DSC) has been widely used to study the gelatinization of starch, a physical transformation that occurs when starch-water slurry is submitted to a heating process [1, 7–11]. Thermogravimetry (TG) can be helpful to show the behavior of starch granules when heating leads to depolymerization [12]. X-ray diffraction has been also used to study the starch granules as well as their structural changes [1, 11, 13, 14].

In order to analyze the enzymatic action on the granules, light microscopy was used to show how the enzyme acts on the granules [1, 5, 13].

The rate of hydrolysis of starch granules strongly depends on the botanical source from which they originated [15]. The aim of this study is to obtain a better understanding of the effect of hydrolysis on granular corn starch due to the action of fungal α-amylase.

Experimental

Samples

Commercial grade granular corn starch (Maizena®, Unilever Bestfoods, Mogi Guaçu SP Brazil) was acquired in a supermarket. Food grade fungal α-amylase (E.C. 3.2.1.1) 4000 SKB (SKB unit per gram is defined as number of grams of soluble starch dextrinized, in the presence of excess β-amylase, per hour at 40°C and pH 5.0) from *Aspergillus oryzae*, called to SPRING ALFA, specially produced for baking processes was purchased from Granotec (Curitiba PR, Brazil).

Methods

Hydrolysis

Samples were identified by hydrolysis reaction time (i.e.one, two or three hours). 3.0 g of starch was added to 8.0 mL of distilled water and the pH was adjusted

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to 5.0 by adding diluted hydrochloric acid solution. The samples were kept in a water bath at 40°C under continuous stirring and 15.0 mg of enzyme preparation was added to each sample. After each hour, up to 3 h, samples were removed from the water bath and the pH of aqueous medium was adjusted to 2.0 using the same hydrochloric acid solution in order to stop the enzyme action. Then, the samples were centrifuged at 3200 rpm for 6 min and the supernatant was stored in a freezer. Solid samples were removed and dried in a vacuum oven at room temperature, then kept in a desiccator over anhydrous calcium chloride.

Thermal analysis

TG, DTG and DSC curves were recorded using a Shimadzu TG 60 and DSC 60 units, under air flow at 100 mL min^{-1} and at a heating rate of 10°C min^{-1}. The initial sample masses were about 5 mg. Alumina crucibles were used for the TG/DTG and sealed aluminum crucibles were used for the DSC experiments. TG was used to measure the mass loss either as a function of time (non-isothermal TG) [14]. DSC studies were carried out in order to study the gelatinization. A 4:1 (water:starch) mixture was prepared and left for two hours in order to equilibrate the moisture content.

Microscopy

Microscopy analysis was carried out using an Olympus stereo microscope SZX9, with polarization filter and Cybernetic's Cool Snap Pro Color camera. The photographs were identified and scaled using Image Pro Plus with N=1000 magnification.

X-ray diffraction

X-ray powder patterns were obtained by using a Siemens D-5000 X-ray diffractometer, with CuK_α radiation (λ=1.544 Å) and a setting of 40 kV and 20 mA.

Results and discussion

Thermal analysis

Table 1 shows corn starch degradation results during hydrolysis. Actually, two-step degradation process occurs in air. According to [13] the thermal treatment of starches normally leads to their degradation when the applied temperature exceeds 300°C. The second major breakdown process does not occur under inert atmosphere and is therefore considered to be the oxidation of the partially decomposed organic material [16].

Thermogravimetry also allows to determine the water content in the sample. Researchers [17] previously used 150°C as temperature limit for such evaluation. It is observed that the loss of moisture and other easily volatile materials and the posterior mass loss of the samples had approximately 2% variation.

TG curves of the starch samples are summarized in Fig. 1. They are in agreement with other results. The onset temperatures decreased (Table 1). It can be explained that enzyme activity leads to a higher available surface area for heating process, consequently the pyrolysis process starts at lower temperatures. Similar results were observed also in other structural change study using chemical oxidation treatment (data not shown).

