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## Development and characterization of innovative spicy persimmon sauces: Opportunities to reduce post-harvest losses

### Desenvolvimento e caracterização de molhos picantes de caqui inovadores: oportunidades para reduzir as perdas pós-colheita

#### Abstract

The persimmon harvest period is short and intensive, which leads to considerable post-harvest losses. Innovative persimmon products with extended shelf life, such as sauces, are one strategy to reduce losses and align with potentially more sustainable approaches in the agricultural industry. This study aimed to develop and characterize the spicy persimmon sauce (S1) and the spicy persimmon sauce with nutmeg, clove and cinnamon (S2). The moisture content of S1 and S2 was  $88.08 \pm 0.01$  and  $87.41 \pm 0.03\%$ , respectively, with an equal pH of  $4.2 \pm 0.12$ . The sauce formulations contained at least 18 and 6.3 g.100 g<sup>-1</sup> carbohydrates and crude fiber, respectively. The sauces showed a reddish color and adequate microbiological quality with thermotolerant coliforms < 3 MPN. g<sup>-1</sup>, absence of *Salmonella* sp and yeast and mold < 10 CFU.g<sup>-1</sup>. The phenolic content was higher in S2 (358.94 µg GAE g<sup>-1</sup>) than in S1 (265.16 µg GAE g<sup>-1</sup>), while the flavonoid content was higher in S1 (729.6 µg RE g<sup>-1</sup>) than in S2 (568.68 µg RE g<sup>-1</sup>). The antioxidant activity by DPPH assay was greater in S2 (IC50: 1,944.88 µg. mL<sup>-1</sup>) than in S1 (IC50: 3,061.33 µg. mL<sup>-1</sup>), but the same in ABTS (IC50: 3,103.08 ± 413.89 µg. mL<sup>-1</sup>) for both sauces. S2 (31 compounds) has a richer volatile composition than S1 (19 compounds). Phenylpropanoids such as elemicin, for example, were detected in S2 samples only. The persimmon sauces presented in this study serve as input for an agroecological product development process with potential functional properties and social benefits.

**Keywords:** Product Development. *Diospyros kaki*. Spicy Sauce. Fruit Sauce. Postharvest Losses.

#### Resumo

O período de colheita do caqui é curto e intenso, o que leva a uma considerável perda pós-colheita. Os produtos inovadores de caqui com um período de conservação prolongado, como os molhos, são uma estratégia para reduzir as perdas e estão em conformidade com abordagens potencialmente mais sustentáveis na indústria agrícola. O objetivo deste estudo foi desenvolver e caracterizar o molho picante de caqui (S1) e o molho picante de caqui com noz-moscada, cravo e canela (S2). O teor de umidade de S1 e S2 foi de  $88,08 \pm 0,01$  e  $87,41 \pm 0,03\%$ , respectivamente, com o mesmo pH de  $4,2 \pm 0,12$ . As formulações dos molhos continham 18 e 6,3 g.100 g<sup>-1</sup> de carboidrato e fibra, respectivamente. Os molhos apresentaram cor avermelhada e qualidade microbiológica adequada com coliformes termotolerantes <3 NMP.g<sup>-1</sup>, ausência de *Salmonella* sp e bolores e leveduras < 10 UFC.g<sup>-1</sup>. O teor de fenólicos foi

maior em S2 (358,94  $\mu\text{g GAE g}^{-1}$ ) do que em S1 (265,16  $\mu\text{g GAE g}^{-1}$ ), enquanto o teor de flavonoides foi maior em S1 (729,6  $\mu\text{g RE g}^{-1}$ ) do que em S2 (568,68  $\mu\text{g RE g}^{-1}$ ). A atividade antioxidante pelo ensaio DPPH foi maior em S2 (IC<sub>50</sub>: 1.944,88  $\mu\text{g. mL}^{-1}$ ) do que em S1 (IC<sub>50</sub>: 3.061,33  $\mu\text{g. mL}^{-1}$ ), mas o mesmo em ABTS (IC<sub>50</sub>: 3.103,08  $\pm$  413,89  $\mu\text{g. mL}^{-1}$ ) para ambos os molhos. O S2 (31 compostos) tem uma composição volátil mais rica do que o S1 (19 compostos). Por exemplo, os fenilpropanoides, como a como a elemicina, só foram detectados nas amostras S2. Os molhos de caqui apresentados neste estudo configuram-se como uma contribuição para o desenvolvimento de produtos agroecológicos com potenciais propriedades funcionais e benefícios sociais.

**Palavras-chave:** Desenvolvimento de Produtos. *Diospyros kaki*. Molho Picante. Molho de Frutas. Perdas Pós-Colheita.

## INTRODUCTION

Persimmon (*Diospyros kaki*) is a widely appreciated and consumed fruit worldwide.<sup>1,2</sup> According to the Food and Agriculture Organization of the United Nations (FAO), global production in 2022 amounted to 4.4 million tons with a cultivated area of 1 million hectares.<sup>3</sup> Brazil, the fifth largest producer of persimmon in the world, produces around 170 thousand tons per year, with an average annual gross value of US\$ 16.5 million.<sup>3</sup> Persimmons (*Diospyros kaki*) are an important crop in Brazil, especially for small and medium-sized family farms, for which production is often the main or only source of income.<sup>4,5</sup>

In the last ten years, persimmon production in Brazil has been characterized by considerable post-harvest losses. According to the FAO, the average annual loss between 2014 and 2019 was around 20% of the total production.<sup>3</sup> Postharvest losses in persimmons are due to several factors, including high perishability, which is generally related to ripening, ethylene production, accelerated respiration and softening of the fruit.<sup>6</sup> In addition, inappropriate handling practices and overproduction during harvest exacerbate the problem and contribute significantly to postharvest losses.<sup>4,7</sup> In addition to improving post-harvest techniques and storage methods,<sup>4</sup> developing new value-added products from persimmon could also help reduce post-harvest losses.<sup>4,8</sup> In China, the world's largest producer of persimmons, post-harvest losses are estimated to be around 5% of total production.<sup>3</sup> China is also the country with the largest number of publications on persimmon-based food products.<sup>9</sup>

In Brazil, the demand for artisanal products has grown significantly as society increasingly values sustainability and prefers locally sourced food.<sup>10</sup> In this context, producing persimmon products is a viable and sustainable strategy for family farms. The use of low-tech processes enables the efficient management of agricultural surpluses, the reduction of post-harvest losses and the increase of added value in local agricultural production. In addition, these products can be easily adapted for home preparation, providing accessible and convenient options that promote dietary diversity and the consumption of products of vegetable origin.<sup>2,4,7,9</sup>

Persimmon is valued for its flavor, nutritional value and phytochemical composition, which makes it suitable for the search for new uses due to its profile of carbohydrates and bioactive compounds such as polyphenols, terpenoids and flavonoids.<sup>2,6,7,9</sup> These properties allow the development of high-quality products with good sensory properties,<sup>9,11</sup> such as wine,<sup>12</sup> vinegar,<sup>13</sup> low-calorie jams,<sup>14</sup> smoothies,<sup>15</sup> chutney and ketchup,<sup>9</sup> beer,<sup>16</sup> juice and jelly.<sup>17</sup> In this respect, developing spicy sauces based on persimmon is a good innovation and product development opportunity.

