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Study of thermal degradation of aspartame and its products of conversion in sweetener using isothermal thermogravimetry and HPLC

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Abstract

Aspartame (APM) has been a center of discussion due to the toxicity of its metabolism products. This work studied the thermal degradation of aspartame and its products of conversion in a sweetener using isothermal thermogravimetry (TG), infrared (FTIR) and high-performance liquid chromatography (HPLC). The TG curves were performed using a Shimadzu Thermobalance, under air atmosphere, flow rate of 20 mL min^{-1} and heating rate of 10 °C min^{-1}. The chromatograms were performed using a Shimadzu Chromatograph with diode-array detector at 210 nm, C18 column, water–acetonitrile (90:10) and 0.1% trifluoroacetic acid. The 5-benzyl-3,6-dioxo-2-piperazineacetic acid (5BZ) was identified as only one product of thermal conversion from aspartame (APM). The 5BZ and APM have been identified and quantified through the comparison of areas and retention time established with standards. The chromatograms had presented limits of quantification (LOQ) and detection (LOD) of 5; 2 μg mL^{-1} and 10; 5 μg mL^{-1} for 5BZ and APM, respectively. The samples have indicated recovery of 100.42 ± 1.34%. The content of APM in the sweetener was 19.08 mg ± 2.22%, this amount is within specifications (19 mg ± 10%). The standard APM was heated at 80–220 °C in TG-isothermal and chromatograms have indicated the total conversion of APM to 5BZ, which showed a maximum rate of conversion in a temperature range of 140–160 °C. In the sweetener the APM was converted to 5BZ in a temperature range of 120–140 °C. The TG-isothermal indicated a decrease in the temperature of conversion to 5BZ and its intensity, probably due to interaction with the lactose excipient contained in the sweetener.

Keywords: Sweetener; Isothermal thermogravimetry; Aspartame thermal degradation

1. Introduction

Aspartame (APM), *N*-L-α-aspartyl-L-phenylalanine methyl ester, is an artificial sweetener that was discovered accidentally in the United States by Schlatter in 1965. It is a white dipeptide, crystalline, low-caloric sweetener, which is 180–200 times sweeter than sucrose [1–4].

The risks of aspartame ingestion would be in the toxicity of its metabolism products (e.g. phenylalanine, methanol and aspartic acid), since one of its metabolites, phenylalanine is not recommended for phenylketonurics. Elevated doses of phenylalanine can cause changes in behavior (e.g. depression, insomnia), alteration in vision and mental retardation, especially in children. An elevated production of methanol can cause acidoses and blindness [5,6].

It is important to study the thermal behavior of foodstuffs because the kinetic factors, temperature and time, can provoke decomposition of components with new compound

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formation, which may be harmful. Chemical interactions can occur during industrial processing, which may necessitate the development of better methodologies applied to quality control [7,8].

Thermal analysis has constituted techniques of great interest in the characterization of foodstuffs and supplying analytical data for industrial process, which decrease time and costs of analyses [9–12]. The association of thermal data with other techniques such as high-performance liquid chromatography (HPLC) and infrared (FTIR) have demonstrated efficiency and quickness to identify and quantify foodstuffs [13,14].

This work studied the thermal degradation process of aspartame and its conversion products in the sweetener using isothermal thermogravimetry (TG), high-performance liquid chromatography and infrared.

2. Experimental

Aspartame, 5-benzyl-3,6-dioxo-2-piperazineacetic acid (5BZ), aspartic acid, phenylalanine and aspartylphenilalanine standards were purchased from Merck. HPLC-grade acetonitrile and trifluoroacetic acid were obtained from Mallinckrodt.

Sweetener tablets were acquired in the local market. The sweetener was composed of aspartame 38%; lactose 57.02%; carboxymethyl celullose sodium 2.4%; polyvinylpyrrolidone 1.5%; silicon dioxide 0.8% and magnesium stearate 0.28%.

