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CONCENTRAÇÃO DE ANTIOXIDANTES NOS SUCOS DOS FRUTOS DE BANANINHA-DO-MATO E ABACAXI

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Trabalho de Conclusão de curso apresentado como requisito parcial para obtenção do título de Bacharel em Ciências Biológicas na Universidade Federal do Rio Grande do Sul.

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“We live in a society exquisitely dependent on science and technology, in which hardly anyone knows anything about science and technology”

Carl Sagan

APRESENTAÇÃO

Trabalho formatado conforme o periódico de referência *Food Chemistry*.

HIGHER CONCENTRATIONS OF ANTIOXIDANTS IN THE JUICE OF THE UNCONVENTIONAL FOOD PLANT *BANANINHA-DO-MATO* COMPARED WITH PINEAPPLE

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ABSTRACT

During February and June of 2017, in a cultivated area 10 fruits of *bananinha-do-mato* were collected from 12 different plants and 12 pineapples were purchased from a street seller in October of 2017, aiming to compare the antioxidant levels in the juice of these two bromeliads, the unconventional food plant *bananinha-do-mato* and the famous worldwide consumed pineapple. Compared with the pineapple juice, *bananinha-do-mato* presented significantly higher non-soluble content, higher levels of the antioxidants Vitamin C and phenols, and higher total antioxidant capacity, proteins and nitrites and nitrates. Vitamin E levels were similar in *bananinha-do-mato* and pineapple juices. In conclusion, the fruit juice of *bananinha-do-mato* has higher antioxidant capacity than the most cultivated and consumed Bromeliaceae in the world, the pineapple.

HIGHLIGHTS

The unconventional food plant *Bananinha-do-mato* is a potential antioxidant source

Antioxidant levels of the bromeliads *Bananinha-do-mato* and pineapple were compared

Bananinha-do-mato has higher levels antioxidants, such as Vitamin C and phenols

The *Bananinha-do-mato* juice is more antioxidant than the pineapple juice

ABREVIATIONS / ACRONYMS

GVC: from Portuguese, *grupos de viveiros comunitários*; UFP: Unconventional Food Plants; TEAC: TROLOX equivalent antioxidant capacity; TE: TROLOX equivalent; GAE: Gallic acid equivalent; BSA: Bovine serum albumin; FDA: U.S. Food and Drug Administration; RS: reactive specie**.**

1. INTRODUCTION

Bromeliaceae is a plant family that is native from tropical and subtropical regions of Americas; except for the species *Pitcairnia feliciana* (Pitcairnioideae), from northern Africa (Givnish et al., 2011; Smith & Downs, 1974). The most cultivated specie of this family is the worldwide consumed *Ananas comosus* Merril, the pineapple (Gonçalves, 2000). The species is originated from South America, where it was domesticated and taken to other tropical regions of the planet, at least 500 years ago (Ctenas and Quast, 2000; Medina, 1978).

South America is very rich in Bromeliaceae species. One of this species is *Bromelia antiacantha* Bertoloni, commonly known as *gravatá, caraguatá* or *bananinha-do-mato.* In a free translation, *bananinha-do-mato* means "little wild banana", due to its fruits appearance, which are yellow/orange berries similar to the common banana (Reitz, 1983).

The *bananinha-do-mato* occurs in the Ombrophylous Dense Forest, in the restinga and in the Ombrophylous Mixed Forest and river banks along the Brazilian south and southeast, Uruguay and Argentina. The flowering and fructification periods of this species occur annually; the flowering starts from October to the beginning of February and the fructification starts from December to June (Fillipon, 2009; Reis, 2010; Reitz, 1983).

Fruits from *bananinha-do-mato* are used in several common products as juices, jellies, liquors and others. B*ananinha-do-mato* is also consumed as cough syrup; in popular culture consider it an expectorant and protective against respiratory infections, being recommended for the treatment of asthma and bronchitis (Filippon, 2009 and 2012; Jorge, 1993; Kinupp, 2007; Mors, 2005; Zanella, 2009). The *bananinha-do-mato* plant is not well-known by the general public, neither is commonly consumed or commercialized, being considered an unconventional food plant (UFP) (Kinupp, 2007).

UFPs have been targets of several research groups due to the increased concern for healthier foods and beverages, as well as drug discovery. Another concern is the consumption of food from a known and reliable origin, therefore, the promotion of organic and agroecological farms that include native plants may contribute to sustainable consumption and environmental conservation (Brack, 2013).

