Physico-chemical characterization and antioxidant content of Physalis fruits

Chaiane Renata Grigolo
Marisa de Cacia Oliveira
Edenes Schroll Loss
Juliane Ropelato
Tatiane Oldoni
Cintia Boeira Batista

Abstract

Physalis berries, from the Solanaceae family, have achieved wide acceptance worldwide due to the flavor of the fruit and its possible medicinal use. This study aimed to evaluate the antioxidant activity, the concentrations of vitamin C, phenolic compounds and sugars of fruits of two species of Physalis (Physalis pubescens L. and Physalis peruviana L.), as well as their variations during storage at two different temperatures. The Physalis was planted in the west of Santa Catarina. The fruits were harvested when the capsules were pale yellow in color and then divided into three groups: fresh, chilled, and frozen. The pH, total soluble solid, total soluble sugars, phenolic compounds, vitamin C and antioxidant activity were evaluated. The fresh fruits of both species presented better results for most of the parameters analyzed compared to the refrigerated and frozen fruits. Antioxidant activity was higher in fresh fruits for the two Physalis species, experiencing a decrease when conditioned at low temperatures. The antioxidant benefits and nutraceutical compounds are best exploited when the fruits are consumed fresh without any storage process at low temperatures.

Keywords: Physalis peruviana, Physalis pubescens, storage.

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Introduction

The search for healthier foods grown without pesticides and with properties that contribute to the proper functioning of the human body has led to an increase in the consumption of various varieties of vegetables and fruits. Among these is Physalis, whose fruit has a mild sweet taste, substantial levels of vitamin A and C, iron, phosphorous, and good levels of phenolic compounds.

Among the more than 120 described species (Li et al., 2008), Physalis peruviana is the only one that is cultivated and marketed worldwide (CABI, 2017). Therefore, P. peruviana is also one of the most studied species with a focus on the production and presence of bioactive compounds (Tomassini et al., 2000; Puente et al., 2011; Olivares-Tenorio et al., 2016).

However, other Physalis species have the potential to produce fruits intended for human consumption and also to be used as a source of compounds with some biological activity (Li et al., 2008; Medina-Medrano et al., 2015), such as the case of Physalis pubescens, which has been given grass status (Lorenzi, 2008), but has the potential to become an alternative crop for small and medium farmers (Bertoncelli et al., 2016; Ariati et al., 2017).

The presence of compounds with antioxidant potential in Physalis fruits, especially vitamin C and phenolic compounds, make the species of this genus an object of interest. Antioxidants are known to act on free radicals that can lead to positive health benefits, such as delaying premature aging and the development of disease, among others (Fontana et al., 2000).

Physalis is mainly consumed fresh, but there is little research, especially on the species Physalis pubescens, on the chemical composition of fruits. In this scenario, the deepest scientific studies are important for its characterization, identification and quantification of compounds present in fruits, thus allowing the development of new products with Physalis as raw material, adding greater value to this fruit tree (Chaves et al., 2004). Due to the low cost of implantation, ease of handling and favorable edaphoclimatic adaptation, the cultivation of Physalis has been gaining ground, allowing an alternative income for rural producers.

The ripening stage of the fruits is related to various chemical and physical alterations and the harvest, as well as the subsequent conservation, are important points to guarantee the best characteristics of the product and its maintenance.

Seeking to explore the production and consumption potential of Physalis pubescens and Physalis peruviana, this study aimed to evaluate the antioxidant activity, the concentrations of vitamin C, phenolic compounds and sugars, as well as their variations during storage at two different temperatures. The hypothesis of the work is based on the fact that Physalis fruits subjected to storage at low temperatures reduce antioxidant activity and lose molecules responsible for the physicochemical characteristics of fresh fruits.
Materials and methods

Obtaining the material

The seedlings of *P. peruviana* and *P. pubescens* were obtained from seeds of plants grown in beds at the Universidade Tecnologica Federal do Parana (UTFPR), *Campus* Pato Branco. The seeds were placed in 250 ml pots containing previously analyzed soil substrate for nutritional determination. As a result of these analyzes, bovine organic fertilizer was used to supplement the macronutrients, according to the studies by Bertoncelli *et al.* (2016); Ariati *et al.* (2017).

