

**Control of lipid oxidation in jerked beef through the replacement of sodium nitrite by natural extracts of yerba mate and propolis as antioxidant agent****Controle da oxidação lipídica em jerked beef através da substituição de nitrito de sódio por extratos naturais de erva-mate e própolis como antioxidante**

DOI:10.34117/bjdv6n1-348

Recebimento dos originais: 30/11/2019

Aceitação para publicação: 30/01/2020

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**ABSTRACT**

This study evaluated the lipid oxidation of Jerked Beef over a period of storage, replacing the sodium nitrite (NO) by natural extracts of yerba mate and propolis, alone or combined. For the production of Jerked Beef 6 different formulations were used: 1 (Control), 2 (NO), 3 (yerba mate - EM), 4 (propolis extract - PRO), 5 (EM+NO) and 6 (PRO+NO). In all formulations, analyzes of proximal composition, analysis of color (L\*, a\* and b\*) and count of Salmonella spp, total coliforms and thermotolerant coliforms and coagulase positive staphylococci at time zero and sixty days of storage were performed. Water activity was monitored at time zero, thirty and sixty days of storage and the quantification of TBARS every 7 days. The activity of natural antioxidants evaluated shows decrease of lipid oxidation of up to 2.5 times when compared to the control and still showed no significant differences between the treatments NO and EM, confirming its potential to minimize lipid oxidation in jerked beef during its storage. The results also showed that EM presents greater antioxidant capacity when compared to PRO and when associated with IN, the values of TBARS became close to the values obtained only for the control samples with the addition of NO. The proximal

composition remained similar to similar products and within specifications of Brazilian legislation, as well as the results obtained for the counting of microorganisms. The samples that differed significantly at 5%, are directly associated with the type of formulation established. Regarding the evaluation of color of the product, it was verified that the intensity of the color red ( $a^*$ ) decreased with storage time and there was also an increase in the intensity of the color yellow ( $b^*$ ) indicating a darkening, already expected from the product, despite the values of luminosity ( $L^*$ ) have also been increased. A final analysis of the results strongly suggests that the use of EM is a good alternative to meat products industry in reducing the addition of curing salts when associated to another antioxidant.

**Keywords:** Natural antioxidant, Salted Meat, Oxidative stability, coagulase-positive Staphylococcus, T

## RESUMO

Jerked beef, típico produto brasileiro, industrializado, adicionado de cloreto de sódio e sais de cura e submetido a um processo de maturação e secagem. Devido aos possíveis efeitos colaterais constatados por testes de laboratório na saúde do consumidor, a substituição de antioxidantes sintéticos por substâncias naturais com potencial antioxidante vem sendo implantada pela indústria cárnea. Este trabalho teve por objetivo avaliar a oxidação lipídica do Jerked beef ao longo do período de armazenamento pela substituição do nitrito de sódio por extratos naturais de própolis e erva-mate. Para a produção do Jerked beef foi utilizada como matéria-prima o corte de ponta de peito em 6 diferentes formulações, formulação 1 (controle – in natura), formulação 2 (nitrito de sódio - NO), formulação 3 (Erva-mate - EM), formulação 4 (Extrato de própolis - PRO), formulação 5 (nitrito de sódio + Erva-Mate – EM+NO) e formulação 6 (extrato de própolis + nitrito de sódio – PRO+NO). A matéria-prima foi submetida a salga úmida, salga seca (tombos), secagem em BOD a 25°C, embalagem e armazenamento em estufa BOD 25°C. Amostras de cada uma das formulações foram retiradas a cada 7 dias para a análise da oxidação lipídica através do método de TBARS. Em todas as formulações, foram realizadas análises de composição proximal no tempo zero e sessenta dias de armazenagem. A atividade de água foi monitorada no tempo zero, trinta e sessenta dias de armazenagem. A análise de cor ( $L^*$ ,  $a^*$ ,  $b^*$ ) foi monitorada no tempo zero e sessenta dias de armazenagem. A contagem de Salmonella spp, Coliformes totais, Coliformes termotolerantes e Estafilococos coagulase positiva foram realizados no tempo zero e sessenta dias. A atividade dos antioxidantes naturais avaliados mostra uma diminuição da oxidação lipídica de até 2,5 vezes quando comparados com o produto in natura e apresentou valores com diferenças não significativas entre os tratamentos NO e EM, confirmando o potencial em minimizar a oxidação lipídica do Jerked beef ao longo dos 60 dias de armazenagem. Os resultados também mostraram que a erva-mate apresenta maior capacidade antioxidante quando comparado à própolis exceto na formulação PRO+NO. Quando associado erva-mate com nitrito de sódio, os valores de TBARS tornam-se próximos dos valores obtidos somente para as amostras de controle com a adição de nitrito de sódio. A composição proximal das formulações manteve-se dentro dos padrões exigidos na IN n022/2000 para Jerked beef. As amostras que diferiram significativamente entre si a 5%, estão diretamente associadas ao tipo de formulação estabelecida. A contagem dos microrganismos esteve dentro dos padrões da RDC n012/2001 exigidos para produtos cárneos maturados. A intensidade do vermelho ( $a^*$ ) diminuiu com o tempo de armazenagem e aumentou a intensidade do amarelo ( $b^*$ ) indicando um escurecimento do produto, apesar de  $L^*$  também ter sofrido aumento. Estes resultados sugerem que a erva-mate é uma boa alternativa para indústria de produtos cárneos na redução da adição de sais de cura quando associada a outro antioxidante.

