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## Acceptability and consumption intention of roasted beef patties seasoned with annatto seed powder (*Bixa orellana* L.)

### Aceitabilidade e intenção de consumo de hambúrgueres de carne bovina assada condimentados com pó de semente de urucum (*Bixa orellana* L.)

#### Abstract

**Introduction:** Annatto is a fruit of Brazilian sociobiodiversity; its seeds, rich in bioactive compounds with antioxidant activity, are used as coloring in traditional Latin American culinary and globally within the food industry. However, the acceptance of roasted beef patties seasoned with this condiment is unknown. **Objective:** Verify the consumption intention and the Acceptability Index (AI) of roasted beef patties seasoned with annatto seed powder and salt. **Methods:** Beef patties were seasoned with the addition of annatto seed powder at 0%, 0.5% or 1%, and salt at 0% or 0.5%, and subsequently prepared by dry cooking. Acceptance test (hedonic scale) was applied, Acceptability Index was calculated and data were submitted to analysis of variance (ANOVA) followed by Student's *t*-test, accepting 5% of significance. **Results:** A total of 160 individuals participated in the sensory analysis. The samples seasoned with 0.5% of salt received higher scores ( $5.7 \pm 1.3$ ) when compared to other treatments ( $p < 0.05$ ); annatto seed powder concentrations of 0.5% and 1.0% resulted in accepted samples, without statistical difference and with intention of occasional consumption; the Acceptability Index for all samples with salt and annatto seed powder was over 70%, result considered satisfactory. **Conclusion:** 1% of annatto seed powder can be used as seasoning of roasted beef patties. Knowing this, future research should explore the benefits of the recognized antioxidant action of this condiment in inhibiting carcinogens formed during thermal processing of beef.

**Keywords:** Meat. Bixaceae. Spices. Cooking. Sensory analysis. Dietetics.

#### Resumo

**Introdução:** O urucum é um fruto da sociobiodiversidade brasileira; suas sementes, ricas em compostos bioativos com atividade antioxidante, são utilizadas como corante na culinária tradicional latino-americana e mundialmente pela indústria alimentícia. No entanto, a aceitação de hambúrgueres de carne assada condimentados com esse ingrediente é desconhecida. **Objetivo:** Verificar a intenção de consumo e o Índice de Aceitabilidade de hambúrgueres bovinos assados condimentados com pó de semente de urucum e sal. **Métodos:** Os hambúrgueres bovinos foram condimentados com pó de semente de urucum a 0%, 0,5% ou 1%, e sal a 0% ou 0,5%, e posteriormente assados. Foi aplicado teste de aceitação (escala hedônica), calculado Índice de Aceitabilidade e os dados foram submetidos à análise de variância (ANOVA) seguida do teste *t* de Student, adotando-se 5% de significância. **Resultados:** A análise sensorial teve 160 participantes. As amostras condimentadas com 0,5% de sal receberam notas

maiores ( $5,7 \pm 1,3$ ) quando comparadas aos demais tratamentos ( $p < 0,05$ ); as concentrações de 0,5% e 1,0% de pó de semente de urucum resultaram em amostras aceitas, sem diferença estatística e com intenção de consumo ocasional; o Índice de Aceitabilidade para todas as amostras com sal e pó de semente de urucum foi superior a 70%, resultado satisfatório. **Conclusão:** 1% de pó de semente de urucum pode ser usado para condimentação de hambúrgueres de carne assada. Sabendo disso, pesquisas futuras devem explorar os benefícios da reconhecida ação antioxidante desse condimento na inibição de carcinógenos formados durante o processamento térmico da carne bovina.

