



## Effect of natural extracts on the oxidative stability of pork hamburgers during refrigerated storage



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### Abstract:

The effect of three natural extracts made from culinary spices with antioxidant activity on the oxidative stability of lipids and proteins, color changes, and sensory quality of cooked pork during 12 days of refrigerated storage was evaluated. Hamburger-type model systems were made with the *Longissimus thoracis et lomborum* muscle, dorsal fat, salt, water and the corresponding extract. The antioxidant activity of the extracts was determined by the DPPH and ABTS+ methods, while the oxidation of lipids and proteins by TBA-RS and DNPH, respectively. For the color evaluation, the parameters of luminosity (L\*) and Hue angle (°h) were used. The sensory analysis was carried out with an untrained panel, which evaluated the attributes of taste, color, smell and texture. The statistical processing of the data obtained

on antioxidant activity, lipid and protein oxidation, as well as color, was performed by an analysis of variance. The sensory evaluation was processed with nonparametric statistics. Extract two had the highest antioxidant activity ( $P \leq 0.05$ ); the three extracts managed to inhibit ( $P \leq 0.05$ ) lipid oxidation in the hamburgers ( $P \leq 0.05$ ); however, none of the three extracts managed to inhibit protein oxidation. There were also no differences ( $P \geq 0.05$ ) with respect to the  $L^*$  parameter, while the values of  $\Delta E^*$  showed that the three extracts managed to preserve the color of the cooked hamburgers during refrigerated storage. Finally, the sensory evaluation showed that none of the three extracts altered the organoleptic quality of the hamburgers.

**Key words:** Natural Extracts, Culinary Spices, Antioxidants, Lipid Oxidation, Protein Oxidation.

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## Introduction

Meat is especially prone to oxidation processes due to its complex structures and composition, including lipids, unsaturated fatty acids and myofibril systems<sup>(1)</sup>. Meat lipids are chemically unstable and easy to oxidize, especially during handling, cooking and *post-mortem* storage<sup>(2)</sup>. Changes associated with lipid oxidation include rancid odor, discoloration, loss of nutritional value, decrease in shelf life and the formation of toxic compounds, which can be harmful to the health of consumers<sup>(2)</sup>. Likewise, protein oxidation implies the loss of nutritional value in meat, causing a decrease in protein bioavailability, a change in amino acid composition, a decrease in protein solubility, loss of proteolytic activity and protein digestibility<sup>(3)</sup>.

Recently, with the outbreak of the coronavirus 2019 (COVID-19) pandemic, some researchers have evaluated the changes in the pattern of purchasing behavior of consumers, reporting an increase in the tendency to change habits, especially food and nutritional ones, towards the consumption of functional and nutraceutical foods, with a tendency towards healthier types of meals, made with natural and homemade preservatives<sup>(4,5)</sup>.

Antioxidant strategies based on the use of natural sources may be a viable option to enrich meat with bioactive compounds that promote health and, in turn, would prevent degradation

due to oxidation. Antioxidant phytochemicals can be applied through the formulation of foods or dietary strategies<sup>(6)</sup>.

The inclusion of natural antioxidants in meat products has been reported by different authors with a positive effect in terms of control of oxidative processes<sup>(7,8)</sup>. Culinary spices, such as cinnamon, cloves, coriander, onion, black pepper, garlic, oregano, bay leaves, turmeric, among others, are an important source of bioactive compounds with antioxidant activity<sup>(9,10)</sup>. Few studies report the inclusion of culinary spice mixtures in pork products<sup>(11,12)</sup>. Therefore, the objective of this study was to evaluate the antioxidant protection of proteins and lipids in processed pork through natural extracts made from mixtures of culinary spices.

## **Material and methods**

### **Preparation of extracts**

A total of three extracts were prepared, considered as treatments #1 (200 g of onion, 20 g of coriander, 15 g of oregano), #2 (200 g of onion, 20 g of coriander, 15 g of bay leaves) and #3 (200 g of onion, 20 g of coriander, 2 g of black pepper, 18 g of green chili, 14 g of garlic, 6 g of salt, 8 g of calyces of green roselle (variety UAN-4) with the different culinary spices, plus 50 ml of a base two-spice mixture and 75 ml of lemon juice. Their preparation consisted of grinding each of the ingredients at the same time with the help of a conventional blender until obtaining a pasty consistency. Subsequently, the paste was placed in 50 ml Falcon tubes for its centrifugation (5,000 rpm, 5 min). The supernatant was used as the extract to be mixed with the meat that was used to prepare the model systems.

