Thermochimica Acta 519 (2011) 50–54

Contents lists available at ScienceDirect

Thermochimica Acta

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

Thermal analysis as a screening technique for the characterization of babassu flour and its solid fractions after acid and enzymatic hydrolysis

Rafael Ramires de Almeida[a,*], Luiz Gustavo Lacerda[b], Fabio Seigi Murakami[b,c], Gilbert Bannach[d], Ivo Mottin Demiate[a], Carlos Ricardo Soccol[c], Marco Aurélio da Silva Carvalho Filho[b], Egon Schnitzler[a]

[a] Universidade Estadual de Ponta-Grossa – UEPG, Av. Carlos Cavalcanti, 4748, CEP 84030-900 Ponta-Grossa, PR, Brazil
[b] Universidade Positivo – UP, Av. Prof. Pedro Viriato Parigot de Souza, 5300, CEP 81280-330 Curitiba, PR, Brazil
[c] Universidade Federal do Paraná – UFPR, R. Cel. Francisco H. dos Santos, 100 – 81531-990 Curitiba, PR, Brazil
[d] Faculdade de Ciências da Universidade Estadual Paulista "Júlio de Mesquita Filho" Campus de Bauru (FC/UNESP-Bauru), Av. Eng. Luiz Edmundo Carrijo Coube, 14-01 17033-360 Bauru, SP, Brazil

ARTICLE INFO

Article history:
Received 6 December 2010
Received in revised form 14 February 2011
Accepted 23 February 2011
Available online 2 March 2011

Keywords:
Babassu flour
Hydrolysis
DSC
TG
Kinetics analysis

ABSTRACT

The babassu (*Orbignya phalerata*) is a native tree found in northern Brazil. Extracts of the babassu coconut have been widely used in industry. Babassu flour has about 60% starch, thus, besides nourishment it can be used as an alternative biofuel source. However, the properties of this starch lack of study and understanding. The main purpose of this study was to investigate the thermal behavior of raw babassu flour and its solid hydrolyzed fraction. The analyses were carried out using SHIMADZU DSC and TG thermic analyzers. The results demonstrated a reduction in thermal stability of the solid hydrolyzed fraction compared to raw matter. The kinetic parameters were investigated using non-isothermal methods and the parameters obtained for its decomposition process were an E_a of 166.86 kJ mol^{-1} and a frequency factor (β) of 6.283 × 1014 min^{-1}; this was determined to be a first order reaction ($n = 1$).

1. Introduction

Babassu (*Orbignya phalerata* Mart.) is a palm tree native to Brazil's northern states (Piaui, Tocantins, Maranhao) that appears between Cerrado and the Amazon rain forest in an ecosystem called Mata dos Cocais [1]. Babassu is a palm tree (up to 20 m high). Usually, each tree produces 15–25 bunches of fruit, each fruit weighting 98–280 g with 3–4 kernels [2]. It reaches fruit production maturity after 10–12 years, and since there are no plantations of these palm trees, the fruits have to be collected from natural woodlands [1]. The annual coconut production of an area with 141–160 palms per hectare is about 2.5 ton/year [3]. The average weights of each component of the babassu coconut are 11% exocarp, 23% mesocarp, 59% endocarp – the hard wood layer – and 7% kernels [4,5]. The presence of tannins renders a brownish color to the flour, whose contents of starch and fibers are 50% and 10% (w/w), on a solid basis, respectively [6].

The use of babassu coconut starch as an energy source was studied in the 1970s, but most of the projects were abandoned due to a lack of interest by the government [7]. Despite this fact, Tobasa Bioindustrial S/A operates in the northern region of Brazil, processing babassu flour, providing babassu oil to the cleaning industry, and ethanol from babassu flour starch [6]. In the past decades, only the babassu kernels were used to produce oil, so, 93% of the fruit become waste. Since the introducing of the industrial processing, the manual labor of breaking the coconut is done only in small scale [4,7] and according to Anderson et al. [8] up to twelve byproducts could be yielded, like charcoal, edible fibers, oil, ethanol and chemical reagents. According to Lima et al. [9] the babassu oil has a composition which is suitable for biodiesel production due to the high concentration of lauric acid.