DTG curves are collected in Fig. 2. In Table 1, the respective (but almost negligible) peak temperature differences from the raw state to the end of enzyme treatment are indicated. Other researchers [14] also observed difference in such result however, using another enzyme and giving more time for its action, they ob-

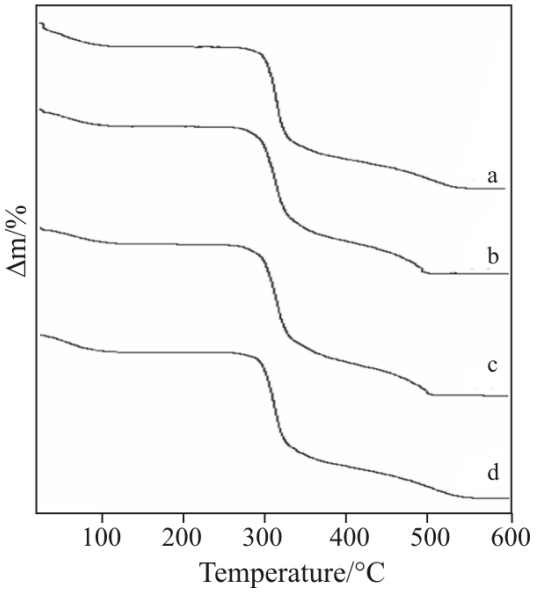

Fig. 1 TG curves of: a – untreated corn starch and after hydrolysis during: b – 1 h, c – 2 h and d – 3 h

Table 1 TG/DTG results for untreated corn starch and during hydrolysis

	Untreated starch	After 1 h	After 2 h	After 3 h
Water content/%	10.78	8.68	8.56	10.14
Total mass loss/%	96.40	97.30	97.69	97.82
On-set/°C	297	292	293	283
DTG peak/°C	312	311	311	310

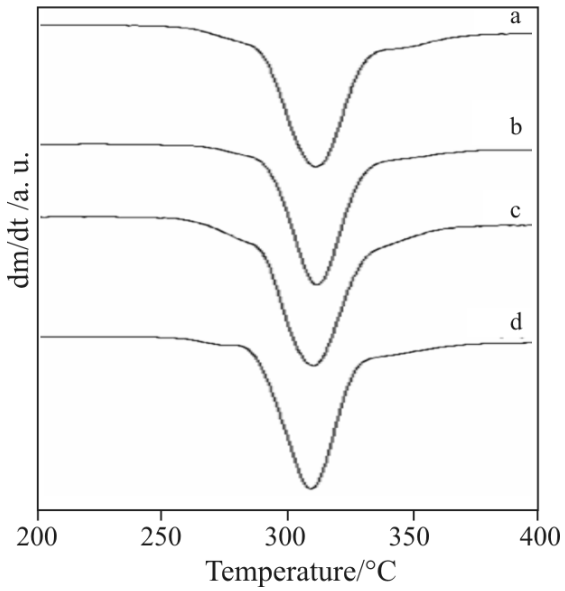

Fig. 2 DTG curves of: a – untreated corn starch and after hydrolysis during: b – 1 h, c – 2 h and d – 3 h

served larger differences. The DTG curve indicates the corresponding temperatures for the beginning and when the reaction rate is maximal [10].

The energy required for the molecular order disrupture differs in the same botanical source, thus, the gelatinization occurs rather in a temperature range than at a definite temperature [1]. According to the DSC analysis (Table 2) the temperature range of gelatinization varies less than 1°C between the samples of same botanical origin.

The increase of enthalpy required to the process is shown in Fig. 3, suggesting an increase in crystalline

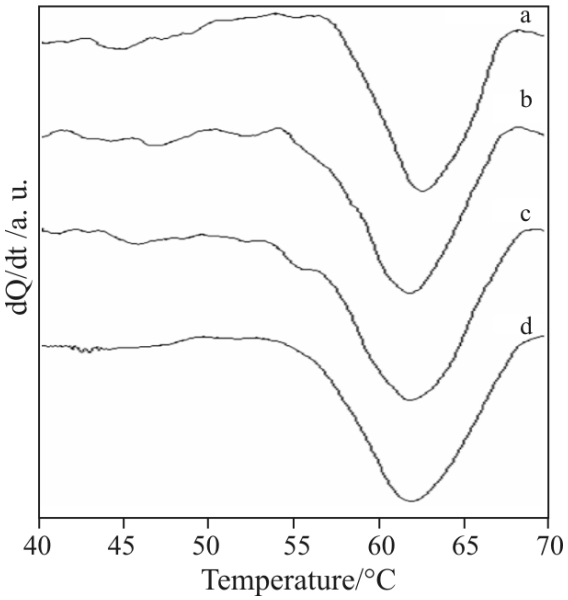

Fig. 3 DSC curves of: a – untreated corn starch and after hydrolysis during: b – 1 h, c – 2 h and d – 3 h