2.1. Thermogravimetric analysis

The TG-dynamic curves were performed using a Shimadzu Thermobalance, under air atmosphere, flow rate of 20 mL min^{-1}, mass of 5 mg and a heating rate of 10 °C min^{-1} up to 900 °C. The TG-isothermal curves were also performed using a Shimadzu Thermobalance, under air atmosphere, flow rate of 20 mL min^{-1} and a heating rate of 10 °C min^{-1}. The TG-isothermal curves for the aspartame standard and sweetener were hold in the temperatures of 80–220 °C for 4 h.

2.2. HPLC analysis

The APM, aspartic acid, 5BZ, phenylalanine and aspartylphenilalanine standards were dissolved in water at 0.4 μg mL^{-1}. The APM thermal residues were prepared in the same concentration.

The analyses were performed using a Shimadzu Chromatograph fitted with a diode-array detector at 210 nm. The Supelcosil C18 column was 50 mm × 4.6 mm and had a 5 μm particle diameter. The samples were mixed in a Quimis Vortex for 10 s and centrifuged in a Revan centrifuge at 9000 rpm for 3 min. The components were eluted isocratically with water–acetonitrile (90:10, v/v) containing 0.1% trifluoroacetic acid, at a flow rate of 2 mL min^{-1} and an injection volume of 5 μL. The mobile phase has degassed prior to analysis.

2.3. Spectroscopic analysis

The infrared spectrum of APM standard, 5BZ standard and degradation product of APM at 160 °C were obtained using a Bomem Spectrometer, model MB-102, in the 4000–400 cm^{-1} wavelengths in KBr pellets.

3. Results and discussion

3.1. TG-dynamic

The TG-dynamic curves (Fig. 1) showed an initial temperature of decomposition for the binary mixture aspartame:lactose (120 °C, curve 2) and sweetener (114 °C, curve 1) smaller than that of the aspartame standard (172 °C, curve 4) and lactose standard (176 °C, curve 3). The thermogravimetric curve for the binary mixture 5BZ:lactose (200 °C, curve 5) also showed a smaller temperature of decomposition than the 5BZ standard (242 °C, curve 6). The aspartame standard presented volatilization processes between 30 and 150 °C followed by three decomposition processes. The sweetener has presented the same volatilization and decomposition processes as the aspartame standard, which included displacement for smaller temperatures and suggesting an interaction with the excipients. The binary mixtures aspartame:lactose and 5BZ:lactose also showed displacement for smaller temperatures suggesting the interaction of aspartame with lactose in the sweete ner.

3.2. TG-isothermal

TG-isothermal curves for the aspartame standard and sweetener have been studied to evaluate the volatilization and decomposition processes in aspartame in the temperature range of 80–200 °C (Figs. 2 and 3).

The TG-isothermal profile for aspartame has presented only one stage of mass loss corresponding to volatilization process (Fig. 2, curves 1–5).

Curves 6–12 in Fig. 2 presented two stages of mass loss; the first stage was similar to curves 1–5 and the second stage corresponded to the decomposition process.

The TG-isothermal profile for the sweetener presented (Fig. 3, curves 1–3) only one stage of mass loss corresponding to volatilization and two stages of mass loss similar to aspartame standard (Fig. 3, curves 4–12).

At the end of the analysis the APM and sweetener residues were collected to identify and quantify the APM and its degradation product.