Plant installation and cultivation of plants

Two seeds were sown per pot and 25 days later, only one plant per pot was maintained after thinning. The Physalis pots were kept in a greenhouse until they reached approximately 15 cm in height and the field capacity of the soil was maintained by manual watering as necessary.

Later, the seedlings were transplanted into a field on a private property in Galvão - SC located at 26° 27’18”S and 52° 41’09” W, with an altitude of 655 m, in October 2016. The weather in the region, confirming with the Köppen classification, is Cfa (humid subtropical climate). According to data from the meteorological website Climatempo (2017), during the period that the plants remained in the field, the average precipitation was 174 mm, with a minimum temperature of 15 ºC and a maximum of 25 ºC.

Before transplanting seedlings into the field, the soil was corrected by liming and fertilizing with livestock manure, as determined by the soil analysis report. Each plant received 1 500 kg of manure, this amount was calculated from the nitrogen, phosphorus and potassium contents determined by the compost analysis. Values were based on the study developed by Bertoncelli *et al.* (2016), which indicated the ratio of 300: 600: 500 kg ha⁻¹ NPK.

Fruit harvest

The fruits were harvested when the capsules were pale yellow, approximately 100 days after planting in the field, and then underwent the following treatments: fresh fruits, which were analyzed as soon as they were collected; fruits stored for 60 days at 4 ºC (refrigerated fruits) and -78 ºC (frozen fruits).

Sample analysis

Hydrogen potential (pH)

For the determination of the pH, first 5 grams of fruit were weighed for each of the samples and then, they were crushed in 50 ml of distilled water. The mixture was filtered to obtain only the juice. Direct reading was done on a 93% calibrated pH meter.
Total soluble sugars

The determination of total soluble sugars was performed using the sulfuric phenol method described by Dubois et al. (1956). Absorbance readings were taken by spectrophotometer at 490 nm and sugar concentrations were determined using a standard glucose curve and expressed in mg of glucose 100 g^{-1} of fruit.

Total soluble solids

The total soluble solids content was obtained by direct readings on a digital refractometer, using filtered and homogenized fruit pulp, at room temperature, obtaining the values in degrees Brix (°Brix).

Total phenolic compounds

The determination of total phenolic compounds was carried out using the Folin-Ciocalten method, described by Singleton (1999). Extraction was performed using 70% ethanol as solvent. The samples were shaken at 60 °C for 30 min. Absorbance readings were taken at 740 nm by spectrophotometer using gallic acid as standard. The results were expressed as mg EAG 100 g^{-1} of fruit.

Vitamin C

The analyzed juices were obtained by manual extraction. The samples were brought to a known volume with 3% metaphosphoric acid, filtered, and placed in amber flasks. Ascorbic acid was determined by high performance liquid chromatography with standard ascorbic acid under the following conditions: C18 ACE column, mobile phase eluting in isocratic mode with phase A composed of water acidified with 0.05% acetic acid and phase B, methanol, in the ratio of 30:70 with a flow of 1.0 mL min^{-1}, column temperature 30 °C and diode array detector (DAD) operating at wavelengths of 210 nm and 243 nm. The injection of 20 µl was made, with a total execution time of 9 minutes. The results were expressed as mg of ascorbic acid 100 g^{-1} of fruit.

Antioxidant activity

Determination of antioxidant activity was based on the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method expressed in reference to Trolox. Aliquots of 0.5 mL of samples were added to 3 mL of ethanol and 0.3 mL of DPPH solution. The negative control was performed under the same conditions, but without the addition of DPPH, using a volume of 3.3 mL of ethanol. The samples were kept in the dark for 30 min. Subsequently, the absorbance readings were taken in a spectrophotometer at 517 nm. The results were expressed in µmol of Trolox per gram of fruit and were calculated from a Trolox curve.

Statistical analysis

The experimental design was completely randomized with four repetitions and three treatments (fresh, chilled and frozen fruits). The same study was carried out with two different species of
Physalis (*P. peruviana* and *P. pubescens*). The data were subjected to analysis of variance and the means were compared using the Tukey test, with a significance level of 95% (*p* < 0.0505), using the OriginPro 8.5 software (2017).

**Results and discussion**

The effect of temperatures on Physalis storage led to a slight accumulation of acids in chilled and frozen fruits. However, only *P. pubescens* had a significantly different pH (Table 1).

