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Myrtaceae leaves grown in Agroforestry: a sustainable alternative to producing natural antioxidants extracts

Folhas de Myrtaceae cultivadas em Agrofloresta: uma alternativa sustentável para produzir extratos antioxidantes naturais

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Myrtaceae species such as uvaia, grumixama, and feijoa have been used in Brazil's Agroforestry Systems (AFS). In AFS, frequent pruning occurs, and leaves are discarded. However, the leaves contain specific phenolic compounds (PC) that, when appropriately extracted, produce extracts with antioxidant activity (AA). These extracts meet the demand for natural and sustainable food ingredients. Therefore, the drying study (40, 50, and 60 °C) of uvaia, grumixama, and feijoa leaves obtained from AFS was carried out, and the effect of drying on the PC profile (LC-ESI-MS/MS) and AA were evaluated. In the uvaia extracts, the major compounds were EGCG, gallic acid, and isoquercitin, accounting for 94% of their PC, and leaves dried at 50 °C promoted a better phenolic extraction. The gallic acid, 2.5 DHBA and isoqueticitrin represent 79% of the relative composition of PC in grumixama extracts, and leaves dried at 60 °C were the condition that allowed a greater extraction. While, epicatechin, catechin, gallic acid and isoquercitrin correspond to 66% of the PC composition of feijoa leaves. Grumixama had the highest values for AA by the FCRC and DPPH while feijoa had significant values for FRAP in all treatments. Dried leaves at 50 °C showed higher AA for all methods, except for grumixama which showed this behavior at 60 °C. These results indicate that these matrices proved promising sustainable sources of various phenolic compounds. The drying process of the leaves at 50 and 60 °C had a greater extraction of compounds with antioxidant activity, with potential industrial applications.
Keywords: *Acca sellowiana*, *Eugenia brasiliensis*, *Eugenia pyriformis*.

Espécies de Myrtaceae como a uvaia, grumixama e a feijoa tem sido utilizada em Sistemas Agroflorestais (SAF) no Brasil. Nos SAF, podas frequentes são realizadas e as folhas são descartadas. No entanto, as folhas possuem uma composição específica de compostos fenólicos (CF) que quando extraídas adequadamente, produzem extratos com atividade antioxidante (AA). Estes extratos atendem à demanda de ingredientes alimentares naturais e sustentáveis. Sendo assim, foi realizado o estudo da secagem (40, 50 e 60 °C) das folhas de uvaia, grumixama e feijoa obtidas da SAF, e foi avaliado o efeito da secagem sobre o perfil de CF (LC-ESI-MS/MS) e AA. Nos extratos de uvaia os compostos majoritários foram EGCG, ácido gálico e isoquercitrina, representando 94% do PC. O ácido gálico, 2,5 DHBA e isoquercitrina representaram 79% da composição relativa do CF nos extratos de grumixama. Enquanto que, epicatequina, catequina, ácido gálico e isoquercitrina correspondem a 66% da composição de CF das folhas de feijoa. A grumixama apresentou os maiores valores de AA pelos métodos FCRC e DPPH enquanto a feijoa apresentou valores significativos para FRAP, em todos os tratamentos. As folhas secas a 50 °C apresentaram maiores valores de CF e AA para todos os métodos, com exceção da grumixama que apresentou esse comportamento a 60 °C. Esses resultados indicam que essas matrizes se mostraram promissoras fontes sustentáveis de vários compostos fenólicos. O processo de secagem das folhas a 50 e 60 °C teve uma maior extração de compostos com atividade antioxidante, com potenciais aplicações industriais.
Palavras-chave: *Acca sellowiana*, *Eugenia brasiliensis*, *Eugenia pyriformis*.

1. INTRODUCTION

Uvaia (*Eugenia pyriformis*), grumixama (*Eugenia brasiliensis*), and feijoa (*Acca sellowiana*) are fruitful and endemic species from Brazil found in the Atlantic Rainforest and belonging to the Myrtaceae family [1]. These species are considered a good alternative for incorporating into Agroforestry Systems (AFS) to recover degraded areas in Brazil [2].

In pruning procedures, the leaves of these species are discarded in the soil. The leaves have great potential as matrices for the extraction of bioactive compounds that have functional properties. In addition, using these specie leaves could be an excellent way to increase the economic value of these crops and promote more sustainable practices.

The functional properties of the extracts have been associated with a set of compounds generated in the secondary metabolism of plants that include PC or other phytochemicals with antioxidant capacities, such as alkaloids, anthraquinones, and phytosterols, saponins, tannins, triterpenoids, and steroids [3]. Among these phytochemicals, PC stand out for acting as hydrogen donors and chelate metal ions, which gives them the ability to inhibit oxidative processes. The literature reported the antimicrobial activity of the uvaia leaves [4], the anti-inflammatory activity of the grumixama leaves [5], and the inhibition of diabetes enzymes from feijoa leaves [6]. Thus, using these leaves as a sustainable and low-cost source for obtaining high-value-added plant extracts may be an alternative to meet the growing demand of consumers seeking sustainable pharmaceutical and food products with natural actives.

On the other hand, fresh leaves have high perishability due to the high moisture content that favors enzymatic and microbial activity and processes that can be delayed or minimized through drying. The dry leaves can be used with infusions or as raw materials in producing extracts for different food, cosmetic or pharmaceutical products. In this context studying the leaves drying process and solid-liquid extraction parameters of extract production are necessary to maintain nutrient quality and minimize the degradation of the functional compounds of interest [7-9].

Therefore, this study aims to investigate the kinetic drying of uvaia (*Eugenia pyriformis*), grumixama (*Eugenia brasiliensis*), and feijoa (*Acca sellowiana*) leaves at 40, 50, and 60°C. Produce extracts from fresh and dried leaves from optimized extraction conditions and evaluate the effect of temperatures on the thermal stability of the phenolic profile by LC-ESI-MS/MS and antioxidant activity *in vitro* (DPPH, FRAP, and Folin-Ciocalteu reducing capacity).

