

## Influence of thermal processing on the characteristics and chemical profile of ora-pro-nobis by PS/MS *paper spray*

Influência do processamento térmico nas características e perfil químico de ora-pro-nóbis por *paper spray* PS/MS

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### Abstract

This work aimed to evaluate the influence of dry and wet heat processing on the antioxidant profile and antioxidant activity in ora-pro-nobis leaves. The leaves were collected, washed, and separated into three groups for the treatments: application of moist heat (hydrothermal cooking at 100 °C / 4 minutes), application of dry heat (70 °C / 8 hours), and control (raw material). The characteristics were evaluated: total soluble solids (SST), total solids (ST), pH, phenolic compounds (CPT), flavonoids (FT), anthocyanins (AT), antioxidant activity (AA). To identify the chemical profile and the chemical profile, fingerprints were obtained using PS / MS *paper spray*. The content of SST and ST decreased with moist heat. The CFT content increased with wet heat ( $12.43 \pm 0.98$ ) and decreased with dry heat ( $4.25 \pm 0.93$  g EAG 100 g<sup>-1</sup>). There was a decrease in the FT and AT content in the leaves processed with dry heat ( $132.67 \pm 20.28$  mg 100 g<sup>-1</sup> and  $19.70 \pm 3.34$  mg CG 100 g<sup>-1</sup>, respectively). AA decreased in both processes, which was higher in ora-pro-nobis leaves processed with dry heat ( $0.82 \pm 0.0033$  μmol ET g<sup>-1</sup>). 26 phenolic compounds were identified in the ora-pro-nob leaves by *paper spray*. The chemical profile, except for the substances 4,5-dimethyl-2,6-octadiene and methyl ester linolenic acid was not affected by thermal processing. Knowing the effect of food processing is important to understand the behavior of compounds that act beneficially on human health.

**Keywords:** Phytochemicals; Bioactive compounds; *Pereskia aculeata* Mill.; PANC.

### Resumo

O objetivo deste trabalho foi avaliar a influência do processamento com calor seco e calor úmido sobre o perfil de antioxidantes e atividade antioxidante em folhas de ora-pro-nobis. As folhas foram coletadas, lavadas e separadas em três grupos destinados aos tratamentos: aplicação de calor úmido (cocção hidrotérmica a 100 °C/ 4 minutos), aplicação de calor seco (70 °C / 8 horas) e controle (material *in natura*). Foram avaliadas as características: sólidos solúveis totais (SST), sólidos totais (ST), pH, compostos fenólicos (CPT), flavonoides (FT), antocianinas (AT), atividade antioxidante (AA). Para identificar o perfil químico e o perfil químico, procedeu-se a obtenção de fingerprints

empregando-se o *paper spray* PS/MS. O teor de SST e ST diminuiu com o calor úmido. O teor CFT aumentou com o calor úmido ( $12,43 \pm 0,98$ ) e diminuiu com o calor seco ( $4,25 \pm 0,93$  g EAG  $100\text{ g}^{-1}$ ). Houve diminuição do teor FT e AT nas folhas processadas com calor seco ( $132,67 \pm 20,28$  mg  $100\text{ g}^{-1}$  e  $19,70 \pm 3,34$  mg CG  $100\text{ g}^{-1}$ , respectivamente). A AA diminuiu em ambos os processamentos, sendo esta, maior nas folhas de ora-pro-nóbis processadas com calor seco ( $0,82 \pm 0,0033$   $\mu\text{mol ET g}^{-1}$  amostra seca). Foram identificados 26 compostos fenólicos nas folhas de ora-pro-nóbis por *paper spray*. O perfil químico, exceto para as substâncias 4,5-dimetil-2,6-octadieno e ácido metilester linolênico, não foi afetado pelo processamento térmico. Conhecer o efeito do processamento em alimentos é importante para entender o comportamento de compostos que atuam de forma benéfica na saúde humana.

**Palavras-chave:** Fitoquímicos; Compostos bioativos; *Pereskia aculeata* Mill.; PANC.

### Resumen

El objetivo de este trabajo fue evaluar la influencia de la aplicación de calor seco y húmedo sobre el perfil de antioxidantes y la actividad antioxidante de las hojas de ora-pro-nobis. Las hojas se recolectaron, lavaron y separaron en tres grupos para los tratamientos: aplicación de calor húmedo (cocción hidrotermal  $100\text{ }^{\circ}\text{C}$  / 4 minutos), aplicación de calor seco ( $70\text{ }^{\circ}\text{C}$  / 8 horas) y control (material fresco). Se evaluaron el contenido de sólidos solubles totales (SST), sólidos totales (ST), pH, fenólicos totales (CPT), flavonoides (FT), antocianinas (AT), actividad antioxidante (AA). Para identificar el perfil químico se tomaron huellas dactilares utilizando el *paper spray*. El contenido de SST y ST disminuyó con el calor húmedo. El contenido de CPT aumentó con el calor húmedo ( $12,43 \pm 0,98$ ) y disminuyó con el calor seco ( $4,25 \pm 0,93$ g EAG  $100\text{ g}^{-1}$ ). Hubo disminución en el contenido de FT y AT en las hojas procesadas con calor seco ( $132,67 \pm 20,28$  mg  $100\text{ g}^{-1}$  y  $19,70 \pm 3,34$  mg CG  $100\text{ g}^{-1}$ , respectivamente). La AA disminuyó en ambos procesos, que fue mayor en las hojas procesadas con calor seco ( $0,82 \pm 0,0033$   $\mu\text{mol ET g}^{-1}$ ). Se identificaron 26 compuestos fenólicos en las hojas mediante *paper spray*. El perfil químico, a excepción del 4,5-dimetil-2,6-octadieno y éster metílico del ácido linolénico, no se vio afectado por el procesamiento térmico. El análisis del efecto del procesamiento en los alimentos es importante para evaluar el comportamiento de los compuestos que actúan de manera beneficiosa en la salud humana.