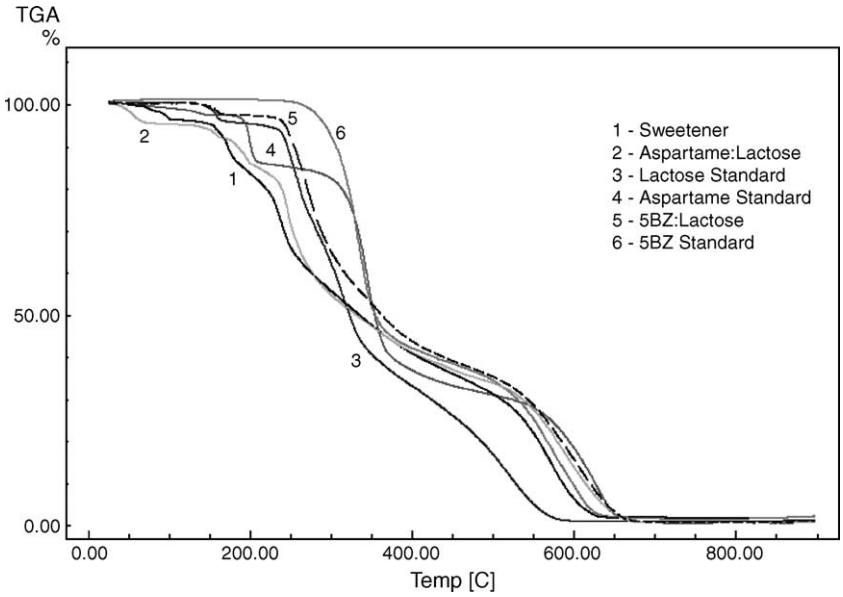

Fig. 1. TG-dynamic curves for aspartame, 5BZ, sweetener, lactose and mixtures.

3.3. Chromatographic analysis

3.3.1. Recovery

The filtration process was performed for products of sweetener degradation due to carbonaceous residues before the injection procedure in HPLC. The samples were filtered in different volumes and compared with centrifuged samples. The best recovery for the filtered samples was 4 mL of filtered volume. Table 1 shows the values for the recovery of aspartame in the sweetener.

3.3.2. Precision and accuracy

The APM content in the sweetener was 19.08 mg ± 2.22%, with quadriplicate determinations. The product is within specifications (19.00 mg ± 10%).

3.3.3. Linearity

Fig. 4 showed good coefficient of correlation for the aspartame and 5BZ standards. The linearity was determined in the concentration range of 5–600 $\mu g\,mL^{-1}$ (5, 10, 20, 40, 200, 400 and 600) with triplicate determinations (Fig. 4).

3.3.4. Detection and quantification limits

The samples were identified and quantified through comparison of areas and retention time established with standards. The chromatograms presented limits of quantification (LOQ) and detection (LOD) of 5; 2 $\mu g\,mL^{-1}$ and 10; 5 $\mu g\,mL^{-1}$ for 5BZ and APM, respectively. The signal-to-noise ratio was 3:1 (LOQ) and 2:1 (LOD).

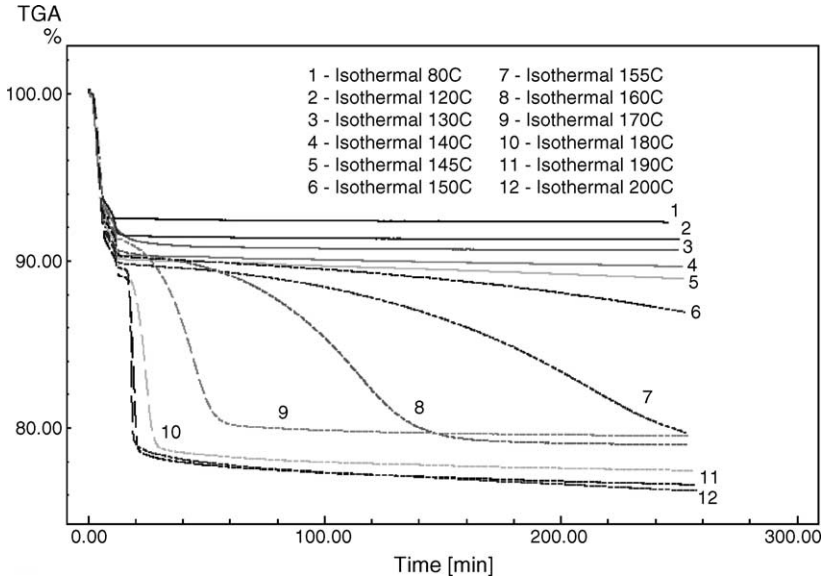

Fig. 2. TG-isothermal curves for the aspartame standard.