Although the starch is the energy reserve polymer in most of the plants, its characteristics change between the sources. Babassu starch granules have structure similar to cereals, but different from roots and tubers – e.g., cassava and potato. The gelatinization temperature, an important step to liquefaction and saccharification processes, sits around 63 °C and 73 °C due to significant amylose content [10]. Papers regarding babassu flour properties and babassu starch are scarce. Furthermore, the mesocarp significant starch content, demands more detailed investigation.

In this study the raw babassu flour starch was treated with acid and amylolytic enzyme hydrolysis. The raw and the hydrolyzed material were submitted to thermal analysis, using thermoanalytical techniques such as simultaneous thermogravimetry and

* Corresponding author. Tel.: +55 41 3317 3116.
E-mail address: rafael.ramires@gmail.com (R.R. de Almeida).

0040-6031/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2011.02.029

differential thermal analysis (TG–DTA) to examine the effect of the enzyme attack on the babassu flour mass loss. The differential scanning calorimetry (DSC) was used to evaluate the impact of the enzyme treatment on the gelatinization temperatures.

2. Materials and methods

2.1. Materials

Babassu flour samples were obtained from an industrial facility located in the State of Maranhao, Brazil. The samples were hydrolyzed with heat-stable *Bacillus licheniformis* α-amylase Termamyl 240L and *Aspergillus niger* amyloglucosidase AMG 300L from Novozymes.

2.2. Composition analysis

The moisture and starch contents were determined by the Association of Official Analytical Chemists (AOAC) [11] analytical methods. Protein, fat, ash and total dietary fiber were determined through the protocols of the AOAC [12].

2.3. Enzymatic hydrolysis

The babassu flour were mixed with room temperature distilled water, resulting in a slurry with solid content of 10% (w/v). Calcium hydroxide was supplemented to the mixture at a ratio of 30 mg/kg. The starch samples were treated with 7.0 μL α-amylase at 95 °C for 1 h and subsequently with 20 μL amyloglucosidase at 55 °C for 12 and 24 h. At the end of the reaction, the suspension was filtered through a Whatman grade no. 42 quantitative filter paper using a vacuum pump. Each fraction – solid and aqueous – was stored for further analysis.

2.4. Acid hydrolysis

The reaction was adapted from Woiciechowski [13] using 2.0 g of babassu flour which was mixed to 20.0 mL of distilled water and 2.0 mL of sulfuric acid 1.0% at 98 °C for 20 min. Afterwards, the material was cooled to room temperature – about 25 °C – and the mixture was neutralized with sodium hydroxide 0.1 M.

2.5. Simultaneous thermogravimetry and differential thermal analysis

The samples were heated from 30 °C to 600 °C using open alumina crucibles with approximately 6.0 mg of sample under a synthetic air flow of 100 mL min^{-1} at a heating rate of 10 °C min^{-1}. The samples were analyzed in a Shimadzu TG-60 thermogravimeter. The equipment was preliminarily calibrated with a standard reference of calcium oxalate monohydrate. The onset temperatures (T_o) as well mass loss percentages were determined using TA-60WS data analysis software. Non-isothermal kinetic investigation was performed from the TG data by application of Ozawa's method [14]. The curves of mass loss versus temperature for five TG curves were obtained at different heating rates (5, 10, 15, 20 and 25 °C min^{-1}) under synthetic air atmospheres.

2.6. Differential scanning calorimetry

Differential scanning calorimetry (DSC-60 SHIMADZU Kyoto, Japan) was used to determine the thermal properties of dry, raw babassu flour by heating the aluminum sealed pans from 30 °C to 250 °C at a rate of 10 °C min^{-1}. The gelatinization study of the raw and hydrolyzed samples was performed weighing 10.0 mg of slurry of babassu flour and distilled water at a ratio of 1:4 (w/v) mixed directly inside the 40 μL aluminum crucibles, which were hermetically sealed with a press. The sealed crucibles were stabilized at room temperature for 1 h in order to homogenize the slurry. The gelatinization behavior of the babassu flour was analyzed in a temperature range of 30–90 °C at a heating rate of 3 °C min^{-1}.

2.7. Microscopy

Dried samples of raw and hydrolyzed babassu flour were mounted on standard glass microscope slides. Microscopy analysis was performed 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.