Previous studies target on potential nutritional advantage of UFPs, for example, evaluating its antioxidant and metal composition (Kinupp, 2008; Krumreich et al., 2015; Santos, 2006). Antioxidants are any substance that prevents the substrate oxidation, being the substrate any molecule found *in vivo*. Antioxidants either may be synthesized by the organisms, or obtained from the diet (Halliwell and Gutteridge, 2015).

Antioxidants obtained from diet improve the redox homeostasis of complex organism that depends on oxygen to live. In that way, the consumption of food rich in nutrients and antioxidants has important health benefits (Brewer, 2011). Compounds with antioxidant activity include phenolic compounds, vitamin C, vitamin E, vitamin A and carotenoids (as β-carotene and licopene) (Papas, 1999).

Considering the *bananinha-do-mato* a potential antioxidant UFP and due to its importance in agroecological systems, we proposed the comparison of antioxidants levels in the juices of *bananinha-do-mato* and pineapple fruits.

2. MATERIAL AND METHODS

2.1. Harvest and storage of fruits

During the *bananinha-do-mato* fructification period (February to June in 2017), from 12 different plants, were randomly collected 10 fruit from each plant. The plants are located in a cultivated area managed by the university plant communitarian nursery group (*Grupo de Viveiros Comunitários*-GVC, from the Institute of Biosciences, in the Valley Campus, UFRGS, Porto Alegre, RS/ Brazil). The coordinates for the four collecting points (M1, M2 M3 and M4) are: M1 = 30°04'05.5"S 51°07'12.4"W, M2 = 30°04'04.6"S 51°07'11.9"W, M3 = 30°04'04.4"S 51°07'11.3"W and M4 = 30°04'04.8"S 51°07'10.4"W.

The fruits were taken to the Oxidative Stress Laboratory, Department of Biophysics in Institute of Biosciences at UFRGS and were stored at environmental temperature by 20 days postharvest (a usual procedure due its climacteric characteristic), reaching the orange mature fruit color. The fruits were washed with Sodium Hypochlorite (200ppm) by 10 minutes, and were rinsed three times with distillated water. Twelve pineapples Pearl cultivar were purchased from a street seller, in October 2017, and promptly, the juices of both *bananinha-do-mato* and pineapple were simultaneously prepared.

2.2. Juice preparation and sample processing

We utilized a usual blender and a 1 mm pore size sieve in the juice preparation and separation of juice soluble and non-soluble content. Each juice was made using a ratio of 100 g of *bananinha-do-mato* fruits (approximately 4 fruits, all from the same plant) and distilled water was added to obtain 150 mL of final juice volume, holding the ratio 2:3. The fruits were cut in small slices (with bark and seeds) and crushed by a usual blender. *Bananinha-do-mato* fruits were crushed in a standard blender and filtered three times (each crushing time followed by filtering), using the juice from the previous crushing for the next one. Each pineapple is formed by coalesced berries (fruits grow together), thus, we simulated a juice preparation similar to *bananinha-do-mato* by taking a central transversal slice of 100 g form each one of the 12 pineapples. The pineapple juice was produced using the same steps and procedure, also holding the same ratio. Finally, we obtained 12 *bananinha-do-mato* and 12 pineapple juices. The non-soluble content separated by the sieve was weighted and compared with the initial fruits mass used in the juice preparation. The juices were aliquoted and stored in freezer -80ºC.

2.3. Analysis

2.3.1. Vitamin C

Vitamin C (ascorbic acid) levels were measured by HPLC (Karatepe, 2004), employing a reversal-phase Supercosil™ LC-18-DB HPLC column (15 cm × 4 mm, 5 µm particle size), using 30 mmol/L monobasic potassium phosphate, pH 3.6, and methanol in a ratio of 90:10 (v/v) as mobile phase, with flow rate of 1 mL/min, and a sample size of 20 µL. The absorbance of the column effluent was monitored at 250 nm. Under these conditions, the retention time of ascorbic acid was 2.1 min and its concentration calculated from an ascorbic acid standard curve (15-400 µmol. L^{-1}). Results are shown as mg per 100 g of fresh fruits.

