Impact of simulated in vitro gastrointestinal digestion on the antioxidant activity and phenolic compounds of soursop peel (Annona muricata L.).

Yasmin Ourives Domingues  
Federal University of Mato Grosso

Geriel Araújo Lemes  
Federal University of Mato Grosso

Fellipe Lopes de Oliveira  
São Paulo State University (UNESP)

Thamara Rosa de Souza  
Federal University of Mato Grosso

Bibiana Silva  
Federal University of Santa Catarina

Maressa Caldeira Morzelle (maressamorzelle@hotmail.com)  
Federal University of Mato Grosso

Research Article

Keywords: functional foods, by-products, accessibility, anonaceas

Posted Date: January 31st, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3891988/v1

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License

Additional Declarations: No competing interests reported.
Abstract

This work aimed to investigate *in vitro* phenolics bioaccessibility and soursop peel's antioxidant activity. Proximate composition, ascorbic acid, total phenolic compounds, and *in vitro* antioxidant activity (DPPH, ABTS, and FRAP assays) were conducted on soursop peel and pulp. The accessibility of total phenolics and antioxidant capacity of soursop peel and pulp was assessed through simulated *in vitro* digestion. Soursop peel had a significant amount of fiber, particularly insoluble fiber, and higher levels of total phenolic compounds and antioxidant capacity than pulp. The pulp has 30% higher levels of ascorbic acid than the peel. Simulated *in vitro* digestion, the total phenolic compounds of the peel exhibited stability throughout the gastric and intestinal stages. In the pulp, there was an increase in total phenolic compounds that persisted until the final stage. After the simulation of *in vitro* digestion, the peel showed stability in DPPH antioxidant capacity analysis and obtained higher accessibility in the enteric II stages (40% and 29%) on ABTS and FRAP assays. Soursop pulp increased accessibility in the gastric stage on DPPH and FRAP assay. In the ABTS method of antioxidant capacity evaluation, the pulp showed the highest values in enteric phase II. These results are essential for human nutrition as they provide information about soursop peel and pulp’s nutritional and bioactive composition. Furthermore, the *in vitro* accessibility of these compounds offers insights into their potential utilization by the organism. This knowledge holds significant implications for nutrition experts, as it can inform the development of healthier dietary habits and promote enhanced health outcomes.

1. Introduction

Soursop fruit (*Annona muricata* L.), also known as Graviola, is a member of the *Anonaceae* family and is found in Central and South America, Africa, Asia, and Australia [1, 2]. It was introduced to Brazil by the Portuguese in the 14th century and is primarily grown in the warm and semi-arid regions of the Northeast [3]. The state of Bahia is the largest producer in Brazil, followed by Pernambuco and Alagoas, with a harvested area of 2.760 hectares and revenues of R$24.323.000 million in 2017 [4].

Soursop has been recently highlighted in national and international commerce due to the studies on its biological effects and consequently the impact of its consumption on human health [5, 6, 7]. The scientific community widely exploits the pulp and leaf of the soursop [8, 9, 6] about their anti-carcinogenic potential.

The fruit is known for its conical shape and comprises pulp, peel, and seeds. The peel is characterized by a dark-green color and the appearance of rigid spines, while the pulp presents a whitish color and soft consistency after maturating. The pulp, generally used to make juices, jams, ice cream, and sweets, has a sweet or sub-acidic taste. The peel comprises much of the fruit’s weight and is often discarded or underused for animal feed [10]. However, although the soursop’s peel corresponds to a large percentage of the fruit’s weight, there are not enough data on the proximate composition and functional composition of the soursop peel, which justifies the development of the present study.

Fruit by-products, such as peels and seeds, have mostly bioactive compounds and nutritional value with beneficial effects on human health superior to those demonstrated by the edible parts [11, 12, 13, 8].
Knowledge of the proximate composition and consequent valorization of fruit by-products can contribute to sustainable development, demonstrating this research's economic, environmental, social, and technological impact.

Therefore, it is essential not only to identify the bioactive compounds but also to investigate the effects of the digestive process on phenolics and antioxidant activity. This is because factors such as pH and enzymes used during gastrointestinal digestion can modify the active substances in the food [14, 15]. Moreover, the accessibility of certain phenolic compounds is modulated by the presence of specific nutrients, such as fiber, which can have a positively or negatively impact on their accessibility [16, 17]. The potential impact of the digestion process on the effective biological action of bioactive compounds in soursop peel has yet to be extensively studied in the scientific community [18, 19].