2. MATERIAL AND METHODS

2.1 Chemicals

All chemical reagents used were of analytical grade, and all analytical standards (purity ≥ 95%) and solvents were in chromatographic grade.

2.2 Raw material

Fresh adult leaves of three species of Myrtaceae: *Eugenia pyriformis* (voucher specimen FLOR69711), *Eugenia brasiliensis* (voucher specimen FLOR69709), and *Acca sellowiana* (voucher specimen FLOR69710), were collected from Agroecological Agroforestry Systems in the state of Santa Catarina (Brazil) in December 2019. After harvest, the leaves were selected, washed, and sanitized with 2.5% sodium hypochlorite for 15 min.

2.3 Drying Experiments

2.3.1 Drying curves

The drying was carried out in a convective oven (TE-394/2, Tecnal, Ourinhos, SP, Brazil) at 40, 50, and 60 °C and an air velocity of 0.77 m/s. The uvaia, grumixama, and feijoa leaves were distributed in monolayers in perforated trays. The mass of leaves was measured by a

semi-analytical balance (AS5500C, Marte, São Paulo, SP, Brazil) until the samples reached the equilibrium moisture condition (constant mass). The initial moisture on the dry basis of the samples was measured by the gravimetric method (n 925.10 AOAC, 2007). All drying tests were performed in triplicate. After drying, the leaves were vacuum-packed (Modulare CV 25, Conceito Vácuo Co., Tatuapé, SP, Brazil) and stored under the light for further analysis.

The constant period drying rate was determined by linearly fitting the experimental data of the drying curves, as suggested by Monteiro et al. (2015) [10]. Rate results were expressed in g water g DM^{-1} min^{-1}.

2.3.2 Moisture diffusion coefficient and activation energy

The experimental moisture data were dimensionless by calculating the moisture ratio (MR) (Eq. 1)

$$MR = \frac{X - X_e}{X_0 - X_e} \quad (1)$$

Where X is the absolute moisture at time t, X_e is the equilibrium moisture, and X_0 is the initial moisture (t= 0).

Fick's second law determined the diffusion coefficient for an infinite plane sheet [11] (Eq. 2), considering the shape of the material as a plane sheet, assuming that the diffusion of leaves moisture occurs in the one-dimensional direction and the measurement of leaves thickness constant.

$$MR = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \, exp\left(-(2n+1)^2 \, \frac{\pi^2 D_{ef}}{2\delta^2} t\right) \quad (2)$$

Where D_{ef} is the moisture diffusion coefficient (assumed to be constant) (m^2 s^{-1}), t is time and δ is the thickness of the leaves. The adjustment of Eq. 2 to the experimental data of the drying curves was performed using four terms in the summation.

The dependence of the D_{ef} about the temperature was represented by the Arrhenius equation (Eq. 3).

$$D_{ef} = D_0 exp\left(\frac{-E_a}{R T_{abs}}\right) \quad (3)$$

Where D_0 is the Arrhenius constant, R is the universal gas constant (8,314 J/ mol K), E_a is the activation energy (kJ/ mol), and T_{abs} is the absolute temperature (K). The activation energy for each species was calculated by linear regression analysis of ln(D_{ef}) versus 1/ Tabs.

2.4 Extract production

Initially, a complete 2^2 factorial was performed to evaluate the effects of time and methanol concentration on the extraction yield (data not shown). The optimized extraction conditions (methanol 80% v/v per 1h) were used to produce extracts from uvaia, grumixama, and feijoa fresh and dried leaves.

2.4.1 Fresh leaves extracts

The extracts were prepared with 50g of uvaia, grumixama and feijoa fresh leaves and immersed in 500 mL of the 80% (v/v) methanol solution and kept at 25 °C in amber flasks. After 15 days, the material was filtered (Whatman n° 1), obtaining the extract that was concentrated at 40 °C (802, Fisatom, Perdizes, SP, Brazil) and lyophilized (L101, Liotop, São Carlos, SP, Brazil) [12].

2.4.2 Dry leaves extracts

The extracts of leaves dried at 40, 50, and 60 °C were prepared with 1g of the sample (35 mesh) dispersed in 10 mL of the 80% (v/v) methanol solution. The extraction was carried out in reactors with magnetic stirring at a temperature of 40 °C for 1h. After the extracts were filtered (Whatman n°1), rotated at 40°C (Fisatom), and lyophilized (Liotop). The extracts were stored in the dark.

2.5 Extracts characterization

2.5.1 Phenolic content determination by LC-ESI-MS/MS

The identification and quantification of PC in the uvaia, grumixama, and feijoa leaves extracts were determined by liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) [13]. Thirty-eight analytical standards were evaluated: apigenin, (+)-catechin, (−)-epicatechin, galangin, (−)-epigallocatechin gallate (EGCG), isoquercitrin, isorhamnetin, kaempferol, luteolin, naringin, pinobanksin, pinocembrin, quercetin, rutin, taxifolin, 2,4-dihydroxybenzoic acid (2,4 DHBA), 2,5-dihydroxybenzoic acid (2,5 DHBA), 3,4-dihydroxybenzaldehyde (3,4 DHB), 3,5-dinitrobenzoic acid (3,5 DNB), 4-methylumbelliferone, caffeic acid, chlorogenic acid, gallic acid, sinapic acid, syringic acid, vanillic acid, benzoic acid, salicylic acid, ferulic acid, coniferaldehyde, chrysin, coumarin, hesperidin, naringenin, p-aminobenzoic acid, p-coumaric acid, sinapaldehyde, and syringaldehyde. The analysis was carried out in a chromatographic system (Agilent 1290 series, Agilent Technologies, Wilmington, DE, USA) coupled with a hybrid quadrupole linear ion trap mass spectrometer QTRAP 5500 (Sciex, Foster City, CA, USA), was equipped with an electrospray ionization source (ESI). Chromatographic separation was accomplished in a Zorbax Eclipse Plus C18 (3.0 x 100 mm, 3.5 μm particle diameter) from Agilent. The flow rate employed was 300 μL/min, and a volume of 5 μL of standards and samples solutions were injected for analysis. The mobile phase (A) and (B) were composed of acidified water (0.1% v/v formic acid), and acetonitrile with 0.1% (v/v) formic acid, respectively. The column temperature was maintained at 40 °C, and the total run time was 17 min, with the column equilibration time between each run being 4 min.