Sauces are defined as products in liquid, pasty, emulsified or suspended form based on spices and/or condiments and/or other ingredients, fermented or unfermented, used to prepare and/or add flavor and aroma to foods and beverages.<sup>18</sup> Among sauces, the production of fruit sauces has increased as they improve valuable functional properties of food as food ingredients.<sup>19</sup>

There are some studies in the literature in which fruit was used as a raw material for sauce production, e.g. for the production of strawberry ripple sauce,<sup>20</sup> strawberry-enriched sauces,<sup>21</sup> partial replacement of tomato by avocado in sweet and sour sauce<sup>22</sup> and assai sauce.<sup>19</sup> When making sauces, spices such as cinnamon, cloves, pepper and others can also be added to increase functionality and create new flavors.<sup>23,24</sup> The present study aimed to develop and characterize an innovative food product – a spicy sauce – using persimmon.

MATERIALS AND METHODS

Raw material and preparation of spicy persimmon sauce formulations

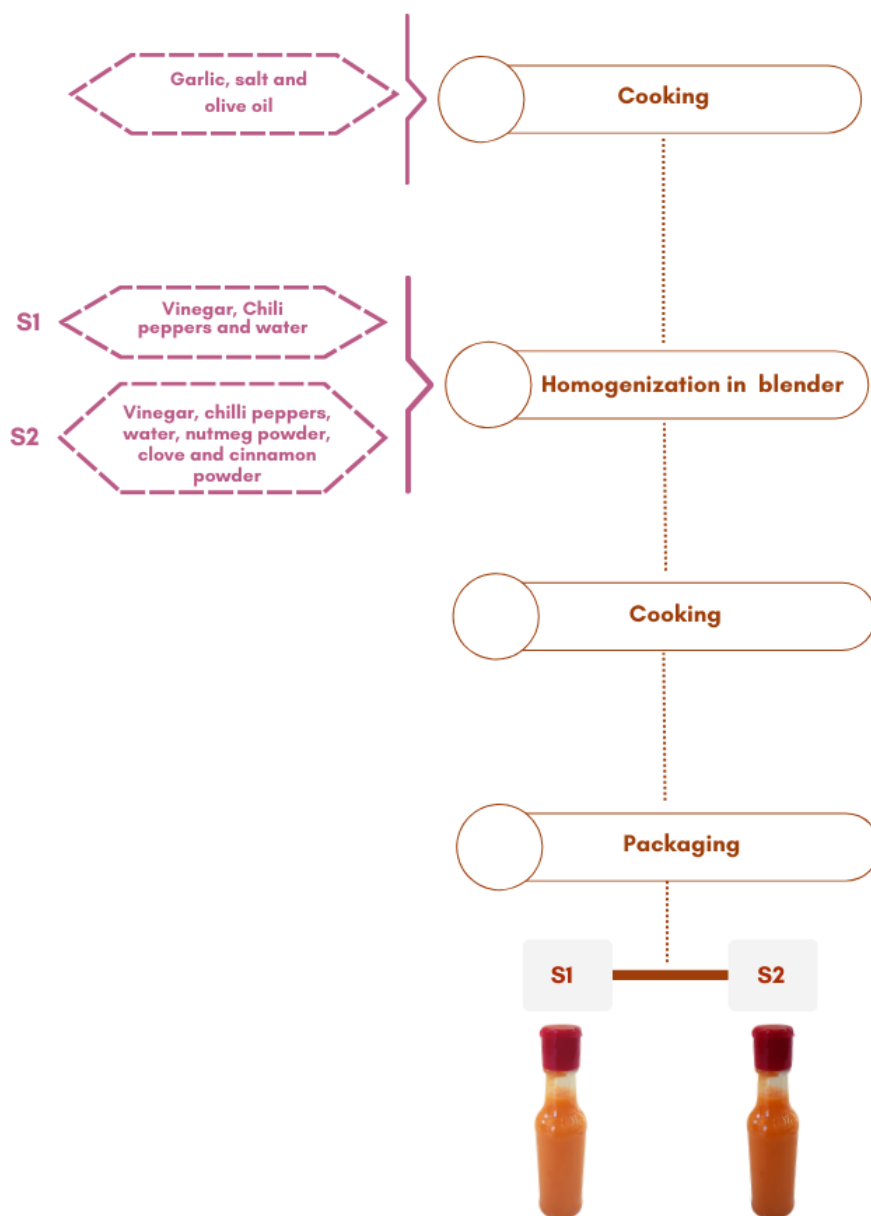
The studied persimmon fruits (*Diospyros kaki* L. ‘Rama Forte’) were cultivated in the municipality of Rio de Janeiro, in Vargem Grande (22.97 latitude; 43.49 longitude), state of Rio de Janeiro, Brazil. The fully ripe fruits were purchased from a local agroecological producer in Rio de Janeiro, Brazil. The other ingredients for the spicy persimmon sauces used in the study were purchased at a local market in Rio de Janeiro, Brazil.

Two recipes for spicy persimmon sauces (S1: Spicy Persimmon Sauce/ S2: Spicy Persimmon Sauce with Spices) were developed (see Table 1). Fresh persimmons were washed with tap water and sanitized, and the calyx was removed. The other ingredients, if applicable, were washed and sanitized; the inedible parts were removed and the edible ones were cut into pieces. Figure 1 shows the preparation steps of the two products (S1 and S2).