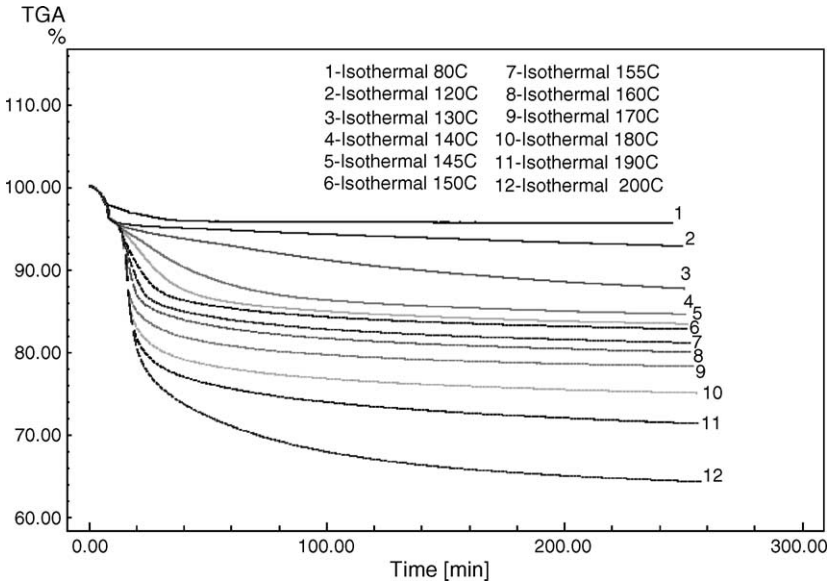

Fig. 3. TG-isothermal curves for the sweetener.

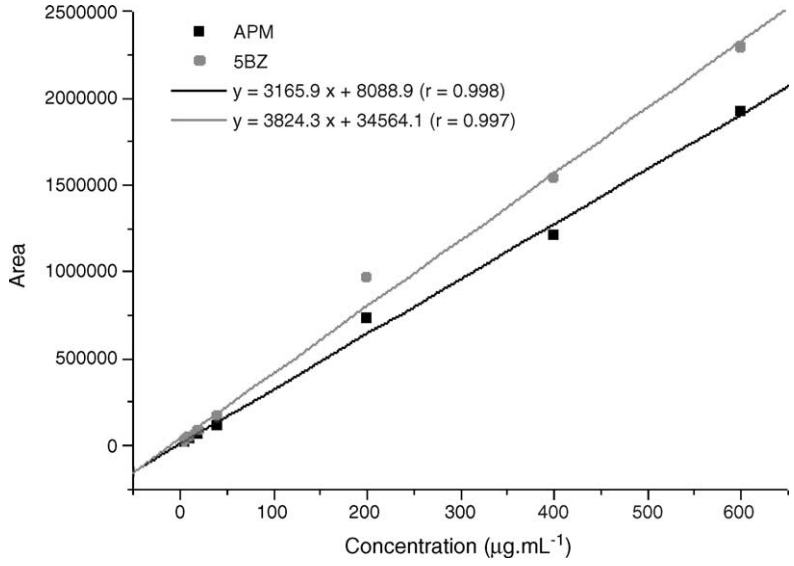

Fig. 4. Calibration curves for the APM and 5BZ standards.

3.4. Identification of the thermal degradation products of aspartame in the sweetener

The 5-benzyl-3.6-dioxo-2-piperazineacetic acid, aspartylphenylalanine, phenylalanine and aspartic acid standards were evaluated. TG-isothermal residues of APM and sweetener were evaluated at all temperatures. Only 5BZ was identified as the thermal conversion product of APM with retention times of 1.4 min (5BZ) and 5.5 min (APM), respectively.

Table 1
Recovery of aspartame solution after sample preparation

Samples	Medium area[a]	Preparation	Recovery (%)	SDR (%)
1	1247997	Filtered 1 mL	95.78	2.71
2	1264882	Filtered 2 mL	97.07	1.98
3	1299919	Filtered 4 mL	99.76	3.14
4	1308580	Centrifuged	100.42	1.34

[a] Quadriplicate determinations.

Fig. 5 showed the aspartame degradation process concomitant with formation of 5BZ at temperatures of 80, 120, 140 and 160 °C. At 120 °C the conversion of aspartame to 5BZ began, which was completed at 160 °C (chromatogram 4).

The aspartame degradation process in the sweetener occurred at lower temperatures than the APM Standard (Fig. 6). At 80 °C the conversion of aspartame to 5BZ began, which was completed at 140 °C (chromatogram 4).