**Palavras-chave:** Carne. Bixaceae. Especiarias. Culinária. Análise sensorial. Dietética.

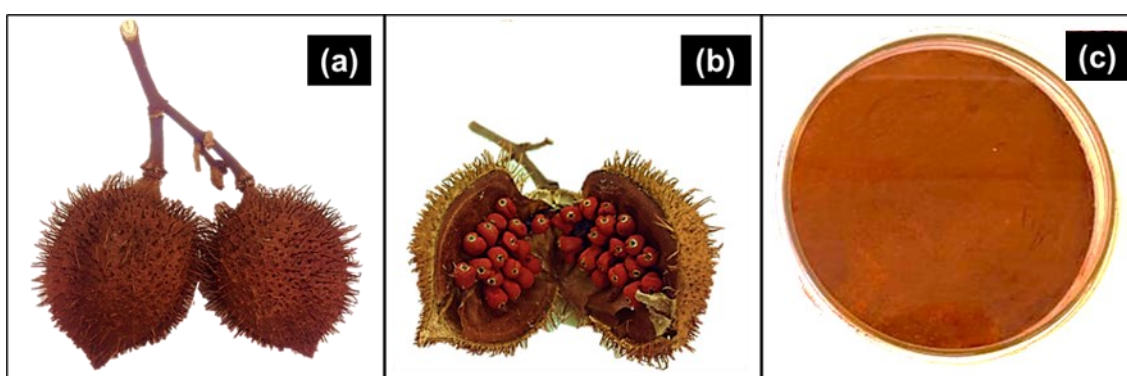
## INTRODUCTION

Brazil is the eighth country to consume red meat, characterized by the muscle meat from mammal, including beef and veal, pork and sheep meat,<sup>1</sup> with consumption of 38.3 kg/person/year, lower only than that of the United States, Chile, Argentina, Korea, Vietnam and China, of 50.2, 49.1, 48.6, 45, 44.4, 42.5 and 38.7 kg/person/year, respectively.<sup>2</sup> This consumption can be considered high, since current health recommendations suggest consumption between approximately 5 and 26 kg/person/year.<sup>1</sup> This fact, coupled with recent studies that prove the association of red meat consumption with an increased risk of developing non-communicable diseases (NCDs), including colorectal cancer, generates great concern and urgency in decision-making in public policies to promote the health of the population.<sup>1,3-5</sup>

Among the possible causes that explain the association of high meat consumption with the emergence of cancer is the formation of thermal processing contaminants with carcinogenic potential, such as heterocyclic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs) due to the combustion of creatinine and fat, respectively, during cooking in temperatures above 100°C and their intensification as time and temperature increase, and humidity decreases.<sup>5-7</sup> In this context, strategies have been investigated to mitigate these compounds, such as the incorporation of natural ingredients rich in bioactive compounds.<sup>7</sup>

Traditional fresh spices from Brazilian culinary culture are food sources of bioactive compounds with a modulating role of oxidative stress and, due to this characteristic, they may have the ability to control the production of the aforementioned carcinogens, while providing sensory quality.<sup>8,9</sup> Nevertheless, the first studies using annatto seeds for this purpose are being conducted by our group. Annatto (*Bixa orellana* L.), shown in Figure 1, is native to Central and South America, but it was distributed in Southeast Asia, Africa, and the Caribbean, and has been cultivated in tropical regions. American Indigenous widely used its seeds to make body paint, lip pigment, arts, crafts, murals, to medicinal ends, to protect the skin against insect bites, and as a principal coloring agent in foods, which has been used to this day in the traditional culinary as a coloring and flavoring agent.<sup>10-13</sup>

**Figure 1.** (a) annatto, (b) annatto seeds in the fruit, and (c) grounded annatto seeds



Bixin, an apocarotenoid derived by from the oxidative cleavage of lycopene, is the main carotenoid that makes up annatto seeds (80%) and provides the reddish color.<sup>10,11</sup> Such a compound has high stability against oxidation.<sup>14</sup> According to Mesquita et al.,<sup>15</sup> carotenoids also have mechanisms of physical or chemical action to neutralize the effects of oxidizing and neoplastic agents, such as HAs and PAHs; as well as phenolic compounds, also present in annatto seeds and which act synergistically for antioxidant benefits.<sup>16</sup>

Knowing this potential for action of bioactive compounds as modulators of oxidative stress, joining them to meat consumption can be a strategy to make the consumption of this food group safer. However, the acceptance of cuts of meat seasoned with annatto seeds is unknown. Thus, this study aimed to verify the consumption intention and the acceptability of roasted beef patties seasoned with different concentrations of annatto seed powder and salt, as a first stage of a study that evaluated the effect of annatto seasoning on the mitigation of thermal processing contaminants in meat.

## METHODS

### Samples preparation

Beef (*Gluteus medius*), used in the basic formulation of patties, and refined salt were purchased at a local market in the city of Santos, state of São Paulo, Brazil. Annatto seeds, of the Piave variety, were received in vacuum packs and protected from light by donation from a producer from Monte Castelo, São Paulo.