**Palabras clave:** Fitoquímicos; Compuestos bioactivos; *Pereskia aculeata* Mill.; PANC.

## 1. Introduction

*Pereskia aculeata* Mill., recognized regionally as food and also as a medicinal plant is considered a food alternative and cultural diversification in agricultural activity, mainly for family farming and low-income urban and rural populations (De Almeida et al., 2016). The lack of knowledge of the population concerning the properties of this vegetable cause it to be underutilized (Bacchetta et al., 2016; Menendez-Baceta et al., 2017).

Belonging to the Cactaceae family, the ora-pro-nobis, as it is best known in Brazil, has a large amount of fiber (39%) and protein (28%) in its leaves, in addition to significant amounts of iron and calcium (Kazama et al., 2012; Rocha et al., 2009; Viana et al., 2015), which are equal or even higher than what can be found in other leafy vegetables (Kinupp & Barros, 2008; Take it et al., 2009, Viana, et al., 2015).

Ora-pro-nobis is not only used for fresh consumption, it can also be introduced in several other dishes such as cakes, loaves of bread, pasta, and ice cream (Martinevski et al. 2013; Mazon et al., 2020; Rosa et al., 2020; Rocha et al., 2009). Also, the mucilage provided by the leaves of the plant can be used in food formulations as a thickening agents, gelling agents, and emulsifiers, in addition to the production of biofilms (Amaral et al., 2018; Conceição et al., 2014; Junqueira, 2018; Oliveira et al., 2019; Takeiti et al., 2009).

A large amount of nutritional components present in species of the genus, *Pereskia* makes this group an important nutritional source combined with health, providing a better quality of life for the population that consumes them (Duarte & Hayashi, 2005; Martin et al., 2017; Pinto & Scio, 2014). Medicinally they are used for antinociceptive, anti-inflammatory, antisiphilitic and healing action (Barbalho et al. 2016; Duarte & Hayashi, 2005; Rosa & Souza, 2003; Sartor et al., 2010; Silva & Fonseca, 2016; Silva Júnior et al., 2010). The extract of the essential oil of the species presents efficient results in the antimicrobial potential in a wide spectrum (Do Carmo Pimenta et al., 2020; Souza et al., 2016).

Bioactive compounds that promote protective action in organisms are related to their antioxidant properties, including phenolic compounds, carotenoids, vitamins A, C, and E (Melo et al., 2006; Namiki, 1990). Such antioxidants naturally present

in vegetables contribute positively to health, and therefore, their consumption is widely recommended. Phenolic compounds are present in the class of phytochemicals with the greatest expression present in ora -pro-nobis (Agostini-Costa et al., 2012; Pinto et al., 2012). These compounds are associated with a reduced risk of the occurrence of degenerative diseases, such as cancer, diabetes, and Alzheimer's disease (Barbalho et al., 2016; Corrêa et al., 2018).

Accordingly, the importance of better understanding the stability of these compounds in food is also increased, especially considering the processing they are submitted to before consumption. According to Torres (2009), the processing will change the matrix of the original product, consequently presenting an effect on the contents of the phytochemicals present in this. The influence of processing on bioactive compounds is still poorly studied, so it is useful to know the relationship between processing and preserving the content of natural antioxidants in food (Campos et al., 2008; Nascimento et al., 2014), especially due to the fact of the growing consumption of industrialized or processed foods in different ways, thus arousing interest in the quality of these products. The aim of the present work was to evaluate the influence of thermal processing with dry heat and moist heat on the antioxidant profile by *paper spray* PS / MS and antioxidant the activity of ora -pro-nobis (*Pereskia aculeata* Mill.).