**Palavras-chave:** Antioxidante natural. Erva-mate. Jerked beef. Oxidação Lipídica. Própolis. TBARS.

## 1 INTRODUCTION

The salting and dehydration are the most ancient forms of meat conservation. Salt was used even before the existence of the cooling system. The curing process is a complex combination of three processes: physical, biochemical and bacteriological, through which the meat absorbs salt, curing agents and other components of the medium, but loses some of its substances, such as proteins, extracts, salts, vitamins, and water for the same curing medium. Curing is not performed only by the action of salt, but also of the sugars and nitrates and nitrites added to the medium. An alternative for the improvement of the quality of the Jerky was the emergence of Jerked Beef (JB), which differs from its predecessor, mainly, by the addition of curing salts at the beginning of the processing and vacuum packaging. These are meat products of intermediate moisture (IU), dehydrated and that maintain their own characteristics without the need of storage under refrigeration, which allow to keep them at room temperature, making them from the economic point of view, very attractive to industry. Because they are stable, they have, at the end of the process, a water activity ( $A_w$ ) between 0.60 and 0.90, which is equivalent to the equilibrium relative humidity (ERH) from 60 to 90% at room temperature. The food industry uses in its processes synthetic additives as the main resource for delaying the lipid oxidation. The complete inhibition of lipids oxidation, until then, is not possible, but this process can be delayed for a long cover period with the use of antioxidants. These, in addition to delaying the oxidative rancidity, protect carotenoids, vitamins A and D and other unsaturated ingredients, improving the quality of the product oxidation, i.e., decreasing the concentration of oxygen, intercepting the singlet oxygen and decomposing the primary products of oxidation for non-radical species, breaking them in chains to prevent the spread of the reaction of hydrogen capture. In some industrial products we have already found the replacement of synthetic antioxidants for natural substances with antioxidant potential in meat products, due to the possible effects detected by laboratory tests in guinea pigs caused by these synthetic products. This study aimed mainly to test some products used in other studies such as antioxidant agents in JB associated or not with sodium nitrite.

## **2 MATERIAL AND METHODS**

For the execution of experiments, cut of meat brisket cut was used as a raw material. The samples of yerba mate extracts were obtained by aqueous extraction with the aid of an ultrasound bath Elmasonic P, brand Elma, using distilled water as a solvent, in the ratio of 1:10 (m/m) yerba mate/solvent (Campos et al., 2008; Beal et al., 2011). Then, the extracts were centrifuged (Routine centrifuge 420R, HettichZentrifugen brand), in a rotational speed of 8000 rpm for 10 minutes at 20°C and the supernatant was filtered. The extracts were dried in a Spray Dryer, LabMaq MSD 1.0 at drying temperature of 150°C and at drying speed of 0.77 L h<sup>-1</sup> and stored in dark bottles and under

refrigeration until the moment of analysis. The samples of alcoholic extract of propolis at 30% were supplied by the company Apis Flora.

The briskets were packed and transported in isothermal boxes to keep the storage temperature. Trim was carried out for removing excess skin and surface fat, thus maintaining the homogeneity of the raw material. For subsequent step of wet salting, which makes it possible to obtain more delicate products and with more homogeneous distribution of salt (Nunes and Pedro, 2011), the parts were divided into 6 parts randomly that were subjected to 6 different formulations. Formulation 1: Control sample; Formulation 2: Addition of 200 ppm of sodium nitrite (Standard); Formulation 3: Addition of 300 ppm of Yerba Mate (EM); Formulation 4: Addition of 400 ppm of Propolis Extract 30% (PRO); Formulation 5: Addition of 100 ppm of sodium nitrite and 150 ppm of extracts of yerba mate (EM+NO) and Formulation 6: Addition of 100 ppm of sodium nitrite and 200 ppm of Propolis extract 30% (PRO+NO).

