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Effect of the addition of brazilian red propolis extract in ground beef

Efeito da adição de extrato de própolis vermelha em carne bovina moída

Efecto de anãdir extracto de propóleo rojo a la carne molida

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Abstract
Meat has important nutritional characteristics for the diet, being a source of high biological value animal protein, and containing important vitamins for the organism. Ground beef is one of the most consumed meat products, which has an increased surface area, and also a high potential for inadequate storing conditions, which reinforces the relevance of innovative technologies for its preservation, such as the use of propolis – a highly diversified product with antimicrobial and antioxidant activities, seen as a component capable of prolonging shelf life. This study evaluated the effects of the addition of Brazilian red propolis extract to ground beef as a natural antioxidant for 0, 7, 14 and 21 days of storage at a temperature of 7 ºC. The physicochemical parameters of water, protein, lipids and ash content remained stable until the 14th day of storage, while the microbiological ones presented peaks of contamination by Coliforms at 35 and 45 ºC in the same period, however, the absence of *Salmonella* sp/25g in the meat analyzed, made it suitable for human consumption, thus confirming the effectiveness of using red propolis extract in its conservation.
Keywords: Processed meat; Brazilian red propolis; Storage.

Resumo

A carne é um alimento que possui características nutricionais importantes para a dieta, tratando-se de uma fonte proteica animal de alto valor biológico, possuindo vitaminas importantes para o organismo. A carne moída é um dos produtos cárneos mais consumidos, tendo uma área de superfície aumentada e um alto potencial a condições inadequadas de armazenamento, o que reforça a relevância de tecnologias inovadoras para a conservação dessa matéria, como a própolis – um produto altamente diversificado, com atividades antimicrobianas e antioxidantes, visto como um componente capaz de prolongar a vida de prateleira. Portanto, foram avaliados os efeitos da adição de extrato de própolis vermelha à carne bovina moída como antioxidante natural durante 0, 7, 14 e 21 dias de armazenamento a uma temperatura de 7 ºC. Os parâmetros físico-químicos de teor de água, proteína, lipídeos e cinzas mantiveram-se estáveis até o 14º dia de armazenamento, já os microbiológicos, apresentaram picos de contaminação por Coliformes a 35 e 45ºC nesse mesmo período, contudo, a ausência de *Salmonella* sp/25g na carne analisada a tornou adequada para o consumo humano, confirmando, assim, a eficácia do uso de extrato de própolis vermelha em sua conservação.
Palavras-chave: Carne processada; Própolis vermelha; Armazenamento.

Resumen

La carne es un alimento que posee importantes características nutricionales para la dieta, siendo una fuente de proteína animal de alto valor biológico, poseyendo importantes vitaminas para el organismo. La carne molida es uno de los productos cárnicos más consumidos, con una mayor superficie y un alto potencial de condiciones de almacenamiento inadecuadas, lo que refuerza la relevancia de tecnologías innovadoras para la conservación de este material, como el propóleo, un producto altamente diversificado, con propiedades antimicrobianas. y actividades antioxidantes, visto como un componente capaz de prolongar la vida útil. Por tanto, se evaluaron los efectos de la adición de extracto de propóleo rojo a la carne molida de res como antioxidante natural durante 0, 7, 14 y 21 días de almacenamiento a una temperatura de 7 ºC. Los parámetros físico-químicos de contenido de agua, proteínas, lípidos y cenizas se mantuvieron estables hasta el día 14 de almacenamiento, mientras que los parámetros microbiológicos mostraron picos de contaminación por Coliformes a 35 y 45ºC en el mismo período, sin embargo, la ausencia de Salmonella sp / 25g en la carne analizada aa la hicieron apta para el consumo humano, confirmando así la eficacia del extracto de propóleo rojo en su conservación.
Palabras clave: Carne processada; Propóleo rojo; Almacenamiento.

1. Introduction

Meat has important nutritional characteristics for the diet of children, adults and older adults. It is source of animal proteins of high biological value, and also has important vitamins, such as vitamins B2 and B12, lipids, minerals and amino acids necessary for the organism (Becker; Kiel, 2011).

According to the Normative Instruction Nº 83 (Brasil, 2003), ground beef is the meat product obtained from the grinding process of the muscle of bovine carcass, followed by cooling or freezing.