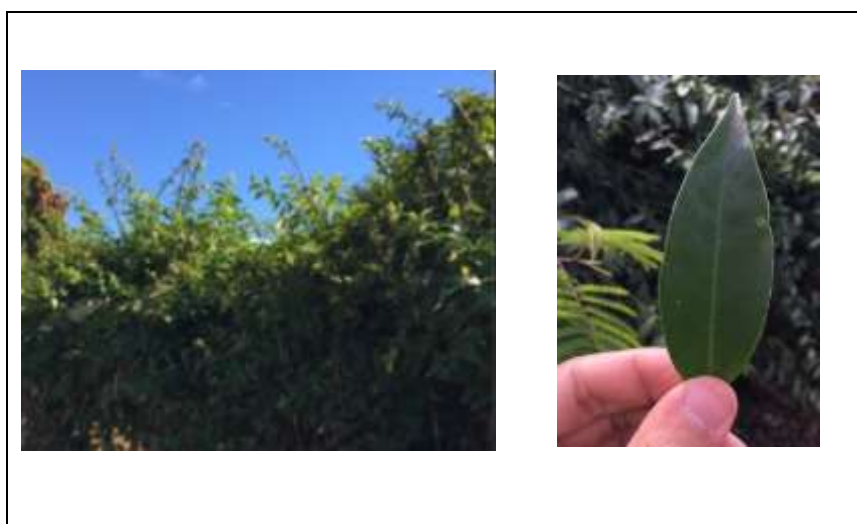
## 2. Material and Methods

### 2.1 Collection and preparation of plant material

Leaves of ora-pro-nobis (*Pereskia aculeata* Mill.) (Figure 1) were collected from the Vegetable Bank of the Agricultural Research Corporation of Minas Gerais (EPAMIG) - Fazenda Santa Rita, located in Prudente de Morais - MG (19 ° 27'13.9 "S 44 ° 09'25.7" O), from cultivation carried out without the application of pesticides.

The leaves of the vegetable were harvested at random, seeking a standardization by size.

**Figure 1.** Ora-pro-nobis leaves (*Pereskia aculeata* Mill.) harvested at the Non-Conventional Vegetable Bank, EPAMIG Midwest Regional Unit in Prudente de Morais - MG.



Source: Authors.

### 2.2 Sample characterization

The leaves were collected in the morning and transported under refrigeration to the Food Conservation Laboratory of the Universidade Federal de São João del-Rei, Campus Sete Lagoas in Sete Lagoas / MG, where the experiment was conducted.

The leaves were washed in running water and separated into three parts, namely, one part was kept *in natura* (control) reserved for comparative analysis with the leaves submitted to thermal processing: hydrothermal heating and dry heat.

### 2.2.1 Dry heat processing

Part of the leaves (60 grams) of ora-pro-nobis were submitted to dry heat using a cabin dehydrator (Sparrow PE 60), with forced air circulation where they remained at 70 °C, for 8 hours. After dehydration, the leaves were ground manually with the aid of a gral and pistil.

### 2.2.2 Processing with application of moist heat

Part of the leaves (60 grams) were submitted to hydrothermal heating, where they were immersed in 1 liter of water at 100 °C for 4 minutes. The experiment was conducted with five replications and all analyzes were performed in triplicate.

## 2.3 Evaluation of physical-chemical characteristics

### 2.3.1 Total soluble solids

The levels of total soluble solids were determined according to the methodology described by AOAC (2016). The samples were crushed, filtered, and placed on the prism of a Reichert, R2 Mini digital refractometer with internal temperature compensation. The results were expressed in ° Brix.

### 2.3.2 Total solids

Approximately 2 g of the homogenized samples were submitted to a temperature of 105 °C in a FANEM 515 sterilization, and drying oven for 48 hours. The percentage of total solids was obtained by the difference between the initial and final masses (after the greenhouse) of the samples, according to the AOAC protocol (2016).

### 2.3.3 Hydrogenionic potential (pH)

The pH value was determined by potentiometry with the aid of a digital pH meter Tekna T-1000, by direct immersion of the electrode in the sample homogenized with 50 milliliters of distilled water (AOAC, 2016).

### 2.3.4 Total titratable acidity

To determine the total titratable acidity, a 0.1 N NaOH solution was used as a standard and phenolphthalein as an indicator, with the assistance of a pH meter. The results were expressed in grams of citric acid per 100 grams of fresh sample (AOAC, 2016). Total titratable acidity was calculated through Equation 1.

$$ATT = \frac{V * N * F * Eq}{10 * M}$$

Equation 1

In which: V = Spent volume of the NaOH solution (liters), N = Normality of the NaOH solution, F = Correction factor obtained from the standardization of NaOH, Eq = Equivalent citric acid, and M = Mass of the sample (grams).

## 2.4 Evaluation of bioactive compounds

### 2.4.1 Total phenolic compounds

The content of total phenolic compounds was determined by the Folin-Ciocalteu method (Singleton et al., 1999) with a comparison of a calibration curve constructed with gallic acid. The absorbance was read on a FENTO 700S spectrophotometer at 740 nanometers. The results were expressed in milligrams of equivalents of gallic acid (EGA) per 100 grams of sample on a dry basis. The content of total phenolic compounds was calculated using Equation 2.

$$CF = \frac{ABS - 0,4864}{0,0024} \quad \text{Equation 2}$$

In which: ABS = Absorbance of the sample and EGA = Equivalent of gallic acid.

### 2.4.2 Total flavonoids

The analysis of the total flavonoid content was performed following the method of Francis (1982). The absorbance was read on a SHIMADZU UV-1800 spectrophotometer at 374 nanometers. The results were expressed in milligrams of flavonoids per 100 grams of sample on a dry basis. The content of total flavonoids was calculated using Equation 3.

$$TF = \frac{\left(\frac{ABS}{76,6} + 100\right)}{\left(\frac{M}{25}\right)} \quad \text{Equation 3}$$

In which: ABS = Absorbance of the sample, TF = Total flavonoids and M = Mass of the sample (grams).

