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Evaluation of the Effect of Antioxidant Moringa *oleifera* Extract in Beef

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Due to the high content of lipids that present in their nutritional composition, meat are highly susceptible to lipid oxidation food. One way to inhibit or retard oxidative deterioration process is the use of antioxidants, which may be natural or synthetic. In view of the concern of the population to the risks that synthetic antioxidants can cause to health, greater attention has been given to the use of antioxidants obtained from nature. Among the natural, there is Moringa *oleifera* plant, known for its antioxidant properties. Therefore, this study aimed to evaluate the antioxidant effect of Moringa *oleifera* leaf extracts when applied on beef. Four treatments were elaborated: Treatment T1 (control - without addition of extract); Treatment T2 (30g leaves / L of water); Treatment T3 (60g of leaves / L of water); Treatment T4 (90g of leaves / L of water). Analysis of pH, color, lipid oxidation (TBARS) and microbiological were made in all treatments. The results obtained for TBARS confirm the ability of the extracts at the three concentrations applied to act as antioxidants to day 7 of storage. The pH values found are within the acceptable range for this type of food. The color analysis showed that the parameters L *, a * and b *, although with significant differences in the level of 5%, were not enough to visually change the color of the samples. Microbiological analysis in turn, demonstrated that the effectiveness of the extract with a concentration of 90g / L minimize coliforms at 35 °C in 7 days storage.

1. Introduction

Meat is the muscle tissue from slaughtered animals, being composed of water, proteins, lipids, minerals and, on a minor proportion, carbohydrates. Because of its composition, meat is highly susceptible to deterioration, being oxidation the main cause for this to occur (Devatkal et al., 2012).
Oxidation occurs due to the loss of eletrons when the meat compounds make contact with the oxygen of the air. To decrease this reaction, antioxidants are used, which can be natural or synthetic (Xiao et al., 2013; Contini et al., 2014).
Among the most used natural antioxidants, there are the Moringa *oleifera* leaves. Moringa *oleifera* is a botanical specie very common in India, Pakistan and Phillipines, Hawaii and parts of Africa, and is highly used for dietary purposes as vegetables (Anwar et al., 2007; Muthukumar et al., 2014). The extract of Moringa *oleifera* leaves has antioxidant activity due to the high amount of phenolic acid and flavonoids (Atawodi, 2010). The aim of the present work was to evaluate the antioxidant effect of Moringa *oleifera* leaf extracts in beef.

2. Materials and Methods

2.1 Obtention and analysis extracts of Moringa *oleifera*

The extracts of Moringa was obtained using the methodology described by Ganesan & Gurumallesh Prabu (2014), slightly modified. First, the leaves was weighted in analytical balance, and distilled water was added, in

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order to extracts in concentrations of 30g/L, 60g/L and 90g/L, which were posteriorly submitted to heating at 80ºC for one hour. Then, the liquids were filtrated and conditioned.

To verify the antioxidant activity of the liquid extract in beef, four treatments were elaborated: T1 (control – without addition of extract); T2 (30g leaves/L of water); T3 (60g leaves/L of water) and T4 (90g leaves/L of water).

The beef was cut in cube pieces of approximately 2cm², and then 1kg of muscle was weighted for each treatment. The samples were immersed in 200 mL of extract. For the control treatment, the samples were immersed in distilled water (200 mL) for 5 minutes, posteriorly the liquid was drained and the samples were vacuum-packed and stored under refrigeration at 4ºC. All the other treatments were submitted to the same procedures.

2.1.1 Phenolic compounds

The extraction of phenolic compounds of the extracts was performed according Anwar, et al (2007), with some modifications.The values were added in a 10,0 mL flask, following the order: first, 5,0 mL of distilled water, then 100 µL of the specific gallic acid diluted solution, then 0,5 mL of Folin-Ciocalteu was added. After 3 minutes was added 2,0 mL of sodium carbonate 15% (m/v), which was prepared 24 hours before and kept under refrigeration, was pipetted. At last, the total volume of the flask was filled using 10,0 mL of distilled water. The flasks were shaken for homogenization. Immediately, all the flasks were placed in a dark place, and the reaction happened during the next 2 hours. All analysis were performed in triplicate.After the 2 hours rest, the absorbance of the samples was read using a spectrophotometer, on a wave-length of 765 nm. The total phenol content was determined through a calibration curve, which was built following the gallic acid pattern and expressed as mg GAE (gallic acid equivalents) per 100 g of extract.