3. Results and discussion

3.1. Composition analysis

The moisture and starch contents were determined to be 14.5% and 64% – dry weight basis – respectively, while protein, fat and ash of babassu flour were 2%, 2.4% and 2.9% – dry weight basis – respectively. Total dietary fiber was 9.7% and was determined by a nonenzymatic gravimetric method [12]. The analyses of the samples corroborated with the results reported by other authors [6].

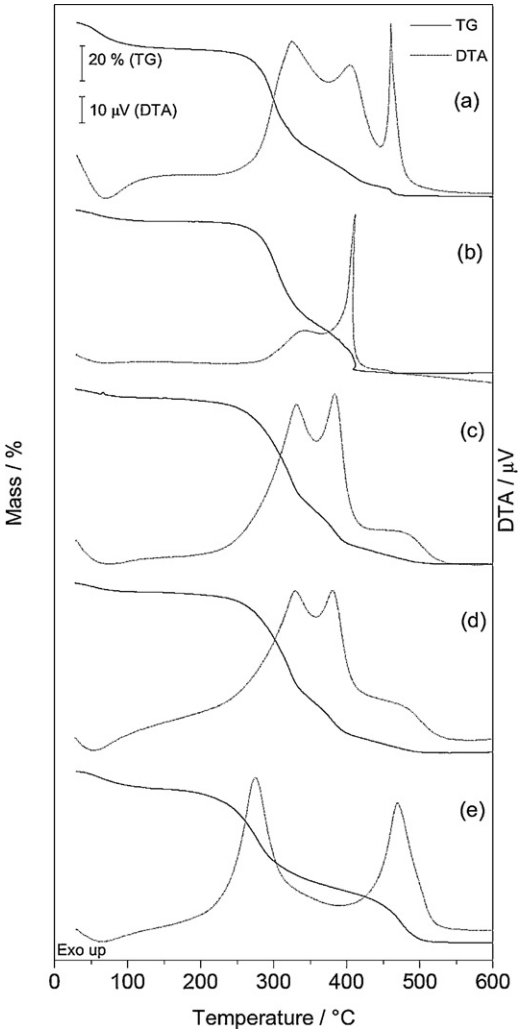

Fig. 1. TG and DTA analyses of the babassu flour. Respectively, (a) native, (b) 1 h α-amylase enzymatic hydrolysis, (c) 12 h α-amylase and amyloglucosidase hydrolysis, (d) 24 h α-amylase and amyloglucosidase hydrolysis and (e) acid hydrolysis.

Table 1
TG analyses of babassu flour.

Samples	TG results			DTA
	Step	Mass loss (%)	Range (°C)	peak (°C)
Native	1st	15	30–150	70 (endo)
	2nd	64	150–375	325 (exo)
	3rd	15	375–445	405 (exo)
	4th	5	445–489	460 (exo)
1 h hydrolysis (Termamyl)	1st	6.5	30–150	71 (endo)
	2nd	59.5	150–358	341 (exo)
	3rd	26	358–415	411 (exo)
12 h hydrolysis (Termamyl + AMG)	1st	4.5	30–150	51 (endo)
	2nd	55.5	150–346	330 (exo)
	3rd	21	346–405	382 (exo)
	4th	10	405–518	472 (exo)
24 h hydrolysis (Termamyl + AMG)	1st	5	30–150	64 (endo)
	2nd	54	150–345	329 (exo)
	3rd	20	345–402	380 (exo)
	4th	9	402–518	475 (exo)
Acid hydrolysis (sulfuric acid 1.0%)	1st	8.5	30–150	73 (endo)
	2nd	46	150–371	274 (exo)
	3rd	27.5	371–529	469 (exo)

3.2. Thermal analysis

The thermogravimetric (TG) and differential thermal analysis (DTA) of the raw and hydrolyzed samples of babassu flour are shown in Fig. 1.

Moisture analyses are important because water is an inherent part of most of biological substances. Water is present in samples either as chemically combined hydrates or as occluded surface adsorbed moisture [15]. Almost all the moisture analyses methods recognized by the AOAC are gravimetrical and take several hours to finish [15,16].

The TG analyses allowed the determination of moisture content in each sample in a temperature range between 30 °C and 150 °C [17]. Both the thermogravimetric as the official methods showed similar results. The thermogravimetric analysis has advantages since it is faster than the gravimetric methods and requires reduced samples quantities (around 5 g).