### 2.4.3 Total anthocyanins

The anthocyanin content analysis was performed following the method of Francis (1982). The absorbance was read on a SHIMADZU UV-1800 spectrophotometer at 535 nanometers. The results were expressed in milligrams of cyanidin-3-glucoside (CG) per 100 grams of sample on a dry basis. The total anthocyanin content was calculated using Equation 4.

$$TA = \frac{\left(\frac{ABS}{98,2} + 100\right)}{\left(\frac{M}{25}\right)} \quad \text{Equation 4}$$

In which: ABS = Absorbance of the sample, TA = Total anthocyanins and M = Mass (grams).

## 2.5 Evaluation of antioxidant activity

The antioxidant activity was determined by the DPPH method (2,2-defenyl-1-picryl-hydrazil), based on the scavenging of free radicals by antioxidant, according to the method of Brand-Williams et al. (1995).

A quantity of 0.2 milligrams of sample was weighed and 15 milliliters of methanol acidified with 1% HCL were added, then they were shaken in the Shaker Novatécnica incubator at 150 rpm for 2 hours. Subsequently, 10 milliliters of this content was transferred to a Falcon tube and it was centrifuged at 3000 rpm for 15 minutes. A 0.1 milliliter aliquot of the supernatant was transferred to a test tube, where 2.9 milliliters of the working radical were added. After the reaction of the solutions in the dark (30 minutes), the reading was performed on a spectrophotometer at 515 nanometers. The results were calculated using Equation 5 and expressed in  $\mu\text{mol}$  of trolox equivalent per gram of sample on a dry basis ( $\mu\text{mol ET} / \text{g}$  sample).

$$AA = \frac{(\Delta ABS - b) * V * D}{a * M} \quad \text{Equation 5}$$

In which:  $\Delta ABS$  = White absorbance - absorbance of the sample,  $b$  = linear coefficient,  $a$  = angular coefficient,  $V$  = Volume used in the extraction (liters),  $M$  = Mass of the sample (grams), and  $D$  = Dilution.

## 2.6 Qualitative assessment of the chemical profile by *paper spray*

The analysis of the chemical profile of the samples was performed using an LCQ Fleet mass spectrometer (Thermo Scientific, San José, CA, USA) equipped with a source of ionization by *paper spray*. The analysis was performed in triplicate in positive and negative ionization modes, according to Silva et al. (2019).

In this analysis, the chromatographic paper was cut into an equilateral triangle shape (1.5 centimeters) and positioned in front of the mass spectrometer entrance. This paper was supported by a metal connector and positioned 0.5 centimeters apart with the aid of a mobile platform (XYZ).

This device was connected to a high voltage source of the spectrometer through a copper wire. Finally, 2.0  $\mu\text{L}$  of the sample methanolic extract was applied to the edge of the triangles, 40.0  $\mu\text{L}$  of methanol transferred to the chromatographic paper was added and the voltage source was connected for data acquisition. For the analysis, the instrument was operated at a voltage of - 3.0 kV (negative ionization mode); capillary voltage of 40 V; transfer tube temperature 275 °C; the voltage of 120 V tube lenses; and mass range from 100 to 1000  $m/z$  (negative ionization mode). The ions and their fragments obtained in this analysis were identified based on the data described in the literature. Collision energies used to fragment the compounds ranged from 15 to 40 V.

The analysis of each sample was performed in triplicate and the presence of the compound was taken into account when it appeared at least twice, the absence when it did not appear or when it appeared only once in the triplicate.

## 2.7 Data analysis

For the statistical analysis, a completely randomized design (CRD) was performed, with 5 replications. The evaluations were carried out in triplicate. The verification of the assumptions of normality and homogeneity of the variances was performed by the Shapiro-Wilk and Levene tests. The data from all analyzes were submitted to analysis of variance (ANOVA) and the means compared by Tukey's test with 5% probability ( $p < 0.05$ ), using the SISVAR 5.6 software.

### 3. Results and Discussion

#### 3.1 Physico-chemical characteristics

Table 1 shows the values for total soluble solids, total solids, pH and total titratable acidity of ora-pro-nobis *in natura* leaves and after thermal processing.

**Table 1.** Average values of the parameters of total soluble solids, total solids, pH and total titratable acidity of ora-pro-nobis *in natura* leaves and post-processing (mean  $\pm$  standard deviation).