### **Preparation of the base two-spice mixture**

The preparation of the base two-spice mixture consisted of macerating 5 g of cinnamon and 5 g of clove powder (separately) in 10 ml of rum (40 % of alcohol) for 24 h. The supernatant obtained from the two macerations was mixed to form the base two-spice mixture.

## Preparation of hamburger-type model systems

Hamburger-type model systems composed of 80 % pork (*Longissimus thoracis et lumborum* muscle), 10 % dorsal fat, 1 % salt, 9 % water (for control hamburgers), and 4.5 % water and 4.5 % extract in the treated hamburgers, were prepared. The preparation of the hamburgers consisted of grinding the meat and fat in a meat grinder with a 1/8" sieve (Torrey® brand, model M12-FS), once the meat was ground, the salt, water and extract (if applicable) were mixed until a homogeneous mixture was obtained. The mixture was packaged under high vacuum to remove any internal air bubbles that might form. From the mixture, portions of 60 g were weighed and with the help of a metal ring of 8 cm in diameter, the hamburger-type model systems were made. The hamburgers were previously cooked at a temperature of 230 °C for 5 min on each side on an Oster Bioceramic® grill

From each treatment (without extract, extract #1, #2 and #3), the hamburger-type model systems (three replications per treatment and per each day of sampling) were made to evaluate the oxidative stability of color, lipids and proteins. The hamburgers were placed in polystyrene trays and covered with transparent oxygen-permeable film paper (14µm thick and 10,445 ml/m<sup>2</sup>/24 h) and stored in refrigeration at 4 ± 2 °C with white fluorescent light (1,620 lux) 24 h. The samplings were carried out on days 0, 3, 6, 9 and 12.

## Determination of the antioxidant activity

Total phenolic compounds were determined by the method of Stintzing *et al*<sup>(13)</sup>. The antioxidant activity based on the 1,1-diphenyl-2-picrilhidrazil (DPPH) method was evaluated according to the procedure reported by Morales and Jiménez-Pérez<sup>(14)</sup>. The antioxidant activity based on the ABTS+ method was evaluated according to the procedure developed by Kuskoski *et al*<sup>(15)</sup>. The TBA-RS technique (thiobarbituric acid reactive substances) was evaluated according to the technique described by Ganhão *et al*<sup>(16)</sup>, which allows the quantitative determination of secondary metabolites of lipid oxidation. The determination of the total carbonyls that are generated during the oxidative processes of meat proteins was based on the DNPH technique described by Ganhão *et al*<sup>(17)</sup>.

## Evaluation of the color of the hamburgers

To determine the deterioration of the color of the meat due to storage over time, color evaluations were carried out by instrumental measurement<sup>(18)</sup> on the surface of the hamburgers treated with the extracts, during the days of storage. A Minolta® colorimeter model CR-410 was used. The measurements were made in three different randomly chosen zones and at room temperature ( $\approx 25$  °C). The CIE-L\*a\*b\* color measurement system was used and the Hue angle (°h) (tone) was calculated as indicated by García-Tejeda *et al*<sup>(19)</sup>: °h=tan-1(b\*/a\*), when a\*>0 and b\*≥0 or °h=180 + tan-1 (b\*/a\*) when a\*<0.

The total color difference ( $\Delta E$ ) was calculated to evaluate the total color changes suffered by the hamburgers as a result of the refrigerated storage days. Therefore,  $\Delta EC-T$  was calculated between the samples of the control group (C) and the treated group (T) using the CIE-L\*a\*b\* color scale for each measurement day as follows:

$$\Delta EC-T = [(L^*T - L^*C)^2 + (a^*T - a^*C)^2 + (b^*T - b^*C)^2]^{1/2}$$

## Sensory analysis

For sensory evaluation, each hamburger cooked under the conditions described above was cut into four parts to offer it to an untrained panel of 35 people. The panelists were instructed to evaluate the attributes of smell, color, taste and texture, marking with an “X” the rating they considered appropriate to assign to each sample, using a hedonic test<sup>(20)</sup> with a seven-point scale where the value of 1 corresponded to “I dislike it very much”, the 2 to “I dislike it a lot”, 3 to “I dislike it a little”, 4 to “I neither like it nor dislike it”, 5 to “I like it a little”, 6 to “I like it a lot” and 7 to “I like it very much”.

