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Evaluation of Cooking Methods in the Phenolic Content and Antioxidant Activity in *Araucaria angustifolia* Seeds

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Araucaria angustifolia seeds (pinhão) are a typical and popular food source in the southern region of Brazil, which contain high amount of carbohydrates and dietary fibers. They are also rich in bioactive compounds highly beneficial to human health. The objective of this study was to evaluate three processing methods of the pinhão with a focus on their centesimal composition and its antioxidant properties, testing different types of solvents in the extraction of the phenolic compounds. The processed pinhão was evaluated in three different forms: raw, cooked flour and grits (toasted cooked pinhão). Centesimal composition, total phenolic content and the antioxidant capacity (DPPH) were measure in three different solvent extractors (0.1 g/mL): aqueous, ethanolic and hydroethanolic. The bioactive compounds obtained in the cooked pinhão flour showed the highest concentration of phenolic compounds and, consequently, the highest antioxidant activity. Comparing the solvents used, the hydroethanolic and aqueous extracts were the most efficient in the extraction of the bioactive compounds from the pinhão.
Key Words: Bioactive compounds, extraction solvents, pinhão, DPPH.

1. Introduction

The *Araucaria angustifolia* is a typical tree species from the southern region of Brazil, which is also found in the south-eastern part of the country and in neighboring countries like Paraguay and Argentina, in mountain range regions and high plains. This species is naturally found in Mixed Ombrophilous Forests, being their most important representative (Silva, 2013). Uncontrolled exploitation of this tree, due to the quality of its wood, resulted in a large-scale impact on the forest of Brazilian pines (*Araucaria angustifolia*), reducing its population considerably. This fact resulted in negative environmental impacts on the biological diversity and on the food chain balance of this ecosystem (Carvalho, 2010).

Usually, pinhão are eaten cooked or roasted, and are used in typical dishes and some delicacies. In spite of its popularity, industrialization of pinhão is almost non-existent, not because of any technical difficulties involved in its processing, but due to the lack of a more industrial culture developed in producing regions, making it difficult to include the seeds in diversified food products (Santos et al., 2002). Pinhão processing studies could enable the commercialization throughout the year, which otherwise would be difficult as they are a seasonal sample. In addition to being an extra source of income, it boosts the production chain by adding value to the seeds, while reinforcing the need of preserving the Brazilian pines, as they are threatened of extinction.

Nowadays, studies have been carried out to determine different forms of pinhão processing. One of the most studied methods is to produce pinhão flour, which may extend their shelf-life by dehydration, reducing physical, chemical and enzymatic deterioration. Additionally, flour and grits are also an interesting alternative for pinhão based products. In this way, pinhão presents a high nutritional value, being a source of starch and dietary fibers, with a low fat content, and also a good source of bioactive compounds, such as phenolic compounds with antioxidant properties (Costa, 2014; Conto et al., 2015; Thys and Cunha, 2015).

Phenolic compounds are secondary metabolites in plants, that presents an important function in the protection and, usually, appearing as a defense mechanism in plants. They present a large structural variety and are

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highly reactive. The most important types are flavonoids, anthocyanins, catechins, tannins (polyphenols) and phenolic acids (simple phenols). These compounds can influence the nutritional value and the sensory quality of foods, giving those colour, texture, bitterness and astringency characteristics (Rockenbach et al., 2008).

Antioxidants are a group of substances that may be composed of vitamins, minerals, natural pigments and other plant compounds, and also by enzymes, which prevent oxidation activity. Some examples of these substances are the phenolic compounds, carotenoids, ascorbic acid (vitamin C), D-alpha tocopherol (vitamin E), among others (Lushchak, 2011; Bianchi and Antunes, 1999; Angelo and Jorge, 2007). These compounds present the capacity to prevent oxidative processes in foods and in living organisms, being also responsible for cellular aging and are closely linked to some chronic degenerative diseases, such as cancer, diabetes and Alzheimer. These disorders are caused by oxidative stress, as a result of the imbalance between the oxidative molecules (in greater number) and antioxidants (Degáspari and Waszczynskyj, 2004).