Mass spectrometry analysis was carried out in positive and negative ionization mode, working in MRM mode, with the following parameters: Ion Spray (IS) voltage: 5500 V; nebulizer gas (GS1) and auxiliary gas (GS2): 55 psi and; curtain gas: 25 psi; source temperature: 400 °C. Using nitrogen as a nebulizer and collision gas. The analyte-specific parameters were optimized through the direct infusion of standard solution for each compound. The compounds were quantified by external calibration and in triplicate.

2.5.2 Antioxidant activity

Lyophilized extracts of uvaia, grumixama, and feijoa leaves were resuspended in methanol 80% (v/v) and determined antioxidant activity by the methods DPPH, FRAP, and Folin-Ciocalteu reducing capacity (FCRC). The free radical scavenging activity of leaves extracts was evaluated by the DPPH method [14]. The inhibition of DPPH free radicals was expressed in Trolox concentration per dry matter (mMol Trolox/ g DM). The reducing capacity of the extracts was determined using the FRAP method described by Benzie and Strain (1996) [15]. The results were expressed in Trolox concentration per dry matter (mMol Trolox/ g DM). FCRC was determined according to the method proposed by Singleton and Rossi (1965) [16]. The results were expressed as gallic acid equivalents (mg GAE/ g DM).

2.6 Statistical analysis

The results of the analyzes (n= 3) were submitted to analysis of variance one-way and Tukey's test ($p \leq 0.05$), using the Statistica 13.3.0 software (TIBCO Statistica™, Palo Alto, CA,

USA). Fick's second law was applied using the curve fitting tool of the MATLAB R2018a software (Mathworks Inc., Natick, MA, EUA).

3. RESULTS AND DISCUSSIONS

3.1 Drying curves

In general, the temperatures of 40, 50, and 60 °C influenced the dry process and, therefore, affected the time for the samples to reach equilibrium moisture, which the distance between the curves can observe as a function of the temperature of drying (Figure 1A and Table 1).

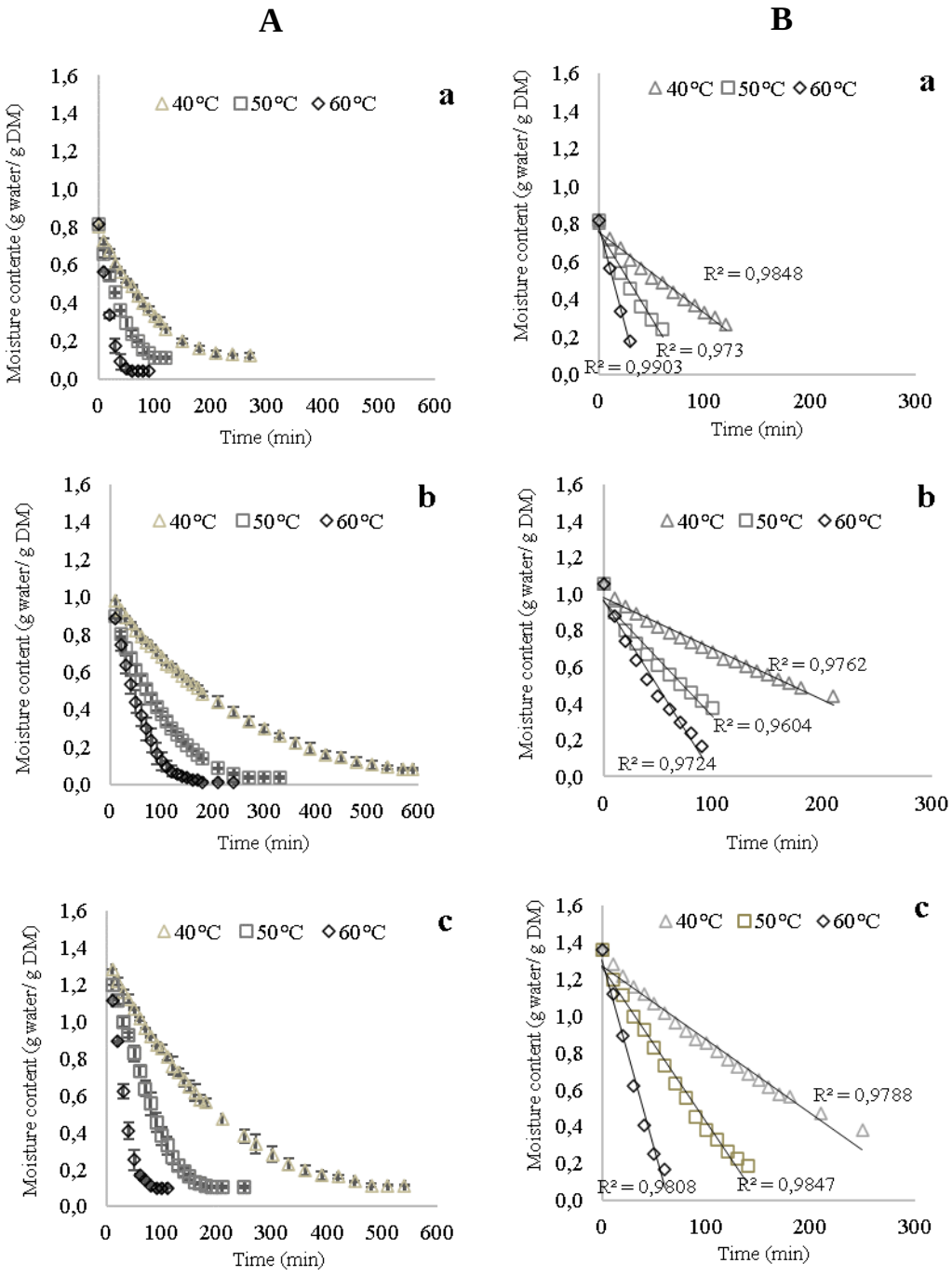

Figure 1: (A) Drying curves at 40, 50, and 60 °C, (B) linear adjustment in the region of a constant rate of the drying curves of uvaia (a), grumixama (b), and feijoa (c) leaves.