This study aims to investigate the impact of *in vitro* digestion on soursop peel's antioxidant activity and phenolic compounds. The bioaccessibility of phenolic compounds will be assessed, as it is essential to understand the potential reuse of discarded fruit parts comprehensively.

### 2. Material and methods

The material and methods section are provided as supplementary materials.

### 3. Results and Discussion

#### 3.1 Sample analysis

Table 1 presents the proximal composition of the soursop peel and pulp, which were analyzed for moisture, digestible carbohydrates, proteins, lipids, ash, and dietary fiber (soluble and insoluble). The fruits analyzed had an average weight of 2.3 kg, with 70.4% of the total weight corresponding to the pulp, 22.5% to the peel, and 7.1% to the seed.

Table 1 - Results are expressed in percentage (%). *¹: Calculated by difference (100 − (moisture + ashes + lipids + protein + total dietary fiber)). *²: Total carbohydrate − total dietary fiber. Different lowercase letters in the row indicate differences by t-test (p < 0.05). Values are mean ± standard deviation. Identical lowercase letters in the same row indicate no difference in the t-test (p < 0.05).

The results indicated that both parts of the soursop were high in moisture and dietary fiber but had lower concentrations of protein, lipids, and ash (Table 1). The peel was found to have a higher caloric value than the pulp, which could be attributed to its higher lipids content (p < 0.05).

No significant difference (p < 0.05) was found in the total carbohydrate between the two parts. The digestible carbohydrate content was higher in the pulp (90%) than in the peel due to the latter's high fiber content. These findings have important implications for the caloric value and potential uses of the two parts of soursop fruit.
Soursop peel presents a significant amount of dietary fiber (59%) compared to the pulp, making it a promising functional ingredient that requires consuming 137 g/day of pulp or 57 g/day of fruit peel. In addition to its health benefits, using the inedible parts of the fruit could help to reduce the environmental impact [11, 12]. In the present study, the peel has the highest content of insoluble fiber (67%), which can aid in promoting a healthy gastrointestinal tract and increasing feelings of fullness [20]. Many studies are being conducted to explore the nutritional value of fruit by-products. These results suggest that soursop peel could be incorporated into new food products as flour to add nutritionally and meet the growing demand for fiber-rich foods in the food industry, ultimately leading to a positive economic impact [21, 22, 23, 24].

Table 2 shows that the soursop pulp fruit contains 29% more ascorbic acid than the peel (p < 0,05). Previous studies also reported high ascorbic acid levels in the Anonaceae family's fruits, including soursop [25, 26]. This high ascorbic acid content may be attributed to the fruit's strong antioxidant capacity, which helps reduce the production of free radicals [26].

Table 2 - Values are mean ± standard deviation. Identical lowercase letters in the same row indicate no difference between them in the t-test (p < 0,05).

Table 2 displays the assessment outcomes conducted to determine the antioxidant capacity and total phenolic compounds of the soursop pulp and peel extracts. The results indicated that the soursop peel extract contained more significant phenolic compounds than the pulp extract. Regarding antioxidant capacity, the peel and pulp extracts exhibited noticeable differences when evaluated using ABTS and DPPH methods but not when measured by the FRAP technique (Table 2).

Results indicated that peel and pulp presented explicit contents of total phenolic compounds, and peel showed a 17% higher content of phenolic compounds than the pulp (Table 2). A higher amount of phenolic compounds in the peel can be explained by the fact that this part of the fruit is more susceptible to environmental stress factors and participate in plant defense mechanisms, such as attack by insects, UV radiation, and temperature. Thus, the phenolic compounds may be generated from the secondary metabolism of the plant [27].

Table 3 - Values are mean ± standard deviation. Identical lowercase letters in the same row indicate no difference between them in the t-test (p < 0,05).

In this study, five phenolic compounds were detected in peel and pulp of soursop, were gallic acid, benzoic acid, coumaric acid, ferulic acid and hesperidin, predominating phenolic acids characterized by HPLC-DAD (Table 3). The phenolic acids are the main phenolic compounds of soursop, according to the literature. The peel showed a 72% higher content of hesperidin than the pulp. In other studies, expressive values of phenolic compounds with antioxidant capacity were also found in fruit peels in Annonas [28, 27].

This study showed a valorization of the by-product in the cosmetic, pharmaceutical, and food industries, being important information for the non-waste of non-edible parts of food. The in vitro antioxidant capacity
methods performed in this study serve as an initial tool, as they are low-cost and high-yielding and can help identify these compounds for later in vivo assays.