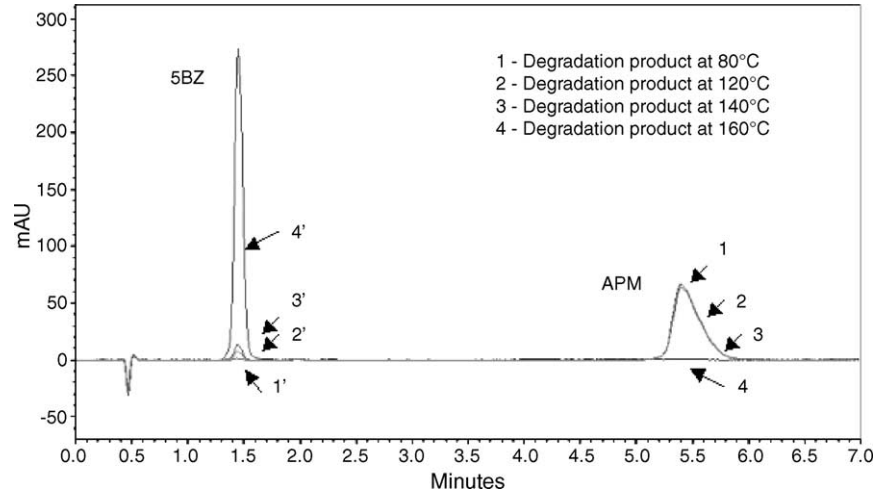

Fig. 5. Chromatogram of aspartame at 80, 120, 140 and 160 °C.

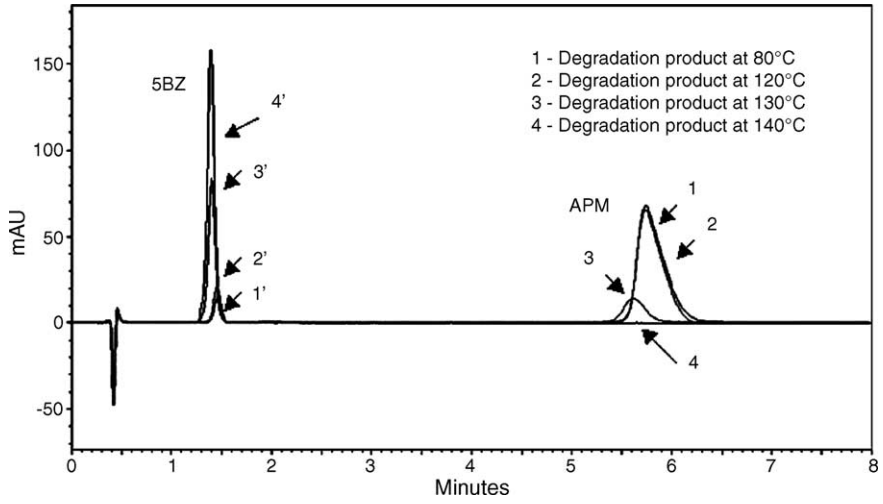

Fig. 6. Chromatogram of sweetener at 80, 120, 130 and 140 °C.

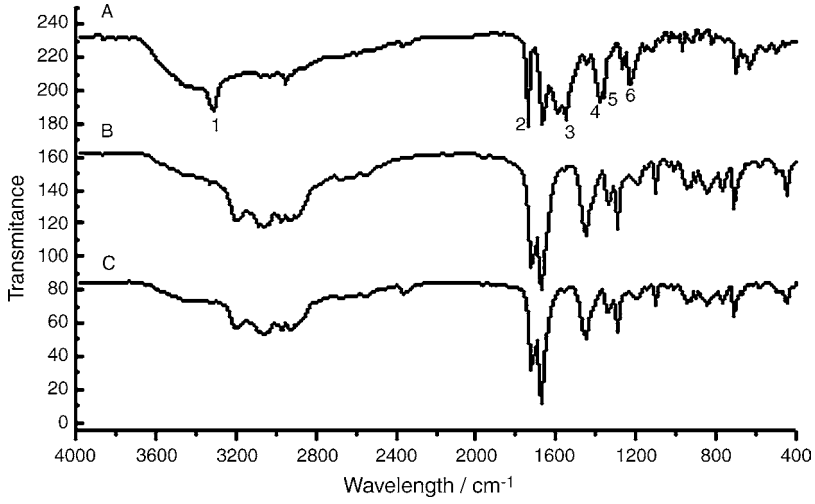

Fig. 7. Infrared spectrum of APM standard (A), degradation product of APM at 160 °C (B) and 5BZ standard (C).