Processing	Parameter			
	SST	ST (U= 100-ST)	pH	ATT
<i>In natura</i>	7,37 $\pm$ 0,30 <sup>a</sup>	15,60 (21,06) $\pm$ 1,09 <sup>b</sup>	5,07 $\pm$ 0,28 <sup>a</sup>	0,11 $\pm$ 0,0066 <sup>b</sup>
Moist heat	5,87 $\pm$ 0,27 <sup>b</sup>	10,01 (84,40) $\pm$ 0,16 <sup>c</sup>	5,10 $\pm$ 0,12 <sup>a</sup>	0,18 $\pm$ 0,0042 <sup>a</sup>
Dry heat	N. r.	78,94 (89,99) $\pm$ 1,79 <sup>a</sup>	5,11 $\pm$ 0,21 <sup>a</sup>	0,14 $\pm$ 0,0038 <sup>a</sup>
CV (%)	12,65	25,76	2,56	10,54

SST= total soluble solids ( $^{\circ}$  Brix), ST= total solids (%), U = moisture (%) ATT= total titratable acidity (citric acid 100 g<sup>-1</sup> fresh sample), N. r. = Unrealized e CV = Coefficient of variation. Averages followed by the same lowercase letter in the columns do not differ significantly from each other, at the significance level of 5% probability by the Tukey test.

Source: Authors.

The leaves of ora-pro-nobis showed to possess a large amount of soluble solids, being higher than in their fruits, in which, Silva et al. (2018) analyzed the SST content at different stages of maturation and pointed out higher levels for ripe fruits (5.65  $^{\circ}$  Brix). After the processing of ora-pro-nobis leaves with moist heat, the content of soluble solids decreased by 1.5  $^{\circ}$  Brix, being still, in higher levels than in the ripe fruits described by Silva et al. (2018).

When performing a chemical characterization of two species of the genus *Pereskia*, De Almeida (2014) reported the 12.46% of total solids for leaves of *Pereskia aculeata* Mill., being slightly lower than the content reported in this work for *in natura* leaves.

The processing of ora-pro-nobis leaves with dry heat decreased the moisture content of the sample since the water present in the leaf tissues of the vegetable evaporated due to the processing conditions. On the other hand, ora-pro-nobis leaves submitted to moist heat processing showed a significantly higher water loss compared to fresh vegetable leaves (5.59%), as can be observed in Table 1, however, still keeping water as its main component.

#### 3.2 Bioactive compounds and antioxidant activity

The ora-pro-nobis leaves submitted to moist heat processing showed an increase of 29.84% in their contents of total phenolic compounds, reaching 12.43  $\pm$  0.98 g EGA 100 g<sup>-1</sup> dry sample. The ora-pro-nobis leaves submitted to processing using dry heat, presented a reduction of just over half of their total phenolic compounds, being detected a total of 4.25  $\pm$  0.93 g EGA 100 g<sup>-1</sup> dry sample (Table 2).

There was a retention of 94.78% of the total flavonoids and 76.81% of the total anthocyanins in the leaves submitted to moist heat, and 10.91% of the total flavonoids and 16.80% of the total anthocyanins in the leaves submitted to dry heat, which shows that leaves submitted to heat for a longer period of time have a large part of their constituents lost.

Tsantili et al. (2010), stated that with the damage suffered by vegetables submitted to processing, the cell membranes are broken, which leads to the leakage of their contents, consequently leading to a decrease in the content of bioactive compounds in the leaves of the processed vegetable.

**Table 2.** Average values of the contents of total phenolic compounds, total flavonoids and total anthocyanins (of ora-pro-nobis *in natura* leaves and post processing (mean  $\pm$  standard deviation)).

Processing	Parameter		
	TPC	TF	TA
<i>In natura</i>	8720 $\pm$ 710 <sup>b</sup>	1215,90 $\pm$ 201,35 <sup>a</sup>	117,23 $\pm$ 19,32 <sup>a</sup>
Moist heat	12430 $\pm$ 980 <sup>a</sup>	1152,47 $\pm$ 197,82 <sup>a</sup>	90,05 $\pm$ 12,89 <sup>b</sup>
Dry heat	4250 $\pm$ 930 <sup>c</sup>	132,67 $\pm$ 20,28 <sup>b</sup>	19,70 $\pm$ 3,34 <sup>c</sup>
CV (%)	11,56	27,76	25,33

TPC= total phenolic compounds (mg EGA 100 g<sup>-1</sup> sample on dry basis), TF= total flavonoids (mg 100 g<sup>-1</sup> sample on dry basis), TA= total anthocyanins (mg cyanidin-3-glycoside 100 g<sup>-1</sup> dry sample), CV = Coefficient of variation. Averages followed by the same lowercase letter in the columns do not differ significantly from each other, at the significance level of 5% probability by the Tukey test.

Source: Authors.

Sultana et al. (2008), studying the influence of different cooking methods on the total phenolic content and antioxidant activity of spinach, carrots, turnips, cabbage, peas, and cauliflower show that the contents of phenolic compounds had a decrease after cooking, reaching up to 70% losses. The leaves of ora-pro-nobis *in natura* and those submitted to hydrothermal heating did not show any difference in the contents of total flavonoids (Table 2).

The cooking process is reported to destabilize the matrix of compounds presents inside plant cells, with losses due to leaching. Boiling water may cause losses of these compounds to the cooking water due to the polarity of the molecules, which may explain the decrease in the contents of the bioactive compounds evaluated and also in the total soluble solids (Table 1) in the leaves of ora-pro-nobis submitted to hydrothermal processing (Lima et al., 2017; Palermo et al. 2014).