<table>
<thead>
<tr>
<th>Storage</th>
<th><em>Physalis pubescens</em></th>
<th><em>Physalis peruviana</em>ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh fruits</td>
<td>4.66 a*</td>
<td>4.38</td>
</tr>
<tr>
<td>Chilled fruits</td>
<td>4.38 b</td>
<td>4.26</td>
</tr>
<tr>
<td>Frozen fruits</td>
<td>4.37 b</td>
<td>4.26</td>
</tr>
</tbody>
</table>

* = mean values of the three replicates. ns = not significant. The means followed by different letters in the same column statistically differ from each other by the Tukey test (*p* < 0.05).

During storage and the respiratory process, metabolization of organic acids is common, resulting in higher pH values (Aguiar *et al.*, 2012; Lima *et al.*, 2013). When fruits are stored at low temperatures, respiration tends to decrease and, as a result, the medium becomes acidic and organic acids accumulate. Muniz *et al*. (2017) observed the same behavior for strawberry and guava fruits, that when stored at 2 °C, the pH was lower (pH = 3.17) than those that were kept at 25 °C ambient (pH = 3.21).

For *P. peruviana*, Lima *et al*. (2013) observed lower pH values, which varied between 3.4 and 3.5 for fruits stored at 4 °C, and these pH values increased slightly over 8 days, but remained lower than those obtained with fruits kept at 20 °C (pH = 3.6).

Similar results were found in studies by Silva *et al*. (2013), when the pH behavior for *P. peruviana* fruits subjected to a temperature of 5 °C showed a strong decrease with increasing storage time, varying from 3.85 to 3.67 at 28 days of storage. The results found by Silva *et al*. (2013) differ from the results found for *P. peruviana* in the present study. Silva *et al*. (2013); Lima *et al*. (2009) found lower pH ranges of 3.52 to 3.61 for fresh fruits of *P. peruviana*, as well as Bertoncelli *et al*. (2016); Ariati *et al*. (2017) that obtained values of 3.4 to 3.8 for *P. pubescens*.

These studies differ from the results found in this work but can be explained by the different growth conditions. For Lanchero *et al*. (2007), the pH of the fruit varies greatly depending on the conditions in which the plants are grown and, therefore, it is not very efficient to help determine the most appropriate harvest time.

In both Physalis species, the concentrations of total soluble sugars in fresh fruits were higher. At low temperatures there were significant differences between the treatments showing that soluble sugars were partially lost in the stored fruits (Table 2).
Table 2. Total soluble sugars (mg of glucose 100 g⁻¹ of fruit) of fruits of two species of Physalis subjected to different storage conditions.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Physalis pubescens</th>
<th>Physalis peruviana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh fruits</td>
<td>39.38 a*</td>
<td>36.9 a</td>
</tr>
<tr>
<td>Chilled fruits</td>
<td>33.25 b</td>
<td>28.4 b</td>
</tr>
<tr>
<td>Frozen fruits</td>
<td>33.6 b</td>
<td>25.95 c</td>
</tr>
</tbody>
</table>

* = mean values of the three replicates. The means followed by different letters in the same column statistically differ from each other by the Tukey test (p < 0.05).

Olivares-Tenorio et al. (2017) evaluated three storage temperatures for *P. peruviana* (4, 8 and 12 °C) and found an increase in glucose and fructose content during the storage period (up to 76 days), while sucrose levels decreased. However, several different results were obtained in studies with different plant species.

In zucchini, in one of the varieties tested there was a higher concentration of soluble sugars throughout the storage period at a temperature of 4 °C, while in the second variety there were no changes. At 20 °C in both varieties there was a decrease in soluble sugars with storage time (Palma et al., 2014). Mango fruits stored at 4 °C and 30 °C showed increases in the concentration of total soluble sugars until day 12 of storage, while at -10 °C the values remained almost unchanged (Hossain et al., 2014). In the specific case of Physalis, the lowest values of total soluble sugars in refrigerated fruits can mean a low and complementary respiratory rate, Gorny et al. (1998) shows that even at low temperatures (0 to 5 °C) respiration persists for various plant species, and that it depends on the concentration of O₂ and CO₂ in the environment.

The reduction of sugars was also observed through the analysis of total soluble solids (Table 3). The fresh fruits of both species presented values of 15 °Brix, with small losses when they were stored at low temperatures, but without significant differences between refrigeration and freezing.