Table 1 shows the TG analysis results. The first step of all samples is the moisture loss. The increasing in the hours of hydrolysis, led to a gradual reduction in the moisture content. This was probably due to the reduced amounts of starch to form hydrogen bonds with water. Above the moisture loss temperature the decompositions occurs in three or four consecutive steps, according to the sample treatment.

The TG analyses of the raw and hydrolyzed samples also aided determining the total mass losses. The residue after heating a starchy sample up to 600 °C was characterized as ash [11,12,18]. The amount of ash for the raw, enzymatic hydrolyses – 1, 12 and

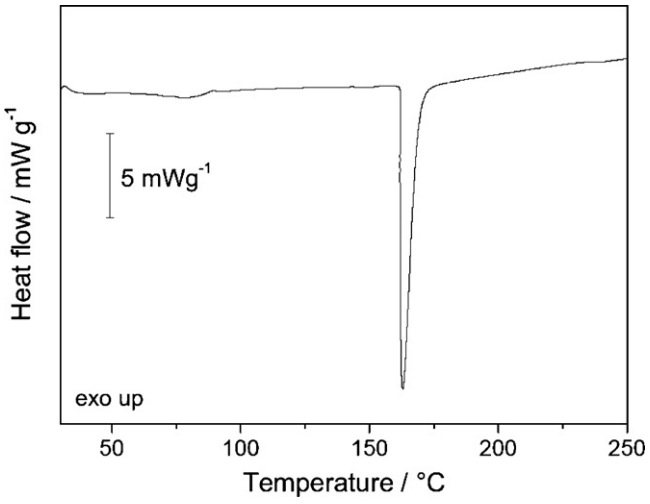

Fig. 2. DSC curve of babassu flour.

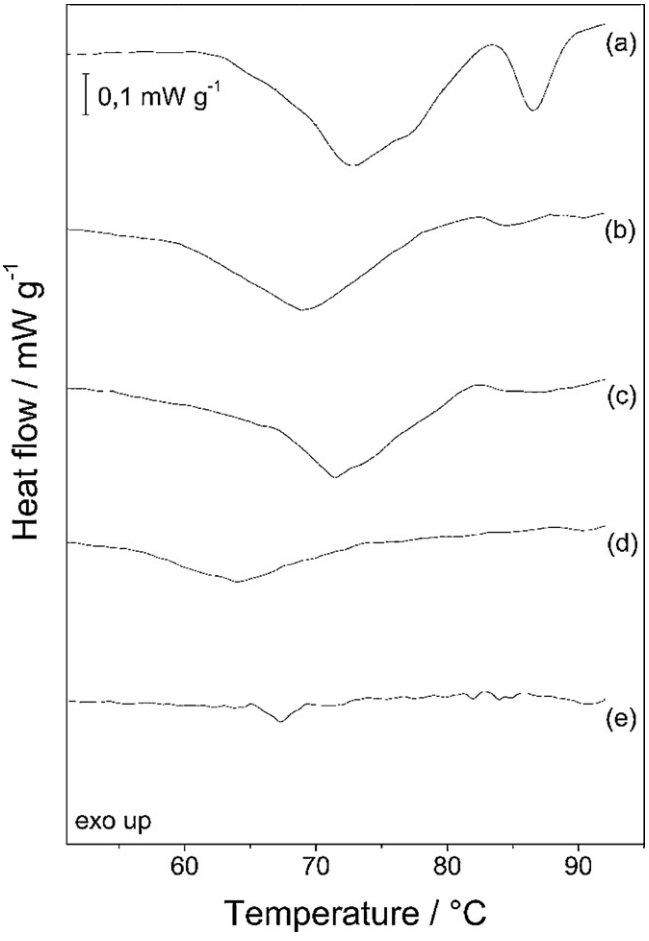

Fig. 3. DSC plot of untreated babassu flour starch, mixed with water in a 2:8 ratio, showing gelatinization of (a) native, (b) 1 h α-amylase enzymatic hydrolysis, (c) 12 h α-amylase and amyloglucosidase hydrolysis, (d) 24 h α-amylase and amyloglucosidase hydrolysis and (e) acid hydrolysis.