Both processes affected the free radical scavenging activity negatively. The activity was drastically reduced in leaves processed with dry heat, whereas in leaves processed with moist heat, the reduction was smaller (Table 3). Likewise, there was less retention of the bioactive compounds in leaves submitted to dry heat, compared to *in natura* leaves (Table 2) and this directly interfered with the antioxidant capacity.

The contents of bioactive compounds showed greater retention when submitted to moist heat, reflecting on its antioxidant activity. Even using a higher temperature (100 °C) during hydrothermal heating, the leaves of ora-pro-nobis were submitted to heat for less time (4 minutes). The leaves processed with dry heat were exposed for 8 hours at a lower temperature (70°C). Therefore, preparations involving hydrothermal processing lead to lower losses.



**Table 3.** Average values of antioxidant activity of ora-pro-nobis *in natura* leaves and post-processing (mean  $\pm$  standard deviation).

Processing	AA
<i>In natura</i>	20,00 $\pm$ 1,29 <sup>a</sup>
Moist heat	12,03 $\pm$ 0,41 <sup>b</sup>
Dry heat	0,82 $\pm$ 0,0033 <sup>c</sup>
CV (%)	13,56

AA = antioxidant activity ( $\mu\text{mol ET} / \text{g dry sample}$ ) e CV = Coefficient of variation. Averages followed by the same lowercase letter in the columns do not differ significantly from each other, at the significance level of 5% probability by the Tukey test.  
Source: Authors.

Heat treatment can present an effect on the antioxidant capacity since it promotes the destruction and/or oxidation of compounds with such potential (Mazzeo et al., 2011, Rajauria et al., 2010). In addition, both the increase in temperature and the residence time to which the leaves are submitted can cause a certain destabilization of plant cells, causing the availability of these compounds to be altered (Gliszczynska-Swiglo et al., 2006). Garcia et al. (2019) reported that the extract of ora-pro-nobis leaves showed high activity when evaluated by different methods (DPPH and ABTS).

Rigueira et al. (2016) evaluated the ability to neutralize free radicals from conventionally and organically grown kale leaves submitted to dry and moist heat treatment and reported 34.8% higher antioxidant activity in lettuce leaves grown in conventional systems, lettuce leaves grown in an organic system showed antioxidant capacity 36% higher in leaves submitted to dry heat compared to leaves submitted to moist heat.

### 3.3 Chemical profile by *paper spray*

Table 4 shows the qualitative analysis of the chemical profile of ora-pro-nobis *in natura* leaves and post-processing. The presence of 56 compounds of different chemical classes in the ora-pro-nobis leaves was detected. Many of these compounds are being reported for the first time in the literature for the leaves of *Pesreskia aculeata*.

Among the identified compounds, 26 of these are present in the class of phenolic compounds: Caffeic acid ( $m/z$  179), Ferulic acid ( $m/z$  193), Sinapic acid ( $m/z$  223), p-Hydroxynonanophenone ( $m/z$  285), Syringic acid acetate ( $m/z$  239), Piscidic acid isomer ( $m/z$  255), Kaempferol ( $m/z$  285), Dalbergioidin ( $m/z$  287), Catechin ( $m/z$  289), Nordihydrocapsiate ( $m/z$  293), Caffeic acid derivative ( $m/z$  295), Quercetin ( $m/z$  301), cis Caftaric acid ( $m/z$  311), Protocatechuic acid hexoxide ( $m/z$  315), Fertaric acid ( $m/z$  325), 1-caffeyolquinic acid ( $m/z$  353), Vacciheine A ( $m/z$  377), Quercetin-O-pentoside ( $m/z$  433), Kaempferol glucoside ( $m/z$  447), Kaempferol-3-O-rutinoside ( $m/z$  593), Quercetin-O-pentoside-O-hexoside ( $m/z$  595), Quercetin-3-O-rutinoside ( $m/z$  609), Isorhamnetin-3-O-rutinoside ( $m/z$  623), Quercetin-O-pentoside-O-rutinoside ( $m/z$  741), Isorhamnetin-O-pentoside-O-rutinoside ( $m/z$  755) e Isorhamnetin dirhamnoside hexoside ( $m/z$  760).

**Table 4.** Qualification of compounds in ora-pro-nobis in natura leaves and post-processing with dry heat and moist heat identified by PS. The letter X in the body of the table represents the presence of the compound in the sample.