Table 3. Total soluble solids (°Brix) of fruits of two Physalis species subject to different storage conditions.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Physalis pubescens</th>
<th>Physalis peruviana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh fruits</td>
<td>15 a*</td>
<td>15 a</td>
</tr>
<tr>
<td>Chilled fruits</td>
<td>14 b</td>
<td>13 b</td>
</tr>
<tr>
<td>Frozen fruits</td>
<td>14 b</td>
<td>12 b</td>
</tr>
</tbody>
</table>

* = mean values of the three replicates. Means followed by different letters in the same column statistically differ from each other by Tukey’s test (p < 0.05).
In *P. peruviana* fruits stored under refrigeration, there was a reduction in the soluble solids content from 14 °Brix to 11 °Brix (Lima *et al.*, 2009). The reduction of these compounds during storage can be attributed, in part, to the maintenance of the respiratory process, for refrigerated fruits (Vieites *et al.*, 2012).

However, because freezing inactivates various enzymes, sugar reduction may have occurred during testing; that is, after thawing the samples for testing. Antunes *et al.* (2003) also observed a reduction in the concentration of soluble solids during the storage period for blackberry cultivars under refrigeration for 12 days.

Codex Stan regulations (2005) establish that Physalis fruits must have at least 14 °Brix to be commercialized. Therefore, *P. pubescens* fruits, regardless of storage treatment, would be suitable for marketing.

However, *P. peruviana* could only be marketed as fresh fruits since the fruits were kept refrigerated and frozen, although they did not show significant differences, they did not meet the minimum standards established by the regulation. Physalis fruits stored at -78 °C showed a significant reduction in the concentration of phenolic compounds, which differ from other treatments (Table 4).

**Table 4. Phenolic compounds (mg EAG* 100 g⁻¹ fruit) of fruits of two Physalis species subjected to different storage conditions.**

<table>
<thead>
<tr>
<th>Storage</th>
<th><em>Physalis pubescens</em></th>
<th><em>Physalis peruviana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh fruits</td>
<td>84 a**</td>
<td>77 a</td>
</tr>
<tr>
<td>Chilled fruits</td>
<td>82 a</td>
<td>75 a</td>
</tr>
<tr>
<td>Frozen fruits</td>
<td>56 b</td>
<td>39 b</td>
</tr>
</tbody>
</table>

*= EAG gallic acid equivalent; **= mean values of the three replicates. Means followed by different letters in the same column statistically differ from each other by Tukey’s test (*p* < 0.05).

Galani *et al.* (2017), when evaluating various plant species, discovered that, although apples and some other horticultural crops had an increase in the content of phenols after 15 days stored at 4 °C, there was a decrease for the same compounds in tomato, banana, grapes and orange juice, compared to levels found at harvest.

In another study, strawberries held at temperatures of 5 °C and 10 °C also had an increase in total phenol content, but at 0 °C it remained constant (Ayala-Zavala *et al.*, 2004). Despite this, for the sapote the storage at high temperatures caused a reduction in the content of phenols and the lowest reductions occurred at 6 °C and 10 °C, in periods of two to twenty days (Camargo *et al.*, 2016).

Phenolic compounds play various roles in plants, giving them specific characteristics such as color, flavor, aroma, etc. and many have antioxidant activity. Therefore, the results show that these substances can be lost during storage at negative temperatures, also reducing the quality of the fruits in relation to their nutritional and nutraceutical characteristics.
It was evident in this study that, regardless of the storage condition of the fruit, both species presented moderate levels of phenolic compounds, which differ from the values reported in the literature for Physalis, which are around 136 and 210 mg EAG 100 g\(^{-1}\) of fruit in fresh fruits of \textit{P. peruviana} (Severo et al., 2010). However, Rockenbach \textit{et al.} (2008) and Puente \textit{et al.} (2011) obtained average values of 40 mg EAG 100 g\(^{-1}\) of sample in \textit{P. peruviana} fruits, being lower than the values found in this study.

Therefore, both the growing conditions and the analysis methodologies used may have contributed to the great variation within or between species. Regarding vitamin C (Figure 1), storage at low temperatures caused its degradation, but a slight increase was observed in the refrigerated fruits of \textit{P. pubescens} (Table 5).