The process of wet salting consisted of the immersion of the samples in saline solution at a concentration of 20% (p/v), at a ratio of 2:1 (saline solution/meat), for a period of 2 hours under stirring. Elapsing the immersion time, the parts were packed in plastic trays identified and covered with coarse salt in the proportion of 2:1 (p/p), forming piles intercalating with layers of coarse salt and beef. This process was kept at room temperature and each period of 24 hours, the piles were inverted, and thus, those parts that were on the top passed to the base and vice versa until the parts reached water activity of 0.75, being the salt changed at every step. After this step, the excess salt was removed, the parts were duly identified, hung with metal staples and then subjected to drying in an oven at 35°C for 24 hours for surface moisture evaporation, then vacuum packed and stored at 25°C until the implementation of the analytical procedures.

Contents of moisture, ash, lipids and proteins were determined according to AOAC (1995). Aw was determined in all stages of processing using an automatic device brand Aqualab-Decagon Devices Inc., model CX-2, at a temperature of 25°C ( $\pm 1$ ). For the evaluation of lipid oxidation, the method of TBARS was used (Reactive substances to thiobarbituric acid-2) according to Crackel et al. (1988), according to the recommendations of Shahidi et al. (1985)

For each batch, the following were verified: presence of *Salmonella* spp, count of *Staphylococcus aureus*, determination of the most probable number of total coliforms and thermotolerant at time zero, thirty and sixty days of storage.

The color analyzes were performed in triplicate, in time 0 and 60 days of samples storage, using Minolta colorimeter calibrated beforehand, taking three different points of reading, in the inner part of the salted product, in which a longitudinal cut for all formulations was carried out. The values of L\*, a\* and b\* are expressed in the CIELab color system.

The results were statistically analyzed with analysis of variance and means testing (Tukey test) at 5% level of significance. For these analyzes, the software Statistica® for Windows version 12.0, from STATSOFT (2015) was used.

### 3 RESULTS AND DISCUSSION

Table 1 presents the results of the proximal composition, being that all samples of JB were within the standards established by the Brazilian legislation (Brasil, 2000) and according to the results obtained by Coelho et al., (2017), Youssef (2003), Garcia (2002). Statistically, the formulations differed significantly at 5%, which is directly associated with the type of formulation established. It is noted that the current legislation and available literature recommend values for moisture, ash and water activity, but the same is not true for lipids and proteins, since they may have variation, because they depend directly on the initial characteristics of the raw materials used for processing. Purnomo (2011) Analyzing Indonesian spicy dried meat obtained results that can be compared with those obtained in this experiment, however some factors are differentiated, but basically the profile is similar.

**Table 1.** Results of the proximal composition of JB in different formulations regarding the initial (T0) and final time of storage (T60 days).

Formulations	Moisture		Ash		Protein		Lipids	
	T0	T60	T0	T60	T0	T60	T0	T60
Control	55,65 <sup>b</sup> (±1,00)	43,94 <sup>ab</sup> (±0,4)	18,34 <sup>b</sup> (±0,42)	20,61 <sup>b</sup> (±0,55)	30,10 <sup>b</sup> (±1,07)	27,34 <sup>d</sup> (±0,67)	3,86 <sup>b</sup> (±0,33)	5,92 <sup>b</sup> (±1,29)
Standard	57,40 <sup>b</sup> (±0,50)	39,57 <sup>c</sup> (±2,51)	20,19 <sup>a</sup> (±0,27)	19,96 <sup>c</sup> (±0,11)	32,08 <sup>a</sup> (±0,85)	33,89 <sup>a</sup> (±0,48)	4,45 <sup>b</sup> (±1,57)	7,43 <sup>ab</sup> (±0,54)
EM	53,01 <sup>c</sup> (±2,9)	40,37 <sup>c</sup> (±0,50)	17,96 <sup>b</sup> (±0,41)	22,67 <sup>a</sup> (±0,05)	26,71 <sup>c</sup> (±1,42)	29,85 <sup>c</sup> (±0,26)	9,08 <sup>a</sup> (±0,53)	9,07 <sup>a</sup> (±1,06)
PRO	60,10 <sup>a</sup> (±0,33)	42,78 <sup>b</sup> (±0,30)	19,79 <sup>a</sup> (±0,11)	21,12 <sup>b</sup> (±0,27)	30,03 <sup>b</sup> (±0,62)	30,77 <sup>c</sup> (±1,90)	3,63 <sup>b</sup> (±0,91)	2,36 <sup>c</sup> (±0,43)
EM + NO	54,91 <sup>bc</sup> (±0,53)	45,01 <sup>a</sup> (±0,53)	19,66 <sup>a</sup> (±0,17)	19,97 <sup>bc</sup> (±0,50)	30,71 <sup>ab</sup> (±0,12)	32,14 <sup>bc</sup> (±0,35)	2,28 <sup>b</sup> (±0,35)	2,63 <sup>c</sup> (±1,08)
PRO + NO	54,79 <sup>bc</sup> (±0,24)	42,69 <sup>b</sup> (±0,39)	20,16 <sup>a</sup> (±0,17)	19,99 <sup>bc</sup> (±0,17)	30,33 <sup>b</sup> (±0,33)	32,86 <sup>ab</sup> (±0,53)	4,97 <sup>b</sup> (±0,80)	2,09 <sup>c</sup> (±0,25)