2.1.2 Determination of the capacity of sequestering free radicals

The sequestering capacity of radicals DPPH (1,1-diphenyl-2-picrylhydrazyl) was done according to Nakbi et al. (2010). Ethanolic solution of DPPH 6.10-5M (3,9 mL) was added to 2,5 mL of solutions of different concentrations, and the samples were then incubated for 30 minutes on room temperature in the dark. The absorbance was read at 515 nm and these values were converted to antioxidant activity (%) through the equation (1):

$$AA\% = \frac{(Abssample - Absblank)}{Absblank} . 100 \quad (1)$$

Where: Abs_{sample} and Abs_{blank} correspond to the absorbances of the sample and white, respectively.

2.2 Physicochemical analysis

2.2.1 pH

The pH was measured using a pHmeter Hanna equipped with a penetration pH-electrode. The pH measure values obtained on days 0, 2, 4 and 6, and the analysis were performed in triplicate.

2.2.2 Color determination

The color was determined using a CR-400 Minolta Chromameter colorimeter, at the CIE L*a*b* color space, where L* refers to luminosity, a* to the red color intensity and b* to the yellow color intensity, calibrated with a white pattern. The measures, made in triplicate, were performed at days 0, 2, 4 and 6.

2.3 Lipid Oxidation (TBARS)

The analysis of the reactive substances to acid 2-barbituric (TBA), determined on days 0, 4 and 7, was performed according to the methodology described by Raharjo et al. (1992), modified by Wang et al. (2002), following the recommendations by Shahidi and Synomiecki (1985) on what refers to the addition of sulphanilamide on the samples that contain nitrite, with some adaptations. It was added 0,5 mL of BHT (di-terc-butilmenil phenol) 0,5% in a test tube that contained 5 g of sample. Following this, it was added 2 mL of sulphanilamide 0,5% and its was left in stand by for 10 min. After this process, 18 mL of TCA (trichloroacetic acid) 5% were added to a 2 mL aliquot of filtered, and then were added 2 mL of TBA 0,08M, and the reaction was conducted in water bath at 40 ºC for 80 min. Posteriorly, the results were checked with an spectrophotometer (Agilent UV-8553) at 531 nm. The quantification was performed using a pattern curve (1. 10^{-8} a 10.10^{-8} mol/mL) of a diethylacetal solution (TEP). The results were expressed in malonaldehyde milligrams by sample kilogram. The analysis were performed in triplicate.

2.4 Microbiological Analysis

Microbiological analysis were performed using the Colony Forming Units (CFU) method for *Salmonella* sp., *Staphylococcus* positive coagulase and coliforms at 35ºC and 45ºC (American Public Health Association, 2001). The analysis were performed at seventh storage day, in duplicate.

2.5 Statistical Analysis

The results were submitted to variance analysis (ANOVA) and compared by the Tukey test, considering the significance level of 5% ($p \leq 0,05$), using the Action – Excel program.

3. Results and Discussion

3.1 Results of the analysis of extracts

Table 1 shown the values of the content of phenolic compounds and the antioxidant activity of the extracts.

Table 1: Content of total phenolic compounds, expressed in equivallent of gallic acid (EGA), and antioxidant activity of the extract of Moringa oleifera leaves.

Extracts g/L	Phenolic compounds (mg EAG/100g extract)	Antioxidant Activity (%)
30	$496,50^{c} \pm 12,54$	$86,96^{a} \pm 0,73$
60	$876,71^{b} \pm 34,27$	$81,01^{b} \pm 0,89$
90	$1164,6^{a} \pm 20,61$	$83,34^{ab} \pm 3,69$

Data referring to the average of three results ± pattern deviation. Same letters on the same columns indicate that there is no significant difference ($p<0,05$) among the averages by the Tukey test.

In Table 1 it is possible to note that content of phenolic compounds varied between 496,50 and 1164,6 for 30 and 90 g / L extract, respectively. The antioxidant activity, in turn, expressed in percentage, varied between 81,01 and 86,96, being the highest percentage referring to the 30 g / L extract.

3.2 Physicochemical analysis

3.2.1 pH

The Table 2 show the pH values of the beef. Considering the results, it can be seen that, generally, the pH values rised as the days passed for the treatments T1, T2 and T4, but not for T3, that had no significant difference on the pH values at a 5% level. The pH values varied from 5,58 to 5,90 for T1, 5,61 to 6,00 for T2 and 5,60 to 6,13 for T4. On T3, it can be noted that there is a pH value of 6,27 on day 0, which is superior to the pH of the other days, what possibly happened due to experimental errors.

According to Elmasry (2012), the pH of beef is, on average, 5,8, and, according to Scheidle (2011), most of the microorganisms have their optimal pH for growing at neutral values, between 7,0 and 7,5. This way, the obtained values on this study have coherent results with the stipulated range, being suitable for comsuption.