The temperature increase favors the vapor pressure of the material's internal water to an unsaturated gas phase, thus facilitating the water removal [17]. The total drying time to reach the equilibrium moisture was shorter for the uvaia, followed by feijoa and grumixama (U <F <G) under the same conditions. When evaluating the drying curves of each species individually, it was observed that the drying time to reach equilibrium moisture decreased from 330 to 90 min, when the air temperature was increased from 40 to 60 °C in uvaia leaves. The feijoa leaves reached equilibrium moisture in 540 min at 40 °C, 250 min at 50 °C, and 110 min at 60 °C. The grumixama leaves showed the most extended drying times 590 min at 40 °C, 330 min at 50 °C, and 240 min at 60 °C (Table 1). The data agree with what was found in the drying rates, where the uvaia leaves showed the highest drying rates at all temperatures followed by feijoa and grumixama. In this study, the uvaia leaves presented the lowest initial moisture and thickness compared to grumixama and feijoa leaves, which may have contributed to these samples having a higher drying rate.

In each species, the temperature had a different effect on drying rates (Figure 1B). For example, changing the temperature from 60 °C to 40 °C reduced the rate values in the constant period of uvaia, grumixama, and feijoa leaves by 80%, 72%, and 81%, respectively. The constant rate period is delimited by X_c and therefore, the drying rate is divided into a long constant rate period, followed by a short decreasing rate period, for all samples (Table 1).

Table 1: Leaves thickness (mm), drying conditions, initial moisture and equilibrium moisture (g H_2O/ g DM), constant drying rate (g H_2O/ g DM^{-1} min^{-1}), critical moisture (X_c) (g H_2O/ g DM), Moisture diffusion coefficient (D_{ef}) (m^2 s^{-1}) and their respective R^2, and activation energy (E_a) (kJ/mol).

Species	Thicknesses	Drying condition	Moisture †	Constant drying rate	X_c	D_{ef} x10^{-12}	R^2	E_a
Uvaia	0.15 ± 0.02	Fresh leaves	0.813 ± 0.088	–	–	–	–	–
		40°C/ 330min	0.120 ± 0.014	0.0043	0.26	0.59	0.94	70.63
		50°C/ 120min	0.111 ± 0.006	0.0095	0.24	1.44	0.95	
		60°C/ 90min	0.039 ± 0.014	0.0216	0.17	3.04	0.96	
Grumixama	0.27 ± 0.02	Fresh leaves	1.057 ± 0.054	–	–	–	–	–
		40°C/ 590min	0.082 ± 0.007	0.0028	0.44	0.95	0.96	62.68
		50°C/ 330min	0.037 ± 0.006	0.0063	0.37	2.43	0.98	
		60°C/ 240min	0.016 ± 0.002	0.0100	0.24	4.02	0.95	
Feijoa	0.26 ± 0.02	Fresh leaves	1.361 ± 0.036	–	–	–	–	–
		40°C/ 540min	0.115 ± 0.001	0.0040	0.38	1.04	0.94	79.94
		50°C/ 250min	0.104 ± 0.004	0.0084	0.19	2.55	0.91	
		60°C/ 110min	0.101 ± 0.003	0.0206	0.17	6.56	0.92	

† Initial moisture content for fresh leaves and equilibrium moisture content for dried leaves.

In addition to the considerations reported above about the drying air temperature effect on drying curves, this parameter can also be a crucial factor in maintaining the quality of extract obtained from them. In this sense, the profile of PC and antioxidant activity of the extract obtained from all samples of dry leaves produced were evaluated.

3.2 Moisture diffusion coefficient and activation energy

Diffusion coefficient (D_{ef}) values represent of the average velocity at which water vapor is transported out of the material (Table 1) [18]. Diffusion is the predominant mass transfer mechanism in the leaves drying. The D_{ef} of the evaluated leaves species ranged from $5.98x10^{-13}$ to $6.56x10^{-12}$ m^2 s^{-1}. This variation was due to drying air temperature and the species. Other authors report similar D_{ef} values $4.77x10^{-13}$ to $2.94x10^{-12}$ m^2 s^{-1} for mint leaves (Park et al., 2002).

The uvaia leaves presented the lowest values for the D_{ef} in all the drying temperatures, followed by grumixama and feijoa (U <G <F). Maroulis et al. (2001) [19] observed an increase in the value of D_{ef} with the increase in the moisture content in the drying process of different samples of agricultural products. In light of this, the initial moisture of uvaia, grumixama, and feijoa leaves (Table 1) exhibited the same trend.

Table 1 shows the E_a and in all leaves' species, a straight line was found in the drying temperature range investigated, with satisfactory R^2, indicating the dependence of Arrhenius (data not shown). That is, there is a uniformity of Def's variation with temperature. According to Corzo et al. (2008) [20], higher E_a values indicate higher sensitivity of the D_{ef} to temperature. The feijoa leaves had the highest values for E_a and were more sensitive to drying temperature. That is, the moisture diffusion in the feijoa leaves happens faster when compared to the uvaia and grumixama leaves. This behavior may be due to weak water interactions with other compounds, making moisture transfer easier.

