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Short communication

Some instrumental methods applied in food chemistry to characterise lactulose and lactobionic acid

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ABSTRACT

Lactose is obtained as a by-product from whey. It is a source of several derivatives, including lactulose and lactobionic acid. These two compounds were analysed by using the following techniques: thermogravimetry/derivative thermogravimetry (TG/DTG), differential scanning calorimetry coupled with optical microscope (DSC-thermomicroscopy), infrared spectroscopy (FTIR) and X-ray diffractometry (XRD). The DSC technique coupled with microscopy made it possible to observe that the lactobionic acid showed several thermal events upon decomposition, which occurred at temperatures higher than 50 °C. The lactulose began to decompose above 180 °C. The DSC curve was used to calculate the purity of the lactulose (according to Van't Hoff equation), which was 98% and the melting point peak occurred at 171 °C. The lactulose showed crystalline behaviour that was different to that of the lactobionic acid, which was attributed to its high hygroscopicity. Purity of lactobionic acid was not calculated because the decomposition occurred in consecutive stages.

1. Introduction

Whey, which is the liquid fraction from the cheese industry, is a by-product with 5–8% of solids that contain around 60–80% of lactose and 10–20% of protein. When this by-product is discarded, without previous treatment, it can cause substantial environmental problems. Thus, several studies have been applied in the industrial field on how to use this waste (Seo, Park, & Han, 2015; Seo, Sung, & Han, 2016).

Lactose (4-O-β-D-galactopyranosyl-D-glucose) is the main carbohydrate in milk. The lactose content in mammalian milk is higher than that of protein and fat. This compound is used as an ingredient in many foods, beverages, bakery and confectionery products, and also infant formula. In the pharmaceutical industry it is employed as diluents in tablets and as a carrier in medicines (Gutiérrez, Hamoudi, & Belkacemi, 2012; Majid & Madadlou, 2016; Seki & Saito, 2012). In addition, lactose can be converted into other high value-added products by chemical or biological processes on the industrial or laboratory scale (Seki & Saito, 2012; Silva, Perez, Minim, Martins, & Minim, 2015; Song, Lee, Park, & Kim, 2013).

Substances, such as lactulose and lactobionic acid, are derived from lactose and can be used as alternatives for the diversification and development of industrial waste (Seki & Saito, 2012; Silva et al., 2015).

Lactulose (4-O-β-D-galactopyranosyl-β-D-fructofuranose) is a ketose, i.e. a semi-synthetic disaccharide consisting of galactose and fructose. It is detected in heated milk and has been consumed by humans over a long period of time. Different methods for the production of lactulose have been described, such as chemical isomerisation and enzymatic isomerisation, among others (Paseephol, Small, & Sherkat, 2008; Seki & Saito, 2012; Seo et al., 2015, 2016; Silva et al., 2015). Increasing attention has been given to lactulose because of its applicability in the food and pharmaceutical sectors. It can be used to cause a reduction in intestinal pH and to improve microbial activity (e.g., *Bifidobacterium*), consequently relieving constipation (Seo et al., 2016).

Lactobionic acid (4-O-β-D-galactopyranosyl-D-gluconic acid) is an aldonic acid, comprising of galactose and gluconic acid. It is obtained through lactose oxidation. Several methods of synthesis have been described (Borodina & Mirgorod, 2014; Gutiérrez et al., 2012; Seki & Saito, 2012; Valle et al., 2013).

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The main characteristics of lactobionic acid include moisturising, antioxidant, stabilising and acidifying capacities, and these features have led to growing interest about the study of the inherent properties of this polyhydroxy acid. Other applications for lactobionic acid include use in medicine and the cosmetics industry (Alonso, Rendueles, & Díaz, 2013).

Thermal analysis techniques, when coupled with other techniques, have proved to be efficient and important tools in the characterisation and study of several substances, the main advantages in the use of thermal analysis are the speed of analysis, low operating cost and simplicity of sample preparation. Such techniques have been employed in studies of pharmaceuticals (Bannach et al., 2010; Gálico, Nova, Guerra, & Bannach, 2015; Schnitzler, Kobelnik, Sotelo, Bannach, & Ionashiro, 2004); oils (Marinho, Bersot, Nogueira, Colman, & Schnitzler, 2015); chemical compounds (Bannach, Schnitzler, Treu-Filho, Utini, & Ionashiro, 2006; Gálico et al., 2011; Leone, Colman, Schnitzler, Ellendersen, & Masson, 2014); starches (Andrade, Oliveira, Colman, Costa, & Schnitzler, 2014; Bicudo et al., 2009; Hornung, Granza, Oliveira, Lazzarotto, & Schnitzler, 2015; Oliveira, Andrade, Colman, Costa, & Schnitzler, 2014) and many others.

A literature survey revealed that there are few existing studies regarding the main properties of these lactose-derived compounds, i.e. lactulose and lactobionic acid. Therefore, the aim of this contribution was to study and characterise these compounds using the following instrumental techniques: TG/DTG; DSC coupled with optical microscope (thermomicroscopy); FTIR and XRD.

2. Materials and methods

2.1. General

The lactulose was acquired from MP Biomedicals (lot M3845) and the lactobionic acid was acquired from Sigma Aldrich (lot BCK9432V), both of which were of analytical grade.