24 h – and acid hydrolysis of the babassu flour after heating up to 600 °C were, respectively, 2.5%, 8%, 9%, 12% and 22%. Using TG analyses we registered results for ash close to those obtained through the official analytical methods [11]. The amount of residue after hydrolysis also tended to increase with the occurrence of enzymatic hydrolysis. This occurred due to the concentration of Ca^{++} used to raise the alpha-amylase stability in the hydrolysis reaction matrix and the concentration of sodium hydroxide to neutralize the acid hydrolysis.

The first exothermic event represents the thermal stability of the samples [19]. The raw, 1, 12, 24 h and acid hydrolyzed samples thermal stability temperatures are respectively 267 °C, 266.5 °C, 259 °C, 256 °C and 216 °C. The observed decrease in the onset temperature of the first exothermic event after 1, 12, 24 h and acid hydrolysis may be due to the increase in the surface area of the starch granules observed in partially hydrolyzed starches, as reported by Aggarwal and Dollimore [20], and Lacerda et al. [19,21]. The acid hydrolysis sample showed the lowest degradation temperature.

The DSC was used to determine melting and gelatinization temperatures of babassu flour. Fig. 2 shows an acute peak at 163 °C, with an onset temperature of 162 °C, and an enthalpy of 160.2 J/g. The melting temperature of the babassu starch at 163 °C confirms another similarity with cereal starch since corn starch melting sits at 168 °C as Ozcan described [22]. Rosenthal and Espindola [10] reported that the gelatinization temperature of the raw babassu flour was in the range of 63–73 °C, and the calculated enthalpy for this event was 6.17 J/g. The higher the gelatinization temperature more perfect are the starch crystals or higher are the co-operative units (longer chains in the crystals or a larger crystal size) [23]. Fig. 3 shows the DSC curves of the gelatinization events of the analyzed samples and Table 1 presents the experimental results obtained for onset temperature (T_o), gelatinization enthalpy (ΔH_{gel}) and peak temperature (T_p). The reduction on the gelatinization temperatures and enthalpies suggests that the hydrolysis process disrupted the crystalline orders by penetration of the acid into the starch granules through channels and cavities. In order to confirm such statement, powder X-ray diffraction analysis should be done [24]. The enzymatic hydrolysis also decreased the enthalpy and gelatinization temperatures. This was based on the observation that α-amylase did not produce an increase in crystallinity. However, crystallinity and gelatinization enthalpy have been shown to decrease during the later stages of α-amylase [25] (Table 2).

Table 2
DSC of babassu flour gelatinization under different treatments showing onset temperature (T_o), peak temperature (T_p) and gelatinization enthalpy (ΔH_{gel}).

Sample treatment	T_o (°C)	T_p (°C)	ΔH_{gel} (J/g)
Native	63	73	6.36
1 h hydrolysis (Termamyl)	62	69	3.60
12 h hydrolysis (Termamyl + AMG)	68	71	2.80
24 h hydrolysis (Termamyl + AMG)	56	64	1.63
Acid hydrolysis (sulfuric acid 1.0%)	65	67	0.20

3.3. Microscopy

The starch granules of raw babassu flour were seen to have a peculiar level of organization, with the granules appearing either isolated or attached in groups of two or three, as observed in Fig. 4.

This agglomeration behavior was similar to rice starch, but with fewer granules attached to each other [26]. Non-aggregated gran-