Table 1. Formulations of spicy persimmon sauces. Rio de Janeiro, RJ, Brazil. 2022.

| Ingredients                                          | Formulations of sauces |     |
|------------------------------------------------------|------------------------|-----|
|                                                      | S1                     | S2  |
| Persimmon ( <i>Diospyros kaki</i> L.) (g)            | 700                    | 700 |
| Fresh garlic ( <i>Allium sativum</i> L.) (g)         | 10                     | 10  |
| Olive oil (g)                                        | 15                     | 15  |
| White wine vinegar (mL)                              | 100                    | 100 |
| Salt (g)                                             | 3                      | 3   |
| Ladyfinger pepper ( <i>Capsicum baccatum</i> L.) (g) | 15                     | 15  |
| Water (mL)                                           | 700                    | 700 |
| Nutmeg ( <i>Myristica fragrans</i> ) powder (g)      | -                      | 1.5 |
| Clove ( <i>Syzygium aromaticum</i> ) (g)             | -                      | 0.2 |
| Cinnamon ( <i>Cinnamomum verum</i> ) powder (g)      | -                      | 0.6 |

**Figure 1.** Flowchart: preparation of spicy persimmon sauces (S1 and S2).



## Physicochemical analysis of spicy persimmon sauces

The analyzes were carried out in duplicate to determine the moisture and pH value of the sauces.<sup>25</sup>

## Estimation of proximate composition of spicy persimmon sauces

The carbohydrate, protein, total fat and crude fiber contents of the spicy persimmon sauces were estimated based on the Brazilian Food Composition Database.<sup>26,27</sup> The macronutrients (carbohydrates, protein, total fat) and crude fiber of the persimmon fruit 'Rama Forte' were determined based on the work of Matheus, Nogueira et al.<sup>28</sup> The energy value (kcal) was estimated according to the Atwater system, taking into account 4 kcal/g of carbohydrates and protein and 9 kcal/g of fat.

## Color measurement

The color values of the samples of persimmon sauces were measured with a CM-5 colorimeter (Konica Minolta, Japan). Color values were indicated by the coordinates lightness ( $L^*$ ), red ( $+a^*$ ), green ( $-a^*$ ), yellow ( $+b^*$ ), blue ( $-b^*$ ), chroma or saturation ( $C^*$ ) and hue ( $H^*$ ). The results were expressed as the average of four measurements. The resulting color parameters were plotted using Microsoft Excel in the color charts proposed by Delgado-González et al.<sup>29</sup>

The total color difference ( $\Delta E^*$ ) (Eq. 1) was calculated for the two samples. This value is a measure of the difference between two colors according to the following classification:  $\Delta E^* = 0-0.5$  trace level difference;  $\Delta E^* = 0.5-1.5$  slight difference;  $\Delta E^* = 1.5-3.0$  noticeable difference;  $\Delta E^* = 3.0-6.0$  appreciable difference;  $\Delta E^* = 6.0-12.0$  large difference; and  $\Delta E^* > 12.0$  very obvious difference.<sup>30</sup>

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (Eq. 1)$$

## Microbiological analysis

To evaluate the microbiological quality of the sauces, the thermotolerant coliforms (expressed as most probable number per gram: MPN  $g^{-1}$ ), the total fungal count (expressed as logarithm of colony forming units per gram: log CFU  $g^{-1}$ ) and *Salmonella* sp. (presence or absence in 25 g) were analyzed. All microbiological analyzes were performed according to the methodology described by the American Public Health Association<sup>31</sup> and Silva et al.<sup>32</sup> Measurements were performed in duplicate for each sample.

## Extraction procedure for total phenolic content, total flavonoid content and antioxidant activity

For the extraction, 3 g of the sauces (S1 and S2) and 40 mL of a water: methanol (1:1, v/v) solution were homogenized and allowed to stand at room temperature for 60 minutes. Then the solution was centrifuged at 3,500 rpm for 15 minutes. Finally, the supernatant was transferred to a 100 mL volumetric flask. For the second extraction, the residue from the previous extraction was used and the same process was carried out with aqueous acetone solution (70%). The supernatant was transferred to the same 100 mL volumetric flask, which was filled with distilled water.<sup>33</sup>

## Total phenolic content assay

The total phenolic content (TPC) of the samples was determined using the spectrophotometric method of Folin-Ciocalteu.<sup>28</sup> The samples were extracted and diluted (1:2 v/v) according to the item "extraction procedures". The TPC was calculated using a calibration curve of gallic acid (5, 10, 25, 50, 75 and 100 mg  $L^{-1}$ ). The results were expressed as  $\mu g$  gallic acid equivalents (GAE)  $g^{-1}$  of sauce. Absorbance was measured at 760 nm.

## Total flavonoid content assay

The total flavonoid content (TFC) of the original solution was evaluated by the spectrophotometric method of aluminum chloride using rutin as a reference compound.<sup>34</sup> The total flavonoid content was

calculated using a calibration curve of rutin (300, 600, 900 and 1200 mg L<sup>-1</sup>). The results were expressed as µg rutin equivalents (RE) g<sup>-1</sup> of sauce. Absorbance was measured at 510 nm.

## Antioxidant activity

The antioxidant activity was evaluated with the ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] test<sup>35</sup> and with the DPPH (2,2-difenil-1-picril-hidrazil) test.<sup>36</sup> New solutions were prepared from the original solutions described under extraction procedures (1,000 µg mL<sup>-1</sup>; 3,000 µg mL<sup>-1</sup>; 6,000 µg mL<sup>-1</sup> and 9,000 µg mL<sup>-1</sup>) to generate the antioxidant curves used to calculate the IC<sub>50</sub> (ABTS) and IC<sub>50</sub> (DPPH) of the sauces. The absorbance was measured at 734 nm for the ABTS assay and at 515 nm for the DPPH assay.

## Isolation, identification and quantification of volatile compounds

The headspace analysis of the volatile components by SPME described by Wang et al.<sup>37</sup> was adopted with minor modifications. A total of 10 g of the sauce was poured into 20 mL clear glass vials, immediately capped and placed in a temperature-controlled water bath at 60°C for 60 minutes. An SPME fiber coated with 100 µm PDMS (100% polydimethylsiloxane) (Supelco®, Bellefonte, PA, USA) was preconditioned at 250°C for 30 minutes and inserted into the headspace above the liquid surface. A system blank with an empty vial was used as a control experiment. The SPME fibers were desorbed at 250°C for five minutes in the injection port of the chromatography system described below.

