

## Research Article

### Rosemary Essential Oil and Lyophilized Extract as Natural Antioxidant Source to Prevent Lipid Oxidation in Pork Sausage

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**Abstract:** The aim of this study was to evaluate the effect of the use of Rosemary Essential Oil (REO) and Lyophilized Rosemary Extract (LRE) on the oxidative stability of pork sausages stored at -12°C. Potential antioxidants in sausages were determined by thiobarbituric acid reactive substances (TBARS) and expressed in mg of malonaldehyde MDA/kg. Measurements were taken one day each week starting from production day until 49 days after. The TBARS values increased over time, starting with a value of 0.45 and ending with 2.89 mg MDA/kg. The control treatment suffered more intense oxidation than those with Sodium Erythorbate (SE) and natural antioxidants. The lipid oxidation of sausage was significantly inhibited at 49 days of frozen storage in the presence of LRE when compared to the control (47.28%). The sausage containing LRE and REO showed a 75.67% and 56.41% acceptance rate, respectively. The LRE exhibited an excellent inhibitory effect on lipid oxidation of sausage and it has potential to use in meat products.

**Keywords:** Antioxidant activity, lipid oxidation, meat products, *Rosmarinus officinalis*, TBARS

## INTRODUCTION

Currently, one of the biggest challenges for the meat products industry is to provide the consumer with fresh food, regarding taste, color and odor of the product. However, the process involving the preparation of meat products such as milling, mixing and cooking, results in lipid oxidation (Shah *et al.*, 2014; Sáyago-Ayerdi *et al.*, 2009; Osawa *et al.*, 2005).

The lipid oxidation, denoted as one of the main degradation reactions, is responsible for the reduction of vitamins and unsaturated fatty acids, production of free radicals, creating undesirable flavors and decreasing the lifetime of the product (Okpala, 2016). It also leads to the formation of several other compounds which have negative effects on the quality of meat and meat products causing changes to the nutritional quality (Falowo *et al.*, 2014; Shah *et al.*, 2014). The use of antioxidants in foods is one method to minimize these problems (Das *et al.*, 2012).

The synthetic antioxidants used in the meat industry, such as butylatedhydroxyanisole (BHA), Butylatedhydroxytoluene (BHT), Tert-Butylhydroquinone (TBHQ) and Propyl Gallate (PG) has been restricted by many countries due to possible

risks to consumer health. The toxicological effects of these substances is growing the interest in studies of using natural substances with antioxidant characteristic in meat (Rocha *et al.*, 2007; Lianhe *et al.*, 2012; Sun-Waterhouse *et al.*, 2011).

The demand for natural products with antioxidant potential has been an objective of scientific studies. Plants are a generous source of bioactive substances and are being evaluated as natural antioxidants to preserve and improve the overall quality of meat and meat products (Shah *et al.*, 2014). Food seasonings are widely known to have bioactive compounds capable of decreasing lipid oxidation and the action of free radicals (Mariutti and Bragagnolo, 2007).

*Rosmarinus officinalis*, commonly known as rosemary (Mariutti and Bragagnolo 2007) has a high content of phenolic compounds, which includes the abundant compounds rosmarinic acid and rosmanol (Windisch *et al.*, 2008) and it has been successfully applied to the oxidation inhibition in food (Sebranek *et al.*, 2005; Keokamnerd *et al.*, 2008). Rosemary Essential Oil (REO) contains many important bioactive compounds, such as monoterpene hydrocarbons (camphene, verbenene,  $\alpha$ -pinene,  $\beta$ -pinene, limonene), oxygenated monoterpenes (1, 8-cineole, linalool,

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camphor, borneol, verbenone) and sesquiterpenes ( $\beta$ -cariofillene) (Napoli *et al.*, 2010). Essential oils and natural extracts of *Rosmarinus officinalis* L. are known to possess antimicrobial and antioxidant activities and some applications of these extracts in food active packaging systems were reported in literature (Realini and Marcos, 2014). It can be used as a natural preservative in order to inhibit lipid oxidation in sunflower oil (Chen *et al.*, 2014), soybean oil (Casarotti and Jorge, 2012), pork-based products (Lara *et al.*, 2011; Hać-Szymańczuk *et al.*, 2011; Hernández-Hernández *et al.*, 2009) and various types of foods, such as sausages, pastas, peanuts and butter. While rosemary essential oil has antioxidant potential, it can be used to inhibit lipid oxidation in sunflower oil, pork meat sausages (Jongberg *et al.*, 2013) and other foods (Lara *et al.*, 2011; Chen *et al.*, 2014; Hać-Szymańczuk *et al.*, 2011; Hernández-Hernández *et al.*, 2009).