Table 2: pH values of the beef submitted to treatments T1, T2, T3 and T4 and stored under refrigeration (4 °C) for 6 days.

Days	T1	T2	T3	T4
0	$5,76^{ABb} \pm 0,19$	$5,90^{Bb} \pm 0,01$	$6,27^{Aa} \pm 0,09$	$5,96^{Bb} \pm 0,02$
2	$5,64^{ABbc} \pm 0,03$	$5,67^{Cb} \pm 0,03$	$6,03^{Aa} \pm 0,04$	$5,60^{Dc} \pm 0,00$
4	$5,58^{Bc} \pm 0,01$	$5,61^{Cc} \pm 0,00$	$6,04^{Aa} \pm 0,01$	$5,69^{Cb} \pm 0,05$
6	$5,90^{Aa} \pm 0,07$	$6,00^{Aa} \pm 0,06$	$6,05^{Aa} \pm 0,28$	$6,13^{Aa} \pm 0,02$

Data refering to the averages of four results ± pattern deviation. The same lowercase letters on the same line and the same uppercase letters on the same column indicate that there is no significant difference ($p<0,05$) among the averages by the Tukey test.

3.2.2 Color

The analysis of the instrumental color values, evaluated for L*, a* and b* are shown on tables 3, 4 and 5.

3.2.2.1 Luminosity (L*)

The average values for L* are shown on Table 3.

*Table 3: Average L*of samples with different extract concentration.*

Days	T1	T2	T3	T4
0	$37,47^{Aa} \pm 0,57$	$31,18^{Bb} \pm 1,57$	$28,3^{Bb} \pm 0,27$	$29,74^{Bb} \pm 1,70$
2	$31,89^{Cb} \pm 0,41$	$34,18^{ABa} \pm 1,19$	$30,67^{ABb} \pm 0,35$	$32,55^{ABab} \pm 0,90$
4	$33,30^{BCbc} \pm 1,20$	$36,81^{Aa} \pm 1,60$	$31,03^{ABc} \pm 0,45$	$34,68^{Aab} \pm 0,92$
6	$33,96^{Ba} \pm 0,53$	$32,53^{ABa} \pm 2,42$	$33,37^{Aa} \pm 2,08$	$36,25^{Aa} \pm 2,92$

Data refering to the average of four results ± standard deviation. The same lowercase letterson the same line and the same uppercase letter on the same column indicate that there is no significant difference ($p<0,05$) among the averages by the Tukey test.

Considering the data on Table 3, it can be verified that there was significant difference at a 5% level between the L* level for each treatment during the time period. Considering the relation among the treatments for each day, it can be verified that there was significant difference on the analysed days, except for day 6. Though, this difference is not enough to visually alter the luminosity of the product, not being noticeable by the consumer.

3.2.2.2 Red color intensity (a*)

The average values for a* are shown on table 4.

Table 4: Average a of samples with different extract concentration.*

Days	T1	T2	T3	T4
0	$9,66^{Bb} \pm 0,65$	$11,65^{Aab} \pm 1,55$	$12,77^{ABa} \pm 0,45$	$11,69^{Bab} \pm 1,30$
2	$14,37^{Aa} \pm 0,36$	$13,52^{Aa} \pm 1,46$	$14,37^{Aa} \pm 1,06$	$12,54^{ABa} \pm 0,92$
4	$13,70^{Aab} \pm 0,84$	$11,62^{Ab} \pm 0,96$	$12,81^{ABab} \pm 0,83$	$14,40^{Aa} \pm 0,64$
6	$12,80^{Aa} \pm 1,82$	$12,12^{Aa} \pm 0,97$	$11,74^{Ba} \pm 1,18$	$12,41^{ABa} \pm 1,06$

Data refering to the averages of four results ± pattern deviation. The same lowercase letters on the same line and the same uppercase letters on the same column indicate that there is no significant difference ($p<0,05$) among the averages by the Tukey test.

Analysing table 4, the results of the variance analysis showed that there was no significant difference on the a* parameter among T1, T2, T3 and T4 on days 2 and 6, while it was not possible to say the same about days 0 and 4. Observing the behaviour of each treatment during the days, it can be seen that only T2 had no significant difference among the storing days, but, for the values that significantly differed among themselves, it cannot be concluded that the difference is high enough to be visually noted.

