

## Hydroalcoholic extraction of antioxidant compounds in Japanese grape pseudofruits

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### Abstract

The pseudofruit of Japanese grape is rich in sugar and phenolic compounds but little explored for extracting antioxidant compounds. The objective of the study was to extract and evaluate the antioxidant capacity of these pseudofruits harvested at two maturation stages: the development phase (DP) and the mature phase (MP). The pseudofruits were evaluated regarding the centesimal composition and physicochemical characteristics and submitted to extraction with 100% water, 50% ethanol, and 100% ethanol. For the extracts, we determined the phenolic compound content and the antioxidant activity using radical capture methods ABTS and DPPH. Pseudofruits may be considered a good source of dietary fiber, regardless of the maturation stage, proving to be a promising raw material for use in foods. Moreover, pseudofruits in the MP presented an increase in the contents of soluble solids, reducing and non-reducing sugars, and titratable acidity, attributed to the formation of the galacturonic acid during the maturation process. Among the solvent used, extraction with 50% ethanol resulted in a larger phenolic compound content and better antioxidant activity, especially for the pseudofruits in the DP, characterizing them as a vegetable matrix of excellent antioxidant capacity and with potential for application in foods and drugs.

**keywords:** ABTS. Phenolic compounds. DPPH. *Hovenia dulcis*. Maturation.

## Extração hidroalcolólica de compostos antioxidantes em pseudofrutos da uva Japão

### Resumo

O pseudofruto da uva Japão é rico em açúcares e compostos fenólicos, mas ainda pouco explorado para extração de compostos antioxidantes. O objetivo do estudo foi extrair e avaliar a capacidade antioxidante desses pseudofrutos colhidos em dois estádios de maturação: a fase de desenvolvimento (FD) e a fase madura (FM). Os pseudofrutos foram avaliados quanto à composição centesimal e características físico-químicas e submetidos à extração com água 100%, etanol 50% e etanol 100%. Para os extratos, determinamos o teor de compostos fenólicos e a atividade antioxidante pelos métodos de captura de radicais ABTS e DPPH. Os pseudofrutos podem ser considerados uma boa fonte de fibra alimentar, independentemente do estágio de maturação, mostrando-se uma matéria-prima promissora para utilização em alimentos. Além disso, os pseudofrutos na FM apresentaram aumento nos teores de sólidos solúveis, açúcares redutores e não redutores e acidez titulável, atribuídos à formação do ácido galacturônico durante o processo de maturação. Dentre os solventes utilizados, a extração com etanol 50% resultou em maior teor de compostos fenólicos

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e melhor atividade antioxidante, principalmente para os pseudofrutos da FD, caracterizando-os como uma matriz vegetal de excelente capacidade antioxidante e com potencial para aplicação em alimentos e fármacos.

**Palavras-Chave:** ABTS. Compostos fenólicos. DPPH. *Hovenia dulcis*. Maturação.

## Introduction

*Hovenia dulcis* T. (Rhamnaceae), commonly known as the Japanese grape, produced pseudofruits, and these edible parts have antialcohol, liver protection, anti-fatigue, anti-steatosis, and anti-inflammatory functions, moreover polysaccharides with hypoglycemic activity (Yang et al., 2022). Thus, pseudofruits can be explored, as they present biocompounds with potential for application in functional foods and pharmaceutical products.

Extraction is the first step to separate biologically active compounds from natural products, and solvent extraction is the most widely used method (Tileuberdi et al., 2022). The main solvents used are of organic origins, such as methanol and ethanol, or also an aqueous solvent, and these may be used separately or combined (Sarfarazi et al., 2019; He et al., 2023). Among the variety of solvents that may be used, ethanol presents some advantages. It is produced by biotechnology, does not generate toxic residues, its flammability degree is intermediary, and it is considered safe for human health, so it may be used for extracting vegetable compounds for application in the most diverse industries such as food, pharmaceuticals, and cosmetics, among others (Nikolić et al., 2023).

Therefore, with the objective of obtaining more knowledge about the functional potential of the pseudofruits of *H. dulcis* as an alternative source of bioactive compounds for the industry, this study performed the hydroalcoholic extraction with pseudofruits harvested at two maturation stages and determined the phenolic compound content and the antioxidant capacity of the extracts. Moreover, the pseudofruits were evaluated for their centesimal composition and physicochemical characteristics.