2.2. Instruments and experimental conditions

The thermogravimetric and derivative thermogravimetric curves (TG/DTG) were obtained using a TGA-50 thermobalance (Shimadzu, Japan). The samples were heated in alpha alumina crucibles from 30 °C to 660 °C, in an air flow of 150 ml min^{-1}. The initial mass of the samples was around 7 mg. The heating rate was 10 °C min^{-1}. The mass losses were calculated using TGA-50 software and by determining the temperatures of the main steps.

The non-isothermal kinetic study was performed using a TGA-50 thermal analysis system (Shimadzu, Japan) that was calibrated according to the manufacturer's recommendations. Using the Flynn-Wall-Ozawa method (Flynn & Wall, 1966; Ozawa, 1965), the samples (5 mg) were heated from 25 to 400 °C at heating rates of 5, 10 and 15 °C min^{-1} under air atmosphere with a flow of 150 ml min^{-1}. The experimental data were adjusted using a linear approximation based on the integral calculus from the Arrhenius equation (first-order-reaction).

A differential scanning calorimeter was used to obtain the DSC curves and the instrument used was a DSC model Q-200 (T.A. Instr. Ltd., USA). The curves were performed and recorded using the instrument's software under an air flow of 50 ml min^{-1} with a heating rate of 10 °C min^{-1}, in aluminium crucibles with a perforated lid and a temperature range of 20–200 °C. The instrument was previously calibrated using 99.99% purity Indium, *m.p.* = 156.6 °C, ΔH = 28.56 J g^{-1} (Andrade et al., 2014; Oliveira et al., 2014).

The micro-images were obtained using a digital microscope coupled with a DSC cell. The microscope was equipped with a colour CMOS sensor and lens glass with 2 M pixel resolution and a magnification of 800 X. AMCAP V9.016 software was used to capture the images (Gálico et al., 2015).

The infrared spectra of each solid compound was obtained by using a Nicolet S10 FTIR spectrophotometer with ATR accessory and Ge window. The FTIR spectra were recorded with 32 scans per spectrum at a resolution of 4 cm^{-1} (Marinho et al., 2015; Silverstein & Webster, 1998; Nicolet TGA Vapor Phase, 2015).

The X-ray powder patterns were obtained using an Ultima – 4 X-ray diffractometer (Rigaku, Japan), employing Cu K α radiation (λ = 1.5418 Å) and settings of 40 kV and 20 mA. The scattered radiation was detected in the angular range of 5°–50° (2θ), with a scanning speed of 8° min^{-1} and a step of 0.06°.

3. Results and discussion

The TG/DTG curves for the lactobionic acid, Fig. 1(a-left) showed that the thermal decomposition occurred in four consecutive stages of mass loss. The first two occurred between 30–119 °C and 119–177 °C with corresponding mass losses of 4.03% and 3.04%, respectively. In the DTG curves the peaks occurred at 88.96 °C and 156.36 °C. The third and fourth steps of mass loss occurred consecutively between 177–388 °C and 388–540 °C with mass losses corresponding to 70.98% and 21.93% (Table 1), and the DTG peaks occurred at 250.97 °C and 488.77 °C, with a final residue from the thermal decomposition corresponding to 0.02% of initial mass. The latter two stages of mass loss were associated with the decomposition and oxidation of the organic matter in air atmosphere. All the TG/DTG results are gathered in Table 1.

The DSC curves for lactobionic acid, Fig. 1(a-right), showed several endothermic peaks in consecutive steps, which corresponds with the TG/DTG curves; the results of which are shown in Table 2.

In relation to the lactulose, the TG/DTG curves in Fig. 1(b-left) show two main steps of mass loss, the first of which was assigned to decomposition, which began at 180 °C. This stage occurred in a consecutive reaction, with shoulders at 190 and 208 °C and a peak at 275 °C in the DTG curve. The second mass loss occurred between 360 and 570 °C in only one stage. The final residue from the thermal decomposition of the lactulose corresponded to 0.52% of the initial mass.

The DSC curve for the lactulose is depicted in Fig. 1(b-right). This shows an endothermic peak at 69 °C, which was attributed to some adsorption water, and a main peak at 171 °C, which corresponded to the melting point. The Van't Hoff equation was used to calculate the purity, which was 98% (Bannach et al., 2010). The other peaks at higher temperatures were due to the decomposition of the compound and all the values are shown in Table 2.

These phenomena were observed simultaneously by using an optical microscope coupled under the DSC cell (thermomicroscopy) and the results can be verified in the Supplementary material.

The kinetic study used the Flynn-Wall-Ozawa method, which made it possible to determine the kinetic parameters for the thermal degradation of the samples. The kinetic parameters obtained for the lactulose were: activation energy (Ea) 70.24 kJ mol^{-1}, the Arrhenius frequency factor (A) was 3.291×10^5 min^{-1}, which was a first-order reaction. The kinetic parameters obtained for the lactobionic acid were: activation energy (E_a) of 232.76 kJ $mol^{-1}$and the Arrhenius frequency factor (A) was 1.682×10^{20} min^{-1} (Flynn & Wall, 1966; Ozawa, 1965). The thermal decomposition occurred as a first-order reaction.

The attenuated total reflectance in relation to the infrared spectroscopic data of the lactobionic acid and lactulose are shown in Table 3. The axial deformation, or stretching bands, for lactobionic acid were observed at: 3360 cm^{-1} (ν $OH_{alcohol}$),