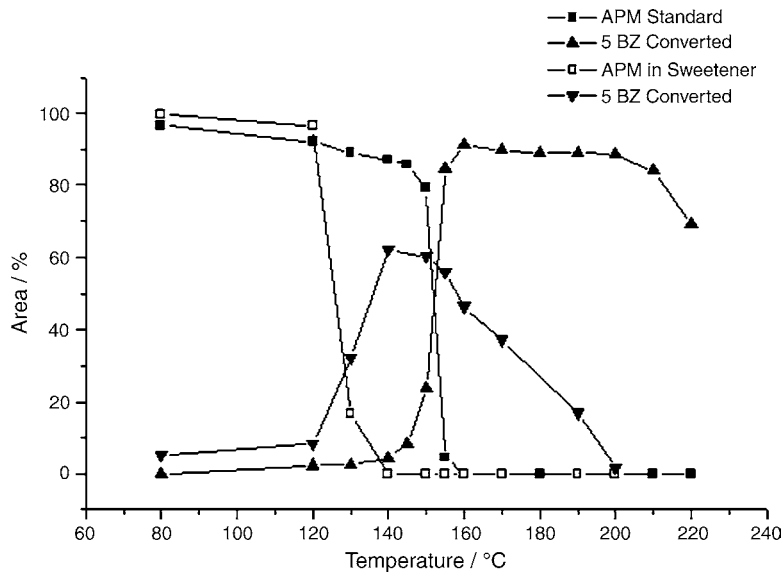

Fig. 8. Conversion of APM (filled and open square) to 5BZ (up and down triangle) in the isothermal degradation residues of APM standard and sweetener, respectively.

3.5. Infrared spectroscopy

The infrared spectrum of the APM degradation product (Fig. 7, spectrum B) confirmed the total conversion from APM to 5BZ due to the absence of bands present in the APM standard (Fig. 7, spectrum A): 3309 cm^{-1} (peak 1: medium intensity) referring to stretching N–H amine; 1736 cm^{-1} (peak 2: strong intensity) referring to stretching C=O ester; 1587 cm^{-1} (peak 3: medium intensity) referring to deformation N–H amine; 1383 and 1376 cm^{-1} (peaks 4 and 5: medium intensity) referring to deformation C–H methyl and 1220 cm^{-1} (peak 6: medium intensity) referring to stretching C–O ester. This data was confirmed with the 5BZ standard (Fig. 7, spectrum C).

3.6. Correlation of TG and HPLC data

Fig. 8 shows quantitatively the aspartame conversion to 5BZ. Starting from 140 °C, the APM standard presented a maximum rate of conversion, which was completed by 160 °C (up triangle). After 200 °C the 5BZ converted has began your decomposition.

In the sweetener the rate of conversion started at 120 °C and was completed at 140 °C (down triangle). This reaction has presented only 62% of the aspartame converted to 5BZ. The temperature of conversion was displaced to a lower temperature, probably due to other interactions with excipients in the sweetener. The 5BZ began to decompose at 150 °C in the sweetener.

The Fig. 8 data has correlated with initial temperatures of decomposition in TG-dynamic studies (Fig. 1) for APM (172 °C), aspartame:lactose (120 °C) and sweetener (114 °C). Probably, aspartame and 5BZ converted interacted with lactose by the Maillard's reaction.

4. Conclusions

The conversion of APM to 5BZ occurred both in the APM standard and sweetener. This conversion presented a maximum rate at 140–160 and 120–140 °C, respectively. This displacement to a lower temperature suggests an interaction with lactose contained in the sweetener.

The isothermal thermogravimetry associated high-performance liquid chromatography and FTIR have demonstrated the efficiency to identify and quantify the APM and its thermal conversion product for both APM and sweetener.

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