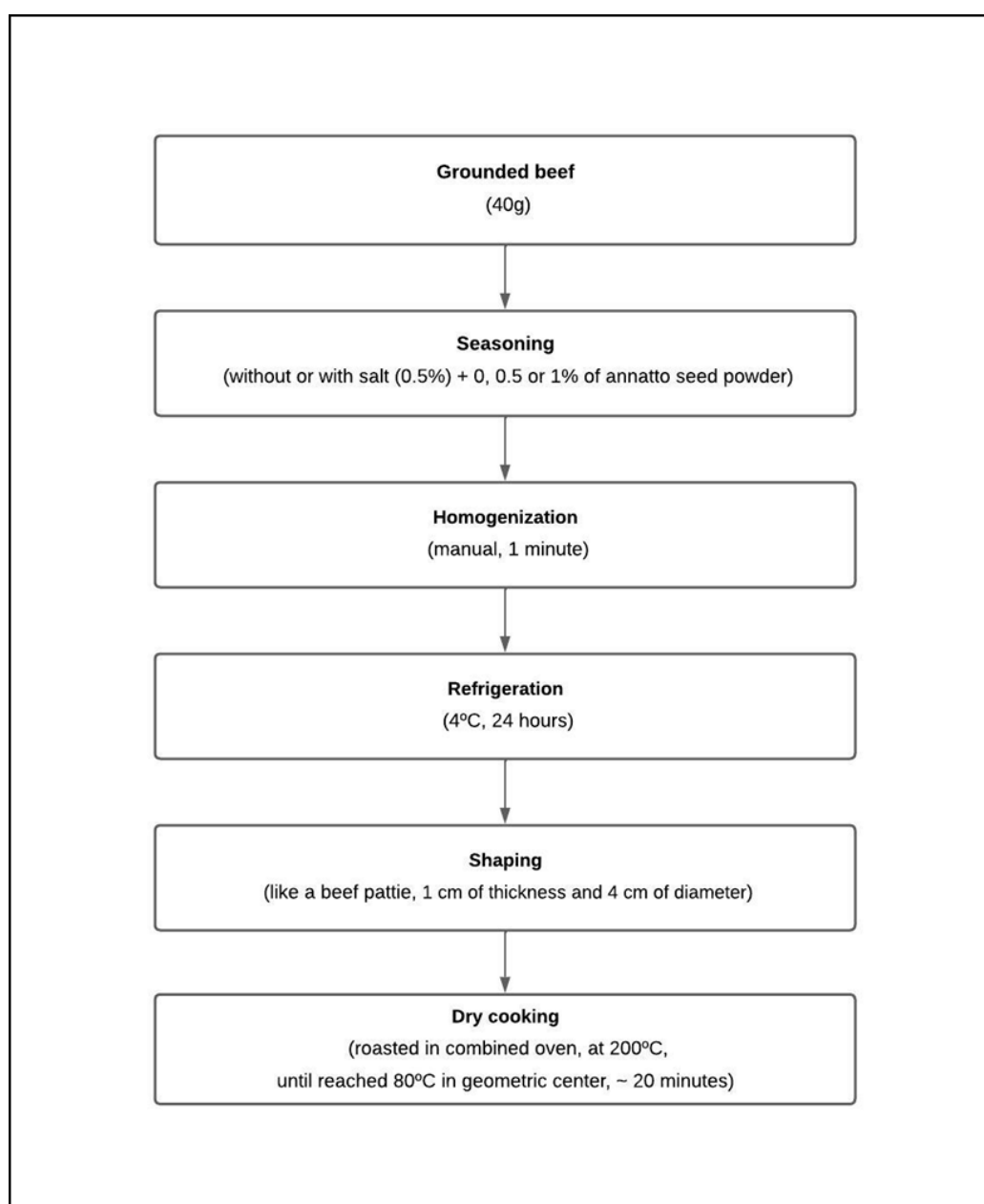
Annatto seeds were ground in a micro-homogenizing mill (Tecnal, TE-645) and sieved through a 125 mm. The product was stored in opaque and vacuum-sealed packaging at -18°C at the Laboratory of Experimental Dietetics (LaDEx) of the Federal University of São Paulo, *campus* Baixada Santista (Unifesp/BS), until it was used to season the beef.

The beef was grounded and seasoned with salt and annatto seed powder, according to the experimental design presented below, and homogenized for one minute; then it was kept at 4°C under refrigeration for approximately 24 hours.

Portions of grounded beef (40g) were shaped in molds (1 cm of thickness, 4 cm of diameter) to obtain regular samples. The beef patties were prepared by dry cooking at 200°C in a combined oven with nine temperature sensors (Prática, EC6/TSC6), in a non-stick roasting pan without the addition of fat, until they reached 80°C in their geometric center, corresponding to approximately 20 minutes, being, therefore, considered well-baked goods.<sup>17</sup> The steps of the beef patties preparation are illustrated in Figure 2. The samples were kept in a heated oven to preserve the temperature of the roasted beef patties between cooking and sensory analysis.