## Statistical analysis

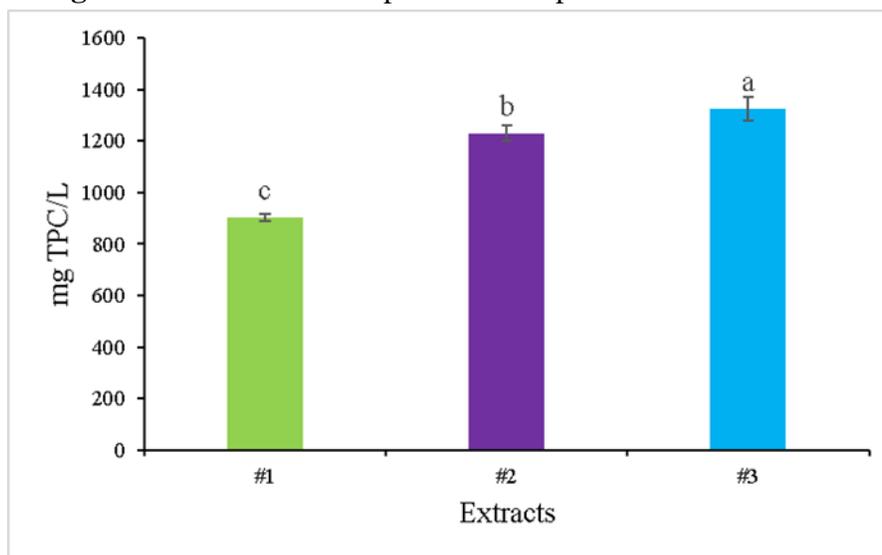
Data on TPC content, antioxidant activity, color determination and inhibition of lipid and protein oxidation were processed using an analysis of variance under a completely randomized design. When the analysis was significant ( $P \leq 0.05$ ), a Tukey mean comparison test was performed. The results of total color difference and sensory analysis were performed using the Kruskal-Wallis Hypothesis test ( $P \leq 0.05$ ). Pearson’s correlation coefficients were calculated to establish linear associations between variables of interest. The statistical package used was Minitab v.16.0.

## Results and discussion

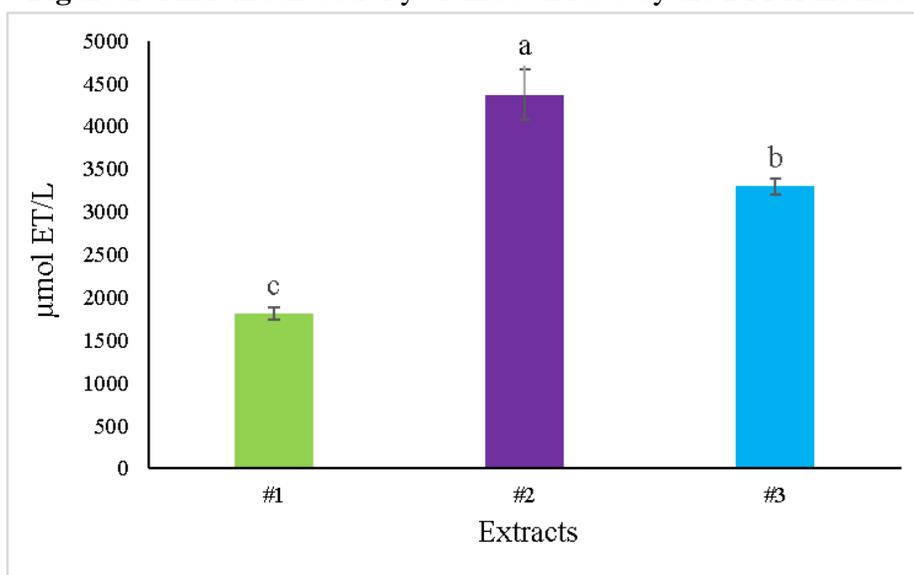
### Content of total phenolic compounds and antioxidant activity