In foods, these antioxidants also participate in reactions, especially lipid oxidation reactions. These reactions cause modifications in sensory characteristics, nutritional loss and the formation of undesirable substances, which may even be toxic, reducing the product shelf life (Araújo, 2011; Barreiros et al., 2006).

A decisive factor in the determination of phenolic compounds is the extraction of these compounds, and its yield and composition depend especially on the solvent used, and the method applied. These parameters may change according to the polarity of the solvent used and its interaction with the sample, since the phenolic compounds have a wide structure variety and high polarity (Rockenbach et al., 2008).

Pinhão presents significant quantities of phenolic compounds, which are concentrated in the coat and move to the seeds after the cooking process. The main group of phenolic compounds in the coat of the pinhão are the condensed and hydrolysable tannins with high molecular weight; these compounds demonstrate higher antioxidant activity when compared to simple phenolic compounds (Koehnlein et al. 2012; Thys and Cunha, 2015).

The aim of this work was to evaluate the centesimal composition of raw pinhão flour, cooked pinhão flour and pinhão grits, as well the presence of phenolic compounds and the antioxidant activity (DPPH) using different extractors solvents.

2. Material and Methods

Samples of pinhão were bought in Lages and Urupema City (Santa Catarina State, Brazil), in 2014 and 2015. Seeds were freeze-stored at -18°C for later use.

2.1 Sample Preparation

Three types of pinhão processing were tested: raw pinhão flour; pinhão grits; and cooked pinhão flour. The raw pinhão flour was obtained after dehulling, crushing, dehydration (60 °C, 12 hours) and final milling, as described by Capella et al. (2009). The pinhão grits were made from cooked, dehulled and ground seeds (for obtaining particles smaller than 0.5 cm), and then they were roasted at 115 °C until constant weight, as described by Conto et al. (2015). The cooked pinhão flour was processed using cooked pinhão, ground and sieved, after dehydration, as described by Capella et al. (2009).

2.2 Determination of the Centesimal Composition

The centesimal composition was determinate by the analyses of moisture, ashes and proteins (AOAC, 2000). The determination of lipids followed the methodology proposed by Bligh and Dyer (1959). The quantification of total carbohydrates was done using the 'difference' method (carbohydrates = 100 – (moisture + ashes + proteins + lipids). All the results were expressed as g/100g.

2.3 Phenolic Extraction

Three extracts solutions were tested, described by Koehnlein et al. (2012) and Sganzerla et al. (2018): hydroethanolic (70% ethanol and 30% water); aqueous (100% water); and ethanolic (100% ethanol). The extracts used in the analyses of bioactive compounds and antioxidant activity were prepared using 10 g of sample, dissolved in 100 mL of the respective solvent, centrifuged for 3 hours (140 rpm) and then filtered. The extracts were kept at -18 °C until the time of analysis.

2.4 Quantification of Total Phenolic Content

The determination of total phenolic content followed the method proposed by Swain and Hillis (1959), with some modifications. Aliquots of 140 µL of each extract were mixed with 1667 µL of distilled water, and afterwards with 104 µL of the reagent Folin–Ciocalteu (0.25 N). After 3 minutes of reaction, 208 µL of Na_2CO_3 1 N was added, and the mixture was incubated for 2 hours at room temperature. A blank was prepared with

104 µL of the solvent used for each extract. The absorbance of the solution was measured at 725 nm and chlorogenic acid was used as standard.