GC/MS analysis of the volatile fractions was performed using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with an HP-5MS 5% phenylmethylsiloxane capillary column (30 m x 0.25 mm, 0.25 µm film thickness; Restek, Bellefonte, PA, USA) equipped with an Agilent HP-5975 mass-selective detector in electron impact mode (ionization energy: 70 eV) and operated under the following conditions. The oven temperature was first held at 50°C for two minutes, then increased to 240°C at a rate of 5°C/min and held at this temperature for ten minutes. The injector and detector temperatures were set at 250°C and 260°C, respectively. The samples were injected in splitless mode. Normalization technique was used to record the quantitative data. The linear retention indexes were calculated for all components using a homologous series of n-alkanes analyzed under the same conditions as the samples. Identification of the components of the volatile fraction was based on retention indices (RI) relative to n-alkanes and computer matching with the Wiley275.L and Wiley7n.L libraries and comparisons of the fragmentation patterns of the mass spectra with data published in the literature.<sup>38</sup> In view of the differences between columns and equipment, the identification criterion with delta < 50 units was adopted.<sup>39</sup>

## Statistical analysis

The study parameters were first subjected to the normality test by D'Agostino & Pearson. For the parameters that passed the normality test, the presence of significant statistical differences ( $p < 0.05$ ) between the groups was assessed using the parametric *t*-test. For the parameters that did not pass the normality test, the non-parametric Mann-Whitney test was performed to check for significant statistical differences between the groups. The probability level of  $p < 0.05$  was considered statistically significant. Correlations between antioxidant capacity, TPC and TFC levels were determined using the Pearson correlation coefficient test. All statistical analyzes were performed using Graph Pad Prism 9.0 software.

RESULTS AND DISCUSSION

Physical-chemical properties of spicy persimmon sauces

Spicy persimmon sauces (S1 and S2) have a pasty and homogeneous texture with a consistency similar to ketchup, which facilitates their use in various culinary preparations. Table 2 shows the results of the physicochemical analysis of the spicy persimmon sauces (S1 and S2). The moisture content was higher in the S1 sauce ( $p<0.05$ ). Despite the statistical difference between these results, they are close to the moisture content of the tomato sauce sauces (89%).<sup>40</sup> Sauces S1 and S2 did not differ ( $p>0.05$ ) in terms of their mean pH values (Table 2). Lower pH values were found in the sweet and sour sauces with partial replacement of tomato by avocado, with values between 3.49 and 3.76.22. The pH values must be below 4.0 to ensure greater product stability by limiting the microbiota to acid-tolerant microorganisms.<sup>19,41</sup> The pH values of the spicy persimmon sauces (S1 and S2) were similar to those of the stability threshold.

Table 2. Physicochemical analysis<sup>1</sup> and estimate of nutritional composition (100 g on wet basis)<sup>2</sup> of spicy persimmon sauces. Rio de Janeiro, RJ, Brasil. 2022

| Physical-chemical Analysis | S1                       | S2                       |
|----------------------------|--------------------------|--------------------------|
| Moisture (%)               | 88.08 ±0.01 <sup>a</sup> | 87.41 ±0.03 <sup>b</sup> |
| pH                         | 4.2 ±0.12 <sup>a</sup>   | 4.12 ±0.01 <sup>a</sup>  |
| Nutritional Composition    | S1                       | S2                       |
| Energy (Kcal)              | 109.67                   | 111.23                   |
| Carbohydrates (%)          | 18.94                    | 19.07                    |
| Protein (%)                | 1.02                     | 1.04                     |
| Lipid (%)                  | 3.31                     | 3.42                     |
| Crude fiber (%)            | 6.33                     | 6.46                     |

<sup>1</sup> Results of moisture and pH were expressed as average ± standard deviation of two replicates. Different superscript letters in the same row indicate significant differences between formulations ( $p < 0.05$ ). <sup>2</sup> Estimate nutritional composition (100 g on wet basis) of spicy persimmon sauces based on TACO (2011), TBCA (2020) food databases and Matheus, Nogueira et al. (2021).

<sup>2</sup> Spicy persimmon sauce 1 (S1) ingredients: persimmon; fresh garlic; olive oil; white wine vinegar; salt; ladyfinger pepper; water. Spicy persimmon sauce 2 (S2) ingredients: persimmon; fresh garlic; olive oil; white wine vinegar; salt; ladyfinger pepper; water; nutmeg powder; clove; cinnamon powder.

Table 2 shows the estimated nutrient composition of the spicy persimmon sauce and the spicy persimmon sauce with spice formulations. The composition is given for 100 g of the formulation for comparison purposes. However, the average portion of the sauce is about 5 g (1 teaspoon), as proposed by Brazilian legislation for this type of sauce.<sup>42</sup> Regarding macronutrients, carbohydrates are worth mentioning, especially because the recipes contain more persimmons. Persimmons contain a good amount of carbohydrates (17.3 to 19.3 g/100 g), which can vary depending on the fruit, harvest and variety (Rama Forte Japanese, Fuyu, Chocolate and Japanese).<sup>1</sup>

Figure 2 shows the color indices of the spicy persimmon sauces. The  $a^*$  and  $b^*$  values show that samples S1 and S2 are more reddish than blue ( $a1^*22.39$ ;  $a2^*21.82$ ) and more yellow than green ( $b1^*61.43$ ;  $b2^*49.29$ ). These colors can mainly be attributed to the original color of the persimmon fruit, which ranges from reddish to yellow depending on the carotene content.<sup>43</sup> The analyzed sauces (S1 and S2) have a light ( $L^*1$ : 61.32;  $L^*2$ : 53.64), slightly intense ( $c^*1$ : 65.39;  $c^*2$ : 53.9) or yellowish-red ( $h1$ : 69.97;  $h2$ : 66.13) color. The obvious difference between the colors of the two samples ( $\Delta E^*14.4$ ) can be explained by the variation between the formulations. Color is generally considered an important attribute for the appearance of a food. For example, before a food is consumed, color attributes help determine expectations of taste, aroma and palatability. The expected color of chili sauce is red, which is closely associated with freshness, ripeness, and desirability.<sup>44-46</sup> The spicy persimmon sauces (S1 and S2) have a reddish color; therefore, it is assumed that the color of our product should be acceptable to consumers of spicy sauces.