Number	Attempted Identification	Formula	m/z	MS/MS	Reference	Processing		
						<i>In natura</i>	Dry heat	Moist heat
1	Malic acid	C4H6O5	133	115	(Cabañas-García et al., 2019)	X	X	X
2	Protocatechuic aldehyde	C7H6O3	137	121, 109	(Cabañas-García et al., 2019)	X	X	X
3	4,5-Dimethyl-2,6-octadiene	C10H18	138	123, 95, 69, 41	(Pinto et al., 2015)	X		
4	Dihydroxy methoxy butanoic acid	-	149	135, 131, 119, 103	(Cabañas-García et al., 2019)	X	X	X
5	Caffeic acid	C9H8O4	179	163, 135, 109	(Cabañas-García et al., 2019)	X	X	X
6	1-Methoxy-p-tolyl-propan-2-ol	-	181	144, 116, 103, 87, 73	(Pinto et al., 2015)	X	X	X
7	Azelaic acid	C9H16O4	187	169, 125	(Cabañas-García et al., 2019)	X	X	X
8	Quinic acid	C7H12O6	191	173, 111, 93, 85	(Rahman et al., 2017)	X	X	X
9	Ferulic acid	C10H10O4	193	149, 134	(Jiménez-Aspee et al., 2014)	X	X	X
10	Sebacic acid	C10H18O4	201	185, 157	(Cabañas-García et al., 2019)	X	X	X
11	Nerolidol	C15H26O	222	204, 161, 93, 69, 41	(Pinto et al., 2015)	X	X	X
12	Sinapic acid	C11H12O5	223	208, 179, 164	(Cabañas-García et al., 2019)	X	X	X
13	p-Hydroxynonanophenone	-	233	219, 167, 135, 121	(Cabañas-García et al., 2019)	X	X	X
14	Syringic acid acetate	-	239	197, 195, 179, 149, 135, 107	(Cabañas-García et al., 2019)	X	X	X
15	Piscidic acid isomer	-	255	193, 165, 135, 119, 107	(Cabañas-García et al., 2019)	X	X	X

16	Palmitic acid	C16H32O2	256	213, 129, 73, 60, 43	(Pinto et al., 2015)	X	X	X
17	Methyl palmitate	C17H34O2	270	227, 143, 87, 74, 55, 43	(Pinto et al., 2015)	X	X	X
18	Buteine	-	271	163, 137, 135, 121, 108	(Cabañas-García et al., 2019)	X	X	X
19	Neophytadiene	C20H38	278	123, 95, 68, 43	(Pinto et al., 2015)	X	X	X
20	Kaempferol	C15H10O6	285	255, 229, 151	(Rahman, 2016)	X	X	X
21	Dalbergioidin	C15H12O6	287	217, 179, 165, 163, 147, 125, 109	(Cabañas-García et al., 2019)	X	X	X
22	Catechin	C15H14O6	289	271, 245, 151, 137	(Rahman, 2016)	X	X	X
23	Linolenic acid methyl ester	C19H32O2	292	108, 95, 79, 67, 55, 41	(Pinto et al., 2015)	X		X
24	Nordihydrocapsiate	C17H26O4	293	277, 263, 247, 157, 153, 141	(Cabañas-García et al., 2019)	X	X	X
25	Caffeic acid derivative	-	295	179, 163, 133	(García, 2019)	X	X	X
26	Phytol	C20H40O	297	123, 95, 81, 71, 57, 43	(Pinto et al., 2015)	X	X	X
27	Methyl octadecanoate	C19H38O2	298	143, 87, 74, 55, 43	(Pinto et al., 2015)	X	X	X
28	Quercetin	C15H10O7	301	273, 179, 151	(Rahman, 2016)	X	X	X
29	3,5-Dihydroxy-4-methyloxolan-2-yl methoxy-6-hydroxymethyl oxane-3,4,5-triol	-	309	293, 279, 147, 131	(Cabañas-García et al., 2019)	X	X	X
30	<i>cis</i> Caftaric acid	C <sub>13</sub> H <sub>12</sub> O <sub>9</sub> -	311	179, 149	(García et al., 2019)	X	X	X
31	Protocatechuic acid hexoside	-	315	255, 211, 153, 137, 121, 109	(Cabañas-García et al., 2019)	X	X	X
32	Tricosane	C23H48	324	113, 99, 85, 71, 57, 43	(Pinto et al., 2015)	X	X	X
33	Fertaric acid	C14H14O9	325	193, 179, 163	(Cabañas-García et al., 2019)	X	X	X

34	Tianshic acid	C18H34O5	329	165, 127	(Cabañas-García et al., 2019)	X	X	X
35	Monogalloyl glucose	C13H16O10	331	271, 211	(Rahman, 2016)	X	X	X
36	Plastoquinone 3	C23H32O2	339	203, 163, 149, 135	(Cabañas-García et al., 2019)	X	X	X
37	1-Caffeoylquinic acid	-	353	191, 179, 135, 93	(Rahman, 2016)	X	X	X
38	Vacciheïn A	C18H18O9	377	361, 347, 319, 289, 125	(Cabañas-García et al., 2019)	X	X	X
39	9,10-dihydroxy-4,7-megastigma dien-3-one hexoside	-	385	223, 205	(Jiménez-Aspee et al., 2014)	X	X	X
40	Campesterol	C28H48O	400	382, 367, 315, 289, 213, 145, 43	(Pinto et al., 2015)	X	X	X
41	Stigmasterol	C29H48O	412	351, 271, 255, 159, 133, 83, 69, 55	(Pinto et al., 2015)	X	X	X
42	Sitosterol	-	414	396, 329, 303, 213, 145, 107, 81, 57, 43	(Pinto et al., 2015)	X	X	X
43	Taraxerol	C30H50O	426	302, 287, 204, 135, 95, 69	(Pinto et al., 2015)	X	X	X
44	Quercetin-O-pentoside	-	433	300, 255, 151	(Rahman, 2016)	X	X	X
45	Lucuminic acid	C19H26O12	445	163, 119, 107	(Cabañas-García et al., 2019)	X	X	X
46	Kaempferol glucoside		447	284, 256, 151	(Rahman, 2016)	X	X	X
47	Quercetin-3-glucoside	C21H20O12	463	300, 179, 151	(Rahman, 2016)	X	X	X
48	Isorhamnetin 3-O- $\beta$ -glucoside	-	477	315	(Jiménez-Aspee et al., 2014)	X	X	X
49	Procyanidin B1	C30H26O12	577	451, 425, 407, 289, 161, 137, 125	(Rahman, 2016)	X	X	X
50	Kaempferol-3-O-rutinoside	C27H30O15	593	285	(Garcia et al., 2019)	X	X	X