The present study aims to evaluate the antioxidant capacity of essential oil and lyophilized rosemary extract when applied to pork sausage meat in order to prevent the lipid oxidation during frozen storage.

## MATERIALS AND METHODS

**Sample:** Rosemary (*Rosmarinus officinalis*) sample was cultivated in Pato Branco, Paraná, Brazil. Was collected in usual flowering period, between January and May 2014. Rosemary leaves were dried in an oven with forced air circulation (Nova Ética 400/D, São Paulo, Brazil) at 40°C for 48 h and ground in an analytical mill (Quimis Q298A21, Diadema, Brazil) to a grain diameter of less than 0.5 mm.

**Chemicals:** Folin-Ciocalteu phenol reagents, 2,2-diphenyl-1-picryl-hydrazyl (DPPH),  $\beta$ -carotene, linoleic acid, Trolox, DPPH (2,2-Diphenyl-1-picryl-hydrazyl), (2,-20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) (ABTS), 2, 4, 6-Tris(2-pyridyl)-s-triazine (TPTZ), 2-thiobarbituric acid, acetone, ferulic acid, gallic acid, catechin, chlorogenic acid, caffeic acid, p-coumaric acid, trans-cinnamic acid, syringic acid, kaempferol, myricetin, rutin, quercetin and resveratrol were obtained from Sigma-Aldrich (Sternheim, Germany). All reagents used were of analytical grade.

**Preparation of Lyophilized Rosemary Extract (LRE) and Rosemary Essential Oil (REO):** A sample containing 10 g of rosemary leaves was subjected to the extraction process with 100 mL of ethanol solution (800 mL/L) in a shaker (SL 222, São Paulo, Brazil) at 40°C for 60 min at a 150 rpm stirring rate. After filtration of the extracts, the supernatants were evaporated in a rotary evaporator (Fisatom 802, São Paulo, Brazil) (vacuum pressure of 600 mm Hg and 40°C) until completely dry and lyophilized (Liotop L101, São Carlos, Brazil). The lyophilized rosemary extract was named LRE.

Essential oil was extracted by hydrodistillation, the rosemary leaves (100 g) were homogenized with 1000 mL of distilled water and placed inside a Clevenger-type apparatus. The heating temperature for the system was controlled to 110°C, until a boil began in the sample flasks began and extraction was carried out for 4 h. The Rosemary Essential Oil (REO) was recovered after decantation and used for analysis.

**Total Phenolic Compounds (TPC):** TPC were quantified by the Folin-Ciocalteu method described by Singleton *et al.* (1999), using gallic acid as a standard. 500  $\mu$ L of the samples was mixed with 2.5 mL of Folin-Ciocalteu and 2 mL of sodium carbonate 40 g/L (v/v). After two hours in darkness at room temperature, the absorbance of the extract was measured at 764 nm in spectrophotometer (UV-VIS Bel Photonics 2000, Piracicaba, Brazil). The results were expressed as mg GAE/g of sample (GAE: gallic acid equivalent,  $y = 0.021x - 0.015$ ,  $R^2 = 0.999$ ). All the assays were carried out in triplicate.