Figure 2. Color parameters and indices of spicy persimmon sauces

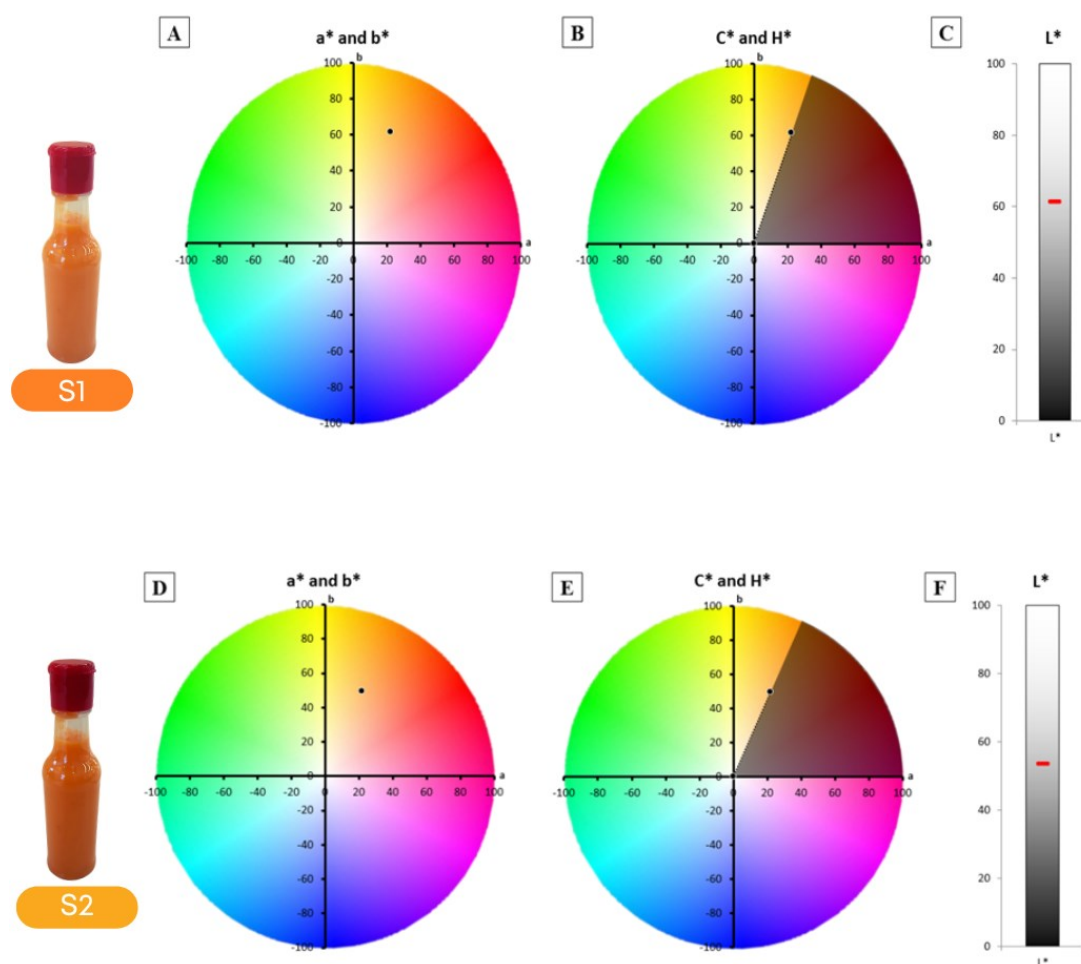


Figure 2.  $a^*$ - $b^*$  graph (A),  $C^*$ - $H^*$  graph (B) and  $L^*$  graph (C) for sauce 1 and  $a^*$ - $b^*$  graph (D),  $C^*$ - $H^*$  graph (E) and  $L^*$  graph (F) for sauce 2. In the  $a^*$ - $b^*$  graph, the black dot represents the sample's point ( $a^*$ ,  $b^*$ ). In the  $C^*$ - $H^*$  graph,  $C^*$  is graphically the length of the dotted line, and  $H^*$  is represented as the angle of the dark sector. In the  $L^*$  graph, the red line represents the  $L^*$  of the sample.

## Microbiological analysis

According to the results, samples S1 and S2 showed a lower content of thermotolerant coliforms than 3 MPN g<sup>-1</sup> and the absence of typical colonies of *Salmonella* sp. in 25 g of the sample. Similar results for the number of thermotolerant coliforms and the analysis of *Salmonella* sp. were reported by Silva et al.<sup>19</sup> in their study with sweet and sour assai sauce.

The microbiological determination of yeasts and molds was carried out because fungi are more tolerant to acidic pH values than bacteria and could therefore spoil the sauce.<sup>19,47</sup> The results for yeasts and molds were below 10 CFU/g in all samples (S1 and S2). Different results were found in the sauces available in Bangladesh; yeast and mold levels ranged from 50 to 500 CFU/g.<sup>48</sup>

The results of this study are in accordance with Brazilian legislation, which requires the absence of typical colonies of *Salmonella* sp. in 25 g of sample and a limit of 10 CFU/g for molds and yeasts for sauces.<sup>49</sup> The application of good food hygiene practices is necessary to ensure food safety from production to consumption.<sup>50</sup> The microbiological characteristics of persimmon seasoning sauces are indicative of appropriate handling practices and satisfactory hygienic conditions in the production process.

## Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of spicy persimmon sauces

Persimmon is the predominant ingredient in sauces (S1 and S2). This fruit is rich in bioactive compounds and therefore known for various properties, such as antioxidant activity.<sup>51</sup> Table 3 shows the results of the ABTS, DPPH, TPC and TFC tests for the formulations.