51	Quercetin-O-pentoside-O-hexoside	-	595	463, 301	(Garcia et al., 2019)	X	X	X
52	Quercetin-3-O-rutinoside	C27H30O16	609	301	(Garcia et al., 2019)	X	X	X
53	Isorhamnetin-3-O-rutinoside	-	623	315	(Garcia et al., 2019)	X	X	X
54	Quercetin-O-pentoside-O-rutinoside	-	741	609, 301	(Garcia et al., 2019)	X	X	X
55	Isorhamnetin-O-pentoside-O-rutinoside	-	755	623, 315	(Garcia et al., 2019)	X	X	X
56	Isorhamnetin dirhamnoside hexoside	-	769	315	(Jiménez-Aspee et al., 2014)	X	X	X

Source: Authors.

Although the content of total phenolic compounds changed after thermal processing (Table 2), there was no variation in the profile of the constituents of this class of compounds in those processed with both dry and moist heat in relation to *in natura* leaves (Table 4). Thus, the processing may have affected quantitatively some of the compounds belonging to this class, which were qualified in this study.

Concerning other classes of compounds, changes in the chemical profile were observed. For example, the substance 4,5-Dimethyl-2,6-octadiene was detected only *in natura* leaves, the compound Linolenic acid methyl ester was not detected in the leaves processed with dry heat. Garcia et al. (2019) identified ten phenolic compounds in ora-pro-nobis leaves from southern Brazil, namely, two phenolic acids (derived from caffeic acid) and eight flavonoids (quercetin, kaempferol, and derivatives of the glycoside isorhamnetin). They also reported that caffeic acid was the main phenolic constituent of the extract, accounting for more than 49% of the phenolic content, followed by quercetin-3-O-rutinoside (14.99%) and isorhamnetin-O-pentoside-O-rutinoside (9.56%), nevertheless the stability of these compounds in ora-pro-nobis leaves after processing with dry heat and moist heat was not known. All the compounds previously mentioned were not affected by any of the processing methods used in this work.

Using ultra-high performance liquid chromatography (UHPLC) to analyze the phytochemical profile of *Coryphantha macromeris* (Cactaceae), Cabañas-García et al. (2019) detected the presence of the compound 3,5-Dihydroxy-4-methyloxolan-2-yl methoxy-6-hydroxymethyl oxane-3,4,5-triol ( $m/z$  309) in the roots of the species. According to Vankudothu & Anwar (2014), this compound presents properties against type 2 diabetes. Teixeira (2018) also found the presence of Caffeic acid ( $m/z$  179) and Ferulic acid ( $m/z$  193) *in natura* leaves of ora-pro-nobis, the Quercetin compound ( $m/z$  301) was not detected.

Souza et al. (2016) detected the presence of Phytol ( $m/z$  297) and Methyl octadecanoate ( $m/z$  298) in the essential oil extracted from *Pereskia aculeata* Mill. leaves collected on the campus of the Universidade Federal do Rio Grande do Sul (Porto Alegre). The former has the potential to treat lung cancer (Islam et al., 2018).

Analyzing the chemical profile and the contents of bioactive compounds present in food are extremely important so that such information can be taken into account in decisions concerning the type of processing to which the food will be submitted. Despite the presence of compounds with high antioxidant capacities in the fresh form, it is important to clarify the behavior of these components under processing conditions.

#### 4. Conclusion

The content of bioactive compounds present in ora-pro-nobis leaves processed with dry heat was drastically reduced, which consequently affected the scavenging activity of free radicals, which showed a decrease of 93.18% when compared to the processing of ora-pro-nobis leaves with moist heat, and 95.90% when compared to unprocessed leaves.

The chemical profile of the leaves was also affected by processing, in which the compound 4,5-Dimethyl-2,6-octadiene was detected only *in natura* leaves, the compound Linolenic acid methyl ester was not detected in the leaves processed with dry heat. The profile of phenolic compounds in ora-pro-nobis leaves was not affected by thermal processing.

The acknowledgement of the effect of processing in food is extremely important in order to understand the behavior of compounds that act in a beneficial way in human health, and thus identify the best way to process and consume them, being also extremely important in the pharmaceutical industry, which uses these compounds for the production of drugs.

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