The three extracts prepared had a high content of TPC (Figure 1) and good antioxidant activity determined by the DPPH (Figure 2) and ABTS+ methods. The ABTS+ test showed the same antioxidant activity behavior as with the DPPH technique. When analyzing the TPC, we can observe that extracts #2 and #3 were the ones that had the highest TPC content, and the highest antioxidant activity is observed with treatment #2, followed by treatment #3. This may be mainly due to the ingredients that differentiate each of these two extracts, the greater antioxidant activity can be attributed mainly to the derivatives of catechin and procyanidins (cinnamtannin B1) and flavonic heterosides derived from kaempferol that have been reported as the majority in the bay leaves present in extract #2<sup>(21)</sup> and to chlorogenic acid and its isomers, caffeic acid and protocatechuic acid derivatives reported as the majority and with high antioxidant activity in green roselle calyces in extract #3<sup>(22)</sup>. These ingredients could exert a greater synergistic effect with the rest of the spices, potentiating their antioxidant activity. So far, the use of green roselle calyces to inhibit lipid and protein oxidation has not been reported, but their antioxidant activity has already been reported<sup>(23)</sup>.

**Figure 1:** Content of total phenolic compounds of three extracts



<sup>abc</sup> Means with a different superscript denote a significant difference ( $P < 0.05$ ).

**Figure 2:** Antioxidant activity of three extracts by the DPPH method

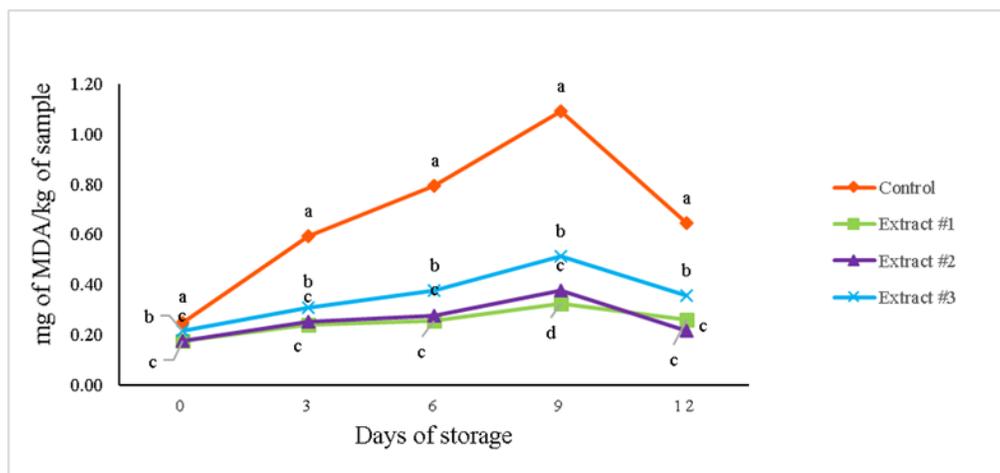
<sup>abc</sup> Means with a different superscript denote a significant difference ( $P < 0.05$ ).

The extracts prepared showed a significant correlation ( $P \leq 0.05$ ) between TPC and antioxidant activity by the DPPH and ABTS+ methods of  $r = 0.798$  and  $0.751$ , respectively. Therefore, it can be said, according to this analysis, that the antioxidant activity of the extracts is a function of the total phenolic compounds.

### Determination of thiobarbituric acid reactive substances

The results of TBA-RS (Figure 3) showed a significant difference ( $P \leq 0.05$ ) between the samples. The three extracts were able to decrease the concentration of malonaldehyde (MDA) with respect to the hamburgers without extract throughout the 12 d of storage, with extracts #1 and #2 being the ones that achieved a significantly better effect in relation to extract #3.

**Figure 3:** Effect of extracts on MDA concentration of pork hamburgers cooked and stored at 4 °C for 12 d



<sup>abc</sup> Means with a different superscript denote a significant difference ( $P < 0.05$ ).