Legend: - Control (salted beef - jerky); Standard (200ppm Nitrite). EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm nitrite); PRO+NO (200ppm Propolis +

100ppm Nitrite); T0 (initial time); T60 (60 days of storage); <sup>a-d</sup> different letters in the same column statically differ among themselves ( $p < 0.05$ ) by the Tukey test; results are averages of triplicate  $\pm$  standard deviation.

It is possible to note in the table above that the addition of propolis causes the biggest changes in proximal composition. Another point to highlight is that it was observed that there was a significant decrease in the values of moisture during storage, this for all the tested formulations. This result suggests that, even after drying, the product still continued suffering dehydration, even packed. The explanations for this fact would be several, which can vary from a thickness of the packaging until the speed of air during the product drying process.

When there is an impact on the values of moisture content of a food product, there is a need to reassess their entire constitution, since the water reduction will tend to concentrate solids. This phenomenon can be observed in this study, indicating that, with the storage, there is an apparent increase of lipids and proteins as reported by Samejima et al., (1992).

Analyzing the results obtained for the color of the products, it should be noted that the values of Luminosity ( $L^*$ ) increased during the 60 days of the product storage. The lowest  $L^*$  value at time zero (darker meat) can be explained by the greater capacity of water retention and less loss of liquids in the medium, associated with the membranes integrity (Koochmaraie et al. 2002). However, the product in question is already part of low  $L^*$  values, i.e., low luminosity when compared to *in natura* meat. Sabadini et al. (2001) found for dried meat that the values for  $L^*$  increased in relation to raw material. These authors mention meat with grayish characteristic found in the previous step (wet salting) not detected in the dry salting and the samples presented a distinctive "glow", characteristic of the product. In Table 2, the evaluated data were observed for the color of the product in initial and final storage times.

**Table 2.** Values of  $L^*$ ,  $a^*$  and  $b^*$  obtained for different formulations of JB between initial time (T0) and final days of storage (T60 days).

Formulation	$L^*$		$a^*$		$b^*$	
	T0	T60	T0	T60	T0	T60
Control	27,30 <sup>b</sup>	40,12 <sup>a</sup>	7,20 <sup>b</sup>	9,00 <sup>a</sup>	3,40 <sup>b</sup>	9,67 <sup>a</sup>
	( $\pm 1,39$ )	( $\pm 2,20$ )	( $\pm 1,32$ )	( $\pm 0,47$ )	( $\pm 0,78$ )	( $\pm 1,04$ )
Standard	31,61 <sup>b</sup>	34,37 <sup>a</sup>	12,09 <sup>a</sup>	9,40 <sup>b</sup>	5,36 <sup>b</sup>	10,82 <sup>a</sup>
	( $\pm 3,67$ )	( $\pm 4,71$ )	( $\pm 0,80$ )	( $\pm 2,64$ )	( $\pm 1,43$ )	( $\pm 3,41$ )
EM	32,04 <sup>b</sup>	41,62 <sup>a</sup>	5,89 <sup>a</sup>	5,03 <sup>b</sup>	5,13 <sup>b</sup>	11,69 <sup>a</sup>
	( $\pm 1,40$ )	( $\pm 4,55$ )	( $\pm 1,11$ )	( $\pm 0,09$ )	( $\pm 0,62$ )	( $\pm 2,42$ )



PRO	29,62 <sup>b</sup> (±0,99)	34,20 <sup>a</sup> (±2,63)	9,48 <sup>a</sup> (±0,46)	4,69 <sup>b</sup> (±1,60)	4,38 <sup>b</sup> (±0,66)	6,72 <sup>a</sup> (±0,75)
EM + NO	31,59 <sup>b</sup> (±1,52)	41,58 <sup>a</sup> (4,20±)	9,75 <sup>a</sup> (±0,67)	8,34 <sup>b</sup> (±1,77)	4,38 <sup>b</sup> (±0,50)	8,79 <sup>a</sup> (±1,87)
PRO + NO	29,55 <sup>b</sup> (±2,33)	42,90 <sup>a</sup> (±3,18)	13,23 <sup>a</sup> (±4,09)	4,90 <sup>b</sup> (±3,19)	5,94 <sup>b</sup> (±1,22)	11,50 <sup>a</sup> (±1,51)

Legend: - Control (salted beef - jerky); Standard (200ppm Nitrite). EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm Nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite); (dp) standard deviation of means; <sup>a-b</sup> different letters in the same column statically differ among themselves ( $p < 0.05$ ) by the Tukey test. Results are averages of triplicate  $\pm$  standard deviation.