The preparation followed good sanitary practices for food handling.<sup>18</sup>

**Figure 2.** Flow sheet of beef patties preparation.



## Experimental design

The factors were studied in two trials using salt (factor A) in two concentrations in relation to the net weight of the meat: 0.5% in trial 1 and 0% in trial 2, and seasoning with annatto seed powder (factor B) in three concentrations, 0%, 0.5% and 1.0% in both assays, having as a response variable the “acceptability”. These combinations of salt and annatto seed powder are shown in Chart 1.

**Chart 1.** Combination of salt and annatto seed powder seasoning used in the preparation of roasted beef patties samples.

Salt 0.5%	Annatto seed powder		
	0	0.5%	1.0%
Yes	A1B0	A1B1	A1B2
No	A0B0	A0B1	A0B2

The addition of 0.5% of salt in relation to the net weight of the meat had as a reference the value sufficient to meet the nutritional recommendations and avoid excess intake.<sup>19</sup> The choice of annatto seed powder seasoning levels (0.5 and 1%) considered the recommendations of the European Food Safety Authority regarding the maximum tolerance for daily bixin intake, which is 6 mg/kg of body weight, or the equivalent of 360 mg for the safe consumption of a 60 kg adult.<sup>20</sup> Considering that the seed is composed of 2% bixin,<sup>21</sup> an average consumption of approximately 100 g of beef seasoned with 1% annatto seed powder per day would result in a total bixin intake of around 20 mg, below the defined safety limits. Furthermore, the use of these seasoning percentages has been observed in other studies that employed condiments and spices with antioxidant potential to mitigate the formation of contaminants during thermal processing,<sup>9</sup> and was demonstrated to be effective in reducing HAs in a recent study conducted by our group.<sup>21</sup>

## Sensory analysis

The study was approved by the Research Ethics Committee of the Federal University of São Paulo (opinion no. 3.008.323).

The affective acceptance and the salt taste sensitivity tests were carried out with untrained volunteers of both sexes, adults, omnivores, and members of the academic community, recruited through an invitation on a social network and in the University's murals, without exclusion criteria (n=160), in two rounds. Samples were presented at random. Sociodemographic variables, such as sex and age, and smoking habits were also investigated. Before data collection, the volunteers were informed about the objectives and methods of the study and expressed their agreement to participate by signing the Free Informed Consent Form.

The tests were conducted by a previously trained team, consisting of undergraduate and graduate students of Nutrition, who had taken the Dietetic Technique classes and received instruction on sensory analysis, along with a pilot training before the official testing. These tests were carried out in individual sensory cabins, designed to be free from external distractions, equipped with a chair, a table, a spittoon, and a wood window for delivery of samples, climate and airflow control, located at the Sensory Analysis Laboratory of Unifesp/BS.

By applying a triangular test, before carrying out test 1, it was possible to estimate the salty taste sensitivity of all the tasters who participated in this first test. For this, samples of solutions for the basic salty taste were prepared with sodium chloride (0.2% of refined salt) and other samples with water.<sup>22</sup> To perform this test, three samples were coded with three-digit random numbers and offered to the tasters randomly in plastic cups, along with the form containing the information that two would be the same and one different. The participant should taste the samples from left to right, identify and annotate the different sample.<sup>23</sup> With the results of this step, it was possible to evaluate the results of the acceptance of the roasted beef patties according to the sensitivity to the salty taste.<sup>24</sup>

The consumption intention of the samples was estimated in trials 1, for samples seasoned with annatto seed powder and with salt, and 2, for samples seasoned with annatto seed powder and without salt, by an affective acceptance test using a 7-point structured hedonic scale, consisting of the verbal notes: "I would always eat" at the upper end, "I would eat occasionally" at the center and "I would never eat" at the lower end. For this test, samples were coded with three-digit random numbers<sup>22,25</sup> and presented to tasters with annatto seed powder seasoning in ascending order from left to right, served on white porcelain plates, with the form that asked them to rate the samples based on the hedonic scale; the response form had space for additional comments.<sup>25</sup>

For the analysis, it was decided to serve 25% of the roasted portion, enough to repeat the test without waste. The tasters received water and were instructed to clean the taste buds between the consumption of the samples, in order to minimize residual effects. Red lighting was used in the cabins to cancel out the color difference between the samples and assess global acceptance, as the tasters were untrained volunteers.