TBA-RS values above 0.5 mg MDA/kg of sample are critical as they indicate a level of products of lipid oxidation that produce a rancid odor and taste that can be easily detected by consumers<sup>(24)</sup>. This level of rancidity was reached after cooking in the hamburgers without extract, increasing its values during subsequent refrigerated storage, indicating that the cooking process may be able to accelerate lipid oxidation rates.

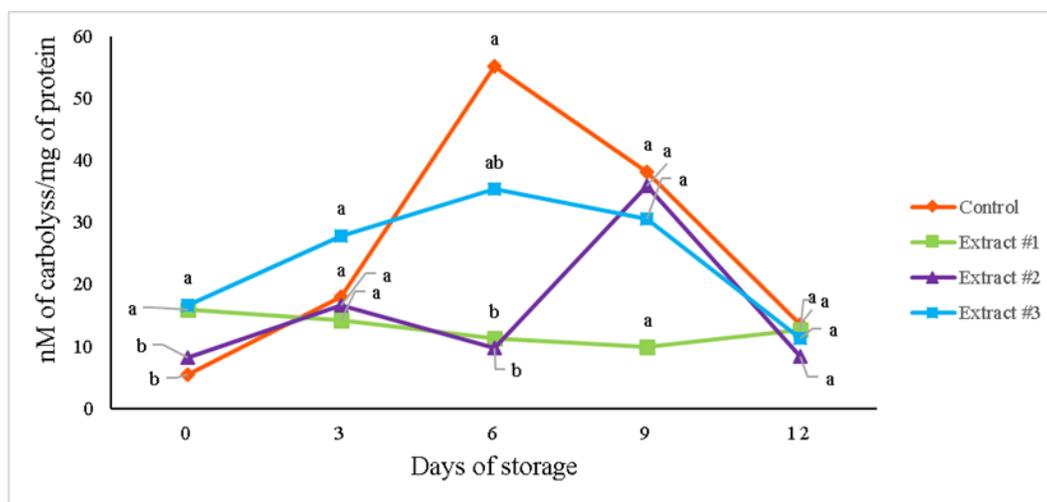
The intense antioxidant activity shown by the extracts in the *in vitro* tests (Figure 2) meant an efficient protective effect of the extracts on lipids in real meat products. Other authors<sup>(12,25)</sup> have obtained similar results, managing to reduce the concentration of TBA-RS by using onion and garlic in pork, as well as tocopherols and ascorbic acid in chicken liver pâté, respectively. However, few studies have attempted to demonstrate the efficacy of natural antioxidant mixtures against lipid oxidation<sup>(26)</sup>. In one of them<sup>(11)</sup>, they used a mixture of essential oils of garlic, cinnamon, cloves and rosemary, and obtained favorable results by inhibiting lipid oxidation in Iberian hams. Some substances, such as MDA, have been reported as compounds with toxic and mutagenic potential for humans<sup>(27)</sup>. So, extracts #1, #2 and #3 can be an efficient strategy to avoid the increase in adverse effects caused by lipid oxidation in cooked pork hamburgers.

### Determination of total protein carbonyls

The results of carbonyls in cooked hamburgers (Figure 4) presented a significant difference ( $P \leq 0.05$ ) between the samples only on d 6 of storage, with extracts #1 and #2 showing a reduction in carbonyls with respect to the hamburgers without extract. However, this effect

was not effective on the other sampling days. So, it could be considered that none of the three extracts applied achieved an efficient inhibitory action on protein oxidation in cooked hamburgers. It is known that the increased susceptibility of cooked meats to protein carbonylation can be attributed to the disruption of myofibrillar tissues as a result of high temperatures, which in turn leads to the release of non-heme (non-protein) iron and a greater incorporation of oxygen into the system. Non-heme iron has been recognized as a major promoter of the formation of carbonyl residues from myofibrillar proteins<sup>(28)</sup>. Compared to the results of the present study, other researchers<sup>(29)</sup> also found high levels of protein carbonyls in pork subjected to cooking and subsequent cold storage, which reveals the impact of high temperatures on the oxidative stability of muscle proteins.