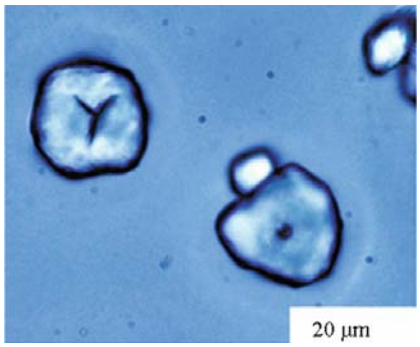

Fig. 4 Photomicrograph of untreated corn starch granules

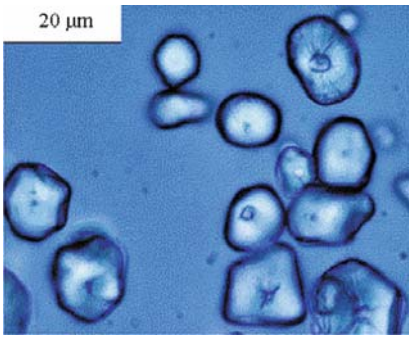

Fig. 5 Photomicrograph of corn starch granules after 3 h under hydrolysis action

Table 2 DSC results for untreated corn starch and during hydrolysis

	Raw starch	After 1 h	After 2 h	After 3 h
On-set/°C	60	59	60	59
Peak/°C	66	65	65	65
ΔH/J g^{-1}	10	11	15	29

characteristic of the material. Starch crystallinity is associated to the presence of amylopectin [18, 19] that probably did not undergo on the same intensity of the enzymatic attack as it has been occurred in the amorphous region of the amylose. The amylose content in regular corn native starch is approximately 27% [17].

Microscopy

Native starch granules of corn observed in Fig. 4 shows differences in their shape and size. Corn presented irregular polyhedral morphology, with diameters between 5 and 20 μm approximately, confirming what was previously reported in [1, 14, 20]. During the time of enzymatic action, the granules gradually underwent morphologic alterations as it can be observed in Fig. 5.

The microscopic study of the morphological alteration of the granules was made with glucoamylase in different starches [10]. This phenomenon

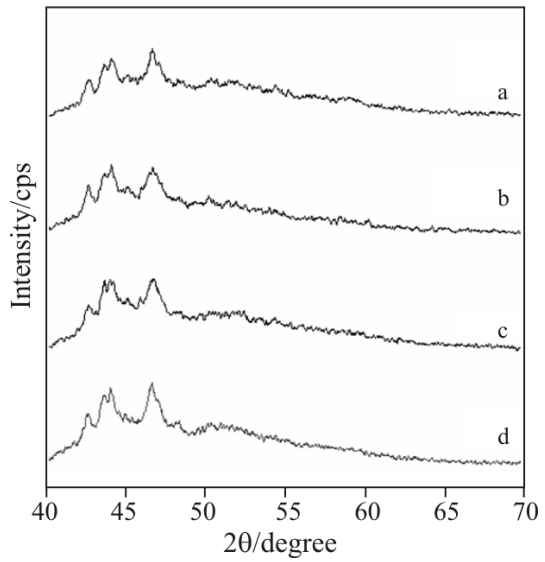

Fig. 6 X-ray diffraction patterns of: a – untreated corn starch and after hydrolysis during: b – 1 h, c – 2 h and d – 3 h

was also mentioned in [19–21] during enzymatic treatment with other amylases in native granules of different botanical sources. Enzyme attacks at first the irregularities of the starch surface. May be this observation can due to the fact that some holes can appear on the surface of the treated substance due to the enzyme action.

X-ray diffraction

Starches tend to present pertinent crystalline arrangements depending on their botanical origin [19]. In accordance with Cereda and Vilpoux [1] the intensity of few diffraction peaks can suggest different characteristic crystallinity patterns. For cereal or A pattern, these peaks appear predominantly as one doublet at 2θ=18° and an only one peak occurring at 2θ=23°. Tuber starches are recognized for the intensity of the corresponding band to one doublet at 2θ=5 and 6°, two singlets at 15 and 17° and one doublet at 2θ=22 and 24° [1]. X-ray diffraction pattern of corn starch is in Fig. 6, showing a typical cereal crystallinity pattern [18, 22].