3.2 Effects of digestion in vitro on TPC

The changes of TPC in soursop peel and pulp during simulated in vitro digestion are shown in Fig. 1. After simulated digestion, the TPC in peel and pulp shows different trends. They were considering the results obtained in evaluating the accessibility of total phenolic compounds of soursop. The results of the total phenolic compounds present in the pulp subjected to simulated in vitro digestion showed that the phenolic compounds were constant in the gastric digestion stage and then increased significantly in the intestinal digestion stage.

For the peel, there is no difference between the gastric and enteric phases of in vitro digestion, as shown in Fig. 1.

Figure 1 – Total phenolic compound content (TPC) of pulp (A) and peel (B) of soursop submitted to the gastric, enteric phase I, and enteric phase II of in vitro simulated digestion. EG: Gastric phase, EEI: Enteric phase I, EEII: Enteric phase II. NS = not significant (p < 0.05); *** = significant differences (p < 0.05). Compared to the Tukey test at 5% probability.

After investigating the effect of in vitro digestion on the phenolic compounds in soursop pulp, there was a 12% increase in accessibility from gastric to enteric phase II and 9% from enteric phase I to enteric phase II. The peel obtained stability between the analyzed phases.

The results were positive, showing good association with the release of total phenolic compounds in the last stage of digestion, thus generating possible impact on human health and showing that the peel is a good product for potential biological effects on the gastrointestinal tract. In the action of proteases, polysaccharides, and peptides, among other nutrients, the total phenolic compounds are released, thus binding with proteins, justifying their increased accessibility of total phenolic compounds [14, 29].

Similar results were found for soursop pulp in the study by Blancas-Benítez et al. [30], where the highest release occurred in the intestinal phase of the in vitro assay. During intestinal digestion, enzymes such as hydrolases, responsible for the hydrolysis of carbohydrates, can react and interact between carbohydrates and compounds, resulting in increased accessibility in the gastrointestinal tract [31]. Furthermore, phytochemicals such as gallic acid (83.5%), chlorogenic acid (6.4%), and caffeic acid (10.1%) are potentially absorbed in the intestinal phase [30].

Some studies have evaluated the accessibility of fruit by-products and their inedible parts, such as the peels, where they showed positive accessibility of total phenolic compounds, showing to be a promising ingredient for the food industry and possible insertion in new healthy products [32, 17]. Depending on the food and its connection to the food matrix, as well as the nutrients of the food, can affect the release of phenolic acids in the intestine [14]. It is worth pointing out that the content of active substances in the food may be reduced due to the pH employed in enteric phases I and II during in vitro intestinal digestion and the interaction between the enzyme's bile and pancreatin with the analyzed fruit [15].
Ferreyra et al. [17] studied fruit by-products, where they found increased accessibility between the oral, gastric, and intestinal phases, showing that they were stable and demonstrating their potential accessibility. Some total phenolic compounds may remain unchanged, and others may change their chemical structure, related to the pH changes in the simulation phases of the *in vitro* digestion [33].

The total phenolic compounds can be bound to proteins in various ways, such as through hydrogen, covalent and hydrophobic bonds, thus justifying their more significant release in the intestinal phase [29, 34].

The accessibility of some phenolic compounds is internally linked to some nutrients, such as fiber, minerals, proteins, carbohydrates, and lipids, and can negatively alter their accessibility, as it happened in the gastric phase [16, 17]. On the other hand, the low pH with the action of the pepsin enzyme in the gastric phase releases phenolic compounds, which bind to carbohydrates [35]. Covalent and non-covalent bonds connect polyphenols and dietary fiber. Some factors, such as ionic strength, temperature, polyphenols, and fiber structure, affect their release [14].

For example, dietary fiber may decrease the accessibility of phenolic compounds in the gastrointestinal tract [36]. The low accessibility in the gastric stage may be related to the presence of soluble fibers, such as pectin, in the soursop pulp and peel, presenting gelation and thus being able to interact with the bioactive compounds, inhibiting their release in the gastrointestinal tract [29, 37]. In addition, some anti-nutritional compounds, such as the phytochemicals saponin, alkaloids, and tannins, present in soursop pulp and peel, can also negatively alter its accessibility [38].

In this study, the stability of accessibility of phenolic compounds to the rind and increase between gastric and enteric phase II was observed. However further studies using high-performance liquid chromatography (HPLC) are needed to identify the individual phenolic compounds after digestion and better understand and justify the decrease, increase or stability of these compounds during the digestive process.