3.3 Influence of temperature on extract composition

3.3.1 Identification and quantification of phenolic compounds

To verify the influence of drying temperature on the profile of PC in the extracts, LC-ESI-MS/MS was performed. Thirty-eight standard PC were tested, and 30 were identified and quantified in uvaia, grumixama, and feijoa leaves. The identified compounds are divided into 15 flavonoids, 9 phenolic acids, and 6 other compounds (1 benzenediol, 1 hydroxybenzoates, 1 guaiacol, 1 benzopyran, 1 cinnamaldehydes, and 1 hydroxybenzaldehyde) (Table 2).

Table 2: Phenolic profile (mg/kg dry matter) of fresh and dried leaves extracts of feijoa, grumixama, and uvaia.

Phenolic Compounds (mg/kg DM)	**Uvaia**				**Grumixama**				**Feijoa**			
	Fresh	**40 °C**	**50 °C**	**60 °C**	***Fresh***	**40 °C**	**50 °C**	**60 °C**	***Fresh***	**40 °C**	**50 °C**	**60 °C**
Flavonoids												
Apigenin	0.46 ± 0.00	<DL	<DL	0.46 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	<DL	0.05 ± 0.00
Catechin	2.11 ± 0.59	2.23 ± 0.47	5.77 ± 0.04	1.03 ± 0.04	3.85 ± 0.53	5.03 ± 1.67	0.81 ± 0.04	5.09 ± 0.08	61.16 ± 0.66	32.11 ± 3.64	59.65 ± 0.67	58.71 ± 0.24
Epicatechin	2.14 ± 0.63	2.19 ± 0.59	5.94 ± 0.47	1.00 ± 0.05	0.42 ± 0.22	1.75 ± 0.36	0.71 ± 0.22	6.16 ± 0.10	64.47 ± 0.98	64.76 ± 1.05	63.25 ± 1.18	62.24 ± 0.07
Galangin	0.41 ± 0.00	0.41 ± 0.00	0.41 ± 0.00	0.41 ± 0.00	0.24 ± 0.10	0.43 ± 0.02	0.14 ± 0.04	0.41 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
EGCG	614.14 ± 29.39	664.66 ± 16.04	1138.20 ± 6.25	448.00 ± 3.39	7.22 ± 0.71	10.22 ± 0.37	3.77 ± 0.97	2.26 ± 0.36	11.24 ± 0.19	2.62 ± 0.09	10.37 ± 0.21	4.02 ± 0.17
Isoquercitrin	21.24 ± 1.22	26.36 ± 3.24	57.45 ± 1.49	20.01 ± 0.15	24.80 ± 1.11	21.73 ± 0.21	13.52 ± 1.72	21.84 ± 0.73	46.56 ± 0.42	61.96 ± 1.62	58.17 ± 0.78	53.33 ± 0.94
Isorhamnetin	<DL	<DL	<DL	<DL	0.08 ± 0.01	0.02 ± 0.00	<DL	0.08 ± 0.01	0.29 ± 0.07	0.54 ± 0.04	0.38 ± 0.03	0.45 ± 0.01
Kaempferol	0.70 ± 0.07	0.69 ± 0.16	2.73 ± 0.13	0.52 ± 0.03	0.41 ± 0.04	0.29 ± 0.01	0.08 ± 0.03	0.44 ± 0.01	9.37 ± 1.24	5.18 ± 0.41	7.71 ± 0.32	9.85 ± 0.14
Luteolin	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.22 ± 0.03	0.03 ± 0.01	0.06 ± 0.01	0.12 ± 0.01
Naringin	<DL	0.41 ± 0.00	0.41 ± 0.00	<DL	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Pinobanksin	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.02 ± 0.01	0.09 ± 0.02	0.09 ± 0.00
Pinocembrin	<DL	<DL	<DL	<DL	6.90 ± 0.31	7.89 ± 0.21	2.33 ± 0.79	8.49 ± 0.17	0.13 ± 0.02	0.24 ± 0.02	0.17 ± 0.01	0.25 ± 0.01
Quercetin	8.92 ± 1.17	10.02 ± 2.05	37.67 ± 0.63	6.72 ± 0.39	6.10 ± 0.25	3.66 ± 0.05	1.48 ± 0.48	4.80 ± 0.26	39.86 ± 1.70	27.85 ± 0.96	30.29 ± 0.24	32.57 ± 0.32
Rutin	1.23 ± 0.19	1.40 ± 0.25	4.07 ± 0.00	1.01 ± 0.06	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.01 ± 0.00	0.13 ± 0.00
Taxifolin	<DL	<DL	<DL	<DL	0.75 ± 0.04	1.64 ± 0.27	0.30 ± 0.10	1.56 ± 0.13	0.92 ± 0.07	0.19 ± 0.01	0.68 ± 0.03	0.58 ± 0.03
Phenolic acid												
2.5 DHBA	20.64 ± 1.41	11.63 ± 0.21	42.19 ± 2.34	10.57 ± 1.75	32.94 ± 0.88	35.82 ± 2.66	12.26 ± 1.76	31.35 ± 1.54	3.43 ± 0.95	7.33 ± 0.41	6.38 ± 0.78	9.23 ± 0.83
Caffeic acid	1.47 ± 0.01	1.45 ± 0.01	1.46 ± 0.02	1.45 ± 0.02	0.16 ± 0.01	0.18 ± 0.02	0.15 ± 0.00	0.19 ± 0.03	14.96 ± 0.31	13.25 ± 0.20	13.52 ± 0.35	13.71 ± 0.49
Chlorogenic acid	0.91 ± 0.04	0.91 ± 0.03	1.00 ± 0.13	0.84 ± 0.04	0.67 ± 0.03	0.94 ± 0.02	0.26 ± 0.06	0.70 ± 0.04	18.64 ± 0.99	14.93 ± 0.44	14.80 ± 0.15	14.38 ± 0.23
Gallic acid	98.44 ± 5.24	22.99 ± 4.09	103.08 ± 3.47	55.43 ± 2.75	51.16 ± 1.70	26.72 ± 0.75	22.06 ± 4.82	60.72 ± 1.51	60.31 ± 2.09	52.11 ± 1.74	101.80 ± 1.97	60.00 ± 1.13
Sinapic acid	3.10 ± 0.00	3.10 ± 0.00	3.10 ± 0.00	3.10 ± 0.00	0.31 ± 0.00	0.31 ± 0.00	0.31 ± 0.00	0.31 ± 0.00	0.31 ± 0.00	0.32 ± 0.00	0.32 ± 0.00	0.32 ± 0.00
Benzoic acid	3.73 ± 0.66	2.87 ± 0.35	11.73 ± 2.48	1.70 ± 0.10	0.09 ± 0.00	0.11 ± 0.06	0.12 ± 0.02	0.09 ± 0.00	3.21 ± 0.34	3.37 ± 0.22	4.28 ± 0.45	4.23 ± 0.29

