Influence of processing methods on the content of polyphenols and anthocyanins and on the antioxidant activity of *Rubus brasiliensis* Mart. fruits

**Abstract**

Little is known about the phytochemical composition and the influence of different processing methods on the concentration of bioactive compounds and on the antioxidant activity of the fruits of *Rubus brasiliensis* Mart., a native plant of Brazil. This work aimed to evaluate the influence of different processing methods on the quantification of phenolic and anthocyanin compounds and on the antioxidant activity of *R. brasiliensis* fruits. The plants were processed by different ways - the extracts of fruits were obtained by Spray Dryer (SD) or Lyophilization (LYO), and the fruits were dried directly in an oven (OD) and were also evaluated after freshly thawed (FT). The processing methods were independent. After processing, the polyphenol and anthocyanin contents and antioxidant activity were evaluated. The Total Phenolic Content (TPC) was assessed using the Folin-Ciocalteu technique. The pH differential method was used for quantification of anthocyanin and the antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. *R. brasiliensis* fruits have a high content of polyphenols and anthocyanins, and an expressive antioxidant activity that can bring benefits to the population. The FT fruits showed the lowest content of total polyphenols. However, the OD fruits showed the most interesting results, since the total polyphenols and anthocyanins contents and the antioxidant activity were similar to the other processing methods performed in this work and were more economically viable. Obtain a bioactive content and adequate antioxidant activity after simple processing such as drying is very interesting when using these fruits for longer, or even, obtaining pharmaceutical formulations. It could
be determined, for the first time, the content of polyphenols and anthocyanins and the antioxidant activity of the fruits of *R. brasiliensis*, as well as the best way to process these fruits by drying them in an oven.

**Keywords:** Amora-do-mato; Blackberry; Antioxidant; Lyophilization; Nutraceuticals; Spray dryer.

**Resumo**

Pouco se sabe sobre a composição fitoquímica e a influência de diferentes métodos de processamento na concentração de compostos bioativos e na atividade antioxidante dos frutos de *Rubus brasiliensis*, uma planta nativa do Brasil. Este trabalho teve como objetivo avaliar a influência de diferentes métodos de processamento na quantificação dos compostos fenólicos e das antocianinas, e na atividade antioxidante dos frutos de *R. brasiliensis*. Frutos de *R. brasiliensis* foram processados de diferentes maneiras – os extratos foram obtidos por spray dryer (SD) ou liofilização (LYO). Os frutos foram secos diretamente em estufa (OD) e avaliados logo após serem descongelados (FT). Os processamentos foram independentes. Após os processamentos, foram realizadas análises dos teores de polifenóis e antocianinas totais, e avaliada a atividade antioxidante. O conteúdo de polifenóis totais foi determinado usando o método de Folin-Ciocalteau. O método de pH diferencial foi utilizado para quantificação de antocianinas e a atividade antioxidante foi determinada pelo método 2,2-difenil-1-picrilhidrazil (DPPH). Frutos de *R. brasiliensis* apresentam alto teor de polifenóis e antocianinas, além de expressiva atividade antioxidante, o que pode trazer benefícios à população. Os frutos FT apresentaram os menores teores de polifenóis totais. Os frutos OD apresentaram os resultados mais interessantes, pois os teores de polifenóis e antocianinas totais, e a atividade antioxidante foram semelhantes aos obtidos nos frutos de outros processamentos realizados neste trabalho, com a vantagem / o diferencial de mostrarem-se mais viáveis economicamente. Obter um conteúdo de bioativos e uma atividade antioxidante adequada após um processamento simples, como a secagem em estufa, é muito interessante quando planejamos o uso desses frutos por mais tempo ou mesmo para obtenção de formulações farmacêuticas. Aqui determinamos, pela primeira vez, o teor de polifenóis e antocianinas totais e a atividade antioxidante dos frutos de *R. brasiliensis*, e demonstramos que a melhor forma de processar esses frutos é por secagem em estufa.

