EVALUATION OF YERBA MATE FRUIT PULP TOTAL AND FRACTIONED SOXHLET EXTRACTION USING THERMAL ANALYSES

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Simultaneous thermal analysis; TGA/MS; different polarity; *Ilex paraguariensis*; extract thermal degradation.

ABSTRACT

Ilex paraguariensis leaves are traditionally used to prepare hot or cold beverages in South America. Yerba mate fruit pulp has no commercial value, nevertheless, its extract showed different biological activities. The fruits contain high concentration of saponins and methylxanthines, besides polyphenols and anthocyanins. Soxhlet extraction using polarity gradient of solvents is used to perform total and fractioned extraction of plant material. Thermal analyses are used to evaluate extracts by revealing their behavior when submitted to temperature variation. The aim was to evaluate by thermogravimetry coupled to mass spectrometry extracts from yerba mate semi-ripe fruit pulp, obtained by Soxhlet extraction using diethyl ether, ethyl acetate, ethanol and water solvents. TGA/MS was applied using air atmosphere. Diethyl ether and ethyl acetate showed thermal behavior similarity, indicating similar composition. Only the aqueous extract showed inorganic compounds with approximately 20% of ashes. Ethanolic extract showed more apparent differences of thermal behavior, with the greater mass loss event related to the release of CO_2, indicating greater amount of more thermal stable compounds. The other extracts showed their main event related to the loss of CO. Ethanolic extract also showed a greater number of events, being the most complex extract.

Introduction

Ilex paraguariensis Aquifoliaceae is a tropical tree from South America, classified by August de Saint Hilaire in 1822. The leaves are traditionally used to prepare hot or cold beverages in Brazil, Argentina, Paraguay and Uruguay. The fruits are globular drupes which contain four seeds and a mucilaginous pulp [1,2]. This pulp has no commercial value, nevertheless, the fruit extracts showed molluscicidal, allelopathic, antimicrobial, antioxidant and anticholesterolemic activities [3-5]. The fruits contain high concentration of saponins, which are different from those found in leaves [6]. Methylxanthines are also found in great concentrations. Caffeine is the main compound found in extracts obtained by pressurized propane and supercritical carbon dioxide [5]. Furthermore, polyphenols and anthocyanins were also observed in *I. paraguariensis* fruits extracts [4]. Soxhlet extraction using polarity gradient of solvents is applied aiming to achieve total extraction of plant material, from more un-polar compounds which are extracted by solvents as hexane or diethyl ether and more polar compounds, which can be extracted by small alcohols and water [7]. Thermal analyses are used to evaluate the behavior of a sample submitted to time and temperature variation. Thermogravimetric analysis (TGA) reveals the mass loss in function of time or temperature, while Differential Scanning Calorimetry (DSC) indicates the differences of temperature between the sample and a reference material [8]. Thermogravimetry analysis coupled to mass spectrometry (MS) allows the identification of released volatiles during samples heating. Therefore, thermal analyses have been used to characterize plant materials and extracts [9]. Additionally, these techniques can be applied in quality control assays because they provide information related to sample stability and composition [10]. The aim of this study was to evaluate extracts from yerba mate semi-ripe fruit pulp obtained by Soxhlet extraction, using diethyl ether, ethyl acetate, ethanol and water solvents. The evaluation of the extracts was performed using thermogravimetry coupled to mass spectrometry.

Material and methods

The fruits were obtained in Embrapa from Ponta Grossa – PR (25º 9.500'S, 50º 4.400'W) in March 2018. Semi-ripe fruits (red-colored) were selected, dried in temperature of 40 ºC for 24 hours and then crushed using a blender (1000 Watts). The pulp was separated from the seeds using 18 mesh sieve. The extracts were obtained from 20.0000 g ± 0.0009 g of pulp powder. The material was supported by a sealed cartridge made of filter paper and placed inside the Soxhlet apparatus. The extraction solvents were used in order of polarity: diethyl ether, ethyl acetate and ethanol. Each extraction was carried out for four hours with 200 mL of each solvent. After the ethanolic extraction, the cartridge content was placed in an Erlenmeyer for the aqueous extraction at 55 ºC for two hours. Organic solvents were reduced to dryness at 60 ºC and water extract was lyophilized. The yield was calculated comparing the mass of solid material obtained from each extraction with the mass of pulp powder placed in the cartridge. TGA/DSC coupled to mass spectrometry were applied to compare chemical compositon of extracts using Simultaneous thermal analyzer coupled to mass spectrometer Q600MS (TA Instruments, USA). A mass of 1.8125 mg ± 0.2690 mg of each extract was heated under air flow of 50 mL min^{-1} and heating rate of 5 ºC min^{-1} until 600 °C. Masses were measured from 1 to 150 Da. Mass loss was obtained using TA 60WS software (Shimadzu, Japan). TG curves were plotted using Origing® Pro 8.0 (OriginLab Research) [9].