With the scores of acceptance of the affective test, the Acceptability Index (AI) was calculated according to the formula described by Bispo et al., as follows: <sup>26</sup>

$$AI (\%) = \frac{\text{Average of the scores attributed} \times 100}{\text{Maximum score attributed}}$$

Samples with results greater than or equal to 70% were considered accepted. <sup>24,26,27</sup>

## Statistical analysis

Data were processed using the Statistical Software for Data Science (STATA) program, version 13, and verified for normality of distribution by measures of asymmetry and kurtosis. The results of the acceptance test were submitted to analysis of variance (ANOVA) followed by Student's *t*-test, accepting 5% of significance.

## RESULTS

Sensory analysis was organized into four sessions: two for test 1, where the triangular test of sensitivity to salty taste also took place, and two for the acceptance test in test 2.

A total of 160 adults participated, 80 in each trial, aged between 18 and 63. The most frequent age group was 21 (17.50%, *n* = 28), and the majority were female (78.13%, *n* = 125). Among the participants, the minority were smokers (9.38%, *n* = 15).

The triangular test analysis of sensitivity to salty taste revealed that almost all participants who participated in the first test were able to perceive it (92.50%, *n* = 74).

The scores of the affective acceptance test using the 7-point structured hedonic scale were given considering the global consumption intention of each participant, without considering the organoleptic properties individually (color, odor, flavor and texture), considering that the tasters were untrained volunteers.

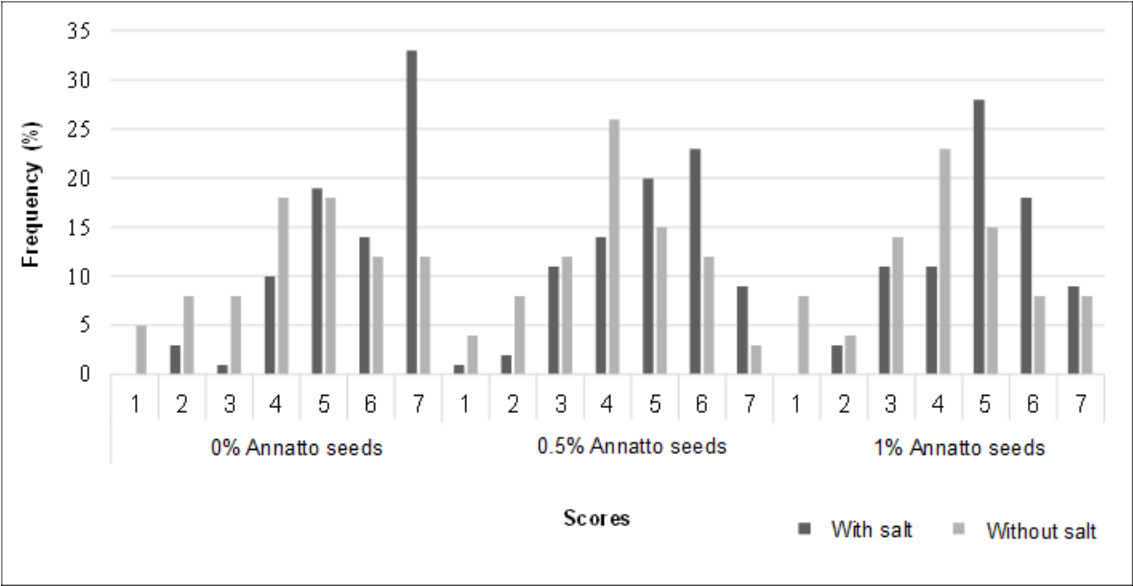
Based on the information available in Table 1, we can say that all preparations are promising, as the averages of the scores obtained ( $\geq 4.1$ ) are higher than the average of the 7-point hedonic scale (3.5), but also that some adjustments can be made to increase acceptance.

The sample with salt and without annatto seed powder obtained the highest average (5.7), corresponding to "I would eat often" ( $p < 0.05$ ), followed by the samples with salt and annatto seed powder at 0.5% and 1.0%, which obtained equal averages (4.9) and reflected the consumption intention with results corresponding to "I would eat occasionally".

The samples without salt and with annatto seed powder obtained the lowest averages (4.1), which, according to the hedonic scale used, would also classify the consumption intention as "I would eat occasionally". The samples without salt and without annatto seed powder obtained the same classification, however, with a slightly higher average (4.5), but without statistical difference between treatments.

The frequency distribution of the scores, illustrated in Figure 3, confirms the above and shows that for the samples with salt, scores 5, 6 and 7 have a higher frequency, while for the samples without salt, the highest frequencies occur with the lowest scores (1, 2, 3 and 4).