2.3.2. Vitamin E

Vitamin E (α-tocopherol) levels was measured by HPLC, employing a C18 (15 cm x 4.6mm) column Nucleosil™ LC-18 HPLC column (15 cm × 4.6 mm, 5 µm particle size) with continuous flow of 96.5:3.5 (v/v) methanol: water at 2 mL per minute (Barbas *et al.*, 1997). Detection of α-tocopherol was performed by fluorescence, excitation at wavelength of 295 nm and an emission at wavelength of 350 nm. The retention time α-tocopherol was 7.5 min and its levels were calculated from a α-tocopherol standard curve. Results are shown as ng per 100 g of fresh fruits.

2.3.3. Total phenolic content

The analysis of the total phenol content was initiated extracting and discarding the lipophilic content and concentrating the hydrophilic, utilizing as solvent n-Hexane, after two subsequent mixing and centrifugation. After, we extracted the phenol content from hydrophilic content using acetone and ethanol (1:1) as solvent, by two hours the samples were mechanically shaken. (Rockenbach *et al*, 2008). Levels of phenol were measured by spectrophotometry (764nm), following the Folin-Ciocalteau's method (Moyer *et al*, 2002; Singleton and Rossi, 1965), using a Gallic acid standard curve (Bora, 2005). Results are shown as mg of Gallic acid equivalents (GAE) per 100 g of fresh fruits.

2.3.4. TROLOX Equivalent Antioxidant Capacity (TEAC)

The antioxidant capacity was performed as Erel, 2004. The discoloration of the samples was observed and quantified. Antioxidants present in the samples are reducing the $ABTS^{\bullet +}$, which is a radical formed from 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) and may be observed the decrease in the absorbance at 660 nm. Using the water-soluble synthetic antioxidant TROLOX (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as a standard antioxidant, the values was calculated using a standard curve of TROLOX. Results are shown as mg of TROLOX equivalent (TE) per 100 g of fresh fruits.

2.3.5. Proteins

Total protein content was quantified using BSA (bovine serum albumin) as a standard (Bradford, 1976). Results are shown as mg of proteins per 100 g of fresh fruits.

2.3.6. Nitrites and Nitrates

The Griess test was used to measure nitrites and nitrates levels (Grisham, 1996). Results are shown as ng of sodium nitrite per 100 g of fresh fruits.

2.4. Statistical analysis

Results were compared using PASW Statistics, Version 18.0 (SPSS Inc.). After the normality test of the samples, the T test was used for parametric samples and the Mann-Whitney U test for nonparametric samples. Differences were considered significant when $p \leq 0.01$. Parametric results are shown as mean ± standard error, and nonparametric results are shown as median (Q25|Q75). All results were normalized by grams of fresh fruits and will be shown per 100 g (150 mL of juice) of fresh fruits.

3. RESULTS AND DISCUSSION

3.1. Antioxidants levels

Antioxidants obtained from the diet are essential for a balanced redox homeostasis, neutralizing reactive species (RS) that may damage biomolecules. Fruit and vegetable juices and other beverages are common sources of antioxidants and bioactive compounds as vitamin C, vitamin E, carotenoids, flavonoids and other phenols (Halliwell and Gutteridge, 2015).

Therefore, a diet rich in fruits and vegetables has important health benefits. Accordingly to Boeing et al. (2012) and Kris-Etherton et al. (2002) there are evidences that the increased intake of vegetables and fruits, as well as bioactive compounds, may decrease the risk of chronic diseases, as stroke, osteoporosis, hypertension and coronary heart disease.

Nutritional characteristics of *bananinha-do-mato* fruits have been previously investigated by Kinupp (2008), Santos (2009) and Krumreich et al., (2015). These studies evaluated the levels of vitamin C, total phenolic content, total antioxidant capacity and total protein content, among others, however performing different fruit processing methods: dehydration, lyophilization and sonication, respectively. For this reason, despite we compare our results with these studies, it is necessary to consider the influence of the different processing methods. The present work proposes a household blender for juice preparation; therefore, there was no laboratory sample processing, as the use of sonicator, Potter or lyophilization. The obtained results are shown in the following table.

Table 1: Antioxidant levels in *bananinha-do-mato* and pineapple juices. Results are shown as unit per 100 g of fresh fruits, or 150 mL of juice. Vitamin C and phenolic content are shown as mean ± standard error. Vitamin E and TEAC are shown as median (Q25|Q75).

Analysis	*Bananinha*	Pineapple	U	Standart
Vitamin C	9.64 ± 0.45*	1.44 ±0.09	mg	Ascorbic acid
Vitamin E	103.45 (23.16|227.39)	35.57 (27.2|68.9)	ng	α-tocopherol
Phenolic content	9.73 ± 0.71*	5.18 ± 0.31	mg GAE [b]	Gallic acid
TEAC	242.29 (188.48|260.59) *	63.41 (54.72|85.08)	mg TE [c]	TROLOX

*Differences were considered significant when $p \leq 0.01$.