2.5 Determination of Antioxidant Activity

The antioxidant activity was determined by removal of free radical DPPH (2.2-diphenyl-1-picrylhydrazyl), as described by Brand-Williams et al. (1995). The reaction was composed by 150 µL of extracts and 2850 µL of DPPH (1 mM), and a blank was prepared with 150 µL of ethanol. After 24 hours of incubation at 25 °C, the reaction was measured at 515 nm and Trolox was used as the standard solution for the calibration curve. DPPH percentage of inhibition was calculated using the Equation 1 described by Koehnlein et al. (2012).

$$\text{DPPH inhibition (\%)} = \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \times 100 \ (1)$$

2.6 Statistical Analysis

All analyses were conducted in triplicate (*n*=3), and the extracts were prepared in triplicate. Data were subjected to Analysis of Variance (ANOVA) and treated statistically using the software STATISTICA 7.0 (StatSoft, Inc., Tulsa, OK, EUA) along with the Tukey's Test at 95% significance level ($p \leq 0.05$).

3. Results and Discussion

Results for moisture content, ashes, proteins, lipids and carbohydrates are shown in Table 1. In all parameters measured there were significant statistical differences depending on the processing method used, with the exception of proteins values.

Table 1: Centesimal composition (%) of raw pinhão flour, cooked pinhão flour and pinhão grits.

Sample	Moisture*	Ashes*	Proteins*	Lipids*	Carbohydrates*
Raw pinhão flour	5.87 ± 0.09^{b}	3.22 ± 0.03^{a}	5.09 ± 0.04^{a}	1.70 ± 0.30^{b}	89.98 ± 0.35^{ab}
Cooked pinhão flour	7.17 ± 0.35^{a}	2.55 ± 0.02^{b}	5.74 ± 0.13^{a}	3.06 ± 0.27^{a}	88.64 ± 0.30^{b}
Pinhão grits	2.30 ± 0.21^{c}	2.35 ± 0.13^{c}	5.50 ± 0.61^{a}	1.73 ± 0.34^{b}	90.75 ± 0.95^{a}

* The results represent the averages of three determinations. Samples followed by the same letters in the same column do not differ significantly ($p > 0.05$).

Cooked pinhão flour presented the highest moisture content, followed by the raw pinhão flour and pinhão grits. The results obtained reflect the processes utilized in each sample. Moisture content was lower than the result obtained by Capella et al. (2009) for raw pinhão flour and cooked pinhão flour (8.29% and 13.88% respectively).

The high level of moisture presented by non-processed pinhão, approximately 50%, makes it highly susceptible to deterioration. Such a condition is ideal for the germination and action of microorganisms, making it very difficult to sell the seeds all year round (Schveitzer et al. 2014). In this way, the flour and grits are good alternatives for the commercialization of the pinhão. The flours produced for this study met the specific requirement concerning the maximum moisture content permitted, 15% according to the Directors' Collegiate Resolution (RDC) nº 263, from September 22nd 2005 (Brasil, 2005).

Ash contents were statistically different ($p < 0.05$) and the highest content of minerals was found in the raw flour, followed by the cooked flour and grits. Similar results were also obtained by Gama (2006) for raw (3.15%) and cooked (2.14%) pinhão. Capella et al. (2009) obtained average ash contents of 2.53% for the raw pinhão flour and 3.01% for the cooked pinhão flour, indicating the effect of processing on the pinhão mineral content.

Proteins values were similar in all three samples evaluated. Similar results were also obtained by Thys and Cunha (2015) for raw (4.29%), cooked (4.22%) and roasted (4.49%) pinhão flour.

Cooked pinhão flour presented the highest lipids content, being statistically different from the other samples ($p < 0.05$). However, the raw flour and grits had similar values for lipids. Thys and Cunha (2015) found different values of lipid contents than the values found in this work, 2.19% for raw pinhão flour and 1.73% for cooked pinhão flour. The difference in the lipid values found can be justified by the method of pinhão preparation, since when passing by high temperatures processing (as in the grits case) the lipids can create bonds with starch, main constituent of this product, being counted in the fraction of carbohydrates; or even lost in the bakery process. Statistical differences were found in carbohydrates contents between the grits and the cooked flour; on the other hand, in the raw flour the results were similar in both samples. Carbohydrate content (on a dry basis) was similar to the content obtained by Capella et al. (2009) for the raw pinhão flour and cooked pinhão flour (88.01% and 88.49%, respectively). Gama (2006) obtained results of carbohydrate (87.26%, 87.17% and 85.25%, for the raw, cooked and roasted pinhão, respectively), dietary fibers (15.70%, 17.34% and 15.09%, respectively) and starch (32.72%, 33.80% and 32.17%, respectively). Among the carbohydrates of the pinhão, there is a predominance of starch and dietary fibers.