Brazil is one of the most important exporters of beef. Recent data show that in the last 22 years the exportations increased by 146%. In the year of 1997, 3,3 million tons of beef were exported, while 8,2 million tons left the country in 2019 (Malafaia et al., 2020).

Between January and April of 2020, the exportations continued increasing, even in a context of world crisis due to the COVID-19 pandemic. During this period, there was an average increase of 620 tons per day when compared to the same period in the previous year. One of the countries that most acquire beef from Brazil is China, purchasing 47% of the beef exported by the country (Brasil, 2020).

The developing of innative technologies for the preservation of ground beef is reinforced by the fact that this is one of the most consumed meat products, which has an increased surface area, and also a high potential for inadequate storing conditions. One of the new food preservatives being currently studied is the Propolis, which is also the focus of this study.

The Brazilian propolis is a highly diversified product, classified in 13 types, which take into consideration the products' chemical composition and its antimicrobial and antioxidant activities. The most recent classified type of propolis can be found along the coast of the sea and rivers of the Northeast of Brazil, and was named "red propolis" due to its intense red color, which originates from the red exudate material harvested by bees in the surface of the *Dalbergia ecastophyllum* (Daugsch et al., 2007; Cabral et al., 2009).

The red propolis from the Northeast of Brazil contains molecules that differentiate it from other types of mentioned in the literature. It is believed that it is the result of a complex mixture of bioactive compounds that are responsible for several biological properties that other types may not present (Gonsales et al., 2006).

Brazil is the third biggest producer of propolis. Although the use of resin in the cosmetic industry has been growing considerably since 2000, the use of propolis in the food industry is still unexpressive, especially the Brazilian red propolis (Silva et al., 2016; Almeida et al., 2018).

With this study, the authors aimed to evaluate the effects of the addition of Brazilian red propolis extract (BRPE) to ground beef as a natural antioxidant through the observation of changes in physicochemical and microbiological characteristics.

2. Methodology

The experiments were conducted in the Technological Vocational Center (CVT) – a laboratory in the Center of Agrifood Sciences and Technology of the Federal University of Campina Grande, in Pombal, PB, Brazil. The BRPE was supplied by the same laboratory.

The raw material (beef *in natura*) used in the experiments was obtained from the local meat commerce of the city of Pombal, PB, and transported in thermic boxes to the laboratory where it was evaluated and ground.

The BRPE was then added to 500g the ground beef in proportions of 3% and 5%, packed in polyethylene trays, covered with food packaging plastic film and refrigerated to a temperature of 7ºC.

The physicochemical analysis – pH, Titratable Acidity (TA), Water Content (W%), Ash content (%), proteins (%), and lipids (%) – followed methodology described by the Adolf Lutz Institute (IAL, 2008). The microbiological analysis (coliforms at 35 and 45ºC, and *salmonella* sp/25g) were performed according to methods described by Silva (2010).

All analysis in this study were conducted in samples containing BRPE in concentrations of 0% (control), 3%, and 5%, in 0, 7, 14 and 21 days of storage, and kept at temperature of 7ºC, performing the Tukey test at 7% of reliability.

3. Results and Discussion

In the Table 1 are presented the averages obtained for the physicochemical parameters from the variance analysis, considering the Extract, Time, and Extract X Time.

Impact of the extract was verified in all the parameters analyzed, except the ash content, which presented nonsignificant results.

For Extract x Time study, the only physicochemical parameter that did not present a statistically significant interaction was also the ash content.