**Table 3.** Total phenolic compounds (TPC), total flavonoid compounds (TFC) and antioxidant capacity (DPPH and ABTS) of spicy persimmon sauces. Rio de Janeiro, RJ, Brazil. 2022

| Analyses                                        | S1 (Avg ± SD)                  | S2 (Avg ± SD)                  |
|-------------------------------------------------|--------------------------------|--------------------------------|
| TPC (µg GAE g <sup>-1</sup> )                   | 265.16 ± 44.06 <sup>a</sup>    | 358.94 ± 8.23 <sup>b</sup>     |
| TFC (µg RE g <sup>-1</sup> )                    | 729.63 ± 52.51 <sup>a</sup>    | 568.68 ± 13.98 <sup>b</sup>    |
| ABTS (IC <sub>50</sub> ) (µg mL <sup>-1</sup> ) | 3,103.08 ± 413.89 <sup>a</sup> | 2,976.31 ± 325.62 <sup>a</sup> |
| DPPH (IC <sub>50</sub> ) (µg mL <sup>-1</sup> ) | 3,061.33 ± 466.09 <sup>a</sup> | 1,944.88 ± 118.17 <sup>b</sup> |

<sup>1</sup>TPC – total phenolic content; GAE - gallic acid equivalents; IC<sub>50</sub> – the concentration of an antioxidant which reduces the free radical ABTS or DPPH by about 50%; Avg – average value; SD – standard deviation. In a specific row of this table, when the extracts of the sauce 1 (S1) were compared with the extracts of sauce 2 (S2) in relation to a parameter that was measured for both, the values marked with different letters in the same line were statistically different (p < 0.05).

<sup>2</sup> Spicy persimmon sauce 1 (S1) ingredients: persimmon; fresh garlic; olive oil; white wine vinegar; salt; ladyfinger pepper; water. Spicy persimmon sauce 2 (S2) ingredients: persimmon; fresh garlic; olive oil; white wine vinegar; salt; ladyfinger pepper; water; nutmeg powder; clove; cinnamon powder.

The mean TPC value of S2 is higher ( $p < 0.05$ ) than the mean TPC value of S1. This discrepancy may be due to the cloves, cinnamon and nutmeg used in the S2 formulation.<sup>52</sup> The TPC values found in the S1 and S2 sauces of the present work are higher than other persimmon products. For example, the TPC of a persimmon ketchup with pepper was reported to be  $73.2 \mu\text{g GAE g}^{-1}$  and the TPC of a persimmon chutney without sugar was  $35.1 \mu\text{g GAE g}^{-1}$ .<sup>9</sup> Such differences between the TPC of persimmon products can be attributed to the different formulations as well as the variability in the production of such products. The mean TFC of S1 is higher ( $p < 0.05$ ) than that of S2. Considering that flavonoid content is strongly influenced by factors such as food preparation and processing, the possible non-standardization of sauce preparation stages may have had an impact on the preservation of these compounds.<sup>53</sup>

The composition of the ingredients used to make the sauce influences the TPC and TFC. The persimmon sauces S1 and S2 had a lower TPC and TFC content than sauces made with other fruits and vegetables such as assai and tomato. TPC values of the assai sauces were 141.00 and 158.16 mg GAE 100 g<sup>-1</sup>.<sup>19</sup> The results for the TPC and TFC content of the tomato sauce were 881 mg GAE 100 g<sup>-1</sup> and 726 mg RE 100 g<sup>-1</sup>, respectively.<sup>40</sup> It is important to emphasize that the limitation of the TPC analysis is that ascorbic acid and sugar are not separated in the sample.<sup>54</sup>

No statistical difference was found between S1 and S2 ( $p > 0.05$ ) in relation to the ABTS analysis. On the other hand, the mean IC<sub>50</sub> (DPPH) value of S1 was higher ( $p < 0.05$ ) than the IC<sub>50</sub> (DPPH) value of S2. In the DPPH analysis, the antioxidant potential of sample S2 was more pronounced than that of sample S1. At least in part, this behavior can be explained by strong antioxidant components in S2, originating from the spices. Thus, eugenol contained in clove essential oil had the strongest antioxidant activity and free radical scavenging effect compared to butylated hydroxyanisole, butylated hydroxytoluene,  $\alpha$ -tocopherol and Trolox.<sup>55</sup> In addition, myristicin, the main aromatic component of the essential oils of *Myristica fragrans* (nutmeg), has a significant antioxidant activity.<sup>56</sup>

## Volatile compounds of spicy persimmon sauces

Table 4 shows the volatile compounds found in the spicy persimmon sauce samples (S1 and S2). Of the 40 compounds listed in Table 4, only three were not identified (they were only detected in the S1 samples). The analysis of the persimmon sauces allowed the detection and identification of 37 volatile compounds, including 4 monoterpenes, 4 oxygenated monoterpenes, 4 organosulfur compounds, 5 aldehydes, 7 phenylpropanoids, 7 sesquiterpenes, 3 carboxylic acids, 1 lactone and 2 alcohols.

**Table 4.** Identification and quantification of volatile compounds in spicy persimmon sauces. Rio de Janeiro, RJ, Brazil. 2022