**Figure 4:** Effect of extracts on carbonyl concentration of pork hamburgers cooked and stored at 4 °C for 12 d



<sup>abc</sup> Means with a different superscript denote a significant difference ( $P < 0.05$ ).

According to the results of the present study, other authors have also reported that certain antioxidant strategies with proven efficacy against lipid oxidation were not effective against protein oxidation<sup>(30)</sup>.

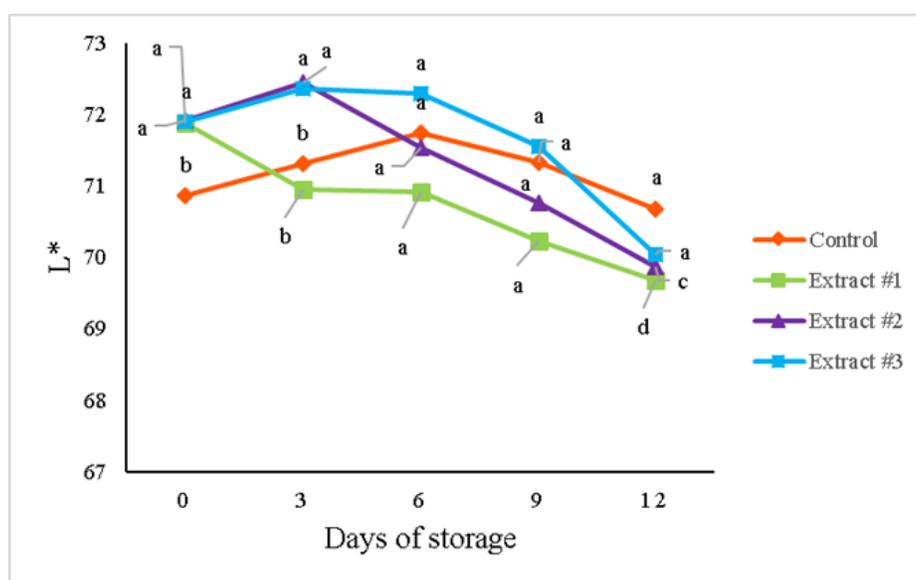
On the other hand, it is known that the formation of lipid oxidation in meat systems occurs more rapidly than the oxidative degradation of myofibrillar proteins<sup>(31)</sup>. The positive correlation ( $r=0.560$ ;  $P=0.000$ ) found in the present study between protein and lipid oxidation in cooked hamburgers supports the theory that lipid and protein oxidation are coupled in food meat systems. In fact, some studies have reported such an interaction between lipids and proteins<sup>(32,33)</sup>, which supports the theory that reactive oxygen species (ROS) formed during the early stages of lipid oxidation can bind to susceptible amino acid residues to trigger their oxidative degradation<sup>(34)</sup>. In contrast to the results of lipid oxidation, the differences between the treatments with respect to protein oxidation were not so clear, possibly due to the complex

structural composition of the proteins that comes to provide some protection and their degradation does not follow a logical pattern, which coincides with other authors<sup>(35,36)</sup>.

## Color evaluation

The results of the luminosity parameter (Figure 5) indicate a slight loss of brightness for all the samples, since the values between them showed a difference of less than 3 points, which ranged from 72.44 to 69.67 during the 12 d of storage, with a significant difference ( $P \leq 0.05$ ) between the samples during the three first days of storage, where the hamburgers with extract had a greater luminosity with respect to the hamburgers without it. However, at the end of the storage period (d 12), all hamburgers with extract lost luminosity significantly ( $P \leq 0.05$ ) with respect to hamburgers without extract.