**DPPH (2, 2 Diphenyl-1-picryl-hydrazyl) Radical Scavenging Assay:** The measurement of DPPH free radical scavenging activity was measured as described by Brand-Williams *et al.* (1995). The reaction medium consisted of 0.5 mL of samples, 3.0 mL of ethanol and 0.3 mL of 0.5 mM DPPH• solution in ethanol. The mixture was incubated at room temperature in the dark for 45 min and the absorbance was read using a spectrophotometer (Bel Photonics 2000, Piracicaba, Brazil) at 517 nm. The  $EC_{50}$  (concentration required to obtain a 50% antioxidant effect) was measured and expressed in  $\mu$ g/mL and the mean percentage of antioxidant activity (%AA), according to the formula used by Carpes *et al.* (2013) (Eq. (1)). The assays were carried out in triplicate.

$$\%AA = \left( \frac{Abs_{sample} - Abs_{blank}}{Abs_{control}} \right) \times 100 \quad (1)$$

**Ferric Reducing Antioxidant Power (FRAP) Assay:** The FRAP assay was performed as described by Pulido *et al.* (2000). The absorbance was measured with a UV/VIS spectrophotometer (Bel Photonics 2000, Piracicaba, Brazil) at 593 nm. Aqueous solutions of ferrous sulfate were used for calibration ( $y = 32.345x - 0.0824$ ,  $R^2 = 0.998$ ) and the results were expressed as mmol of  $Fe^{+2}$ /g. All tests were carried out in triplicate.

**ABTS (2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid)) Assay:** The ABTS method was performed as described by Re *et al.* (1999). The stock solutions included 7.4 mM ABTS<sup>•+</sup> and 2.6 mM potassium persulfate. The solution was prepared by mixing 1 mL ABTS<sup>•+</sup> solution with 60 mL ethanol to obtain an absorbance of 0.70 at 734 nm using the spectrophotometer (UV-VIS Bel Photonics 2000 Piracicaba, Brazil). The results were expressed in  $\mu$ mol/g of TEAC (Trolox-equivalent antioxidant

capacity,  $y = 10,666x - 0,5757$ ,  $R^2 = 0,999$ ). The assays were carried out in triplicate.

**Coupled oxidation of  $\beta$ -carotene and linoleic acid assay:** The  $\beta$ -carotene bleaching assays were conducted as previously described by Ahn *et al.* (2004). For emulsion stock, 40 mg of linoleic acid and 400 mg of Tween 40 were diluted in 3 mL of  $\beta$ -carotene chloroform solution (0.1 mg/mL). Chloroform was removed with nitrogen gas and was then added to oxygenated distilled water (100 mL) to form an emulsion. Absorbance of the samples at 470 nm were measured in triplicate every 20 min until the color of  $\beta$ -carotene disappeared in the control reaction ( $t = 60$  min). The antioxidant activity was determined by the inhibited percent in comparison to the control sample.

**Preparation of the pork sausages:** The preparation of the sausages was conducted under formulation and procedures recommended by Brasil (2000). Fresh pork lean and fresh pork backfat were ground through a plate with 0.6 and 0.8 cm diameter holes, respectively and homogenized in a meat mixer with salt (1.8%), garlic, onion powder and dried parsley (all 0.1%). This basic formulation was divided into 4 lots. The first was designated as the control and no additional antioxidants were included. The second lot was designated as a positive control and was prepared by adding sodium erythorbate (0.2%), the third lot added LRE (0.2%) and the fourth lot added REO (0.2%). Separately, the meat lots were stuffed into natural pork casings, packed and stored at  $-15^{\circ}\text{C}$  for 49 days. The sausages were analyzed for TBARS content and sensory analysis.

**Oxidative Stability-Thiobarbituric Acid Reactive Substances (TBARS) Assay:** TBARS were measured by Raharjo *et al.* (1992). Measurements were made after processing and after 7, 14, 21, 28, 35, 42 and 49 days of frozen storage. Tetramethoxypropane (TMP) was used as a standard reference (Pateiro *et al.*, 2014; Sáyago-Ayerdi *et al.*, 2009). The results were expressed as mg of malonaldehyde MDA/Kg of the sample. The lipid oxidation was assessed in triplicate.

**Sensorial analysis:** The stored frozen sausages were evaluated for sensory characteristics after 49 days. The color, odor, flavor, texture and overall acceptability were analyzed using a hedonic scale of 9 points, ranging from 1 ("I disliked very much") to 9 ("I liked very much") (Dutcosky, 2007). The samples randomly coded with three-digit numbers, were served in a monadic form in individual booths. Unsalted crackers and room temperature water were provided between samples to clean the palate. The percentages of agreement were analyzed of the grades assigned during the sensory analysis by testers. 115 panelists, including untrained students and staff members (75 females and

40 males), of Federal Technological University of Paraná (UTFPR) in Brazil participated in the test for the sensory evaluation. The members ranged in age from 19 to 45 years old.