Analyzing the other parameters of color, intensity of red color ( $a^*$ ) decreased with storage time, which may have occurred as a result of changes in the status of the chemical form of heme pigments (Mancini and Hunt, 2005). Luciano et al. (2009) describe that the intensity of red was reduced over the maturation period (1 to 14 days) for *in natura* beef and went to values around 12 for values below 8 and, concomitantly, these authors observed significant reductions in the amount of heme pigments and an increase in the percentage of metmyoglobin. The increase in the intensity of the yellow ( $b^*$ ) is due to the fact that the stored meat has brownish color, because there is on the product surface a predominance of metmyoglobin (Lawrie 2005).

A dark color can be observed in products that undergo superficial dehydration, which promotes the concentration of pigments and at the same time changes the optical properties of muscle fibers (Faria et al. 2001). This is exactly what can be observed in this study, once values of  $a^*$  and  $b^*$  indicate that there was a darkening of the product, although  $L^*$  also had an increase. This increase of  $L^*$  does not necessarily indicate that the sample was lighter, but rather an increase in its luminosity, which in turn can be associated with possible dehydration that the product undergoes during its storage, causing even more drastic change in the proteins conformation. It was also verified, a darkening of the samples due to excessive addition of nitrite, which fosters the myoglobin oxidation, leading to the metmyoglobin formation (Walshe and Rose, 1956), which can cause the product greening (Judge et al. 1989), fact that was not observed in this study. On the other hand, the low availability of nitric oxide, resulting from the addition of insufficient quantities of nitrite or reducing agents during the curing, generating a low residual concentration of nitric oxide in the final product, could result in products with low coloration, little stable (Towsend and Bard, 1971).

The obtained values of water activity ( $A_w$ ) (Table 3) are in line with those found in the literature. However, additional points should be discussed, since there was a significant difference for  $A_w$  in relation to storage time and type of formulation. It is observed in Table 3 that for times of

0 days and 30 days of storage there is variation in the product  $A_w$  and there is stabilization only for the time of 60 days. This can be directly associated with the fact that there has been the same for moisture contents, which decreased over time, being statistically different between the initial time and time 60 days of storage. Youssef et al., (2013) indicated that the water activity in the JB samples at time zero was 0.77. The values of the sun-dried meat  $A_w$  had an amplitude from 0.87 to 0.95. Lira and Shimokomaki (1998) observed an  $A_w$  of 0.92 for sun dried meat. Considering that the mean average of  $a_w$  studied was below 0.75, all formulations are close to the recommended value for the product storage without refrigeration.

**Table 3.** Results obtained for water activity ( $A_w$ ) for different formulations of JB stored for 0, 30 and 60 days.

Formulations	Storage Time 25°C (days)		
	T0	T30	T60
Control	0,724 <sup>a</sup> (± 0,006)	0,722 <sup>b</sup> (±0,014)	0,713 <sup>a</sup> (±0,006)
Standard	0,714 <sup>b</sup> (±0,003)	0,733 <sup>a</sup> (±0,07)	0,712 <sup>a</sup> (±0,012)
EM	0,717 <sup>a</sup> (±0,005)	0,70 <sup>d</sup> (±0,01)	0,714 <sup>a</sup> (±0,002)
PRO	0,722 <sup>a</sup> (±0,002)	0,707 <sup>c</sup> (±0,008)	0,712 <sup>a</sup> (±0,001)
EM + NO	0,724 <sup>a</sup> (±0,001)	0,717 <sup>a,b,c,d</sup> (±0,003)	0,711 <sup>a</sup> (±0,004)
PRO + NO	0,721 <sup>a</sup> (±0,003)	0,718 <sup>a,b,c,d</sup> (±0,003)	0,720 <sup>a</sup> (±0,013)

Legend: - Control (salted beef - jerky); Standard (200ppm Nitrite). EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm Nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite); (dp) standard deviation of means; <sup>a-b</sup> different letters in the same column statically differ among themselves ( $p < 0.05$ ) by the Tukey test. Results are averages of triplicate  $\pm$  standard deviation.

$A_w$  and the concentration of salt are the main parameters to ensure the JB stability. According to Sabadini et al. (2001), in mass transfer studies, verified that  $A_w$  was directly influenced by the salt penetration and water loss, which occurred simultaneously. Whereas Biscontini et al. (1995) observed shrinkage of muscle fibers and formation of intra- and extracellular spaces, a fact that resulted in the exit of water and other components, characterizing reductions in moisture contents and  $A_w$ . In



another study, Torres et al. (1994) found that the value of 0.75 for  $A_w$  characterizes point of balance between the diffusion of sodium chloride to the interior of the meat, with stabilization of the product moisture content.