**Palavras-chave:** Amora-do-mato; Amora-preta; Antioxidante; Liofilização; Nutracêuticos; Secador por aspersão.

**Highlights**

- Phenolic compounds and antioxidant activity were determined in *Rubus brasiliensis* Mart. fruits.
- Fruits dried in oven present the same properties than using spray dryer or lyophilization.
- Drying in oven is the best way to processing the fruits because it's cheaper and faster.

**1 Introduction**

The genus *Rubus* is widely diversified and widespread in the world. There are approximately 400 to 500 species of raspberry and mulberry trees in America, Europe, Africa and Asia (Poling, 1997). In Brazil, seven native species of the genus *Rubus* were found, among them the *Rubus brasiliensis* Mart. is the one with the greatest geographical distribution, occurring in the Northeast, Midwest and Southeast regions (Barcelos & Heiden, 2015). In fact, *R. brasiliensis* Mart. belongs to the Rosaceae family and is popularly known as *amora-do-mato* or *amora silvestre*. Indeed, it is a native species of Brazil, occurring from Pernambuco (PE) to Rio Grande do Sul (RS) (Alice et al., 1995).

Functional foods provide physiological benefits and reduce the risk of diseases, besides nurturing (Domínguez Díaz et al., 2019). Nutraceuticals are isolated nutrients and dietary supplements with a...
concentrated content of bioactive compounds processed as a pharmaceutical’s products, thus providing health benefits, as well as preventing and treating diseases (Durazzo et al., 2020).

Processing a product from a food or plant can provide benefits and improve the biological properties of the compounds present. Among the many processes, the spray dryer process consists of spraying the liquid product inside a chamber subjected to a controlled stream of hot air, evaporating water ultra-rapidly, with the minimum degradation of the product, then obtaining a powder (Souza et al., 2013). Lyophilization removes the moisture contained in the material through freezing, sublimation and desorption. Thus, it avoids protein denaturation, the degradation of thermo sensitive vitamins, the proliferation of microorganisms, loss of volatile compounds (flavor and aroma) and preserves the chemical and/or physical properties (Pisano et al., 2019).

Genotypes of Rubus sp. (amora preta) are already known for their antioxidant activity (Vizzotto et al., 2012). There are few reports on R. brasiliensis regarding this activity. In this sense, this work aimed to evaluate the influence of the processing methods on the polyphenol and total anthocyanin contents and to evaluate the antioxidant activity of R. brasiliensis fruits.

2 Materials and methods

2.1 Preparation of the samples

The fruits of R. brasiliensis were collected in the state of Rio Grande do Sul (RS) (Arvorezinha, RS, Brazil, 28° 52' 46.2" S; 52° 12' 28.3" W). The voucher specimen was identified and deposited in the Museum of the Institute of Biological Sciences of the University of Passo Fundo (Universidade de Passo Fundo) and notified under the register number RSPF 14,243.

The fruits were washed, and subdivided into 4 parts, which were submitted to different processing. One part of the fruit was dried at 35 °C for 3 days in an Oven Dried (OD) (Marconi/ Piracicaba/ SP/ Brazil) and powered in a Willey mill (this sample was called OD). The second part was grinded with distilled water in blender in the proportion 1:10 (fruits: water) for 15 minutes. After, the material was filtered, frozen and lyophilized (LYO) (this sample was called LYO). The third part was grinded with distilled water in blender in the proportion 1:10 (fruits: water) for 15 minutes. After the material was filtered, the resulting extract was subjected to Spray Dryer (SD) (LM Labmaq/MSD 1.0) according to the conditions as following: 0.8 mm nozzle, 0.6 L/h flow, at 110 °C per approximately 30 minutes (this sample was called SD). The last part of the fruit was directly frozen for 30 days at -20°C. The fruits were evaluated immediately after thawing (FT) (this sample was called freshly thawed - FT).

The samples were submitted to total polyphenol and anthocyanin quantifications and antioxidant activity.