3.2.2.3 Yellow color intensity (b*)

Table 5: Average b of samples with different extract concentration.*

Days	T1	T2	T3	T4
0	$7,90^{Aa} \pm 0,48$	$5,34^{Bc} \pm 0,45$	$7,28^{BCab} \pm 0,48$	$5,90^{Abc} \pm 0,77$
2	$8,31^{Aa} \pm 0,04$	$8,14^{Aab} \pm 0,46$	$8,51^{Aa} \pm 0,36$	$7,17^{Ab} \pm 0,47$
4	$7,12^{Aab} \pm 0,58$	$6,29^{Bb} \pm 0,62$	$7,79^{ABab} \pm 0,39$	$8,08^{Aa} \pm 0,69$
6	$7,08^{Aa} \pm 1,48$	$5,76^{Ba} \pm 0,81$	$6,24^{Ca} \pm 0,41$	$8,05^{Aa} \pm 1,31$

Data refering to the averages of four results ± pattern deviation. The same lowercase letters on the same line and the same uppercase letters on the same column indicate that there is no significant difference ($p<0,05$) among the averages by the Tukey test.

The average values for b* are shown on Table 5. Observing the results for the b* values, it is possible to notice that there was significant difference among the treatments at a 5% level on different analysis days. Though, this difference is not viasually perceptible.

3.2.3 Lipid oxidation

Table 6 displays the results related to the TBARS analysis.

Table 6: Concentration of malonaldehyde (mg/kg of sample) of samples with different extract concentration.

Days	T1	T2	T3	T4
0	$0,13^{a} \pm 0,00$	$0,05^{b} \pm 0,02$	$0,05^{b} \pm 0,00$	$0,07^{ab} \pm 0,02$
4	$0,07^{a} \pm 0,00$	$0,05^{a} \pm 0,01$	$0,06^{a} \pm 0,01$	$0,08^{a} \pm 0,00$
7	$0,14^{a} \pm 0,01$	$0,11^{ab} \pm 0,01$	$0,10^{b} \pm 0,02$	$0,11^{ab} \pm 0,00$

Data refering to the averages of three results ± pattern deviation. The same lowercase letters on the same line column indicate that there is no significant difference ($p<0,05$) among the averages by the Tukey test.

According to Table 6, it can be seen that the TBARS values for T1 were superior to the other treatments values, showing that the extracts were effective on inhibiting the lipid oxidation. On day 0, T1 had a value of 0,13, and the other treatments had values between 0,05 and 0,07. On the same way, on storing day 7, T1 had a value of 0,14, and the other treatments had values between 0,10 and 0,11.

According Trindade (2009), foods with values that are superior to 2,00mg of malonaldehyde/kg of sample can be harmful to the consumer's health. The amount of malonaldehyde/kg of sample were all inferior to 2,00 on this study, varying between 0,05 and 0,14 mg.

Generally, the presented results confirm the existence of antioxidant activity of the extracts with concentrations of 30 g/L, 60 g/L and 90 g/L of Moringa *oleifera* leaves on the beef samples.

3.2.4 Microbiological analysis

The results of the microbiological analyzes for beef are shown in the table 7.

Table 7: Microbiological characteristics during storage of beef.

Treatment	Coliforms at 35°C (CFU/mL)	Coliforms at 45°C (CFU/mL)	*Salmonella* sp	Positive Coagulase Staphylococcus
T1	$10 \times 10^{1\ bc}$	< 3	Abs	Abs
T2	$24,5 \times 10^{1\ ab}$	< 3	Abs	Abs
T3	$30 \times 10^{1\ a}$	< 3	Abs	Abs
T4	$6 \times 10^{1\ c}$	< 3	Abs	Abs

Data refering to the averages of four results ± pattern deviation. The same letters on the same column indicate that there is no significant difference ($p<0,05$) among the averages by the Tukey test. *Aus – Absence

The microbiological food patterns must follow the RDC nº 12 Resolution, created by the Brazil Sanitary Vigilance National Agency (ANVISA). For fresh beef, this resolution establishes that *Salmonella* must be totally absent, and that the limit for *in natura* coliforms must be $5x10^{3}$ UFC at 45 ºC. It does not determine criteria for coliforms at 35 ºC and positive coagulase Staphylococcus. Thus, the obtained results showed that all the analysed samples follow the patterns stipulated by the legislation, being good for consumption.

Although the legislation does not establish parameters for coliforms at 35 ºC, it is possible to observe on table 7 that T4 had a lower results for these microorganisms than T1, what attests the effectiveness of T4 on the reduction of the coliform growing at 35 ºC.

4. Conclusions

With the results of the presented study, it can be concluded that all the tried liquid extract concentrations of Moringa *oleifera* leaves were effective on the inhibition of lipidic oxidation of beef. The microbiological analysis results showed that all the subjected samples are within the microbiological standards established by legislation for Salmonella and coliforms at 45ºC. Thus, the utilized extract of Moringa *oleifera* leaves satisfactorily reduced the oxidative deterioration on beef, but it's still necessary to perform more studies to better understand its performance.

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