## Material and Methods

### Sample collection

The pseudofruits were harvested from the same tree in the city of Chapecó, Santa Catarina, Brazil (latitude 27°05'08.7" S and longitude 52°36'58.9" W), in two periods: February, the development phase (DP), and April, the mature phase (MP). After the harvesting, the pseudofruits were selected, sanitized in running water then immersed in a 200 ppm hypochlorite solution for 15 minutes. Next, they were separated into two batches: the first was used for characterizing the pseudofruits *in natura*, while the second was frozen at -86 °C in an Ultrafreezer (ULT 335/710 D Vertical, Indrel) involved

in a dark-colored plastic packaging until the antioxidant capacity analyses were performed.

### Characterization of the pseudofruits in natura

The centesimal composition of the pseudofruits was determined through moisture, ash, lipid, protein, and total dietary fiber analyses, as per methodologies 934.06, 940.26, 920.39, 960.52, and 985.29 by AOAC (2016), respectively. The carbohydrate content was calculated by difference.

For the pH, titratable acidity, soluble solids, reducing and non-reducing sugars, the samples *in natura* were crushed in a mixer (250W, Walita) for 30 s. To determine the pH, 10 g of the crushed samples were quantified on an analytical balance and diluted in 100 mL of water and the reading was carried out with a previously calibrated pH meter (MPA210, TecnoPON). The titratable acidity was determined as per methodology no. 962.19 (AOAC, 2016). For determining the total soluble solids, 3 to 4 drops of the samples homogenized in 10 mL of water were transferred to the refractometer prism (RFM 732, Bellingham Stanley). The analyses of reducing sugars in glucose and non-reducing sugars in sucrose were carried out employing the Lane-Eynon method, using Fehling's solution (AOAC, 2016).

### Extraction of antioxidant compounds

For extracting the antioxidant compounds, the methodology proposed by Larrauri et al. (1997) was followed with modifications. The extractions were carried out in batch duplicate with extractor solvents 100% water, 100% ethanol, and ethanol-water (50:50, v/v) in the pseudofruit-solvent proportion of 1.5:10 (w/v). We used a shaker incubator (Luca-223, Lucadema) at 30 ± 1 °C with an agitation speed of 40 rpm for 60 min. The mixtures were filtered with quantitative filter paper 'Whatman' no. 40, and the filtrate was stored in an amber bottle involved with aluminum foil at 4.0 ± 1 °C for 24 h in a refrigerator (CRD37EB, Consul) until the analyses were performed.

### Determination of the total phenolic compounds and antioxidant activity through the capture of free radicals DPPH and ABTS+

The quantification of the phenolic compounds was carried out in triplicate using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). The

quantitative evaluation of the antioxidant activity was determined by the free radical capture of DPPH methodology (Rufino et al., 2007a). The results were expressed in EC50. The total antioxidant activity was determined by the free radical capture of ABTS according to the method described by Rufino et al. (2007b). The results were expressed in  $\mu\text{M Trolox.g}^{-1}$  of dry sample.

### Statistical analysis

The results of the quantifications that presented a null hypothesis occurrence probability lower than 5% ( $p < 0.05$ ) applying an ANOVA were considered statistically different, followed by multiple comparisons by the

Tukey test. All analyses were conducted using the trial version of software trial STATISTICA 14 (Statsoft).

## Results and Discussions

### Characterization of the pseudofruits in natura

Significant differences ( $p < 0.05$ ) were observed between the two maturation stages (Figure 1) for all pseudofruit characterization analyses performed (Table 1), except for the protein content. For the total dietary fiber content, a reduction was observed from the DP to the MP. This change may be attributed to the increase in the solubility of the pectic polysaccharides, with this being an alteration observed in the maturation of fleshy fruits (Liu et al., 2023).

Table 1 – Characterization (dry base) of Japanese grape pseudofruits at different maturation stages

Analyses	Maturation stage	
	Development phase	Mature phase
Moisture (%)	64.45 $\pm$ 0.20 <sup>b</sup>	70.18 $\pm$ 0.93 <sup>a</sup>
Ash (%)	1.96 $\pm$ 0.08 <sup>a</sup>	1.52 $\pm$ 0.09 <sup>b</sup>
Protein (%)	7.19 $\pm$ 0.62 <sup>a</sup>	7.97 $\pm$ 0.48 <sup>a</sup>
Lipids (%)	1.39 $\pm$ 0.07 <sup>a</sup>	1.17 $\pm$ 0.06 <sup>b</sup>
Total dietary fiber (%)†	61.74 $\pm$ 3.66 <sup>a</sup>	41.66 $\pm$ 3.68 <sup>b</sup>
Carbohydrates (by difference) (%)	89.46	89.34
Total soluble solids (°Brix)	4.50 $\pm$ 0.10 <sup>b</sup>	10.10 $\pm$ 0.20 <sup>a</sup>
Reducing sugars (%)	5.33 $\pm$ 0.18 <sup>b</sup>	7.97 $\pm$ 0.18 <sup>a</sup>
Non-reducing sugars (%)	2.84 $\pm$ 0.67 <sup>b</sup>	6.54 $\pm$ 0.16 <sup>a</sup>
Titrateable acidity (%)	4.17 $\pm$ 0.11 <sup>b</sup>	5.00 $\pm$ 0.21 <sup>a</sup>
pH	6.49 $\pm$ 0.01 <sup>b</sup>	6.68 $\pm$ 0.04 <sup>a</sup>

Values expressed as averages  $\pm$  standard deviation. †inserted in the carbohydrate fraction; Averages on the same line followed by equal letters do not present significant differences according to the Tukey test ( $p < 0.05$ ).