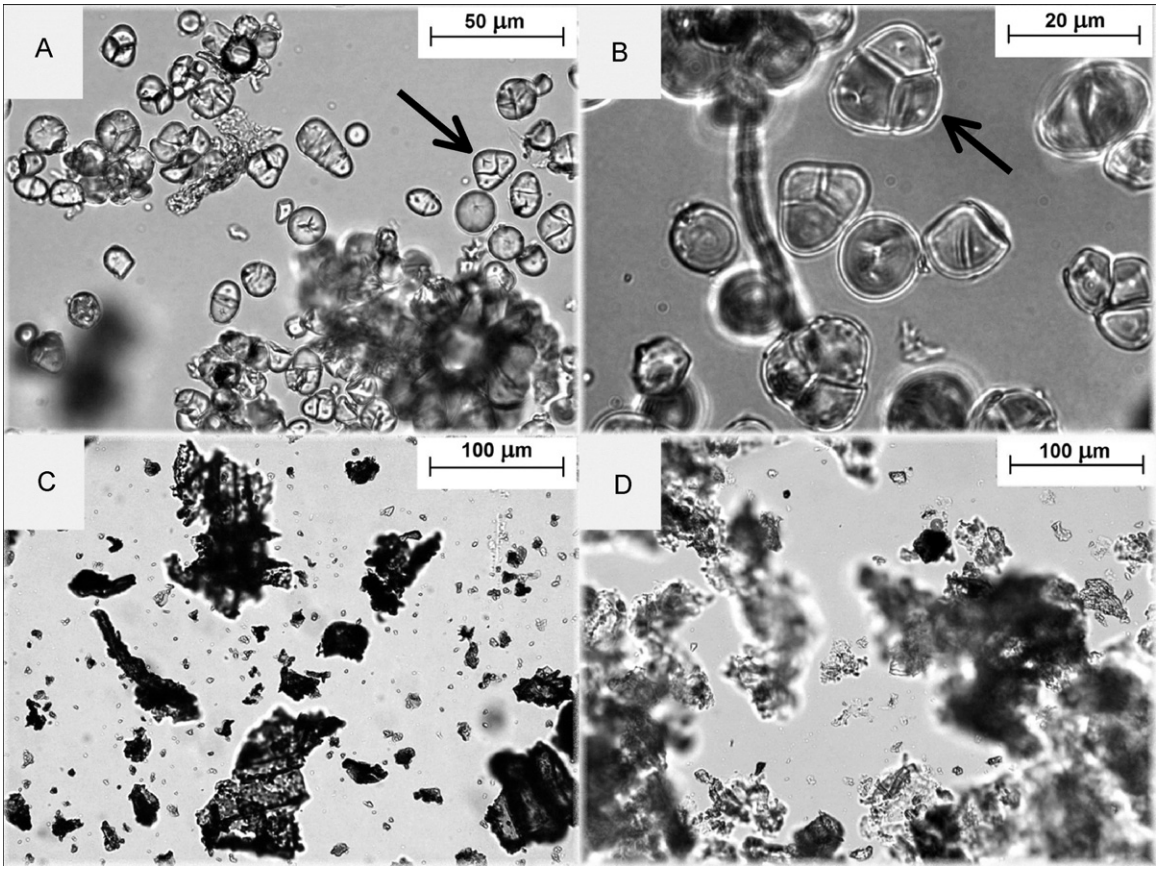

Fig. 4. Microscopy identification of babassu flour starch granules: (A) native babassu flour with arrow showing starch granule and fiber material at 400× magnification, (B) arrow showing starch granule in the native babassu flour with fibers (1000×), (C) after acid hydrolysis showing degraded fiber material (400×) and (D) after enzymatic hydrolysis showing high amounts of fibrous material (400×).

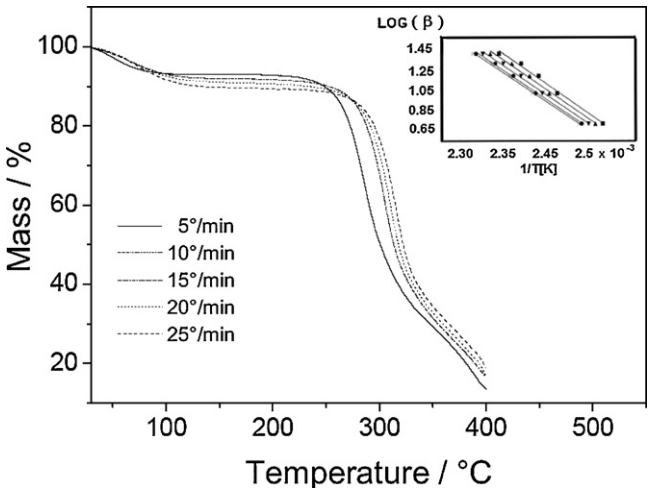

Fig. 5. TG curves obtained at different heating rates under a dynamic synthetic air atmosphere. The inset figure shows Ozawa plot with a linear correlation of the five curves.

ules had an average diameter of about 10 μm. The presence of fiber among the granules was also visible at any magnification. After acid treatment and 12 and 24 h of enzymatic hydrolysis, the resulting solid phase displayed reduced amounts of starch, only the fibrous material remained changeless.