[a] Unit of measurement

[b] Gallic acid equivalent

[c] TROLOX equivalent

The present study aimed to evaluate the levels of antioxidants in juices of *bananinha-do-mato,* to promote the use and knowledge about this UFP. For this reason, we compared *bananinha-do-mato* with another Bromeliaceae, the worldwide consumed pineapple. Evaluating the juices antioxidants levels, we observed that *bananinha-do-mato* has higher levels of vitamin C, Phenolic content and TEAC when compared with the pineapple juice.

Vitamin C is an essential antioxidant for human diet, as well as for all primates, which do not synthesise this molecule (Burns, 1959). Besides its antioxidant function, vitamin C has a fundamental role in the hydroxylation reactions during collagen synthesis. Moreover, vitamin C facilitates intestine iron absorption (Levine, 1986).

Previously, Krumreich et al. (2015) utilized the sonication processing to obtain the *bananinha-do-mato* juice and shown 60 mg of vitamin C per 100 g of ripe fruits. This result represents 100% of the recommended daily dosage of Vitamin C for adult human, according to the FDA. Vitamin C rich sources are: oranges, lemons, guava, berries, broccoli and peppers (Halliwell and Gutteridge, 2015). Our *bananinha-do-mato* juice has lower levels of Vitamin C, representing 16% of the daily recommended Vitamin C intake for human adults (Food and Drug Administration-FDA). However, our juice may be easily prepared at home; also *bananinha-do-mato* juice has 6 times more vitamin C than pineapple juice.

There is a wide range of monophenols and polyphenols obtained from plant diet with high antioxidant activity, directly neutralizing RS (Rice-Evans, 1996). Accordingly to Duthie (2000) and Tapiero (2002), high phenolic intake has been related to decreased risk of cardiovascular diseases and some types of cancer.

The major sources of phenolic compounds in human diet are vegetables, fruits and beverages. Among beverages, wine and grape juice have higher phenolic compound levels. Concerning the richest beverages in phenolic content, fresh grape juice presents 172 mg of gallic acid equivalents per 100 mL, and Italian wine presents 330 to 420 mg of gallic acid equivalents per 100 mL, as reported by Balasundram, Sundram and Samman (2006). In *bananinha-do-mato*, Santos (2009) and Krumreich, *et al* (2015) reported 50 mg and 70 mg of phenolic compounds per 100 g of fresh fruits, respectively, using lyophilization and sonication as processing methods to obtain the juice. Despite we obtained lower levels than reported, the phenolic content of *bananinha-do-mato juice* was 2 times higher compared with pineapple.

The TEAC assay is used to measure a broad range of antioxidants present in foods. Therefore, we evaluated a general antioxidant profile, using the TROLOX antioxidant as standard, enabling the estimation of total hydrophilic antioxidant activity. Krumreich et al. (2015) reported 178.56 mg of TROLOX equivalent per 100 g of *bananinha-do-mato* fresh fruits, and suggest that the alcoholic extraction used could have influenced their results. In our study we used an aqueous extract to prevent this possible interference. The TEAC was approximately 4 times higher in *bananinha-do-mato* than in pineapple being higher than previously reported (**Table 1**) showing that *bananinha-do-mato* juice is more antioxidant than the pineapple juice.

Vitamin E is a common term used to refer the tocopherol isomers, being the most known the α-tocopherol. These compounds have important role in redox profile maintenance, due the capacity of decrease the lipid peroxidation in cells membrane. Food sources of Vitamin E are nuts, dark green leafy vegetables and oils from vegetable origin (Galli et al., 2017; Sundl et al., 2007).

Due to the lipophilic characteristic of Vitamin E, it is possible that our preparation method using a polar solvent was not appropriated to extract the Vitamin E in the juice. In this way, we obtained low vitamin E levels in both *bananinha-do-mato* and pineapple juices. According FDA, the levels obtained represent approximately 1% (100 ng per 100 g of ripe fruits) and 0.3% (30 ng per 100 g of ripe fruits) of the recommended daily dosage of Vitamin E, to *bananinha-do-mato* and pineapple juices, respectively, however without significative difference.