Carbohydrates constitute the majority component of the pseudofruits of the Japanese grape. During the maturation, an increase occurred in contents of total soluble solids and reducing and non-reducing sugars due to the hydrolysis of the starch, which is present in greater amounts in immature fruits (Ali et al., 2022). Over the maturation stage, an increase in pH and titrateable acidity was observed. In fruit maturation, there is usually an increase in the ratio of soluble solids to titrateable acidity resulting from the increase in the soluble solid content and decrease in titrateable acidity; however, in this study, this reduction was not observed.

### Evaluation of the hydroalcoholic extracts of pseudofruits

The study of the extraction conditions, among them the type of solvent and concentration thereof, is

extremely important to quantify the antioxidant potential of vegetable extracts (Table 2). It was observed that the content of total phenolic compounds (TPC) differed significantly ( $p < 0.05$ ) among the three solvent systems evaluated, being higher for solvent 50% ethanol (v/v) in both maturation stages (DP and MP).

The physicochemical characteristics of the diluted ethanolic solvent system possibly resemble, to a higher degree, the characteristics of most phenolic compounds present in the samples evaluated. The type of solvent and the polarity may affect the transference of electrons and hydrogen atoms, which is a key aspect in evaluating the phenolic compounds and antioxidant capacity (González-Cardoso et al., 2023).

The values for TPC obtained in the extractions with different systems were influenced by the maturation

of the pseudofruits. Regardless of the extractor solvent used, the DP presented a higher content ( $p < 0.05$ ) of phenolic compounds than the MP. This difference between the stages was more significant when the solvent used was 100% ethanol, with the TPC content in the DP

being 3.5 times that of the MP. It is worth stressing that the hydroalcoholic extract (50% ethanol) of the Japanese grape pseudofruit proves to be an alternative for extracting a high amount of phenolic compounds.

Figure 1 – Maturation stages of the *Hovenia dulcis* pseudofruits

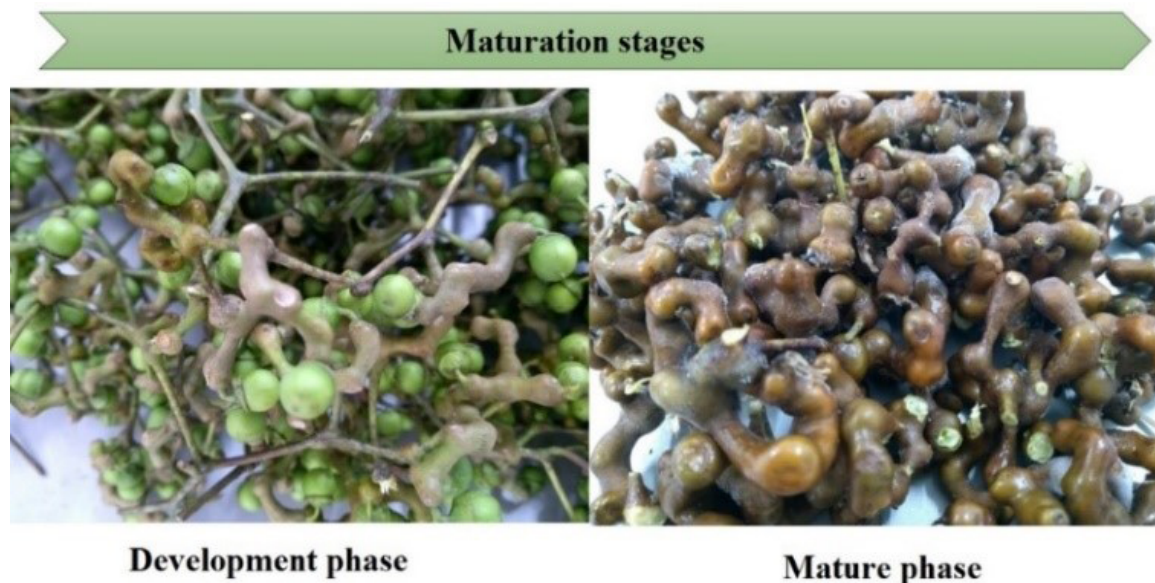


Table 2 – Total phenolic compounds and antioxidant activity of the extracts of Japanese grape pseudofruits in different maturation stages, obtained with different solvents.