| Volatile Compounds     | LRI<br>(calculated) | LRI<br>(literature) | S1 (Avg $\pm$ SD) %           | S2 (Avg $\pm$ SD) %          | Odor description                   |
|------------------------|---------------------|---------------------|-------------------------------|------------------------------|------------------------------------|
| <b>Monoterpene</b>     |                     |                     |                               |                              |                                    |
| Limonene               | 1029                | 1031 $\Phi$         | 9.92 $\pm$ 1.00 <sup>a</sup>  | 0.76 $\pm$ 1.08 <sup>b</sup> | lemon, orange                      |
| m-cymene               | 1023                | 1026 *              | Nd                            | 1.05 $\pm$ 0.01              | solvent, gasoline, citrus          |
| $\gamma$ -terpinene    | 1063                | 1062 *              | Nd                            | 1.73 $\pm$ 0.06              | gasoline, turpentine               |
| $\alpha$ -terpinolene  | 1087                | 1086 $\S$           | Nd                            | 0.61 $\pm$ 0.02              | -                                  |
| <b>Oxygenated</b>      |                     |                     |                               |                              |                                    |
| <b>Oxmonoterpene</b>   |                     |                     |                               |                              |                                    |
| Eucalyptol             | 1033                | 1033 $\Phi$         | 15.87 $\pm$ 0.04 <sup>a</sup> | 1.04 $\pm$ 0.09 <sup>b</sup> | mint, sweet<br>turpentine, nutmeg, |
| terpinen-4-ol          | 1182                | 1179 $\Phi$         | Nd                            | 11.28 $\pm$ 0.84             | must                               |
| $\alpha$ -terpineol    | 1192                | 1198 $\Phi$         | Nd                            | 1.04 $\pm$ 0.00              | oil, anise, mint                   |
| geranylacetone         | 1447                | 1448 $\Phi$         | 1.36 $\pm$ 0.17               | Nd                           | -                                  |
| <b>Organosulfur</b>    |                     |                     |                               |                              |                                    |
| diallyl disulfide      | 1067                | 1077 *              | 3.26 $\pm$ 0.01 <sup>a</sup>  | 0.84 $\pm$ 0.04 <sup>b</sup> | -                                  |
| diallyl trisulfide     | 1289                | 1289 $\S$           | 10.9 $\pm$ 0.07 <sup>a</sup>  | 2.73 $\pm$ 0.04 <sup>b</sup> | -                                  |
| 3-Vinyl-1,2-           |                     |                     |                               |                              | -                                  |
| dithiacyclohex-4-ene   | 1190                | 1190 *              | 5.32 $\pm$ 0.49 <sup>a</sup>  | 1.20 $\pm$ 0.13              |                                    |
| 3-Vinyl-1,2-           |                     |                     |                               |                              | -                                  |
| dithiacyclohex-5-ene   | 1197                | 1197 *              | 8.73 $\pm$ 0.11 <sup>a</sup>  | 1.59 $\pm$ 0.06 <sup>b</sup> |                                    |
| <b>Aldehyde</b>        |                     |                     |                               |                              |                                    |
| Nonanal                | 1097                | 1098 $\Phi$         | 2.01 $\pm$ 0.02               | Nd                           | fat, citrus, green                 |
| 2-decenal              | 1255                | 1260 *              | 9.17 $\pm$ 0.32 <sup>a</sup>  | 0.71 $\pm$ 0.01 <sup>b</sup> | tallow, orange                     |
| Undecanal              | 1340                | 1365 $\Phi$         | 1.35 $\pm$ 0.08               | Nd                           | oil, pungent, sweet                |
| Cinnamaldehyde         | 1266                | 1266 $\S$           | Nd                            | 17.36 $\pm$ 1.90             | cinnamon, paint                    |
| 2-                     |                     |                     |                               |                              | -                                  |
| Methoxycinnamaldehyd   |                     |                     |                               |                              |                                    |
| e                      | 1512                | 1505 *              | Nd                            | 0.52 $\pm$ 0.04              |                                    |
| <b>Phenylpropanoid</b> |                     |                     |                               |                              |                                    |
| Safrol                 | 1285                | 1286 *              | Nd                            | 2.76 $\pm$ 0.12              | spice, sweet, warm                 |
| eugenol                | 1355                | 1356 $\Phi$         | Nd                            | 3.89 $\pm$ 0.03              | clove, honey                       |
| methyl isoeugenol      | 1495                | 1495 *              | Nd                            | 1.66 $\pm$ 0.11              | -                                  |
| methyl eugenol         | 1400                | 1401 *              | Nd                            | 6.07 $\pm$ 0.09              | clove, spice                       |
| myristicin             | 1511                | 1515 $\S$           | Nd                            | 15.46 $\pm$ 0.26             | spice, warm, balsamic              |
| elemicin               | 1540                | 1540 $\Phi$         | Nd                            | 16.35 $\pm$ 1.11             | spice, flower                      |
| isoelemicin            | 1580                | 1581 $\S$           | Nd                            | 0.89 $\pm$ 0.01              | spice, flower                      |

**Table 4.** Identification and quantification of volatile compounds in spicy persimmon sauces. Rio de Janeiro, RJ, Brazil. 2022 (continues).

| Volatile Compounds                        | LRI<br>(calculated) | LRI<br>(literature) | S1 (Avg $\pm$ SD) %          | S2 (Avg $\pm$ SD) %          | Odor description |
|-------------------------------------------|---------------------|---------------------|------------------------------|------------------------------|------------------|
| <b>Sesquiterpene</b>                      |                     |                     |                              |                              |                  |
| Cadalene                                  | 1610                | 1636 $\Phi$         | 4.87 $\pm$ 2.74              | Nd                           | -                |
| $\alpha$ -copaene                         | 1375                | 1376 *              | Nd                           | 1.84 $\pm$ 0.18              | wood, spice      |
| trans-caryophyllene                       | 1418                | 1418 $\S$           | Nd                           | 1.07 $\pm$ 0.25              | -                |
| $\alpha$ -bergamotene                     | 1435                | 1436 $\S$           | Nd                           | 0.36 $\pm$ 0.11              | wood, warm, tea  |
| $\alpha$ -muurolene                       | 1499                | 1499 *              | Nd                           | 1.03 $\pm$ 0.14              | wood             |
| $\beta$ -bisabolene                       | 1509                | 1506 *              | Nd                           | 0.53 $\pm$ 0.13              | balsamic         |
| 1,4-Cadinadiene                           | 1525                | 1531 $\Phi$         | Nd                           | 0.36 $\pm$ 0.13              | -                |
| <b>Carboxylic acid</b>                    |                     |                     |                              |                              |                  |
| dodecanoic (lauric) acid                  | 1565                | 1568 $\Phi$         | 7.28 $\pm$ 2.28 <sup>a</sup> | 1.55 $\pm$ 0.22 <sup>b</sup> | -                |
| tetradecanoic (myristic) acid             | 1760                | 1751 *              | 7.09 $\pm$ 1.28 <sup>a</sup> | 2.66 $\pm$ 1.14 <sup>b</sup> | -                |
| hexadecanoic (palmitic) acid              | 1970                | 1970 *              | 4.51 $\pm$ 0.19 <sup>a</sup> | 0.55 $\pm$ 0.58 <sup>b</sup> | -                |
| <b>Lactone</b>                            |                     |                     |                              |                              |                  |
| Coumarin                                  | 1428                | 1428 $\Phi$         | Nd                           | 0.51 $\pm$ 0.02              | green, sweet     |
| <b>Alcohol</b>                            |                     |                     |                              |                              |                  |
| 1-octanol                                 | 1065                | 1070 *              | 1.60 $\pm$ 0.07              | Nd                           | -                |
| Nonadecanol                               | 2130                | 2175 $\Phi$         | 0.76 $\pm$ 0.1               | Nd                           | -                |
| <b>Compounds that were not identified</b> |                     |                     |                              |                              |                  |
| NI                                        | 1090                | -                   | 2.41 $\pm$ 0.13              | Nd                           | -                |
| NI                                        | 1105                | -                   | 2.41 $\pm$ 0.09              | Nd                           | -                |
| NI                                        | 2135                | -                   | 1.22 $\pm$ 0.07              | Nd                           | -                |