**Statistical analysis:** The profiles were presented as the mean value $\pm$ SD (standard deviation). The data was processed by one-way Analysis of Variance (ANOVA). The averages were compared by Tukey test and  $t$  test, considering the significance level of 95% ( $p < 0.05$ ), using the STATISTICA program 8.0 version (StatSoft, USA).

## RESULTS AND DISCUSSION

**Total Phenolic (TPC) Compounds:** The mean value of total phenolic compounds in LRE and REO was  $45.67 \pm 1.08$  mg GAE/g and  $0.21 \pm 0.08$  mg GAE/g, respectively. Afonso *et al.* (2013) reported  $16.67 \pm 0.40$  mg GAE/g aqueous rosemary extract and Erkan *et al.* (2008) found 162 mg GAE/g for the methanol rosemary extract, higher than what was found in aqueous and ethanol extract. This study demonstrates that; for the extraction of phenolic compounds present in rosemary, a less polar solvent, such as methanol, is the most efficient. However, other studies show that the mixture of methanol and water are highly efficient in extracting rosemary phenolic compounds (Lapornik *et al.*, 2005; Švarc-Gajić *et al.*, 2013). Olmedo *et al.* (2015) found 8.3 mg GAE/g for the essential oil of rosemary, much higher than found in this study ( $0.21 \pm 0.08$  mg GAE/g). This difference can be attributed to differences in the conditions of extraction and the season in which the harvest was taken.

In previous studies, gallic acid was found in rosemary extracts from Czech Republic (Dvorackova *et al.*, 2014), Murcia (Herrero *et al.*, 2010) and Turkey (Hossain *et al.*, 2011). Maldini *et al.* (2016) studied methanol rosemary extracts of different parts of Sarnidia and detected the presence of kaempferol, quercetin and rutin but the most abundant was the kaempferol ( $1.68 \pm 0.12$  mg/g). Vallverdú-Queralt *et al.* (2014) also found ferulic acid in hydroalcoholic extract of rosemary, although to a lesser extent ( $1.99 \pm 0.08$   $\mu\text{g/g}$ ) than the present study. Mulinacci *et al.* (2011) identified thirty phenolic compounds, including rosmarinic acid, caffeic acid, carnosol, carnosic acid and cirsimaritin and assigns these compounds antioxidant activity exhibited by the extract.

**Antioxidant capacities:** The antioxidant activity was determined by four distinct *in vitro* methods due to the complexity of constituents of LRE and REO. It was found that there is a significant difference ( $p < 0.05$ ) between the results for the extract and oil (Table 1).

The concentration of the extract required to obtain a 50% antioxidant effect against DPPH radical is

Table 1: Antioxidant activity by different methods in LRE, REO and commercial antioxidants

Samples	EC <sub>50</sub> (µg mL <sup>-1</sup> )	FRAP (µmol de Fe <sup>2+</sup> g <sup>-1</sup> )	ABTS (µmol Trolox g <sup>-1</sup> )	β-caroteno (%)
LRE	127.33±0.04 <sup>aB</sup>	112.14±2.80 <sup>dC</sup>	198.07±0.09 <sup>dA</sup>	92.31±0.17 <sup>aD</sup>
REO	79.63±0.03 <sup>cB</sup>	14.61±0.48 <sup>eC</sup>	6.42±0.00 <sup>dD</sup>	89.10±0.11 <sup>bA</sup>
BHT	114.66±0.07 <sup>bC</sup>	2310.11±70.59 <sup>bB</sup>	4277.33±33.53 <sup>cA</sup>	63.68±0.18 <sup>cD</sup>
BHA	72.25±0.07 <sup>dC</sup>	2717.79±71.46 <sup>aB</sup>	4743.04±51.37 <sup>bA</sup>	68.06±0.22 <sup>dD</sup>
Alfa-tocoferol	61.22±0.06 <sup>eD</sup>	1328.80±22.09 <sup>cB</sup>	5270.03±57.27 <sup>aA</sup>	70.29±0.17 <sup>cC</sup>