As a result, obtained for microbiological analyzes performed in this study, all the tested samples for thermotolerant coliforms were negative. A similar result occurred for determination of salmonella, where all the tested samples tested exhibited absence of this microorganism. Table 4 and Table 5 indicate the microbiological study conducted at time 0, 30 and 60 days of JB storage. It should be noted that the microbiological quality of the product is in accordance with the recommended procedure for legislation by the parameters stipulated for matured meat products such as raw hams, salami, desiccated sausages, jerky, JB and alike (Brasil, 2001).

**Table 4.** Survey on Total Coliforms (MPN/g) in different JB formulations in function of the storage period.

Formulation	T0	T30	T60
Standard	<3,0	<3,0	<3,0
EM	<3,0	<3,0	<3,0
PRO	<3,0	<3,0	<3,0
EM + NO	<3,0	<3,0	<3,0
PRO + NO	9,2	<3,0	<3,0

Legend: Standard (200ppm Nitrite); EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite).

**Table 5.** Survey of *Staphylococcus* positive coagulase (UFC/g) in different JB formulations in function of the storage period.

Legend:	Formulation	T0	T30	T60	Standard
	Standard	<10	<10	<10	
	EM	<10	<10	<10	
	PRO	<10	<10	<10	
	EM + NO	absent	<10	<10	
	PRO + NO	<10	<10	<10	

(200ppm Nitrite); EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite).

The microorganisms capable of developing into meat products of intermediate water activity are molds, yeasts and Halophile bacteria (Franco and Landgraf, 1996), the latter belonging to the genus *Halobacterium* and *Halococcus* (Buchanan and Gibbons, 1974). These bacteria produce red

pigments and a putrefactive alteration, called "redness". On the other hand, these bacteria have slow growth, even if the environmental conditions are ideal and these, strictly aerobic (Buchanan and Gibbons, 1974). The vacuum packaging prevents the development of halophilic bacteria as well as yeasts and molds (Geisen et al. 1992).

According to Lara et al. (2003) the salted meat products may present pathogenic microorganisms, this being corroborated by Santana and Azeredo (2005), who detected the presence of *Staphylococcus aureus* in salted meat. Also, in this issue of microbiological quality other authors as Pinto et al. (2002) indicate that the group of coliforms, such as *Escherichia coli*, may be resistant to mild concentrations of salt. This author indicates that up to 5% of salt of this microorganism is viable.

According to Franco and Landgraf (2008), the determination of deteriorating and pathogenic micro-organisms in sun dried meat serves as a basis for establishing microbiological standards. Among the bacteria of the coliform group, especially *Escherichia coli* has been used as the most known indicator of fecal contamination. Studies conducted by Coelho et al., (2017) indicated that sun dried meat samples, a product similar to those studied in this work, resulted in an average count of *Staphylococcus aureus* exceeding 5.0UFC/g and these values represent a considerable risk for the presence of enterotoxins, which may result in cases of food poisoning. These authors also suggest that these organisms are halotolerant, and they can bear up to 15% of salt and are favored, since there is a decrease of other competing microorganisms, which enables their growth in an easier way. The results obtained for TBARS during 60 days of storage are shown in Table 6. Through the TBARS test the malondialdehyde is quantified, one of the main products of the decomposition of hydroperoxides of poly-unsaturated fatty acids, formed during the oxidative process.

**Table 6.** Values of TBARS in JB (mg of malondialdehyde/kg of sample) for treatments in different periods of storage.

Formulation	periods of storage at 25°C (days)								
	T0	T7	T14	T21	T30	T37	T45	T52	T60
Control	1,298 <sup>a</sup> (±0,022)	1,760 <sup>a</sup> (±0,029)	2,096 <sup>a</sup> (±0,020)	2,646 <sup>a</sup> (±0,031)	3,448 <sup>a</sup> (±0,027)	3,512 <sup>a</sup> (±0,019)	3,639 <sup>a</sup> (±0,065)	4,266 <sup>a</sup> (±0,069)	4,332 <sup>a</sup> (±0,175)
Standard (NO)	0,739 <sup>b</sup> (±0,021)	0,853 <sup>b</sup> (±0,017)	0,880 <sup>c</sup> (±0,017)	1,113 <sup>c</sup> (±0,019)	1,340 <sup>b</sup> (±0,032)	1,295 <sup>c</sup> (±0,013)	1,383 <sup>c</sup> (±0,023)	1,580 <sup>c</sup> (±0,038)	1,619 <sup>b</sup> (±0,029)
EM	0,895 <sup>c</sup> (±0,018)	1,020 <sup>c</sup> (±0,021)	1,019 <sup>c</sup> (±0,026)	1,090 <sup>b</sup> (±0,014)	1,272 <sup>e</sup> (±0,017)	1,483 <sup>b</sup> (±0,017)	1,552 <sup>b</sup> (±0,030)	1,785 <sup>b</sup> (±0,023)	1,843 <sup>b</sup> (±0,051)
PRO	1,055 <sup>d</sup>	1,183 <sup>d</sup>	1,216 <sup>b</sup>	1,671 <sup>d</sup>	1,855 <sup>c</sup>	1,895 <sup>c</sup>	1,976 <sup>c</sup>	2,026 <sup>c</sup>	2,121 <sup>c</sup>