![HPLC chromatogram of the ascorbic acid standard for frozen, chilled and fresh fruits of \textit{P. peruviana} and \textit{P. pubescens}.](image)

Although several studies point to a decrease in ascorbic acid content during storage, the small increase may be justified by the variability between plants, even if grown under identical conditions. Galani \textit{et al.} (2017) state that the wide variation in vitamin C content may be related to extraction and other applied methodologies, citing several studies that show different results. These authors tried different methods of analysis and also found variations in the levels of this vitamin.
Table 5. Vitamin C (mg of ascorbic acid 100 g⁻¹ of fruit) from fruits of two species of Physalis subjected to different storage conditions.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Physalis pubescens</th>
<th>Physalis peruviana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh fruits</td>
<td>25.11 b*</td>
<td>28.78 a</td>
</tr>
<tr>
<td>Chilled fruits</td>
<td>27.39 a</td>
<td>27.6 b</td>
</tr>
<tr>
<td>Frozen fruits</td>
<td>20.93 c</td>
<td>20.58 c</td>
</tr>
</tbody>
</table>

* = mean values of the three replicates. Means followed by different letters in the same column statistically differ from each other by Tukey’s test (p < 0.05).

When using the titration method, of the 19 plants (including fruit and broad leaves) stored at 4 °C for 15 days, only the pomegranate had a 34.35% increase in vitamin C content, while the other plants showed losses in this content, being the tomato that now has the highest rate of vitamin C loss (71.8%). Through the DNP method (2,4-dinitrophenylhydrazine), cabbage, cauliflower, sapote and orange had insignificant increases in the content of ascorbic acid. Therefore, variations can be found according to species and different methodologies.

When antioxidant activity was evaluated, fresh fruit *P. pubescens* showed the highest rate and was significantly different from fruits stored at low temperatures. Despite the lower activity, the same pattern was observed for *P. peruviana* (Table 6).

Table 6. Antioxidant activity (µmol of Trolox g⁻¹ of fruit) of fruits of two species of Physalis subjected to different storage conditions.

<table>
<thead>
<tr>
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<th>Physalis peruviana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh fruits</td>
<td>3.75 a*</td>
<td>1.69 a</td>
</tr>
<tr>
<td>Chilled fruits</td>
<td>1.37 b</td>
<td>1.46 b</td>
</tr>
<tr>
<td>Frozen fruits</td>
<td>1.01 b</td>
<td>1.15 b</td>
</tr>
</tbody>
</table>

* = mean values of the three replicates. Means followed by different letters in the same column statistically differ from each other by Tukey’s test (p < 0.05).

*Galani et al. (2017)*, tested the antioxidant activity of several species, including tomato, and verified the reduction of this activity with storage at 4 °C for 15 days. However, reducing antioxidant capacity is not the rule. Some studies point to different results with small fruits, such as strawberry, raspberry, red currant and others, where both the decrease and improvement of antioxidant activity were found and depended on the species, temperature (-20 °C, 4 °C or 25 °C) storage time and analysis methodology (Šamec et al., 2011, 2015).

The low antioxidant activity obtained in *P. peruviana* has been described by Severo et al. (2010); Vasco et al. (2008), with values ranging from 1.14 to 1.73 µmol TE g⁻¹ and 0.7 µmol TE g⁻¹, respectively, so it is described that the fruits of this species have a reduced antioxidant capacity, compared to blackberry, guava and mango. However, in other studies, such as de Rutz et al. (2012), values of approximately 9.71 µmol TE g⁻¹ were obtained in fresh fruits.
In addition to the different methodologies used, the growth conditions, storage and other intrinsic factors are responsible for the variation in the responses found for the same species (Silva, 2013).

Conclusions

In general, the storage of fruits of both Physalis species, at low temperatures, caused losses of molecules responsible for some of the characteristics of fresh fruits, and freezing, despite being considered a way to maintain the viability of the fruit, it may not be indicated for the conservation of important compounds in human nutrition. It is evident that very low temperatures, such as -78 °C, are restricted to extreme conditions and do not represent the equipment found in consumers’ homes, but it helps to understand the behavior and maintenance of the bioactive compounds present in fresh food.

For both Physalis species, the refrigerated and frozen storage methods caused a decrease in the antioxidant capacity of the fruits and loss of physical-chemical characteristics of fresh fruits. However, it is known that other compounds can act as antioxidants, for example phenolic compounds. Therefore, more studies are required to prove or not these findings.

Acknowledgments

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Cited literature