<sup>1</sup> RI, modified Kovats index (Van Den Dool and Kratz, 1963), calculated using using C<sub>9</sub>-C<sub>26</sub> alkanes; Nd – not detected; (Avg  $\pm$  SD)% - percentage of the compound in relation to the total area of the chromatogram (normalization technique) expressed as average value  $\pm$  standard deviation; Avg - the average value determined in the analyses of the samples; Sd- standard deviation; All compounds were identified with the aid of the mass spectra data and by comparing the calculated LRI with the theoretical LRI (LRI obtained from the literature). In a specific row of the Table, the values marked with different letters are statistically different (p < 0.05); References for LRI-literature: \*NIST;  $\Phi$  PHEROBASE;  $\S$  PUBCHEM. Odor description: collected from the website: <https://www.flavornet.org/flavornet.html>

<sup>2</sup> spicy persimmon sauce 1 (S1) ingredients: persimmon; fresh garlic; olive oil; white wine vinegar; salt; ladyfinger pepper; water. spicy persimmon sauce 2 (S2) ingredients: persimmon; fresh garlic; olive oil; white wine vinegar; salt; ladyfinger pepper; water; nutmeg powder; clove; cinnamon powder.

Ten common compounds were found in the volatile fraction of both sauces: Limonene, eucalyptol, diallyl disulfide, diallyl trisulfide, 3-vinyl-1,2-dithiacyclohex-4-ene, 3-vinyl-1,2-dithiacyclohex-5-ene, 2-decenal, dodecanoic acid, tetradecanoic acid and hexadecanoic acid. It is important to note that the common ingredients of S1 and S2 – persimmon, garlic, olive oil, and ladyfinger pepper – can be associated with the above compounds. For example, limonene, eucalyptol and 2-decenal have already been identified as components of the volatile fraction of persimmon.<sup>57-60</sup> Limonene and eucalyptol were also found in the volatile fraction of ladyfinger pepper.<sup>61</sup> The olive oil component can be linked to carboxylic acids in both sauces. The compounds diallyl disulfide, diallyl trisulfide, 3-vinyl-1,2-dithiacyclohex-4-ene and 3-vinyl-1,2-dithiacyclohex-5-ene are responsible for the characteristic aroma of garlic. In addition, these compounds have been associated with anticancer and antitumorigenic activities.<sup>62-66</sup>

In addition to the ingredients contained in sauce S1, the formulation of sample S2 contains spices such as nutmeg, clove and cinnamon. For this reason, S2 has a richer composition (31 compounds) than S1 (19 compounds). Spices are often used as preservatives and to flavor food; they are also known as the aromatic plants group.

In this work, phenylpropanoid and sesquiterpene compounds are associated with spices.<sup>67-69</sup> According to the quantitative analysis of the chemical composition of sauce S2, the compounds myristicin, elemicin and cinnamaldehyde are noteworthy because they are present in large quantities. According to Butzge et al.,<sup>70</sup> cinnamaldehyde, myristicin and elemicin are probably responsible for the antifungal effect of the essential oils of *Cinnamomum cassia* (cinnamon), *Syzygium aromaticum* (clove) and *Myristica fragrans* (nutmeg).

Aroma is one of the most important factors in consumer preference and the choice of certain products. A broad spectrum of different volatile compounds is responsible for the aroma. The sensory relevance of compounds can be assessed using the odor threshold (OT). This parameter is defined as the minimum concentration of a volatile compound at which it can be perceived by the human sense of smell.<sup>71,72</sup>

In this study, the aroma of sample S1 may be influenced by the presence of limonene, eucalyptol and diallyl trisulfide due to their relatively high concentration and low odor threshold.<sup>68</sup> Limonene has a fresh lemon and orange odor and an OT in water of  $10 \mu\text{g L}^{-1}$ , while eucalyptol has a low OT in water ( $12 \mu\text{g L}^{-1}$ ) and its aroma is usually associated with mint and sweet.<sup>73</sup> Diallyl trisulfide present in sauce S1 contributes to the pungent, herbaceous and metallic notes (OT:  $0.34 \mu\text{g L}^{-1}$ ).<sup>74</sup> Carboxylic acids, such as dodecanoic acid (lauric acid) and tetradecanoic acid (myristic acid), are often considered off-flavors in foods, as their flavors are usually associated with unpleasant, cheesy and rancid notes.<sup>75</sup> These carboxylic acids were found in considerable amounts in sample S1, but probably cannot be considered as main off-flavor compounds in this sample as their OT is  $10,000 \mu\text{g L}^{-1}$ .<sup>76</sup> The high relative concentrations of elemicin and myristicin and the low OT make their contribution to the flavor of sauce S2 relevant. The sensory properties of these compounds have been described as spicy, warm, balsamic and floral, with an odor threshold of 25 ppb in water.<sup>77,78</sup> Despite the high concentration of cinnamaldehyde, its OT ( $750 \mu\text{g L}^{-1}$ ) is classified as high. Therefore, it cannot be classified as an odor-active compound of this sample.<sup>79</sup>

## CONCLUSION

The development of persimmon-based sauces has proven to be an alternative to using this fruit, contributing to increase the fiber and antioxidant compounds content in products such as food sauces.

The use of persimmon in the formulation of sauces is an innovative and effective approach to promote the consumption of this fruit even in the off-season and reduce post-harvest losses, strengthening its

potential as a functional ingredient in new foods. Both sauces showed adequate physicochemical and microbiological properties.

The samples showed high antioxidant activity. The addition of spices (nutmeg, clove and cinnamon) to persimmon-based sauces influenced the sensory (color and aroma) and bioactive properties (antioxidant activity) of the product.

Further research is needed to investigate the sensory profile and acceptance of the developed sauces to increase their innovation potential and market acceptance. In addition, future studies to analyze volatile compounds in more detail using internal standards are recommended to explore the possible extension of this work beyond the field of Dietetics.

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## Contributors

Maragoni-Santos C contributed to developing the product, collected the data, performed the analysis, wrote the paper, contributed data and analysis tools; Santana I developed the product, collected the data and contributed with analysis tools; Ribeiro LO collected the data and performed the analysis; Mora BM, Martins BAC, Ferreira C, Cardoso LLS, Santos LM, de Oliveira LS contributed to the product development; Jung EP contributed analysis tools; Moreira RFA contribution performed the analysis, wrote the paper, contributed with analysis tools; Fai AEC conceived and designed analysis, wrote the paper, revision and approval of the final version.

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