However, after 3 h of the enzymatic treatment it can be observed that the crystallinity was more defined, a time that the separation of the peaks at 18° is more defined and the peak at 23° is more acute. All the characteristic peaks of this botanical source are more intense in comparison to the raw state of these starch granules. Using another de-branching enzyme on several starches, Agarwall and Dollimore [13] also observed this behavior.

As it was previously described in [1, 19, 23] higher amylopectin concentration causes higher crystallinity. The X-ray diffraction pattern of the hydrolyzed material showed higher degree of crystallinity compared to the native. Consequently it was concluded that the enzyme attacks rather the amorphous regions of the starch. Probably it can be explained that upon hydrolysis, crystallinity alterations were observed, once α-amylase acts mainly at amorphous region the granules.

Conclusions

Microscopy assisted to understand that the enzyme acts initially on the surface of the granules and especially in their imperfections. Thermogravimetry helped to observe the hygroscopicity of starch, the thermal stability of raw state and how it decreased during hydrolysis due to the higher surface area.

Using DSC, the starch gelatinization was followed, which has great importance for the industry. According to the DSC, no peak temperature displacement was found in comparison the raw and hydrolyzed state. However, the increase in the enthalpy required evidences the higher amount of crystalline fraction of the substance.

X-ray diffraction confirmed the characteristic native pattern and the increase of intensity of main peaks suggesting that the crystalline part was not influenced by the enzyme action. The crystallinity of the starch is associated to the amylopectin fraction that probably did not suffer the same intensity of the enzymatic attack as it occurred in the amorphous region.

Acknowledgements

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References

1 M. P. Cereda and O . Vilpoux, Tecnologia, Usos e Potencialidades de Tuberosas Amiláceas Latino Americanas, Fundação Cargill, São Paulo 2003, p. 711.
2 S. Jobling, Plant Biotech., 7 (2004) 210.
3 Z. Konsula and M. Kyriakides, Process Biochem., 39 (2003) 1745.
4 L. Kandra, J. Mol. Struct. Theochem., 666–667 (2003) 487.
5 J. Li, T. Vasanthan, R. Hoover and B. Rossnagel, Food Chem., 84 (2004) 621.
6 R. Gupta, P. Gigras, H. Mohapatra, V. K. Goswami and B. Chauhan, Process Biochem., 38 (2003) 1559.
7 M. J. E. C. Van der Maarel, B. van der Veen, J. C. M. Uitdehaag, H. Leemhuis and L. Dijkhuizen, J. Biotechnol., 94 (2002) 137.
8 W. J. Sichina, Use of DSC for the Characterization of Starches, PerkinElmer Instruments, 2000.
9 J. Farkas and C. Mohácsi-Farkas, J. Thermal Anal., 47 (1996) 1787.

10 M. Ionashiro, 'Giolito' Fundamentos de Termogravimetria e Análise Térmica Diferencial/Calorimetria Exploratória Diferencial, Giz, São Paulo 2005.
11 V. Garcia, P. Colonna, D. Lourdin, A. Buleon, H. Bizot and M. Ollivon, J. Thermal Anal., 47 (1996) 1213.
12 E. Rudnik, G. Matuschek, N. Milanov and A. Kettrup, J. Therm. Anal. Cal., 85 (2006) 267.
13 P. Aggarwall and D. Dollimore, Thermochim. Acta, 319 (1998) 17.
14 C. G. Mothé, D. Z. Correia, F. P. de França and A. T. Riga, J. Therm. Anal. Cal., 85 (2006) 31.
15 S. Srichuwong, N. Isono, T. Mishima and M. Hisamatsu, Int. J. Biol. Macromol., 37 (2005) 115.
16 P. Aggarwall, D. Dollimore and K. Heon, J. Thermal Anal., 50 (1997) 7.
17 A. Soliman, N. A. EL-Shinnavy and F. Morabak, Thermochim. Acta, 296 (1997) 149.
18 L. Yu and G. Christie, Carbohydr. Polym., 46 (2001) 179.
19 G. Zhang, Z. Ao and B. R. Hamaker, Biomacromol., 7 (2006) 3252.
20 Y. Ma, C. Cai, J. Wang and D. W. Sun, J. Food Eng., 73 (2006) 297.
21 E. Sarikaya, T. Higasa, M. Adachi and B. Mikami, Process Biochem., 35 (2000) 711.
22 C. M. L. Franco and C. F. Ciacco, Ciência e Tecnol. de Alim., 15 (1995) 112.
23 C. G. Biliaderis, Food Tech., 46 (1992) 98.

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