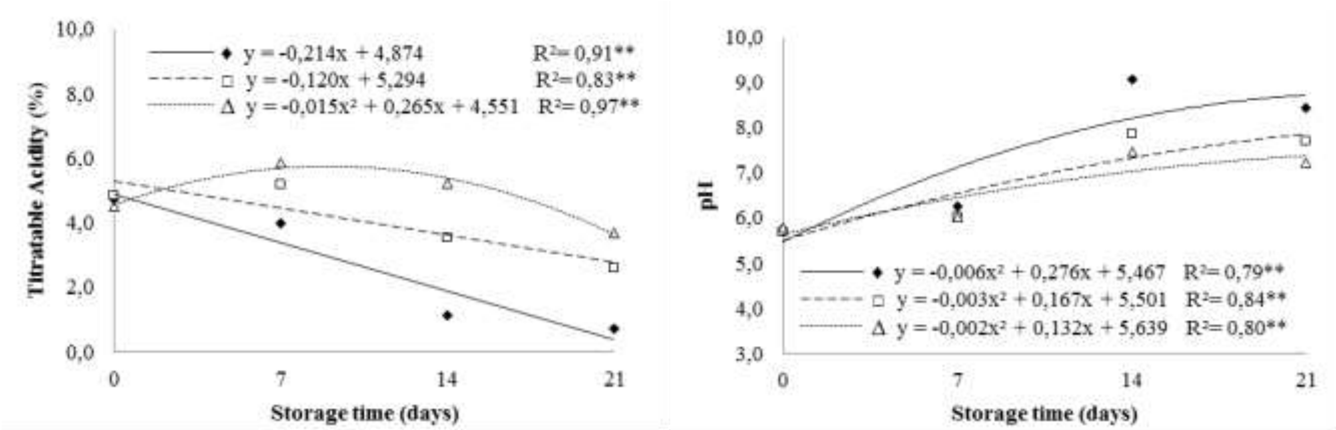
Table 1 – Variance Analysis of the physicochemical parameters of ground beef.

Variation Sources	DF	Average Results					
		TA	**pH**	**W%**	**Proteins**	**Lipids**	**Ash**
Extract (E)	2	14,640**	1,851**	15,745*	2,338*	20,508**	0,172^{ns}
Time (T)	3	13,993**	12,895**	24,743**	8,393**	12,133**	0,347**
E x T	6	2,577*	0,470**	15,645*	2,419**	5,717**	0,073^{ns}
Error	24	0,711	0,024	4,585	0,534	0,518	0,056
CV (%)	-	22,06	2,25	2,84	17,48	18,22	19,00

*statistically significant (p<0,05). **statistically significant (p<0,01). ^{ns}not statistically significant. Source: Authors (2021).

The Figure 1 brings the results obtained for the Titratable Acidity and pH of the ground beef, considering constant temperature and time of storage.

Figure 1 – Titratable Acidity and pH of ground beef containing concentrations of 0% (control), 3% and 5% of BRPE during storage at 7ºC, respectively.



♦ = 0% BRPE; □ = 3% BRPE; Δ = 5% BRPE. **statistically significant (p<0,01). Source: Authors (2021).

Titratable Acidity was affected by different treatments through time of storage, which can be observed in the Figure 2 that shows different behaviors between the control sample (0% BRPE) and the samples containing BRPE. The control sample presented a constant drop as time passed, reaching almost zero percent by the 21st day of storage. The 3%-BRPE sample showed a lower and constant decrease in the acidity. The curve of the sample containing 5% BRPE had an elevation until around the 7th day of storage, followed by stability and finally by a slight drop.

During food storage, it is possible to identify the increase in water content present in it, as can be seen in Figure 2, being a possible cause of the variation in the titratable acidity in the samples of ground meat analyzed.

The changes in the pH of the samples were considerably similar. The pH presented a higher increase (to almost 9) in the 0%-BRPE sample, which may be justified by the absence of BRPE.

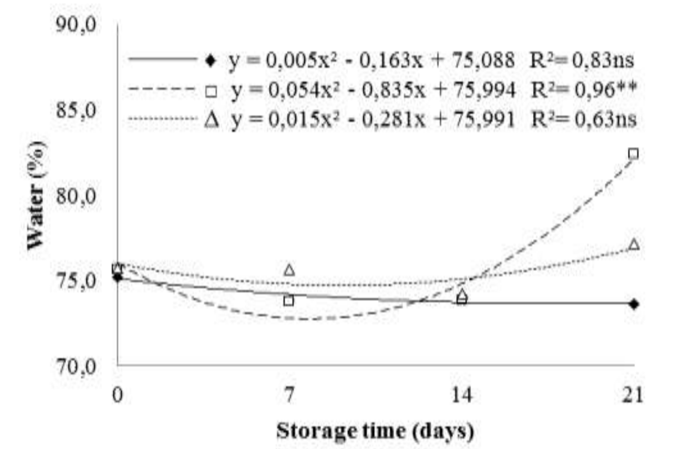
The pH in values within 5,8-6,2 is still able to maintain the characteristics and nutritional properties of the meat, such as water retaining, texture, moist and microbiological stability (Costa, 2014).