The consumption of foods with high starch content contributes to increase the sense of satiety, resulting in lower blood sugar levels after meals (Gama, 2006). The fiber consumption presents an important role in the functioning of the human gastrointestinal system, and they are also associated with the reduction of cholesterol and glucose control, and consequently of diabetes (Gama, 2006; Pereda, 2005). The differences in the results were already expected, were in different maturation stages, there were from different varieties and origins, and also due to experimental differences and environmental conditions.

Table 2 shows the results for the determination of total phenolic compounds and antioxidant activity (DPPH) of the samples of raw and cooked pinhão flour and pinhão grits, according to each type of extract.

Table 2: Determination of total phenolic compounds and antioxidant activity (DPPH) of the raw pinhão flour, cooked pinhão flour and pinhão grits.

Sample	Extract	Total phenolics (g eq. chlorogenic acid /100mL extract)	Total phenolics (g eq. chlorogenic acid /100g sample)	DPPH µg Trolox equivalent/100 mL extract	DPPH % inhibition
Raw pinhão flour	Aqueous	0.17 ± 0.01^{c}	1.71 ± 0.08^{c}	8.35 ± 0.08^{d}	41.12 ± 0.39^{b}
	Hydroethanolic	0.09 ± 0.00^{e}	0.89 ± 0.02^{e}	2.43 ± 0.12^{f}	16.82 ± 0.46^{e}
	Ethanolic	0.04 ± 0.00^{g}	0.35 ± 0.03^{g}	1.22 ± 0.05^{g}	4.88 ± 0.22^{f}
Cooked pinhão flour	Aqueous	0.24 ± 0.01^{b}	2.43 ± 0.06^{b}	16.18 ± 0.05^{b}	79.72 ± 0.25^{a}
	Hydroethanolic	0.27 ± 0.01^{a}	2.72 ± 0.08^{a}	20.13 ± 0.19^{a}	82.14 ± 0.78^{a}
	Ethanolic	0.06 ± 0.00^{f}	0.61 ± 0.02^{f}	1.62 ± 0.09^{g}	6.49 ± 0.34^{f}
Pinhão grits	Aqueous	0.15 ± 0.00^{d}	1.46 ± 0.04^{d}	5.89 ± 0.31^{e}	29.04 ± 1.53^{d}
	Hydroethanolic	0.08 ± 0.00^{e}	0.85 ± 0.04^{e}	9.00 ± 0.47^{c}	36.73 ± 1.90^{c}
	Ethanolic	0.00 ± 0.00^{h}	0.04 ± 0.05^{h}	0.12 ± 0.02^{h}	0.54 ± 0.01^{g}

The results represent the averages of three determinations. Samples followed by the same letters in the same column do not differ significantly ($p > 0.05$).

Analysis of variance (ANOVA) revealed that the cooked pinhão flour yielded the best results, as well for total phenolic compounds as for DPPH, when the mixture of ethanol and water (70:30 ratio by weight) was used, followed by water. It can be explained by the migration of the bioactive compounds from the coat to the seeds during the cooking process. This fact was also demonstrated in the study about total phenolic conducted by Darolt and Helm (2012), where the fresh coat had 4198.75 mg eq. chlorogenic acid / 100 g sample, and after the cooking process only 1215.82 mg eq. chlorogenic acid / 100 g sample remained in the seed coat.

The higher total phenolic content observed in the cooked seeds is explained by the fact that the cooking process opens the membranes of the cell wall of the seed coat, facilitating the migration of these compounds into the seeds (Thys and Cunha, 2015). The partial hydrolysis of the tannins present in the seed coat can also occur, producing simple phenolic that migrate to the seed more easily (Koehnlein et al., 2012). Thys and Cunha (2015), when determining the total phenolic compounds obtained the highest contents (22.09 mg GAE/100 g of sample) for the cooked flour, when compared to the raw flour (5.75 mg GAE/100 g of sample).