LRE: Lyophilized Rosemary Extract; REO: Rosemary Essential Oil; EC<sub>50</sub>: Concentration required obtaining a 50% antioxidant effect; Values are presented as mean±SD (n = 3); Different lower-case letter in the same column indicate significant difference (p<0.05) by the Tukey test; Different capital letters in the same row indicate significant difference (p<0.05) by the Tukey test; BHT: Butylatedhydroxytoluene; BHA: Butylated Hydroxyanisole

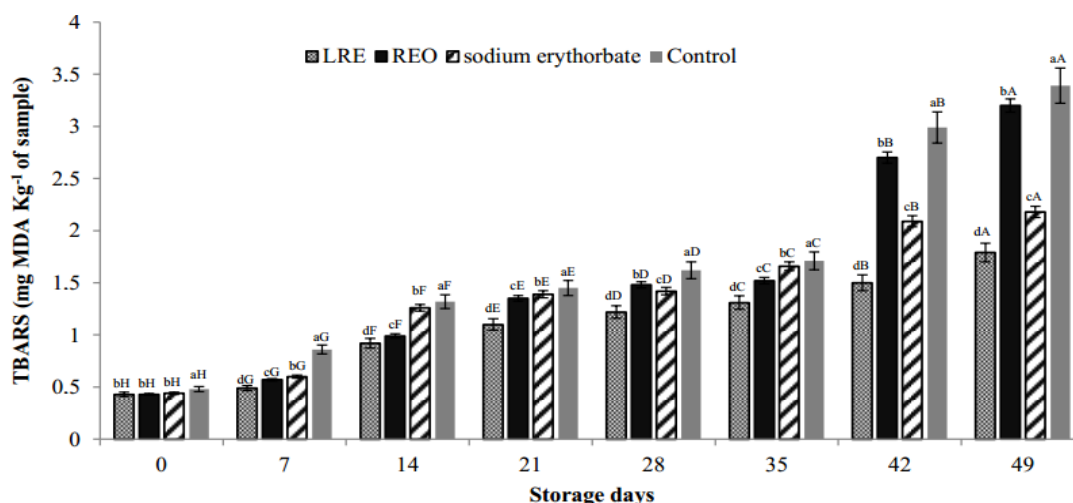


Fig. 1: Effect of LRE (Lyophilized Rosemary Extract), REO (Rosemary Essential Oil) and sodium erythorbate in oxidative stability (TBARS) of sausage during 49 days of frozen storage. Different lowercase letter indicate significant differences between treatments (p<0.05) by the Tukey test. Different capital letters indicate significant differences between the storage time (p<0.05) by the Tukey test

defined as EC<sub>50</sub>. It is important to note that high values of EC<sub>50</sub> are negatively related to the antioxidant activity, in other words, EC<sub>50</sub> value is inversely proportional to the antioxidant capacity of the compounds (Almeida *et al.*, 2017; Kontogianni *et al.*, 2013; Mishra *et al.*, 2012; Vicente *et al.*, 2013). Given all these considerations, the LRE had lower antioxidant activities by DPPH method EC<sub>50</sub> = 127.33 µg/mL than the REO by EC<sub>50</sub> = 79.63 µg/mL (Table 1). Similar to LRE, EC<sub>50</sub> value was found by Rocha *et al.* (2007) (125.0 µg/mL) for ethanolic extracts of rosemary and Maldini *et al.* (2016) (29.86 µg/mL) in methanolic extract. Zaouali *et al.* (2010) reported the antioxidant capacity of six essential oils of the species *Rosmarinus officinalis*, obtained DPPH values ranging from 6 to 28.5 µL/mL and Yosr *et al.* (2013) obtained values ranging from 7.73 to 12.8 µL/mL.

Different than what is observed in the EC<sub>50</sub> FRAP and ABTS the LRE and REO had lower antioxidant activity when compared to the commercial antioxidants by FRAP and ABTS methods (Table 1). The FRAP values found for the REO and LRE were 14.61 and 112.14 µmol Fe<sup>2+</sup>/g respectively. Yosr *et al.* (2013) evaluated the essential rosemary oil and obtained FRAP levels between 13.28 and 17.75 mM Fe<sup>2+</sup>/g, similar to present study results (Table 1). The antioxidant activity by ABTS' method for REO and LRE were 6.42 and

198.07 µmolTrolox/g of the sample, respectively (Table 1). These results are similar to Aouadi *et al.* (2014) who studied the effect of dietary supplementation in lambs containing essential oil of rosemary (*Rosmarinus officinalis*) and found ABTS value of 12.29 µmolTrolox/mL of the sample.