3.2. Protein content and nitrites and nitrates levels

Table 2: Protein content and nitrites and nitrates levels in *bananinha-do-mato* and pineapple juice. Results are shown as unit per 100 g of fresh fruits, or 150 mL of juice.

Analysis	*Bananinha*	Pineapple	U[a]	Standard
Total protein	75.07 ± 4.18*	36.81 ± 3.93	mg	BSA[b]
Nitrites and nitrates	125.75 (112.99|149.32)*	17.26 (8.64|41.49)	ng	Sodium nitrite

*Significantly higher, p≤0.01.

[a] Unit of measurement

[b] Bovine serum albumin

Proteins are macronutrients obtained from diet and approximately 50 g per day are required for adults (FDA). Vegetables that provide higher protein content are grains as soybeans and chickpea. Richest sources of proteins, with higher bioavailability on diet are animal meat (Millward, 2008). In this way, juices are not rich sources of proteins. As expected, *bananinha-do-mato* and pineapple juices are protein poor. Although the low protein levels, *bananinha-do-mato* juice has 2 times more protein content than pineapple.

Nitrites (NO_2^-) and nitrates (NO_3^-) are controversial inorganic molecules due the well-known effects caused by high levels of NO_2^- intake from cured meat, where it is added to prevent microorganism contamination. NO_3^- sources on diet are usually vegetables. NO_2^- and NO_3^- are precursors of nitric oxide (NO) and, despite NO is considered a less reactive free radical in the 'reactivity scale', it may cause oxidative damage after reacting to superoxide (Halliwell and Gutteridge, 2015).

On the other hand, there are evidences reporting that the NO derived from diet nitrites and nitrates has beneficial vasodilation effect, decreasing blood pressure, arterial ageing, increasing the exercise performance and pulmonary circulation (Lidder and Webb, 2012). The acceptable daily intake for nitrates is 3.7 g per kilograms do body weight. Data on nitrites and nitrates levels in *Bananinha-do-mato* and pineapple juice were never previously reported, and we found low levels of nitrites and nitrates on the evaluated juices. Vegetables containing high nitrate levels are rocket, spinach and beetroot (150 to 260 mg per 100 g, respectively).

3.3. Soluble and non-soluble content

Fruits from Bromeliaceae plants, as the pineapple, are often characterized as having a citric taste, which is not different to *bananinha* fruits. Fruits mass, soluble content and non-soluble content were also evaluated in *bananinha-do-mato* and pineapple fruits.

Table 3: Values of soluble and non-soluble content in *bananinha-do-mato* and pineapple juices, shown in g per 100 g of fruits.

	Soluble content (g)	Non-soluble content (g)
Bananinha	50.2 ± 12.2	49.8 ± 12.2 *
Pineapple	79.6 ± 5.7*	20.4 ± 5.7

Results are shown as mean ± standard deviation.

*Higher content according our preparation method and sieve filtering (1 mm)

Bananinha-do-mato fruits are more fibrous then pineapple fruits. *Bananinha* and pineapple fruits have approximately 50% and 20% of non-soluble content, respectively; therefore, *bananinha-do-mato* fruits have significantly higher non-soluble content compared with the pineapple fruits. Consequently, pineapple fruits have approximately 1.5 times more soluble content than *bananinha-do-mato* fruits (**Table 3**).

Fioravante et al. (2016) studied a similar plant from Brazilian Cerrado biome, the *Bromelia balansae* Mez, however considering the consumption of the non-soluble content as flour, which preserves antioxidants and minerals of the fruit. Therefore, the flour preparation from the non-soluble content of the *bananinha-do-mato* might permit its use in other products preparations, also enabling the use of the whole fruit.

4. CONCLUSION

The UFP *bananinha-do-mato* has an important value to the south Brazilian region, where it may be cultivated as a native crop, and may be used in ecosystem restoration and sustainable consumption projects. *Bananinha-do-mato* may result in many products from ripe fruits, including an antioxidant- rich juice. Others studies may be useful to reinforce the potential use of *bananinha-do-mato* as an alternative antioxidant source, promoting its cultivation in agricultural systems. After comparing the *bananinha-do-mato* with the pineapple juice, we concluded that we are studying a great value plant. Our *bananinha-do-mato* juice has higher antioxidant capacity than the most known Bromeliaceae plant in Brazil, the pineapple, and using Pearl cultivate, the most produced.

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6. CONFLICT INTERESTING

The authors have declared no conflict of interest.

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8. FOUNDING

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