### 3.2 Effects of simulated digestion on antioxidant capacity

There was stability for the peel between the gastric, enteric I, and enteric II phases in the antioxidant capacity DPPH. There was a significant difference between the first and last phases for the antioxidant capacity ABTS. From the above results, we can conclude that the antioxidant activity of soursop peel and pulp measured by the FRAP method showed a similar trend during the simulated digestion *in vitro*, as there increased after gastric digestion and decreased during intestinal digestion.

**Figure 2** – Content antioxidant capacity (DPPH, ABTS e FRAP) of pulp (A) and peel (B) of soursop submitted to the gastric, enteric phase I, and enteric phase II of *in vitro* simulated digestion. EG: Gastric phase, EEI: Enteric phase I, EEII: Enteric phase II. NS = not significant (p < 0,05); *** = significant differences (p < 0,05). Compared to the Tukey test at 5% probability.

These methods were employed to evaluate the changes in antioxidant activity during simulated *in vitro* digestion. In DPPH analysis, there was stability for the peel in all three phases of digestion. However, a decline can still be seen for the pulp accessibility at 4% from gastric to enteric phase I and stability between
enteric phase I and II. The reduction from the gastric to the enteric phase in the pulp may be due to the composition of the active metabolites of the fruit by the gut microbiota [39].

In the ABTS analysis, there was a significant change between the three phases for the pulp, increasing from the gastric phase to enteric phase II considerably by 57%, showing a higher antioxidant capacity. In comparison, for the peel, the first two phases remained stable, and a 40% increase in accessibility between enteric phase I and II. Many polyphenols are released in the gastric phase because, during intestinal digestion at neutral or weakly alkaline pH, most compounds are lost by incubation with bile and pancreatic salts. For this reason, the phenolic compounds are degraded or converted to new compounds. Another answer to the low antioxidant capacity in the gastric stage is because of its short duration of contact with enzymes in the stomach. The increase in antioxidant activity between the stages also occurs by the change from acidic pH to basic pH, thus the release of phenolic compounds, and this occurs because there is deprotonation of the hydroxyl group on the aromatic ring. Still, of all the assays, the most suitable for assessing digestion in the gut is the ABTS method due to its pH of 7–7.5 [14].

Sollano-Mendieta et al. [40], when analyzing the antioxidant capacity in the in vitro digestion of fruits, obtained expressive results in ABTS and DPPH analysis in the in vitro intestinal digestion phase, which the pH variation in the enteric phase digestion can explain. Studies have reported that the transition from acidic to alkaline pH can increase the release of total phenolic compounds and flavonoids, thus contributing to the increase of antioxidant capacity, occurring by deprotonation of hydroxyl groups that are present in the aromatic rings [41, 42].

In the FRAP analysis, there was stability in both parts analyzed between the gastric and enteric phase II, and in both decline between the gastric and enteric phase I, being pulp with 26% and peel with 15%. Other studies performed with fruit by-products have shown similar results [43]. This reduction may be related to the pH changes in the in vitro digestion simulation phases [33].

The properties of these functional components in vitro are related to their accessibility after food consumption. They are affected by several factors, such as the extent to which they are released from the food matrix in the gastrointestinal tract, their digestive stabilities, and their metabolism in the human body [18, 44]. The stability of these phytochemicals is related to interfering environmental factors during human digestion, such as pH, digestive enzymes, and temperature [18, 19, 40].

Although all the antioxidant activity quantification methods are direct (DPPH, ABTS, and FRAP), there was a difference between the values found in each method because each method has its particularities and evaluates the antioxidant capacity in different ways.

4. Conclusion

Soursop peel has expressive dietary fiber values, indicating that its flour can be a promising food in the composition of new foods in the food industry. As for the antioxidant capacity and content of total phenolic compounds, the peel obtained the highest values, demonstrating its potential to be explored as a functional ingredient. Simulated digestion results for TPC and antioxidant capacity demonstrated the stability of the
bioactive antioxidant compounds until the end of the assay, thus demonstrating their potential beneficial effect on human health.

Declarations

Author contributions Conceptualization: YOD and MCM; Investigation: YOD, MCM, GAL, FLO, TRS and BS; Writing – original draft: YOD and MCM; Writing – review and editing: YOD and MCM; Supervising: MCM.

Funding Not applicable.

Data Availability Data and material may be provided on request by the corresponding authors.

Ethical Approval Not applicable.

Conflict of Interest The authors declare no conflict of interest.