In the β-carotene/linoleic acid test, the free radicals attacked the highly unsaturated β-carotene molecules and as a result of this, the β-carotene was oxidized, smaller molecules were broken and the system lost its color. Therefore, the antioxidants inhibit the formation of peroxyradicals, thereby inhibiting the oxidation of β-carotene (Seneviratne *et al.*, 2016).

The antioxidant activity of LRE by different methods was higher than REO (Table 1).

These results showed that LRE and REO contain compounds which are responsible for capturing free radicals and therefore can be used to stabilize and slow down lipid oxidation. According to Pérez-Fons *et al.* (2010) and Rašković *et al.* (2014), the antioxidant activity of ethanolic rosemary extracts is mainly due to phenolic compounds (phenolic acids and flavonoids).

#### Thiobarbituric Acid-Reactive Substances (TBARS):

The results were obtained by TBARS to assess lipid oxidation in the pork sausages frozen for 7 weeks in storage (Fig. 1). During the storage the control

Table 2: Percent decrease in TBARS values in sausage treated with antioxidants (LRE and REO) during storage

		Percent decrease in TBARS compared to control (T1) <sup>a</sup>							
		Days of storage							
Conditions	Treatment	0	7	14	21	28	35	42	49
Frozen	T2	8.33	30.23	4.54	4.14	12.34	2.92	30.10	35.69
	T3	10.42	43.02	30.30	24.14	24.69	23.39	49.83	47.19
	T4	10.42	33.72	25.00	6.89	8.64	11.11	9.69	5.60

<sup>a</sup>Data not statistically analysed, but percentages were computed from the statistical data in Table 3; T1: Control -without antioxidant; T2: synthetic antioxidant-sodium erythorbate; T3: Lyophilized Rosemary Extract (LRE); T4: Rosemary Essential Oil (REO)

Table 3: Sensory evaluation of sausage in different treatments at 49 days frozen storage

Treatments	Color	Flavor	Texture	Taste	Global impression
T1	6.67±1.38 <sup>b</sup>	6.09±1.59 <sup>c</sup>	6.76±1.54 <sup>b</sup>	6.95±1.38 <sup>b</sup>	6.78±1.42 <sup>b</sup>
T2	7.03±1.14 <sup>a</sup>	6.58±1.50 <sup>b</sup>	7.21±1.20 <sup>a</sup>	7.38±1.26 <sup>a</sup>	7.29±1.21 <sup>a</sup>
T3	6.04±1.82 <sup>d</sup>	6.01±1.66 <sup>d</sup>	6.19±1.79 <sup>d</sup>	5.92±2.10 <sup>c</sup>	6.04±1.83 <sup>c</sup>
T4	6.59±1.54 <sup>c</sup>	6.65±1.81 <sup>a</sup>	6.68±1.69 <sup>c</sup>	5.27±2.61 <sup>d</sup>	5.68±2.26 <sup>d</sup>
Average	6.58±0.40	6.33±0.33	6.71±0.42	6.38±0.96	6.45±0.42

T1: Control -without antioxidant; T2: commercial antioxidant-sodium erythorbate; T3: Lyophilized rosemary extract; T4: Rosemary essential oil; Values are presented as mean±SD (n = 3); Different lower-case letter in the same column indicate significant difference (p<0.05) by the Tukey test

treatment had significantly higher TBARS values (p<0.05) and there wasn't a significant difference in the malonaldehyde values when compared to other treatments with antioxidants (T2, T3 and T4) in processing day (Fig. 1).

There was significant difference (p<0.05) between treatments with synthetic antioxidant (SE) and treatments with natural antioxidant (LRE and REO) throughout the storage period (Fig. 1). After 7 storage days there was an increase in TBARS values on all treatment. In this point the samples treated with LRE (T4) had significantly (p<0.05) lower MDA values than the control (T1), sodium erythorbate (T2) and REO (T3) (Fig. 1).