2.2 Determination of total polyphenols

Approximately 20 mg of the all samples processed were weighed into a 5 mL volumetric flask, diluted with distilled water, in a final concentration of about 4 mg/mL. An aliquot of 0.5 mL was taken from this solution and mixed with 2.5 mL of Folin reagent (1:10 in water). After 5 minutes, 3 mL of 14% aqueous sodium carbonate solution (p/v) was added. Samples were placed in the dark and at room temperature for 2 hours. The reading was taken at 750 nm (Lambda 20/Perkin Elmer spectrophotometer). A solution containing Folin reagent (1:10 in water) and 14% of sodium carbonate was used as blank (Sousa et al., 2007). Determinations were performed in triplicate. The standard curve was obtained with solutions of Gallic Acid in the range of 5 to 50 μg/mL (results of samples were expressed in mg equivalent to Gallic Acid Equivalent (GAE)/ 100g of dry matter or dry extract).
2.3 Determination of total anthocyanins (TA)

The pH differential method was used for quantification of TA. The absorbance was measured in a spectrophotometer at 540 and 700 nm in buffers at pH 1.0 and 4.5. The amount of TA was calculated using the Equation 1:

\[
TA = \frac{\left( A_{\text{max, pH 700}} - A_{\text{pH 1.0}} \right) - \left( A_{\text{max, pH 4.5}} - A_{\text{pH 1.0}} \right)}{\lambda_{\text{max}} MW DF \epsilon \times 10^3}
\]

Where MW is related to the Molecular Weight of 449.2 g/mol for cyanidin-3-glucoside; DF is associated with the Dilution Factor, and \( \epsilon \) is the molar extinction coefficient of cyanidin-3-glucoside (26,900); and \( 10^3 \) is the factor for conversion from g to mg. The results were expressed in mg equivalent to cyanidin/100g of dry matter or dry extract (Giusti & Wrolstad, 2001; Falcão et al., 2007; Lee et al., 2005).

2.4 Antioxidant activity

The scavenging activity of DPPH (1,1-diphenyl-2-picrylhydrazyl) was determined using the method of Brand-Williams et al. (1995) modified by Kim et al. (2002). Indeed, 0.1 mM DPPH was prepared in 80% of methanol. The samples were prepared at the concentration of 1000 μg/mL in 80% of methanol, and an aliquot of 0.1 mL was added in 2.9 mL of DPPH, homogenized and kept at room temperature in the dark for 30 min. The decrease in absorbance of the resulting solution was monitored at 517 nm. The blank was a solution containing 0.1 mL of 80% methanol and 2.9 mL of the DPPH. The calibration curve was obtained with vitamin C solutions (20 to 100 μg/mL) reacted with DPPH. The results were expressed as % of antioxidant capacity equivalent to vitamin C (VCEAC) after 30 minutes of reaction (Brand-Williams et al., 1995; Kim et al., 2002).

2.5 Statistical analysis

Data were submitted to normality analysis by the Shapiro-wilk test (Graph Pad Prisma 7). Data with a non-normal distribution were analyzed by Kruskall-Wallis test followed by the Dunn post-test, considering a margin of error of 5%.

3 Results and Discussion

The major finding of this study was associated with the high antioxidant potential of \textit{R. brasiliensis} fruits, as shown by total polyphenol and anthocyanin contents, and also antioxidant activity (Figure 1). Moreover, we could highlight that air Oven Drying (OD) was the gold standard protocol considering the maintenance of the antioxidant potential and economical aspects.

![Figure 1](https://example.com/figure1.png)  
**Figure 1.** Determinations performed in the \textit{R. brasiliensis} fruits submitted to different processing. Data represent the mean of three replicates. A) Total polyphenols. Results were expressed in equivalent to gallic acid, mg/100 g of dry matter or dry extract.
matter for OD and FT, whereas mg/100 g of dry extract were expressed for SD and LYO. B) Total anthocyanin. Results expressed in equivalent to cyanidin. C) Antioxidant activity. Results expressed in equivalents to vitamin C. *Statistically significant (FT was different from LYO) in A (Kruskall-Wallis test followed by the Dunn post-test, \( p < 0.05 \)). SD= Spray Dryer. OD= air Oven Dried. LYO= Lyophilized. FT = Freshly Thawed.