	(±0,015)	(±0,020)	(±0,021)	(±0,019)	(±0,037)	(±0,018)	(±0,043)	(±0,034)	(±0,031)
EM + NO	0,767 <sup>b</sup>	0,887 <sup>b</sup>	0,821 <sup>f</sup>	1,054 <sup>b,c</sup>	1,344 <sup>b</sup>	1,460 <sup>b</sup>	1,506 <sup>b</sup>	1,694 <sup>d</sup>	1,758 <sup>b</sup>
	(±0,020)	(±0,031)	(±0,024)	(±0,018)	(±0,028)	(±0,020)	(±0,041)	(±0,027)	(±0,038)
PRO + NO	0,860 <sup>c</sup>	0,880 <sup>b</sup>	0,975 <sup>d</sup>	1,178 <sup>d</sup>	1,595 <sup>d</sup>	1,663 <sup>d</sup>	1,718 <sup>d</sup>	1,800 <sup>b</sup>	2,010 <sup>c</sup>
	(±0,029)	(±0,014)	(±0,020)	(±0,031)	(±0,036)	(±0,021)	(±0,030)	(±0,022)	(±0,031)

Legend: - Control (salted beef - jerky); Standard (200ppm Nitrite). EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm Nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite); (dp) standard deviation of means; <sup>a-b</sup> different letters in the same column statically differ among themselves ( $p < 0.05$ ) by the Tukey test. Results are averages of triplicate  $\pm$  standard deviation.

As it can be observed in Table 6, the starting point for comparison among the formulations is the presence of sodium nitrite (NO), i.e., is the sample set as Standard. The formulations were processed with 200ppm of sodium nitrite values suggested by legislation that limits a residual of 150ppm of this salt for cured products, in this case JB. The maximum allowed value of nitrite in cured Chinese ham, at the end of processing, is 20ppm, according to Zhu (1998), and by the Brazilian legislation is maximum 150ppm of product (ABIA, 1998).

It should be noted that over 60 days of storage, there was an increase in the TBARS values. A formulation control was performed in order to be able to show clearly the action of these different types of antioxidants in salted meat product. The values obtained in this experiment are, for JB, within the ones provided by the literature, remembering that the control formulation presented values much higher than the others, since it does not have any type of antioxidant product in its formulation, and thus it may not be directly compared to the others. However, it indicates how a salted meat product without curing behaves concerning to their oxidation rates when compared to a meat salted and cured product.

Pearson et al., (1977) suggested that the antioxidant effect of sodium nitrite in cured meat products is interesting. However, the use of salt should be minimized, because its consumption is associated with the occurrence of toxic effects (Lijinsky 1999). The use of sodium nitrate in the cured products behaves like a reserve, restoring the levels of sodium nitrite through the action of reducing bacteria, prolonging the salts antioxidant action (Cassens, 1997). Authors such as Nassu et al. (2003), Trindade et al. (2008) and Bertolin et al. (2010) studied the action of synthetic and natural antioxidants, indicating that they can minimize lipid oxidation in meat products.

The work of Torres and Okani (1997) present values of TBARS per group of foods, being that the group of meat ranged between 0.20 to 1.25mg/kg of malondialdehyde/kg of sample. TBARS values of 1,240mg/kg of malondialdehyde of sample were obtained by Pinto (1998), at the end of the

processing of JB. Youssef et al. (2003) obtained mean values of TBARS in samples of JB ranging from 0.23 to 1.38 mg TBARS.

Analyzing the results obtained in this study, it is noted that JB with addition of EM in its formulation presented values of TBARS lower when compared to the PRO added formulation (Han and Park, 2002). Govaris et al., (2004) researcher with natural product based on oregano also obtained positive result in decreasing lipid oxidation rates.

It is observed that when a blend with EM with sodium nitrite (NO) was formulated, even though there was a reduction of 50% of the amount of NO used (200 ppm to 100 ppm of NO), the values of TBARS become close to the values obtained only for NO. These data suggest that there may be a reduction in addition to curing salts when associated to another antioxidant, in this case EM. This hypothesis comes hand in hand with what is currently looked for industry of cured products, i.e., a reduction in the addition of curing salts with the goal of making these products more attractive to the general public, which is increasingly concerned with the production of potentially disease-causing products, such as nitrosamines and other products originated from the meat curing process. The comparative evolution of lipid oxidation during the storage period is presented in Figure 1.