Analysis	Maturation stage	Solvents		
		100% Water	50% Ethanol	100% Ethanol
TPC	DP	182.27 ± 1.74 <sup>ba</sup>	347.49 ± 0.66 <sup>aA</sup>	28.18 ± 3.00 <sup>ca</sup>
	MP	153.85 ± 4.35 <sup>bb</sup>	272.47 ± 2.94 <sup>ab</sup>	7.97 ± 3.42 <sup>cb</sup>
EC50	DP	494.4 ± 62.9 <sup>bb</sup>	66.7 ± 7.9 <sup>cb</sup>	53443.8 ± 88.4 <sup>ab</sup>
	MP	16240.0 ± 63.6 <sup>ba</sup>	2865.0 ± 28.3 <sup>ca</sup>	101387.5 ± 141.4 <sup>aA</sup>
ABTS	DP	11.15 ± 1.02 <sup>ba</sup>	345.34 ± 3.51 <sup>aA</sup>	2.52 ± 0.10 <sup>ca</sup>
	MP	7.10 ± 0.41 <sup>bb</sup>	24.55 ± 1.10 <sup>ab</sup>	1.62 ± 0.10 <sup>cb</sup>

Values expressed as averages ± standard deviation. DP: development phase; MP: mature phase; Different lower-case letters on the same line and different upper-case letters on the same columns, for the same analysis, indicate significant differences from the others ( $p < 0.05$ ); TPC: total phenolic compounds expressed in mg of gallic acid equivalent per 100 g of dry sample; ABTS expressed in  $\mu\text{M}$  of Trolox per g of dry sample; EC50: extract concentration in  $\mu\text{g}/\text{mL}$  capable of reacting with 50% of the radical present in the DPPH solution.

For the ABTS and EC50 analyses (extract concentration in  $\mu\text{g}/\text{mL}$  capable of reacting with 50% of the radical present in the DPPH solution), in which lower EC50 values result in higher antioxidant activity, the extraction with 50% ethanol (v/v) rendered more considerable antioxidant activity in both maturation stages (Table 2). The lowest efficiency was found for the 100% ethanol solvent, while water presented intermediary efficiency. When a higher number of OH groups is added, stemming from ethanol, an increment in the hydrogen bonds between the solute and water occurs, and, with this, there is an increase in the solubility of some bioactive compounds of *Hovenia dulcis* (Martins et al., 2013).

However, possibly, when a larger number of OH groups is added (100% ethanol), the solubility of such compounds decreases considerably.

The antioxidant activity was significantly different ( $p < 0.05$ ) between the two maturation stages for all solvents used, being higher for the DP. These results can be attributed to the phenolic compound content; however, as the fruits mature, their phenols undergo oxidation processes by polyphenol oxidase and participate in the biosynthesis of anthocyanins, which accumulate during fruit maturation, increasing the antioxidant activity or keeping it high (Schwartz et al., 2009). Non-phenolic

compounds such as acids (ascorbic, tartaric, and citric) found in developing fruits, or their synergic action with the phenolic compounds, may also have contributed to the higher antioxidant activity of the pseudofruits at this phase (Maieves et al., 2015).

## Conclusions

The solvent system used for extraction and the maturation stage of pseudofruits from the Japanese grape influenced the total phenolic compound content and the antioxidant activity of the extracts directly, with 50% ethanol (v/v) proving to be the best solvent for extracting phenolic compounds and antioxidant activity (evaluated through the ABTS radical capture method and extract capable of reacting with 50% of the DPPH radical). Pseudofruits in the development phase (DP) are richer in phenolic compounds and present more considerable antioxidant activity compared to those in the mature phase (MP) when extracted with the solvents 100% water, 50% ethanol (v/v), and 100% ethanol. Moreover, the pseudofruits of *Hovenia dulcis* T. present a high amount of dietary fiber at the two maturation stages evaluated, proving to be a promising source of this nutrient for use in the food industry, along with its antioxidant potential.

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## Full Disclosure

The authors also declare that there is no conflict of interest in the research and publication of the manuscript.

## Authors contribution

GCM - Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft; SVS - Investigation; AMPA - Investigation; ER - Funding acquisition, Methodology; GARS - Project administration, Visualization, Writing - Review & Editing; Methodology, Conceptualization; DC - Supervision, Visualization, Writing - Review & Editing; Methodology, Conceptualization.

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