**Figure 3.** Frequency of consumption intention scores of roasted beef patties seasoned with salt and/or annatto seed powder.



Regarding the Acceptability Index (Table 1), the sample seasoned only with salt obtained the highest result (81.43%) for global acceptance. The samples with salt and annatto seed powder at different concentrations obtained AI equal to 70%, which demonstrates a satisfactory result and that the product can also be considered accepted. For the samples without salt, the values were lower than 70%, which indicates non-acceptance.

**Table 1.** Mean values and standard deviations (SD) of the consumption intention and Acceptability Index (%) of roasted beef patties seasoned with salt and/or annatto seed powder. Santos-SP, 2019.

With salt						Without salt					
Annatto	n	AI (%)	Mean	SD	CI (%)	n	AI (%)	Mean	SD	CI (%)	p-value <sup>1</sup>
0	80	81.43	5.7 <sup>a</sup>	1.3	95	80	64.29	4.5 <sup>a</sup>	1.7	95	0.000
0.5%	80	70.00	4.9 <sup>b</sup>	1.4	95	80	58.57	4.1 <sup>a</sup>	1.5	95	0.000
1.0%	80	70.00	4.9 <sup>b</sup>	1.3	95	80	58.57	4.1 <sup>a</sup>	1.7	95	0.001

Acceptability Index (AI).  
Standard deviations (SD).  
Confidence Interval (CI).  
Different letters in the column indicate a statistically significant difference (p<0.05).  
<sup>1</sup>Student's *t*-test for samples with and without salt.

DISCUSSION

The roasting method reproduces the usual condition of meat preparation according to conventional procedures of Brazilian cuisine,<sup>17,19,22</sup> and as dry cooking poses a greater risk to health, given the potential for the formation of HA and PAH, it has been used in studies of this nature.<sup>28</sup> The use of modulators of



oxidative stress, like the bixin and phenolic compounds in annatto seeds composition, whether in pre-preparation or preparation, is a promising strategy to reduce the formation of HAs and PAHs, thanks to the ability to interact in reactions and inhibit the formation of such compounds.<sup>8,9,29</sup> In this context, the sensory analysis is an important tool for studying consumer perceptions of food, including its acceptance or rejection,<sup>19,22,30</sup>. It must be used before studies that require more resources to investigate the efficiency of ingredients in mitigation of thermal processing contaminants.

The averages obtained in the affective test and the frequency distribution of the scores, presented in the results of this study, demonstrate that the samples of roasted beef patties with salt obtained greater consumption intention, regardless of the annatto seed powder concentration. The role of salt in the acceptance of culinary preparations was also discussed by Otto et al.,<sup>31</sup> who, evaluating the acceptance of soup with different salt concentrations, observed that the higher the salt content, the higher the averages obtained for the sample, with the formulation without salt being the less accepted. Borjes et al.,<sup>32</sup> in a sensory analysis of beans with reduced salt and use of herbs and spices in the preparation, also obtained higher AI scores for samples directly related to increased salt content, which the authors associate with the culturally established practice of using salt in abundance.

The salty taste is, in fact, an attraction for food consumption. The use of refined salt is justified by its conservation properties, to meet the nutritional recommendations of sodium, for its fortification with iodine, an important strategy to combat goiter endemic in many areas around the globe, and in moderate amounts in meats as it favors not only the flavor, but also the tenderness.<sup>33</sup> However, exaggerated consumption can modify the taste and be a risk factor for cardiovascular diseases.<sup>32</sup>

The *Dietary Guidelines for the Brazilian Population* also include salt in the group of foods that should be used in small amounts to create culinary preparations, and suggest the use of natural seasonings as a way of adapting this use and keeping foods tasty, including meats.<sup>34</sup> Specifically for this food group, the addition of spices may improve color and flavor characteristics, and be associated with higher scores in the sensory evaluation.<sup>35</sup>

Annatto was incorporated into was later incorporated into European culture by the colonizers as a way of replacing saffron.<sup>36</sup> Currently, it is well used in traditional cuisine and in the food, cosmetics, pharmaceutical and textile industries,<sup>11</sup> and Brazil is considered its largest producer,<sup>10</sup> with 12,000 tons in the year 2022.<sup>37</sup> In addition, its cultivation has no negative impact on the environment<sup>38</sup> and in Brazil annatto is on the list of native species of sociobiodiversity with food value, that is, it is a product of biodiversity that generates income, promotes the improvement and quality of life of traditional communities and family farmers.<sup>39</sup>