Ferulic acid	<DL	<DL	0.49 ± 0.11	<DL	0.29 ± 0.03	0.24 ± 0.01	0.04 ± 0.02	0.30 ± 0.02	8.51 ± 0.57	6.15 ± 0.12	8.03 ± 0.12	7.20 ± 0.23
p-aminobenzoic acid	<DL	<DL	<DL	0.97 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.11 ± 0.01	0.11 ± 0.00	0.11 ± 0.00
p-coumaric acid	<DL	<DL	<DL	<DL	0.35 ± 0.02	<DL	<DL	0.11 ± 0.01	5.06 ± 0.22	0.68 ± 0.02	1.27 ± 0.02	0.78 ± 0.04
Others												
3.4-DHB	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.12 ± 0.00	0.56 ± 0.04	0.68 ± 0.01	1.87 ± 0.04	3.01 ± 0.13
Salicylic acid	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.88 ± 0.14	1.33 ± 0.04	1.74 ± 0.05	1.61 ± 0.02
Coniferaldehyde	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.22 ± 0.02	0.08 ± 0.01	<DL
Coumarin	<DL	<DL	<DL	<DL	0.02 ± 0.01	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.02	0.55 ± 0.06	0.08 ± 0.01	0.02 ± 0.01	0.04 ± 0.00
Sinapaldehyde	0.98 ± 0.00	<DL	0.98 ± 0.00	0.98 ± 0.00	0.10 ± 0.00	0.11 ± 0.01	0.10 ± 0.00	0.10 ± 0.01	0.12 ± 0.02	0.17 ± 0.00	0.16 ± 0.00	0.12 ± 0.02
Syringaldehyde	0.06 ± 0.03	<DL	<DL	<DL	0.57 ± 0.04	0.17 ± 0.12	0.08 ± 0.02	0.22 ± 0.01	0.70 ± 0.01	0.44 ± 0.04	0.43 ± 0.02	0.38 ± 0.06
Total phenolic compounds	780.69	751.31	1416.66	554.20	137.62	117.46	58.72	145.55	352.72	296.84	385.73	337.57

Notes: The data are presented as the means ± SD (mg/kg DM). <DL indicates that concentration was lower than the detection limit.

The main compounds of the fresh leaves' extracts of uvaia, grumixama, and feijoa, account for 74, 79, and 66% of the concentration of total PC, respectively (Figure 2A). The fresh leaves extract of uvaia showed as the main component (-)-epigallocatechin gallate (ECGC), followed by gallic acid, isoquecitrin, and 2,5 DHBA. In the fresh leaves extract of grumixama, gallic acid > 2.5 DHBA > isoquecitrin stood out. The fresh leaves extract of feijoa had higher concentrations of epicatechin > catechin > gallic acid > isoquercetin. However, the drying temperature had a significant effect ($p \leq 0.05$) on the stability of these compounds (Figure 2B).