Total phenol content and antioxidant activity of fruits and leaves of other Rubus species were previously evaluated as following: *R. idaeus* (about 13 mg GAE/ g dry matter); *R. brigantinus* (about 39 mg GAE/ g dry matter); *R. genevieri* (about 27 mg GAE/ g dry matter); *R. sampaioanus* (about 34 mg GAE/ g dry matter); and *R. vigoi* (about 36 mg GAE/ g dry matter) (Santos et al., 2011). The polyphenol content found in our study was lower than the aforementioned results when the data was expressed in mg/g of dry matter (mean (mg GAE/ g of dry matter): SD 1.62; OD 2.11; LYO: 2.17; FT: 0.72). However, this study was the first report describing antioxidant characterization of the fruits of *R. brasiliensis*.

Phenolic and anthocyanin contents, antioxidant capacity and *in vitro* intestinal bioaccessibility were determined in the *R. fruticosus* cv. Tupy. In fact, lackberry Residues (BR) and Ultrasonicated Blackberry Residues (US-BR) were used. BR had higher polyphenol, anthocyanin contents and antioxidant capacity than US-BR. Phenolic and anthocyanin contents obtained by US-BR were more bioaccessible compared to BR. The ultrasound aided the extraction of phenolic compounds and improved their bioaccessibility (Zafra-Rojas et al., 2020). In this work, we tested more than one processing methods in other species of Rubus.

In fact, *R. brasiliensis* fruits presented an amount of phenols similar to cashew, or *caju* (*Anacardium occidentale* L.), smaller than acerola (*Malpighia emarginata* DC.), and higher than bacuri (*Platonia insignis* Mart.), cajá (*Spondias mombin* L.), guava, or *goiaba* (*Psidium guajava* L.) and tamarind (*Tamarindus indica* L.) (Vieira et al., 2011).

The blackberries cv Xavante and purple pitanga presented the highest content of total phenols and antioxidant activities among the native Brazilian fruits as araçá (*Psidium cattleianum* Sabine), butiá (*Butia eriospatha* (Mart. ex Drude) Becc), even as different colors (purple, red and orange) of pitanga (*Eugenia uniflora* L.) and blackberries (*Rubus* sp.; cv Xavante and Cherokee). Quercetin, quercitrin, isoquercitina and cyaniding derivates were also found in these fruits (Denardin et al., 2015).

The hexanic fraction of *R. brasiliensis* leaves has hypnotic, anticonvulsant, and muscle relaxant actions. These effects was related to the GABA A system and was demonstrated using 300 mg/kg in rats. However, using lower doses these effects were not observed (Nogueira & Vassilieff, 2000).

Considering different protocols of processing, small differences between samples are not significant, except in polyphenol content. The FT form presented a statistically lower content of polyphenols than the LYO form. Probably this occurs because the FT were not subjected to drying methods that may stabilize enzymatic process (Simões et al., 2016).

Indeed, the LYO and SD were used to concentrate the active compounds to obtain extract. However, these compounds were not observed. In addition, the SD processing used high drying temperature that may have decreased the levels of polyphenol and anthocyanin contents when compared to the other processes.

Kyi et al. (2005) proved the decline of polyphenol content in cocoa beans during drying at 40°C to 60 °C and 50% to 80% of relative humidity for 5h. The SD uses higher temperatures for shorter times. In this study we used a temperature of 110 °C for approximately 30 minutes, which probably reduced some bioactive compounds.

Thus, it is more economically feasible to process the fruits directly, without a process of extraction. To obtain a pharmaceutical form containing *R. brasiliensis*, ideally, we recommend the OD method (which would stabilize the enzymatic processes), since the antioxidant compounds could be preserved and the processing method was much easier and cheaper than the others.
4 Conclusion

This study was the first report describing antioxidant activity, polyphenol and anthocyanin contents of *Rubus brasilienis* Mart. fruits. In general, processing method did not affect the content of bioactive compounds, only the freshly thawed processing method presented lower amount of polyphenols than the lyophilization. In this way, fruit dried directly in an oven can be considered the most appropriate processing method, considering the amount of bioactive and antioxidant capacity, as well as facilitating carrying out the study and also being economically viable.

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