3.4. Kinetics investigation

The kinetics study was performed in order to determine the kinetic decomposition of babassu flour. The non-isothermal Ozawa method was applied in this study, which is an integral method for determining the activation energies from dynamic heating experiments [27,28]. The kinetics data were calculated by plotting mass loss versus temperature for five TG curves obtained at different heating rates.

Fig. 5 demonstrates the superposition of thermogravimetric curves, which were shifted towards higher temperatures when the heating rates increased. It demonstrates the linear correlation of the five curves. The activation energy (E_a) was obtained from a plot of the logarithms of heating rates as an inverse function of the temperature ($1/T$) for a constant $g(\alpha)$, where $g(\alpha)$ is the integrated form of the conversion dependence function, $f(x)$. The kinetics parameters obtained were an E_a of 166.86 kJ mol^{-1} and a frequency factor (β) of 6.283×10^{14} min^{-1}, and the process was observed to be first order ($n = 1$).

4. Conclusions

Carried analyses provided some new thermal behavior information. TG-DTA analyses showed notable difference in the degradation temperature probably due to the surface reduction area of starch granules after acid and enzymatic hydrolysis. Thermogravimetry could be used for moisture and ash determinations since the results were similar to those obtained by using AOAC official methods and require less analysis time and sample preparation. DSC analyses illustrated with precision the gelatinization of babassu flour starch, a particular structural change of great importance, and the results were within the range described in the literature. Furthermore, DSC of babassu flour showed a notable peak with an endothermic event at temperatures near to those observed for corn starch, which confirms the similarity between babassu starch granules and those of cereal starches. There are only few studies regarding the chemical properties of babassu starch so far. Thus, more efforts would be needed to increase knowledge regarding babassu flour.