In our study, the addition of annatto seed powder as a condiment in the roasted beef patties samples that contained salt, regardless of concentration, resulted in scores corresponding to the consumption intention "I would eat occasionally", and could be considered accepted due to the AI of 70%. Through the comments that the participants made in the sensory analysis forms, it was possible to perceive that the texture was the aspect that least pleased them and may have interfered with the result obtained, since properties such as juiciness and texture are desirable in meat products and those that do not have these characteristics are commonly rejected by consumers.<sup>40</sup>

Silva et al.<sup>41</sup> carried out a study that compared hamburgers produced from beef and buffalo, and showed that the beef preparation presented better scores in terms of juiciness, which is justified by the greater presence of fat in its composition. Sedlacek-Bassani et al.,<sup>30</sup> in their work with beef burgers seasoned with 1% annatto seed powder, obtained averages for aroma and flavor that did not differ from the standard formulation, as well as for texture that was not influenced by the addition of the condiment. However, the

higher addition of salt may have obtained these results, 2% in relation to the net weight of the meat, and the inclusion of fat, respectively. The authors also mention that for appearance and color, the formulation with annatto obtained lower scores and, in the comments, consumers reported that these samples were raw, which may be related to the color provided by the bixin in annatto. In contrast, the addition of 500 ppm of bixin in fresh sausage increased the color intensity and made the annatto taste detected; however, it was preferred by the tasters.<sup>42</sup> Our beef patties also showed more intense color proportional to the amount of annatto seed powder used; this aspect was controlled in our study with the use of red light in the sensory analysis cabins, and this approach ensured that tasters focused on the overall sensory experience without being influenced by visual cues.

In breads, the annatto dye concentration also affected the flavor and acceptance scores. Participants reported a bitter taste with a higher percentage of dye (1%), but even so, these samples received scores above average and were considered accepted. On the other hand, adding the dye significantly decreased the bread's moisture due to water loss.<sup>43</sup> The same was observed in grilled beef seasoned with 1.5% of Sichuan pepper, black or red pepper, and was associated with texture modification due to increased osmotic pressure induced by spices, which would lead to water loss and cooking hardness.<sup>35,44</sup>

In our study, the participants' opinion regarding the texture of the meat may be associated with this aspect of seasoning, but not only that, the standardization in the molds may also have contributed to greater water loss in meats exposed to the heat of cooking and, consequently, it made them less succulent. This suggests that mold modification and the dietetic strategy of adding fat may be interesting for further research. A specific evaluation for each organoleptic characteristic (color, odor, flavor and texture) could also lead to a better understanding of consumption intention and favor the creation of more specific strategies to increase the acceptability of beef seasoned with annatto seed powder.

In addition to the evaluation of sensory characteristics, the literature highlights other advantages of using annatto seeds, including reducing lipid oxidation and increasing shelf life with better sensory characteristics in meats, bread and other food products.<sup>30,43,45,46</sup> This effect can be attributed to the presence of bixin, whose conjugated double bonds in the structure can provide modulating properties of the oxidative system, by suppressing reactive species, through electron transfer and capturing hydrogen via the functional molecule of the apocarotenoid, which can break the structure of the reactive compounds. Additionally, although present in smaller amounts, phenolic compounds found in annatto seeds, such as chlorogenic acid, hypolaetin,<sup>47</sup> catechin, chrysin, butein, licochalcone A and xanthohumol,<sup>16</sup> contain hydroxyl groups that enable them to capture electrons, quench singlet oxygen, and deactivate the excited triplet state of sensitizers, properties associated with the mitigation of photosensitization and free radical scavenging during their transition states.<sup>16,48-50</sup>

In a previous study conducted by our group,<sup>21</sup> the quantification of bixin (2.08 (0.44) g/100 g) and phenolic compounds (8.56 (0.26) mg expressed as gallic acid equivalents (GAE)/g) of the annatto seed powder sample used for seasoning the beef patties was presented. These results are consistent with the literature, as the bixin content can vary from 0.75 to 5.5 g/100 g,<sup>47,48,51-55</sup> and the phenolic compounds range from 0.30 to 9.65 mg of GAE/g,<sup>16,47,48,52,56</sup> depending on the variety of the plant, the geographic location of the crop, the altitude of the harvesting area, soil conditions, climate, harvesting methods, storage, and the analytical techniques chosen for determination.<sup>47,52,54,55</sup>