References


### Tables

**Table 1** – Proximate composition (% w/w) of Soursop pulp and peel (mean ± Standard deviation).

<table>
<thead>
<tr>
<th></th>
<th>Peel</th>
<th>Pulp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energetic Value (kcal 100 g⁻¹)</td>
<td>97,97</td>
<td>72,30</td>
</tr>
<tr>
<td>Moisture</td>
<td>76,44 ± 1,86</td>
<td>81,41 ± 0,58</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>18,62</td>
<td>16,41</td>
</tr>
<tr>
<td>Digestible carbohydrate</td>
<td>0,94</td>
<td>9,09</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>17,68</td>
<td>7,32</td>
</tr>
<tr>
<td>Insoluble fiber</td>
<td>16,67 ± 1,41</td>
<td>5,40 ± 0,97</td>
</tr>
<tr>
<td>Soluble fiber</td>
<td>1,01 ± 1,38</td>
<td>1,76 ± 0,36</td>
</tr>
<tr>
<td>Protein</td>
<td>1,75 ± 0,10</td>
<td>1,43 ± 0,06</td>
</tr>
<tr>
<td>Lipids</td>
<td>2,25 ± 0,07</td>
<td>0,10 ± 0,00</td>
</tr>
<tr>
<td>Ash</td>
<td>0,94 ± 0,03</td>
<td>0,64 ± 0,05</td>
</tr>
</tbody>
</table>

Results are expressed in percentage (%). *¹: Calculated by difference (100 − (moisture + ashes + lipids + protein + total dietary fiber)). *²: Total carbohydrate – total dietary fiber. Different lowercase letters in the row indicate differences by t-test (p<0,05). Values are mean ± standard deviation. Identical lowercase letters in the same row indicate no difference in the t-test (p<0,05).

**Table 2** – Total phenolic compound content (µg EAG 100 g⁻¹), in vitro antioxidant capacity by DPPH (µM ET g⁻¹), ABTS (µM ET g⁻¹), FRAP (µM ferrous sulfate g⁻¹) and Ascorbic acid (mg ascorbic acid 100 g⁻¹) methods of soursop peel and pulp extracts.
<table>
<thead>
<tr>
<th></th>
<th>Peel</th>
<th>Pulp</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFC</td>
<td>645 ± 54</td>
<td>532 ± 18</td>
</tr>
<tr>
<td>DPPH</td>
<td>3280 ± 158</td>
<td>2330 ± 134</td>
</tr>
<tr>
<td>ABTS</td>
<td>5768 ± 386</td>
<td>4401 ± 133</td>
</tr>
<tr>
<td>FRAP</td>
<td>8997 ± 886</td>
<td>7727 ± 649</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>40,2 ± 0,9</td>
<td>56,4 ± 3,8</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation. Identical lowercase letters in the same row indicate no difference between them in the t-test (p<0,05).

**Table 3** – Content of phenolic compounds of the peel and pulp extracts.

<table>
<thead>
<tr>
<th></th>
<th>Peel</th>
<th>Pulp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galic acid</td>
<td>44,26 ± 0,47</td>
<td>43,70 ± 0,25</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>21,51 ± 0,33</td>
<td>20,09 ± 0,59</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>30,65 ± 1,22</td>
<td>27,66 ± 0,40</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>Traces</td>
<td>17,09 ± 0,55</td>
</tr>
<tr>
<td>3,4 Dihdroxibenzoic</td>
<td>n.d</td>
<td>16,51 ± 2,08</td>
</tr>
<tr>
<td>Abscisic acid</td>
<td>7,38 ± 1,61</td>
<td>n.d</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>61,08 ± 6,17</td>
<td>17,02 ± 0,91</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation. Identical lowercase letters in the same row indicate no difference between them in the t-test (p<0,05).

**Figures**
Figure 1

Total phenolic compound content (TPC) of pulp (A) and peel (B) of soursop submitted to the gastric, enteric phase I, and enteric phase II of *in vitro* simulated digestion. EG: Gastric phase, EEI: Enteric phase I, EEII: Enteric phase II. NS = not significant (p<0.05); *** = significant differences (p<0.05). Compared to the Tukey test at 5% probability.
Figure 2

Content antioxidant capacity (DPPH, ABTS e FRAP) of pulp (A) and peel (B) of soursop submitted to the gastric, enteric phase I, and enteric phase II of *in vitro* simulated digestion. EG: Gastric phase, EEI: Enteric phase I, EEII: Enteric phase II. NS = not significant (p<0.05); *** = significant differences (p<0.05). Compared to the Tukey test at 5% probability.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterialandMethods.docx