When meat has a pH lower than 5,8, it generally preserves important characteristics to its quality, like tenderness, color, and it presents satisfactory flavor. Differences are seen in meat with a pH higher than 6,4, which was a value surpassed by all samples after the 7th day of storage. The darkening and hardening of the samples are usually related to the increase in their pH values.

With the decrease in acidity present in the samples, an increase in pH is common, since there is also an increase in water present in the same. The pH analysis are not a qualitative determinant, and it should not be used as an isolated parameter to evaluate if the product is or is not adequate for human consumption, it should rather be determined through microbiological analysis.

The Figure 2 brings the water content of the samples through storage time.

Figure 2 – Water content in ground beef containing 0%, 3%, and 5% of BRPE during storage at 7ºC.



♦ = 0% BRPE; □ = 3% BRPE; Δ = 5% BRPE. **statistically significant ($p<0,01$). [ns]not statistically significant. Source: Authors (2021).

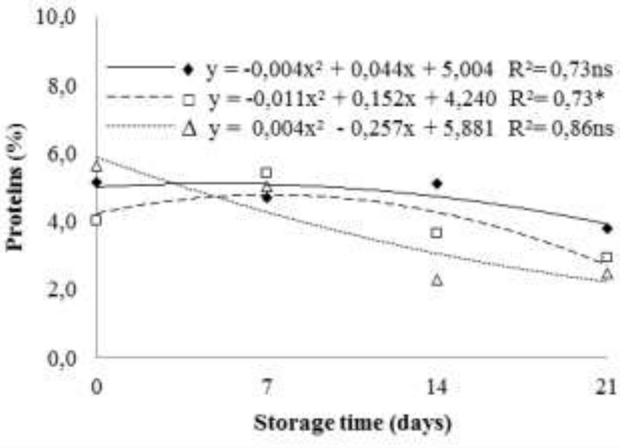
The control sample (0% BRPE) and the 5%-BRPE sample had similar behavior regarding the changes in their water content through the days of storage, presenting slight variation until the 14th day, when values started to increase for the 5%-BRPE sample.

As displayed in the Figure 2, the addition of BRPE at 3% concentration showed a different behavior for the water content when compared to the other samples. For this specific sample, the water percentage presented a drop, followed by an increase after the 7th day.

Knowing the water content is related to the amount of free water in the sample, the results indicate that although all samples had relatively close W% by the 14th day of storage, the samples with the presence of BRPE had a higher W% by the 21st day. This may be explained by the interaction of the BRPE compounds with water molecules.

The Figure 3 brings the results for the Protein analysis of the sample through storage time.

Figure 3 – Proteins in ground beef containing 0%, 3%, and 5% of BRPE during storage at 7ºC.

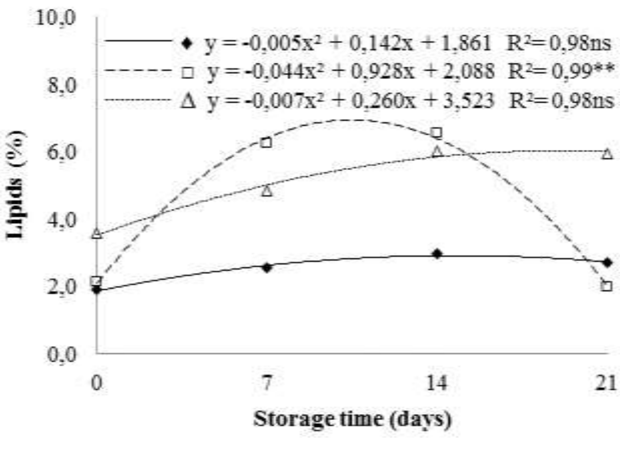


♦ = 0% BRPE; □ = 3% BRPE; Δ = 5% BRPE. *statistically significant ($p<0,05$). [ns]not statistically significant. Source: Authors (2021).