References

[1] D. Albiero, Proposta de uma máquina para colheita mecanizada de babaçú para agricultura familiar (Solutions to a machine for mechanical harvesting of babassu for family farms), Acta Amaz. 37 (2007) 337–346 (in Portuguese).
[2] M.A. Teixeira, Heat and power demands in babaçu palm oil extraction industry in Brazil, Energy Convers. Manage. 46 (2005) 2068–2074.
[3] P.H. May, Palmeiras em chamas: transformação agrária e justiça social na zona do babaçu (Palm Trees in Flames: Agrarian Transformation and Social Justice in the Area of Babassu), EMAPA/FINEP/Fundação Ford, São Luís, 1990 (in Portuguese).
[4] M.A. Teixeira, Babassu – a new approach for an ancient Brazilian biomass, Biomass Bioenergy 32 (2008) 857–864.
[5] A. Vivacqua Filho, Babaçu: Aspectos sócio-econômicos e tecnológicos (Babassu: Social Economical and Technological Aspects), Universidade de Brasília, Brasília, 1968 (in Portuguese).
[6] E.A. Baruque Filho, M.G.A. Baruque, G.L. Sant'anna Jr., Babassu coconut starch liquefaction: an industrial scale approach to improve conversion yield, Bioresour. Technol. 75 (2000) 49–55.
[7] E.A. Baruque Filho, M.G.A. Baruque, D.M.G. Freire, G.L. Sant'anna Jr., Ethanol from babassu coconut starch. Technical and economical aspects, Appl. Biochem. Biotechnol. 70–72 (1998) 877–886.
[8] A.B. Anderson, P.H. May, M.J. Balick, The Subsidy from Nature: Palm Forests Peasantry and Development on an Amazon Frontier, Columbia University Press, New York, 1991.
[9] J.R.O. Lima, R.B. Silva, C.C.M. Silva, L.S.S. Santos, J.R. Santos Jr., E.M. Moura, C.V.R. Moura, Biodiesel de babaçu (*Orbignya sp.*) obtido por via etanólica (Biodiesel from babassu (*Orbignya sp.*) obtained through alcoholic way), Quim. Nova 30 (2007) 600–603 (in Portuguese).
[10] F. Rosenthal, A.C. Espindola, O amido do coco de babaçú: algumas propriedades dos grânulos e das pastas (The babassu coconut starch: some properties of the granules and pastes), Rev. Bras. Technol. 6 (1975) 307–315 (in Portuguese).
[11] Association Official Analytical Chemists, Official Methods of Analysis of AOAC International, 17th ed., 2000 (v. II, Method 945.39).
[12] B.W. Li, M.S. Cardozo, Nonenzymatic–gravimetric determination of total dietary fiber in fruits and vegetables, J. AOAC Int. 75 (2) (1992) 372–376.
[13] A.L. Woiciechowski, S.N. Rocha, C.R. Soccol, A. Pandey, Enzymatic hydrolysis to recover reducing sugars from cassava bagasse: an economic study, Braz. Arch. Biol. Technol. 45 (2002) 393–400.
[14] T. Ozawa, Non-isothermal kinetics of consecutive reactions, J. Therm. Anal. Calorim. 60 (2000) 887–894.
[15] R.E. Wrolstad, E.A. Decker, S.J. Schwartz, P. Sporns, Handbook of Food Analytical Chemistry, Water, Proteins, Enzymes, Lipids and Carbohydrates, Wiley-Interscience, Hoboken, NJ, 2005.
[16] K.E. Ileleji, A.A. Garcia, A.R. Kingsly, C.L. Clementson, Comparison of standard moisture loss-on-drying methods for the determination of moisture content of corn distillers dried grains with solubles, J. AOAC Int. 93 (2010) 825–832.
[17] A.A.A. Soliman, N.A. El-Shinnawy, F. Mobarak, Thermal behavior of starch and oxidized starch, Thermochim. Acta 296 (1997) 149–153.
[18] M. Pérez-Hidalgo, E. Guerra-Hernández, B. García-Villanova, Determination of insoluble dietary fiber compounds: cellulose, hemicellulose and lignin in legumes, Ars Pharm. 38 (4) (1997) 357–364.
[19] L.G. Lacerda, J.A.M. Azevedo, M.A.S. Carvalho Filho, I.M. Demiate, E. Schnitzler, L.P.S. Vandenberghe, C.R. Soccol, Thermal characterization of partially hydrolyzed cassava (*Manihot esculenta*) starch granules, Braz. Arch. Biol. Technol. 51 (2008) 1209–1216.
[20] P. Aggarwal, D. Dollimore, A thermal analysis investigation of partially hydrolyzed starch, Thermochim. Acta 319 (1998) 17–25.
[21] L.G. Lacerda, M.A.S. Carvalho Filho, I.M. Demiate, G. Bannach, M. Ionashiro, E. Schnitzler, Thermal behaviour of corn starch granules under action of fungal alpha-amylase, J. Therm. Anal. Calorim. 93 (2008) 445–449.
[22] S. Ozcan, D.S. Jackson, Functionality behavior of raw and extruded corn starch mixtures, Cereal Chem. 82 (2) (2005) 223–227.
[23] M. Miao, B. Jiang, T. Zhang, Z. Jin, W. Mu, Impact of mild acid hydrolysis on structure and digestion properties of waxy maize starch, Food Chem. 126 (2011) 506–513.
[24] M.J. Kim, S.J. Choi, S.I. Shin, M.R. Sohn, C.J. Lee, Y. Kim, W.I. Cho, T.W. Moon, Resistant glutarate starch from adlay: preparation and properties, Carbohydr. Polym. 74 (2008) 787–796.
[25] M. Lauro, P.M. Forsell, M.T. Suortti, S.H.D. Hulleman, K.S. Poutanen, α-Amylolysis of large barley starch granules, Cereal Chem. 76 (1999) 925–930.
[26] H. Cheng, M. Lai, Fermentation of resistant rice starch produces propionate reducing serum and hepatic cholesterol in rats, J. Nutr. 130 (2000) 1991–1995.
[27] F.S. Murakami, K.L. Lang, C. Mendes, A.P. Cruz, M.A.S. Carvalho Filho, Physico-chemical solid-state characterization of omeprazole sodium: thermal, spectroscopic and crystallinity studies, J. Pharm. Biomed. 49 (2009) 72–80.
[28] T. Osawa, A new method of analyzing thermogravimetric data, Bull. Chem. Soc. Jpn. 38 (1965) 1881–1886.