Several methods and solvent systems are being studied for the extraction of bioactive compounds, making it possible its quantification and the determination of the antioxidant capacity. The polarity of the solvent is the most important factor, considering that phenolic compounds have different polarities (varying from simple to highly polarized). That being the case, solubility in a given solvent is a distinctive characteristic of the compound. Due to this fact, there isn't any specific extraction system that would be satisfactory to analyse the varied group of compounds due to the diversity of the chemical structures and the variations of the sensitivity of the compounds according to the extraction conditions and the environment (Rockenbach et al., 2008).

After all samples analysed were compared, it was found that when the seeds were processed as grits they presented a lower content of bioactive compounds and lower antioxidant activity when the solvent ethanol was used. To obtain grits, the pinhão are cooked and roasted at 115 °C, which was possibly the cause for the degradation of the phenolic compounds. Furthermore, it should be taken into consideration that the ethanol, by virtue of its polarity, may not have extracted effectively all compounds present in the seeds.

In the case of the raw pinhão flour, the aqueous extract was the most efficient in the extraction of the phenolic compounds, followed by the hydroethanolic and ethanolic extracts, showing a similar behaviour to the grits. When the antioxidant activity was measured, the same order of effectiveness of the solvents in the raw flour was observed. However, in grits the hydroethanolic extract exhibited the highest scavenging capacity of the DPPH radical.

The content of total phenolic compounds obtained was higher than the one obtained by Koehnlein et al. (2012) 0.29 and 1.20 g eq. gallic acid/100 g for raw and cooked seeds, respectively, using ethanol 70% for the extraction. While the DPPH inhibition percentage obtained was lower than the ones obtained by Thys and Cunha (2015) with raw (72.71%), cooked (141.04%) and roasted (67.33%) seeds flour, but it should be taken into consideration that methanol and acetone 70% were used for the extraction of the compounds.

Rockenbach et al. (2008), analysing total phenolic compounds in grape pomace with different extraction solvents, obtained the highest concentration by using ethanol 50% (7.32 g GAE/100 g of dry weight), followed by ethanol 70% (5.86 g GAE/100 g of dry weight), ethanol 100% (2.73 g GAE/100 g of dry weight), and water (1.48 g GAE/100 g of dry weight). Vieira et al. (2009) studied the total phenolic content in residues from the industrialization of *Ilex paraguariensis*, by different solvents extracts. They observed the methanolic extract (80%) exhibited the highest content of total phenolic compounds (11.51 g GAE/100 g), followed by the ethanolic extract (80% - 2.8 g GAE/100 g) and water extract (1.23 g GAE/100 g).

In this way, the best solvent for extraction is the one that shares the most physical and chemical characteristics with phenolic compounds present in the different samples evaluated. In addition to that, foods have a complex matrix of different components, which can establish among themselves and with the solvents, a wide range of different forms of interactions (Rockenbach et al. 2008).

4. Conclusion

With a view to preserving the *Araucaria angustifolia* and raising the shelf life of pinhão, the effect of processing could be observed, and it is possible to conclude that the processing of the pinhão influences its composition, maintaining the protein content. However, ash content was higher in raw flour; the cooked flour revealed the highest content of moisture and lipids; and the highest carbohydrate content was found in grits and raw flour. With regard to the bioactive compounds, cooked pinhão flour had the highest concentration of phenolic compounds and consequently the highest antioxidant activity, probably caused by the migration of these compounds from the coat to the seed during the cooking process. As for the solvents used, the hydroethanolic and aqueous extracts were the most efficient in the extraction of the bioactive compounds from the pinhão. It is due possibly to the polarity of the compounds and their interaction with the solvent. Based on these facts, the pinhão processing is an interesting technological alternative, since they have nutritional and antioxidant properties, in addition to being a way of adding value to the seeds.

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