**Figure 5:** Effect of extracts on the luminosity or brightness of pork hamburgers cooked and stored at 4 °C for 12 d

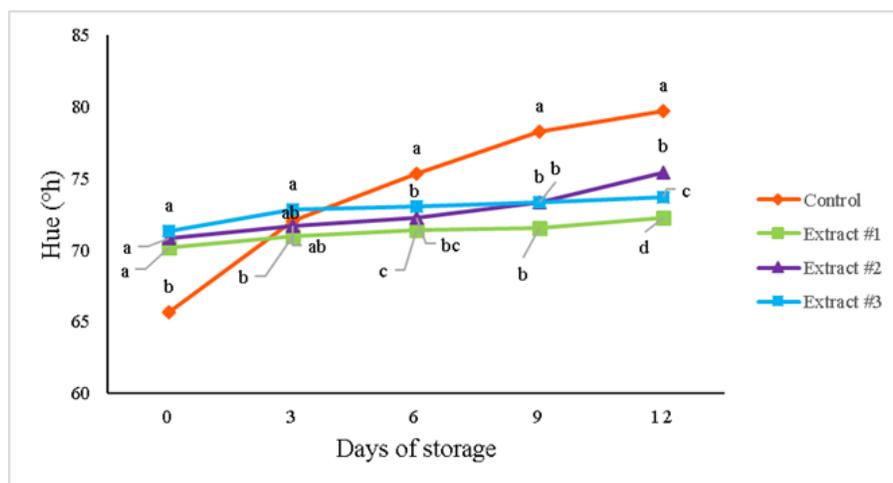


<sup>abc</sup> Means with a different superscript denote a significant difference ( $P < 0.05$ ).

For the values obtained of the Hue angle (Figure 6), it can be observed that the addition of the studied extracts had a significant effect ( $P < 0.05$ ) with respect to the hamburgers without extract, since the extracts had a faint orange color with slight touches of brown as a result of the extraction of pigments from the spices and the base two-spice mixture used. The pigments were probably transferred to the hamburgers during their preparation, causing the modification of their color and intensifying it after the cooking process, thus causing a brown coloration with slight touches of gold, or in other words a toasted shade. During the initial

day of storage, the hamburgers without extract showed significantly ( $P \leq 0.05$ ) a lower brown or toasted shade. During the third day of storage, the hamburgers without extract matched the color with respect to the treated hamburgers. However, from d 6 of storage, the hamburgers without extract significantly increased ( $P \leq 0.05$ ) the Hue angle value, with an upward trend standing out, and showing a yellow shade with greenish touches with respect to the hamburgers with extracts. It is highlighted that hamburgers with extract #1 better protect toasted coloration throughout the 12 d of storage.

**Figure 6:** Effect of extracts on the color of pork hamburgers cooked and stored at 4 °C for 12 d



<sup>abcd</sup> Means with a different superscript denote a significant difference ( $P < 0.05$ ).

The protection given by the extracts may be due to their antioxidant defense, which may be responsible for protecting heme pigments against oxidative processes in cooked hamburgers, which is confirmed by the significant correlation of  $r=0.690$  ( $P=0.001$ ) between color and lipid oxidation. That is, the bioactive compounds present in the extracts could possibly inhibit the formation of primary products of lipid oxidation (mainly hydroperoxides), which oxidize ferrous iron ( $Fe^{2+}$ ) of oxymyoglobin to its ferric form ( $Fe^{3+}$ ) present in metmyoglobin (responsible for discoloration)<sup>(37)</sup>, thus inhibiting the discoloration of hamburgers.

Table 1 shows the total numerical color difference ( $\Delta E$ ) between hamburgers without extract and treated hamburgers (with extract #1, #2 and #3) during d 0, 3, 6, 9 and 12 of refrigerated storage. According to some authors<sup>(38)</sup>, color modifications measured instrumentally between two given meat samples can be considered as notable visual changes when  $\Delta E$  values are greater than 2. In the case of cooked hamburgers, a  $\Delta E$  greater than 2 was found for hamburgers with extract #1 on d 12 of storage. However, statistically, there was a significant difference ( $P \leq 0.05$ ) in the  $\Delta E$  between the hamburgers treated from d 3 of storage, where the hamburgers with extract #1 showed the greatest color differential during d 6, 9 and 12 of storage. This coincides with the results obtained of the Hue angle of the cooked hamburgers,

where, from d 6 of storage, all the hamburgers with extract presented a significant difference with respect to the hamburgers without it, being precisely the hamburgers with extract #1 the ones that presented the greatest difference. That is, extract #1 showed significantly greater efficacy ( $P \leq 0.05$ ) in preserving the toasted color of cooked hamburgers throughout the 12 d of storage.