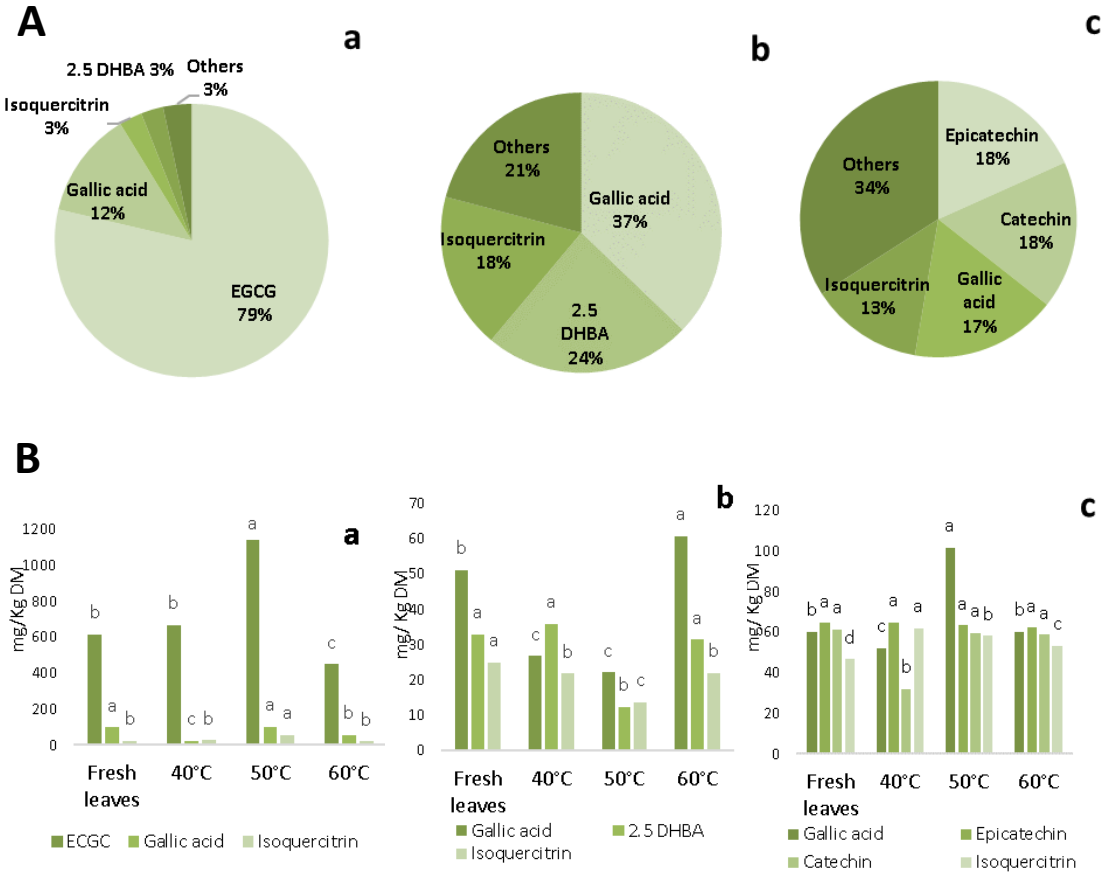

Figure 2: (A) Main compounds of the fresh leaves extract, and (B) effect of the temperature drying in the (a) uvaia, (b) grumixama, and (c) feijoa extracts. Lowercase letters correspond to comparing the same compound at different temperatures ($p \leq 0.05$).

This study reports the PC profile of uvaia leaves (*E. pyriformis*) for the first time. Seventeen PC were identified in the fresh leaves extract of uvaia, 15 compounds in dried leaves at 40 °C, and 17 compounds in dried leaves at 50 and 60 °C. Regarding total PC, the best results were obtained from extracts of dried uvaia leaves at 50 °C (TPC= 1416.66 mg/kg DM) (Table 2). The flavonoid EGCG was the main responsible for this result since in dried leaves at 50 °C, its concentration (1138.20 mg/kg DM) was 2.5 times higher than that found in dried leaves at 60 °C (448.00 mg/ kg DM). According to Wang et al. (2008) [21] EGCG degradation and epimerization reactions occur simultaneously and are potentiated with increasing temperature and time.

Twenty-five PC were identified in the fresh leaves' extracts of grumixama. Still when submitted to the drying process, twenty-four and twenty-three compounds were identified for temperatures 40 and 50 °C, respectively. However, in the grumixama leaves dried at 60 °C, twenty-six PC were found with the highest concentration (TPC= 152.30 mg/ Kg DM) compared to the other treatments. Siebert et al. (2017) [22] identified seven PC in the extracts of

grumixama leaves. Among these, catechin, isoquercetin, rutin, quercetin, and galangin were also identified in the present study.

Gallic acid was the main compound quantified in grumixama leaves and had higher retention in the samples dried at 60 °C (60.72 mg/kg DM). Gallic acid synthesis may be due to the degradation of ester-catechins, such as (-)-epicatechin gallate (ECG), EGCG and gallocatechin gallate [23]. Among these, we noticed a reduction in the EGCG concentration of grumixama leaves dried at 60 ºC, which may indicate biotransformation into gallic acid.

The feijoa leaves showed the highest number of compounds identified compared to the uvaia and grumixama leaves under the same conditions. In the feijoa extracts were identified 28, 30, 29, and 29 PC for the fresh and dried leaves at 40, 50 and 60 °C, respectively. Regarding concentration, the PC of feijoa leaves were better preserved in the drying at 50 °C for 250 min (Table 2) and the gallic acid was the major compound in this treatment. A recent study by Poodi et al. (2018) [24] also report gallic acid as the principal constituent of feijoa leaves corroborating the data found in this study.

The time-temperature binomial has an important role in the thermal stability of PC in plant materials. The temperature used in drying can break or rupture the bonds between the lignin and the PC, releasing them from the matrix and facilitating extraction. On the other hand, long drying times may promote the thermal degradation of PC [25].

Finally, time and temperature conditions of 50 °C for 120min, 60 °C for 330 min, and 50 °C for 250 min are suitable for convective drying of uvaia, grumixama, and feijoa leaves, respectively. Because under these conditions, there was a lower loss of individual PC and higher concentrations of total PC in these matrices.

3.3.2 Antioxidant activity (AA)

The AA of uvaia, grumixama and feijoa leaves are significantly different ($p \leq 0.05$) (capital letters). Grumixama had the highest values for AA by the FCRC and DPPH methods, while feijoa had the highest values in the FRAP method in all treatments (Figure 3). This behavior may be associated with the different mechanisms of action of PC or the presence of other phytochemicals with antioxidant capacities, such as alkaloids, anthraquinones, phytosterols, saponins, tannins, triterpenoids, and steroids [3, 26]. Grumixama extracts act strongly on the sequestration and reduction of free radicals, while feijoa extracts have greater potential to chelate metal ions, forming stable complexes.

The drying temperature significantly influenced ($p \leq 0.05$) the AA of extracts of uvaia, grumixama, and feijoa leaves (lowercase letters). In all the methods evaluated, the uvaia leaves extracts had higher AA values when dried at 50 °C. These results agree with the PC profile of the uvaia extracts.

The extracts of grumixama leaves dried at 60 ºC presented higher values of AA determined by the FCRC method (642.7 ± 7.0 mg GAE/g DM). On the other hand, the DPPH results of leaves dried at 60 °C indicated a reduction of 7% when compared to fresh leaves, no difference was observed in AA grumixama leaves dried by FRAP. The high concentration of gallic acid in grumixama leaves dried at 60°C may have corroborated these results.

The data of the AA feijoa by FCRC, DPPH, and FRAP methods had the same behavior, where the extract of fresh leaves showed the highest values, followed by leaves dried at 50 °C. The thermal degradation process depends on the time-temperature binomial. The time to reach the equilibrium moisture at 40 °C was much longer (590min) than other temperatures, and drying at 50 °C reduced this time by 54%. Likewise, drying at 60°C had the shortest residence time (240 min), but the temperature was sufficient to cause thermal degradation of PC and AA. Poodi et al. (2018) [24] found that the feijoa leaves extract has an inhibition percentage for DPPH radicals of 88.12%. The leaves dried at 50 °C presented a reduced 86.5% of the DPPH radical, indicating an important inhibition percentage similar that to reported by these authors.