These factors may also influence the antioxidant activity, that can be proportional to the amount of bioactive compounds present in the food matrix, and which, in the case of the cited study with our annatto seed powder sample,<sup>21</sup> was measured using three different radical-scavenging methods, resulting in 30.40 (2.62) mg of trolox equivalent antioxidant capacity (TEAC)/g of seed in the ABTS<sup>•+</sup> assay, 18.30 (0.66) mg of

TEAC/g of seed in the DPPH<sup>•</sup> assay, and 17.44 mg of ferrous sulfate equivalents (FSE)/g of seed in the FRAP assay; values that are difficult to compare with the literature due to the lack of standardization in the measurement units for antioxidant potential.<sup>49</sup> Despite this, other authors have reported the high antioxidant activity of annatto extracts *in vitro*, which have the capacity to eliminate reactive oxygen and nitrogen species, usually associated with a series of metabolic disorders, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), peroxy radicals (ROO<sup>•</sup>), hypochlorous acid (HOCl), singlet oxygen (<sup>1</sup>O<sub>2</sub>), nitric oxide (<sup>•</sup>NO), and peroxynitrite (ONOO<sup>-</sup>);<sup>57</sup> which can explain the *in vivo* effects of bixin reported in the literature, such as anti-inflammatory and anticancer, through the suppression of oxidative stress and regulation of specific metabolic pathways.<sup>58</sup> In food applications, the use of annatto has shown stability of antioxidant activity and beneficial effects after storage and thermal processing.<sup>21,54,59</sup>

Sancho et al.<sup>59</sup> and Cuong and Chin<sup>54</sup> applied annatto seed powder to fish meatballs (0.1%) and pork patties (0.25 and 0.5%) and observed the reduction of lipid oxidation over 120 and 14 days of refrigerated storage, respectively. This action is likely associated with the donation of electrons and protons from bixin and phenolic compounds, which could prevent the initiation and propagation of oxidation, inhibit radical reactions caused by the chelation of transition metal ions, and prevent oxygen attacks in radical chains.

In the previous study conducted by our group, the addition of annatto seed powder (0.5% and 1%) demonstrated the maintenance of antioxidant characteristics after cooking using different times and methods, achieving a reduction of up to 91% in the total content of heterocyclic amines; the most pronounced impact was observed at the 1% addition level, regardless of cooking time or method, and the effectiveness persisted even under harsher cooking conditions with higher thermal exposure.<sup>21</sup> This likely occurred due to the potential of bioactive compounds in annatto seed powder to inhibit oxidation linked to Maillard reaction through scavenging of reactive species, eliminate intermediates and degrade amino acids necessary for the formation of these carcinogens.<sup>9,21</sup>

Finally, the acceptance of meats seasoned with annatto seed powder also reveals a good perspective to expand research with this food matrix, that demonstrates not only culinary and sensory, but also technological and for health.<sup>10-12,14,15,21,30,43,45,60</sup> Therefore, future research should continue investigating the effects of annatto seasoning on the production of the aforementioned carcinogens - HAs and PAHs - in thermally processed beef and other food, as understanding this relationship could lead to safer processing practices and reduce cancer risks associated with dietary exposure. Furthermore, encouraging the consumption of this fruit also contributes to the development of the local economy of small and medium-sized producers and favors the rescue and maintenance of traditional cuisine.<sup>39</sup>

Additionally, the levels of each factor were established to obtain the greatest distances between the minimum and maximum values to meet nutritional safety criteria (sodium and bixin intake); however, exclusive seasoning with annatto showed no difference in acceptability between the levels, contradicting the expectation of linearity of the outcome in response to the effect of the independent variables. For this reason, other studies should include the central point in the experimental design.

## CONCLUSION

The results of this work allow us to conclude that seasoning with annatto seed powder at a concentration of 1% can be used in the preparation of roasted beef patties, given that the participants indicated intention of occasional consumption, that the samples were accepted by the Acceptability Index and that acceptance was equal to those with half the concentration. As well as use of 0.5% salt significantly

improves acceptance, and that the inclusion of fats and/or other strategies that may impact texture should be explored.

Then, annatto seasoning is sensorially accepted in meat preparations and associated with the presence of its bioactive compounds, bixin and phenolics, has cultural, economic, biological and technological importance. Knowing this acceptance is essential to enhance your application in culinary and future research; for our group, this result translates into an important step towards carrying out studies to evaluate the effect of annatto seed powder on mitigating thermal processing contaminants.

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### Contributors

Novoa GG and Domene SMA worked at all stages from the conception and design of the study to the review and approval of the final version of the article; Neves-Gonçalves TM participated in the acquisition, analysis and interpretation of data, writing, review and of final approval of the article.

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