It is possible to see that percentage of proteins had an impact caused by the different treatments (0%, 3% and 5% BRPE) through storage time. The control sample (0% BRPE) was the one that present the most constant protein content through time, being constant until the 7th day, and showing a slight dropping after that day. Similar behavior was seen for the sample containing 3% BRPE, however, the 5%-BRPE sample presented distinct behavior, showing a decrease in the protein content since the first day of storage. This may have happened due to errors in the storage or the concentration of BRPE in the sample, what might be responsible for an intensification of the metabolic reactions leading to a faster degradation of proteins and, therefore, to a faster decline of these compounds in the sample.

In the Figure 4 are shown the results of the lipid analysis of the samples through storage time.

Figure 4 – Lipids in ground beef containing 0%, 3%, and 5% of BRPE during storage at 7ºC.

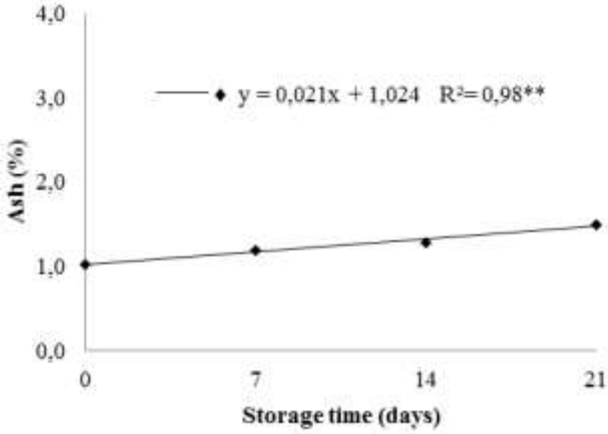


♦ = 0% BRPE; □ = 3% BRPE; Δ = 5% BRPE. **statistically significant ($p<0,01$). [ns]not statistically significant. Source: Authors (2021).

According to the curve of lipids through storage time, the control sample presented the smallest changes, showing a slight increase. The 5%-BRPE sample showed an increase on the lipid content through the days of storage and reaching nearly 6% of lipids in the sample. The increase of lipids in the sample may be related to the natural composition of the raw material (beef). On the other hand, the 3%-BRPE sample had a different behavior than the other ones, increasing the lipid content until the 14th day, and then declining. These interactions indicate that the use of BRPE in a concentration of 3% leads to an increase of lipids higher than the other samples, at least until the 14th day, and this finding is relevant since lipids are known for influencing the taste of food.

The Figure 5 shows the results of the ash content of the samples through storage time.

Figure 5 – Ash content in ground beef containing 0%, 3%, and 5% of BRPE during storage at 7ºC.



♦ = 0% BRPE. **statistically significant (p<0,01). Source: Authors (2021).

No interaction was observed between the variants regarding the ash content; thus, no difference was associated to the different concentrations of BRPE in the samples.

The Table 2 brings the results of the microbiological analysis of Coliforms at 35ºC in ground beef stored at 7ºC, through storage time.

Table 2 – Most Probable Number (MPN/g) of Coliforms at 35°C in ground beef containing 0%, 3%, and 5% of BRPE during storage at 7ºC.

	Days of storage			
Sample	**0**	**7**	**14**	**21**
S1 (0% BRPE)	>1100	160	>1100	110
S2 (3% BRPE)	210	9	>1100	>1100
S3 (5% BRPE)	290	3,6	>1100	460

Source: Authors (2021).

Although the maximum limits of acceptance for coliforms at 35ºC are not determined in the Brazilian legislation, this analysis is necessary for it serves as an indicator of unsatisfactory sanitary conditions (Livoni et al., 2013). Usually, the high number of coliforms in ground beef is likely related to inadequate storage temperature and faster proliferation enhanced by long periods of exposure of the beef to room temperature.

The process through with meat is submitted to produce ground beef facilitates the occurring of contamination by microorganisms, once the grinding increases the surface area of the product and often allow the incorporation of residues from previous grindings (Almeida et al, 2002).

In the Table 3 are the results of the microbiological analysis of Coliforms at 45ºC in ground beef stored at 7ºC, through storage time.

Table 3 – Most Probable Number (MPN/g) of Coliforms at 45°C in ground beef containing 0%, 3%, and 5% of BRPE during storage at 7ºC.