However, drying the leaves of uvaia, grumixama, and feijoa at 50, 60, and 50 °C, respectively, made it possible to maintain the AA in all the methods evaluated. Furthermore, these results complement those found in the PC profile for all species, confirming the positive effect of these temperatures on conserving the leaves and their secondary metabolites.

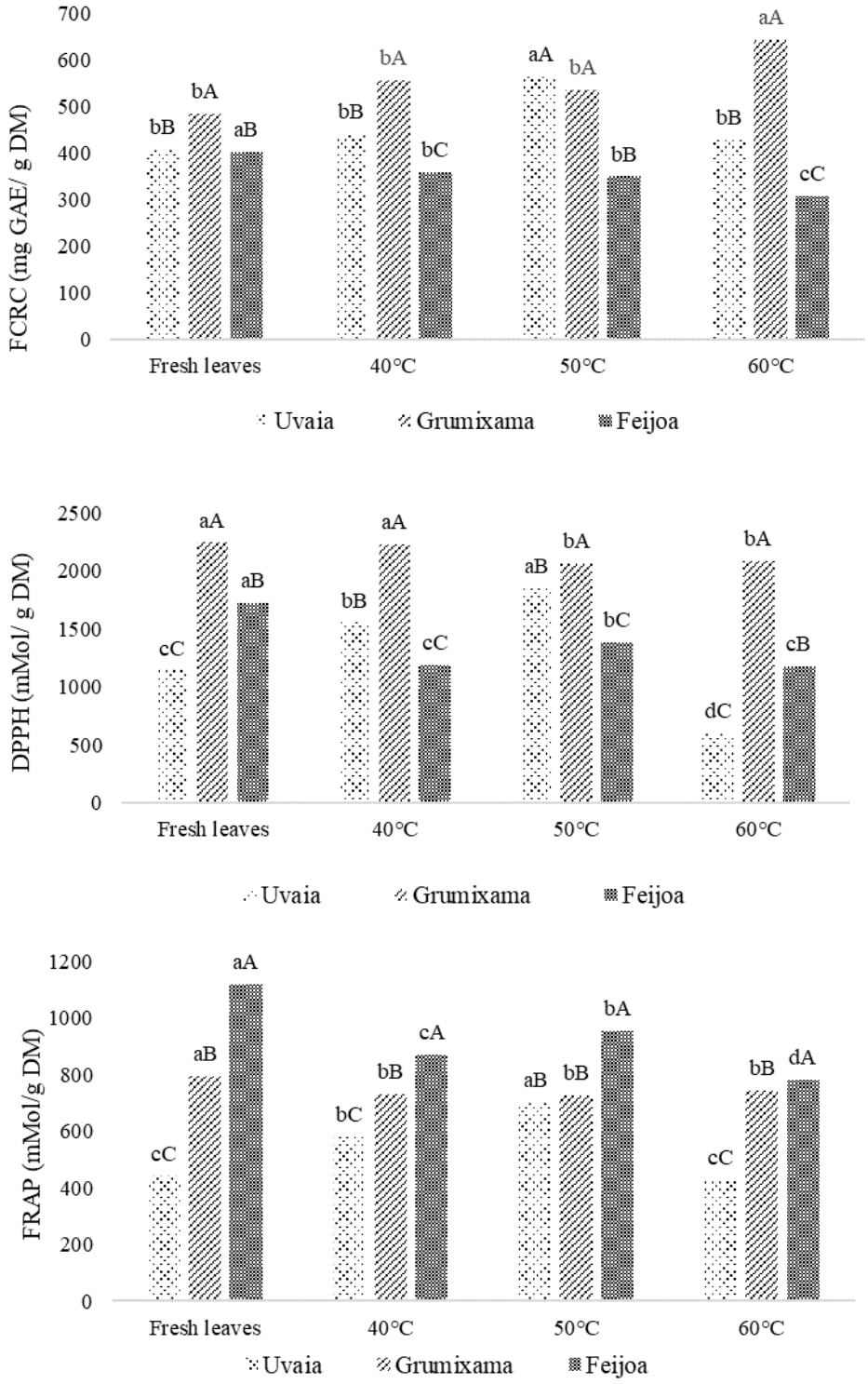

Figure 3: Effect of temperature on the antioxidant activity of uvaia, grumixama, and feijoa leaves by FCRC, DPPH, and FRAP methods. Lowercase letters correspond to the comparison of the same specie at different temperatures ($p \leq 0.05$). Capital letters correspond to the comparison of the different species at the same temperature ($p \leq 0.05$).

4. CONCLUSION

Increasing the drying air temperature reduced the drying time of all leaves, with a consequent increase in the drying rate in the constant rate period. The constant rate period was predominant in the drying curves. The Def increased with increasing drying temperature in all leaves and ranged from 0.59 to $6.56.10^{-12}$ m²/s. The uvaia leaves showed the lowest values for Def at all temperatures.

Thirty-eight standard PC were tested, and 30 were identified in extracts evaluated. In the uvaia extracts, the major compounds were EGCG, gallic acid, and isoquercitin. The gallic acid, 2.5DHBA and isoqueticitrin are the main PC in grumixama extracts. Feijoa extracts showed a more diverse composition, in which the compounds epicatechin, catechin, gallic acid, and isoquercitrin stood out. Grumixama had the highest values for AA by the FCRC and DPPH methods while feijoa had the highest values in the FRAP method, in all treatments. These results indicate that drying the uvaia and feijoa leaves at 50 °C, and 60 °C for grumixama leaves promoted a greater extraction of compounds with AA. These extracts produced from by-products sustainable have the potential for use in different applications in the food, chemical, and pharmaceutical industries.

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