In general, the TBARS values increased over time with an average of 0.45 mg MDA/kg at the beginning of the experiment and an average of 2.89 mg MDA/kg at the end of the experiment (49 days) (Fig. 1).

After 14 days the TBARS level was significantly lower (p<0.05) to the LRE treatment (T4) when compared to the control treatment (Fig. 1). On the same date, the sausage containing lyophilized extract and essential rosemary oil reached 30.30% and 25.00%, respectively, of the inhibition of malonaldehyde formed in the control sample (Table 2).

After 28 days of storage the control treatment suffered more intense oxidation than those with sodium erythorbate (T2) and natural antioxidants (T3 and T4) (Table 2). Haminiuk *et al.* (2011) proved that storage time and fat content were important factors that affected the degree of oxidation in the sausage in a study showing the effects of fat replacement of fermented sausages during 4 weeks of storage.

The lipid peroxidation of sausage was significantly inhibited (p<0.05) at 49 days of frozen storage in the presence of LRE (1.79mg MDA/kg) when compared to the control (3.39 mg MDA/kg) (Fig. 1). This could be proven by the decrease of malonaldehyde production (47.28%), indicating that LRE had antioxidant activity (Table 2) and is able to increase the oxidative stability of the sausages.

Some authors have reported that the addition of REO and LRE are efficient in lipid oxidation in meat (Jongberg *et al.*, 2013; Sebranek *et al.*, 2005; Han and Rhee, 2005). Naveena *et al.* (2013) evaluated the effect of carnosic acid dried rosemary added to chicken patties during refrigerated storage and observed reduction of TBARS (37% to 87%) in comparison with the control sample. Teruel *et al.* (2015) showed the effectiveness of the rosemary extract as antioxidants, after 9 months of storage of chicken nuggets. Sebranek *et al.* (2005), showed the rosemary extract was more effective than BHA/BHT for preventing increased TBARS values in precooked-frozen sausage and showed similar results as that of BHA/BHT in refrigerated sausage.

**Sensory evaluation:** Mean sensory scores for odor, taste, flavor, textural attributes and global impression for the control sausage (T1) and sodium erythorbate (T2) and natural antioxidants (T3 and T4) are shown in Table 3.

Among the sausage containing natural antioxidant, the most liked sausage was that containing the LRE (75.67%) and the least acceptable was the sausage containing REO (56.41%). T3 and T4 differ from other treatments in all attributes. The sample T2 obtained higher average values close to 7.0 ("like regular") for attributes such as color, texture, taste and global impression (Table 3).

According to the panelists, the treatment containing the lyophilized extract showed green color change and a bitter taste. Similar results were found by Savadkoochi *et al.* (2014) in his study with beef sausage containing tomato pomace. Color intensity scales for beef sausages containing tomato residue were lower than control, resulting in an increased shade of orange in the sample.

According to the grades obtained, it was observed that the two samples of sausage containing natural antioxidant (REO and LRE) were accepted. However, the averages of sausages with synthetic antioxidants and control had higher values. Therefore, the panelists indicated that the pork sausage containing the synthetic

antioxidants and control got better sensory acceptance than the sausages containing REO and LRE.

In study by Venturini *et al.* (2011) consumer acceptability for meat products is related to their color, odor, appearance, flavor and juiciness. Regarding the acceptability rate of the product, it was observed that treatment 3, with an addition of 0.2% rosemary essential oil, showed the lowest acceptance rate in the global impression of the product.

### CONCLUSION

The LRE was more effective in the lipid oxidation inhibition in pork sausage than REO. However, this study confirms that the use of LRE and REO as a natural antioxidant, combined with storage under low temperatures, can be considered an effective method to retard lipid oxidation in pork sausage. This protection may be due to high antioxidant activity and the presence of phenolic compounds in LRE and REO. The sausage containing LRE obtained greater acceptance (75.67%) among the tasters than the sausage containing REO (56.41%). Considering the interest in finding natural antioxidants that have similar performance to synthetic antioxidants in meat products, rosemary showed promising results. The sausage with rosemary extract can be an alternative substitution for synthetic antioxidants, showing the possibility of using in meat products resulting with satisfactory acceptance.

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