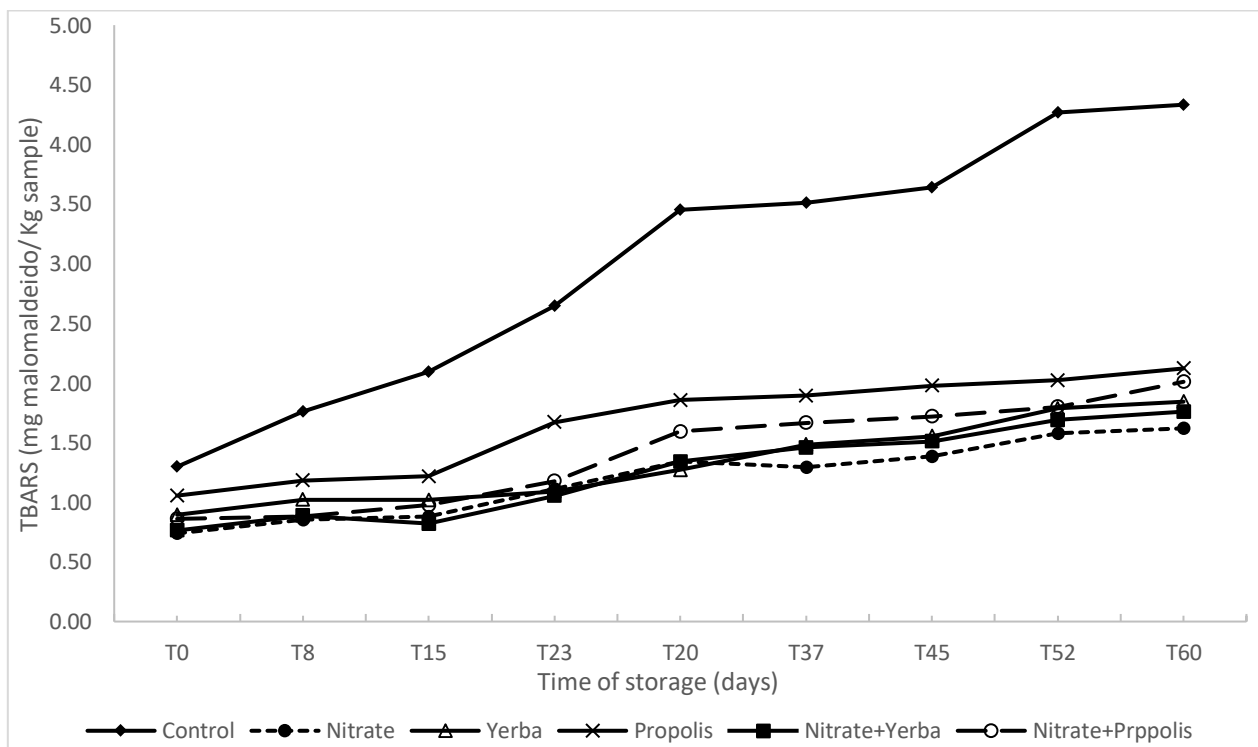


Figure 1. Evolution of lipid oxidation by the number of TBARS in function of the storage time in JB.

Legend: Control (salted meat-jerky); Standard (200ppm Nitrite);EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite).

Among the different formulations with tested natural antioxidants, one may realize that the least satisfactory response was observed with the addition of PRO, once more can be compared to the work of Hans and Park (2002), who upon examining the antioxidant capacity of PRO, concludes that between this and the first showed the worst performance.

#### **4 CONCLUSION**

It can be observed that the lipid oxidation of Jerked Beef remained constant during all the 60 days of storage and that the partial replacement of sodium nitrite per extract of Yerba Mate resulted in a decrease in the speed of the oxidative rancidity comparable to action of isolated sodium nitrite. Neither microbiological contamination nor changes of the proximal composition during the experiment were detected, confirming that a product is safe for consumption even in storage conditions without refrigeration. It was concluded, therefore, that the replacement of synthetic antioxidant agents by natural alternatives are feasible in the control of lipid oxidation in Jerked Beef and may become a plausible alternative to the industry to meet the desires of a portion of the consumer market.

#### **ACKNOWLEDGEMENTS**

The authors are thankful for the granting of Productivity Scholarships in Industrial Development (DT) and Scientific Initiation (PIBIT and PIBIC) granted by CNPq, Fundação Araucária de Amparo a Pesquisa and **LabMulti** - UTFPR Campus Londrina

#### **DISCLOSURE STATEMENT**

No potential conflict of interest was reported by the authors

#### **FUNDING**

This work was supported by CNPq, UTFPR Campus Londrina and Fundação Araucária.

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