Results and discussion

Primarily, the seeds were separated and the pulp powder mass obtained corresponded to 28.4029% ± 2.9461% in relation to dried fruit mass. Soxhlet extractions were performed using polarity order of solvents (diethyl ether, ethyl acetate and ethanol) and an aqueous extraction was carried out at the end. The extracts were lyophilized or reduced to dryness and the yields were calculated compared to yerba mate fruit pulp. The obtained yield result was 5.2988% for diethyl ether, 0.3365% for ethyl acetate, 10.2275% for ethanol and 12.8769% for water extraction. This result showed that the semi-ripe dried fruit pulp from yerba mate had more hydrophilic compounds. The lyophilized extracts were evaluated using SDT coupled to mass spectrometer equipment in air atmosphere (Fig. 1). Diethyl ether and ethyl acetate showed three events on TG curves. Ethanolic extract showed four events, being the first related to the loss of water and volatile hygroscopic compounds (77-91 ºC). In aqueous extract this compounds were absent and only one event was observed. The single event of aqueous extract showed similar temperature to the first event of diethyl ether and ethyl acetate extracts and the second event of ethanolic extract. This event occurred at 245-340 ºC (46% - diethyl ether), 267-332 ºC (35% - ethyl acetate), 268-313 ºC (20% - ethanol) and 240-312 ºC (43% - water), Table 1. This mass loss can be related to the degradation of carbohydrates and other carbon compounds in addition to volatilization [11]. The next event occurred at 393-409 ºC (7% - diethyl ether), 375-427 ºC (14% - ethyl acetate) and 399–454 ºC (24% - ethanol). This mass loss is probably due to oxidation, degradation of more stable compounds and initial formation of carbonaceous compounds [9]. The last event occurred at 459-512 ºC (16% - diethyl ether), 453-500 ºC (22% - ethyl acetate) and 464-524 ºC (10% - ethanol). This event is related to the formation of ashes. Only the aqueous extract showed remaining ashes of 20.4368%, which are inorganic compounds extracted in aqueous solution.

Figure 1 - TGA and DSC curves of yerba mate pulp fruit extracts obtained with different solvents using air atmosphere. Diethyl ether (E1), ethyl acetate (E2), ethanol (E3) and water (E4).

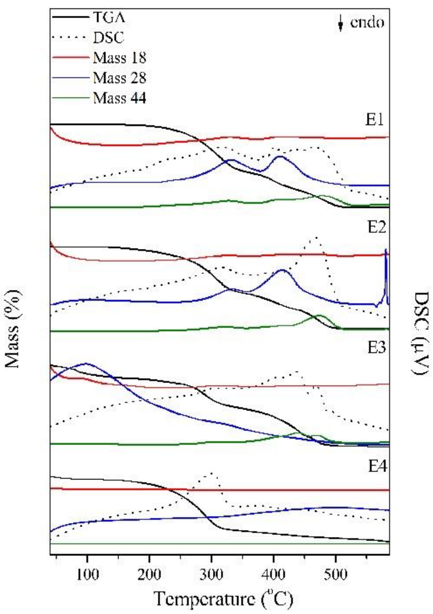

Table 1 - TGA data, related DSC and MS data of yerba mate pulp fruit extracts obtained with different solvents: diethyl ether (E1), ethyl acetate (E2), ethanol (E3) and water (E4).

Extracts	TG			DSC
	Events	Δm (%)	ΔT (°C)	T_p (°C)
(E1)	1st	46	245-340	316 (exo)
	2nd	7	393-409	399 (exo)
	3rd	16	459-512	463 (exo)
(E2)	1st	35	267-332	315 (exo)
	2nd	14	375-427	426 (exo)
	3rd	22	453-500	468 (exo)
(E3)	1st	4	77-91	267 (shoulder)
	2nd	20	268-313	294 (shoulder)
	3rd	24	399-454	438 (exo)
	4th	10	464-524	468 (exo)
(E4)	Single	43	240-312	302 (exo)

Δm: mass loss (%), ΔT: temperature range, T_p: peak temperature.

The extracts showed one event of mass loss in similar, which is related to the degradation of alkanes [11]. Diethyl ether and ethyl acetate showed thermal behavior similarity, indicating similar composition, because they contain the compounds of lower polarity [7]. Ethanolic extract showed more events than the other extracts, which indicates greater complexity. The aqueous extract showed a single event (the simplest extract). CO, CO_2 and H_2O fragments are the main products from organic compounds degradation and oxidation processes in air atmosphere [12]. These fragments (CO_2-44 Da, CO-28 Da and H_2O-18 Da) were monitored by mass spectrometry during the TGA events. Mass 18 showed a peak during thermal degradation of ethanolic extract. Mass 28 was observed with the major TIC for all first events of mass loss of the extracts. It showed a peak related to the events observed, except for aqueous extract single event. Mass 28 also showed a peak in diethyl ether, ethyl acetate and ethanolic extracts second events. The CO fragment has 28 Da, but this mass can also be related to CH_2N and C_2H_4 fragments [13]. Thus, the first event of ethanolic extract thermal degradation is due to the release of a greater mass of volatiles than to water molecules. These volatile compounds were not observed in other extracts. Mass 44 showed a peak during third event of all organic solvents extracts and fourth event of ethanolic extract. CO_2 fragment has 44 Da, but this mass can also be related to N_2O, CH_2NO, CH_4N_2, C_2H_4O, C_2H_6N and C_3H_8 [13]. The events of greater mass losses can be mainly related to the release of CO fragment mass for diethyl ether, ethyl acetate and aqueous extracts and CO_2 fragment mass for ethanolic extract. This indicates that ethanolic extract may show more thermal resistant compounds, that were not completely carbonized. Notwithstanding that the last event of organic solvents extracts also showed a carbon dioxide fragment mass peak.

Conclusion

Thermogravimetry coupled to mass spectrometry allowed the evaluation of total and fractioned Soxhlet extraction of yerba mate fruit pulp using different polarity extracts. Only the aqueous extract showed inorganic compounds (20% of ashes). Diethyl ether and ethyl acetate showed more similar thermal behavior which indicates more similar composition. Ethanolic extract showed similar events to the other organic solvents extracts except for the greater mass loss related to the release of CO_2, which indicates greater amount of more thermal stable compounds. Ethanolic extract also showed greater complexity compared to other extracts.

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