**Table 1:** Total color difference ( $\Delta E$ ) between the sample without extract and the samples treated in pork hamburgers cooked and stored at 4 °C for 12 d

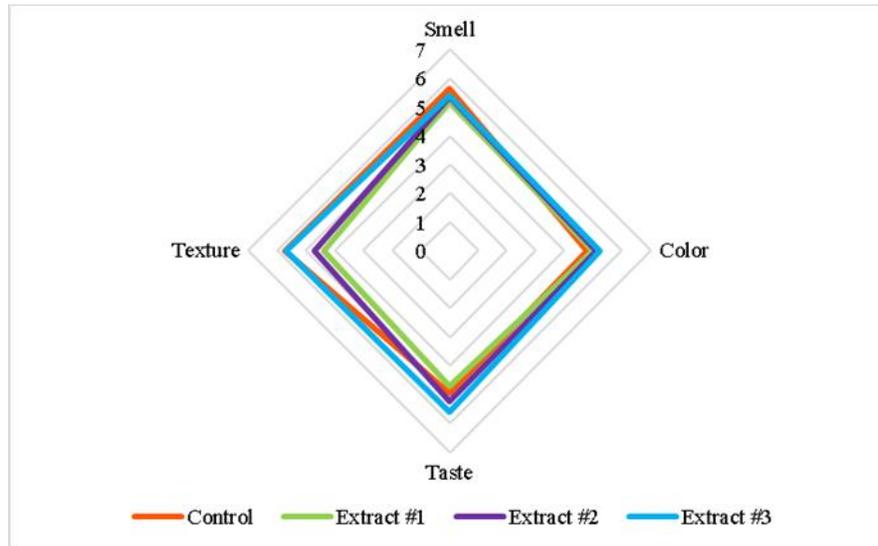
Samples	Days				
	0	3	6	9	12
Extract #1	1.51 <sup>a</sup>	0.56 <sup>c</sup>	1.35 <sup>a</sup>	1.95 <sup>a</sup>	2.32 <sup>a</sup>
Extract #2	1.57 <sup>a</sup>	1.10 <sup>a</sup>	0.94 <sup>b</sup>	1.36 <sup>b</sup>	1.49 <sup>c</sup>
Extract #3	1.80 <sup>a</sup>	1.09 <sup>b</sup>	0.76 <sup>c</sup>	1.09 <sup>c</sup>	1.50 <sup>b</sup>

Means with a different superscript within one day of storage denote a significant difference between extracts ( $P \leq 0.05$ ).

### Sensory analysis

The results of the sensory evaluation (Figure 7) indicate that, in relation to the attributes of smell and color, there was no significant difference ( $P > 0.05$ ) between the treatments, that is, the application of the extracts did not perceptively modify the smell or the color of a cooked meat. In the evaluation corresponding to the attribute of taste, there were significant differences ( $P \leq 0.05$ ) between the different sources of variation used in the experimental design, with hamburgers with extract #3 being the ones that had the highest preference among the panelists, surpassing the hamburgers without extract, while the ones with the lowest acceptance were the hamburgers with extract #1. Regarding the attribute of texture, there were statistically significant differences between treatments, being the hamburgers added with extract # 3 the ones that showed greater acceptability for this attribute and equalizing the texture of the hamburgers that did not have it. While the hamburgers that were added with extract #1 were the ones that had the least acceptance among the tasters. In general, the addition of the three extracts to the hamburgers did not have a negative effect on the preference of the tasters, since the results of the four attributes (smell, color, taste and texture) evaluated were on the scale of 4 to 6, which ranges from “I neither like it nor dislike it” to “I like it very much”, with the preference for hamburgers with extract #3 among diners standing out.

**Figure 7:** Sensory evaluation of the attributes of smell, color, texture and taste of cooked pork hamburgers



## Conclusions and implications

The protective effect of extracts #1, #2 and #3 on lipid oxidation and color deterioration in pork hamburgers coked and stored in refrigeration can be attributed to the phenolic compounds present in the culinary spices present in the extracts, which showed antioxidant activity. The three extracts can be an efficient strategy that causes an increase in the shelf life of meat products without causing damage to nutritional attributes, and without presenting anomalous alterations in sensory perceptions by consumers.

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