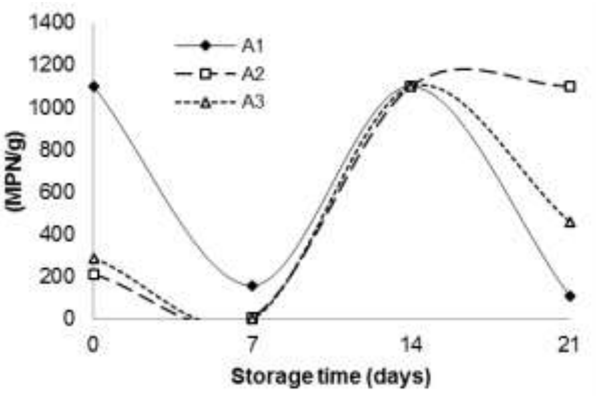
	Days of storage			
Samples	**0**	**7**	**14**	**21**
S1 (0% BRPE)	15	3,6	>1100	150
S2 (3% BRPE)	9,1	3	>1100	>1100
S3 (5% BRPE)	3	<3,0	>1100	93

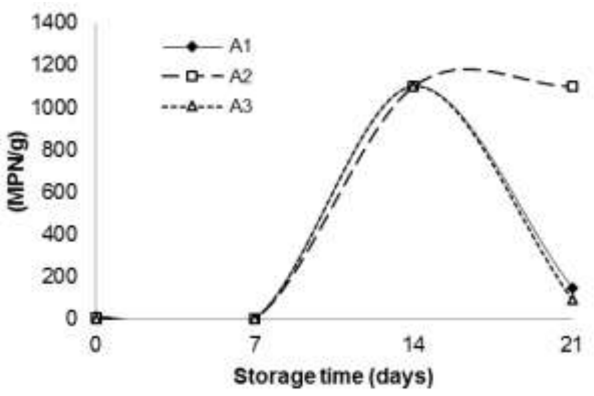
Source: Authors (2021).

The presence of coliforms at 45ºC in food products provides information on the sanitary conditions to which the products have been exposed, indicating fecal contamination (FRANCO; LANDGRAF, 2002).

All samples presented a reduction in the microbiological contamination until the 7th day of storage (Figure 6); this may be related to the antimicrobial action of the BRPE. After the 7th day, however, there was an increase in the contamination and the BRPE seems to not have been able to be as effective as in early storage days.

Figure 6 – Most Probable Number (MPN/g) of Coliforms at 35°C and 45ºC in ground beef containing 0%, 3%, and 5% of BRPE during storage at 7ºC, respectively.





♦ = 0% BRPE; □ = 3% BRPE; Δ = 5% BRPE. Source: Authors (2021).

The ground beef analyzed (with or without BRPE) presented satisfactory sanitary results. The samples had the absence of *Salmonella* sp. confirmed. According to a Brazilian regulation (Brasil, 2019) – which determines the technical regulation for the microbiological standards in food – meat products kept unfrozen should have negative results for the presence of *Salmonella* sp./25 g.

The absence of these bacteria does not guarantee the safety of the meat product, since other microorganisms, such as *Staphylococcus aureus* and thermotolerant coliforms may be related to foodborne illness associated with the consumption of meat (Abreu et al., 2011).

In Brazil, Salmonella are the bacteria responsible for the most outbreaks caused by contamination through food ingestion (Caetano; Pagano, 2019), ground beef, for instance, is one of the food products with the highest microbiological contamination.

There are several studies approaching the microbiological quality of commercial ground beef (Monteiro et al., 2018; Barbosa et al., 2019) highlighting its frequent contamination due to unsanitary and unhygienic conditions in different moments, such as in the receiving, processing, exposing, and storing during commercialization, until the consumer's house.

Rosina et al. (2013) and Damer et al., (2014) found results similar to the ones of this study. Both documented satisfactory results regarding the absence of Salmonella in their evaluation of ground beef.

4. Conclusion

The Brazilian Red Propolis Extract showed greater efficiency in microbial inhibition during the first 7 days of ground meat storage, in addition to positively affecting its physical-chemical characteristics. It is recommended to store this type of meat at lower temperatures, however, it was possible to identify the efficiency of the extract in another storage range. Therefore, the use of this extract should be encouraged, especially in a product such as ground meat that goes through a grinding process that leads to the rupture of the tissue, making it more sensitive to degradation.

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