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FOOD TECHNOLOGY

# Pomegranate (*Punica granatum* L.) peel lyophilized extract delays lipid oxidation in tuscan sausages

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**ABSTRACT**: *Pomegranate (Punica granatum L.) contains a high concentration of antioxidant and phenolic compounds. Pomegranate peel extracts at different concentrations were used as natural antioxidant to increased the lipid stability of fresh Tuscan sausages, comparing with sodium erythorbate (SE). Peels were subjected to ultrasound-assisted aqueous extraction and lyophilization. The extract was previously characterized throught phenol and flavonoids determination. The Tuscan Sausages were prepared, and color, pH, peroxides, and thiobarbituric acid reactive substances were assessed at 1, 15, and 30 days of storage, at a mean temperature of 5 ± 1 ºC and under a 12-hour light cycle/day. Sausages containing 0.05 and 0.1% of peel extract showed results similar to sodium erythorbate in lipid peroxidation. Sausages treated with pomegranate peel extracts showed an adequate global acceptability level in the sensory analysis. Pomegranate peel extracts are; therefore, a promising natural alternative for maintaining the lipid stability of Tuscan sausages, promoting the protection of the meat and in addition, being able to bring beneficial of the pomegranate to the human health.*
**Key words**: *meat product, lipid, oxidation, storage, sensory analysis.*

## Estabilidade oxidativa de linguiça tipo Toscana com extrato liofilizado da casca de romã (*Punica granatum* L.)

**RESUMO**: *A romã (Punica granatum L.) contém uma alta concentração de compostos antioxidantes e fenólicos. Extratos de casca de romã em diferentes concentrações foram utilizados como antioxidante natural para aumentar a estabilidade lipídica de linguiça tipo Toscana, comparados ao eritorbato de sódio. As cascas foram submetidas à extração e liofilização aquosas por ultrassom. O extrato foi caracterizado por determinação de fenol e flavonóides. Os parâmetros cor, pH, peróxidos e ácido tiobarbitúrico foram avaliadas nos dias 1, 15 e 30 pós armazenamento, a temperatura de 5 ± 1 ºC e ciclo de luz de 12 horas/dia. As linguiças contendo 0,05 e 0,1% de extrato de casca apresentaram resultados semelhantes ao eritorbato de sódio quanto a peroxidação lipídica. Também apresentaram aceitabilidade global adequada na análise sensorial. Os extratos de casca de romã são, portanto, uma alternativa natural promissora para manter a estabilidade lipídica das linguiças toscanas, promovendo a proteção e podendo trazer benefícios da romã à saúde humana.*
**Palavras-chave**: *produtos cárneos, lipídios, oxidação, armazenamento, análise sensorial.*

## INTRODUCTION

The use of synthetic antioxidants in foods is associated with the increase in the incidence of chronic and degenerative diseases. Therefore, researchers and the meat industry have sought alternatives to synthetic antioxidants, using natural compounds that can be produced on an industrial scale (SHIMOKOMAKI, 2006).

Fresh sausages, such as Tuscan sausages, are widely accepted by consumers due to ease to prepare and consume. Lipids present in these sausages are accountable for desirable characteristics such as flavor, juiciness, nutritional value, and aroma. However, lipid oxidation can affect the quality of these products, leading to rancidification and reducing their shelf life and sensory acceptability. Oxidation can be delayed by the use of antioxidants. Quite often,

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synthetic antioxidants such as sodium erythorbate (SE) are used at concentrations until the desirable effect is attained (*quantum satis*). Nevertheless, natural antioxidants are a feasible and interesting option for increasing the shelf life of meats and their derivatives, since they promote health (JIANG & XIONG, 2016).

Pomegranate (*Punica granatum* L.) has high levels of phenolic and antioxidant compounds, and in countries whith extensive production, it is processed into juices. Nonetheless, the peels are thrown away for being regarded as industrial waste. The antioxidant and antimicrobial potential of pomegranate is distributed all over the plant (FISCHER et al., 2011; GONZÁLEZ-MOLINA et al., 2009; LANSKY & NEWMAN, 2007; TEHRANIFAR et al., 2011), but the peel contains higher amounts of nutritional and bioactive compounds when compared to edible parts and to other fruits (ORGIL et al., 2014; SESTILI et al., 2007), indicating the potential use of this raw material in the pharmaceutical, cosmetic, and food industries (AMYRGIALAKI et al., 2014).

This research aimed to produce Tuscan sausages using pomegranate peel lyophilized extract (PPLE) and to assess the potential of this natural antioxidant for delaying lipid oxidation reactions and providing (or not) good sensory properties during storage.

## MATERIALS AND METHODS

*Preparation of pomegranate peel extracts*

Pomegranates (*Punica granatum* L) were purchased in the local market in Getúlio Vargas, Rio Grande do Sul State, Brazil, in July 2017. The fruits were washed in drinkable water and immersed in sodium hypochlorite (2.5%) for 15 minutes, weighed whole, and sorted into peel, pulp, and seeds for yield estimation. The peels were cut into 3-cm slices and dried at 40±5 ºC for 72 hours in an air circulating oven (Splabor, M- SPL 102). Peels were weighed again and ground in a cyclone mill (Marconi, MA-020) with a 20-mesh sieve, and the resultant powder was stored in a hermetically sealed container at room temperature until analysis.

The phenolic compounds, were extracted by an ultrasonic-assisted method (NIN - 001-13, SB5200 DTDN - UltrasonicCleaner) at 40 KHz and at a maximum temperature of 40 ºC for 2 hours obtaining the pomegranate peel aqueous extract (PPAE). Thereafter, the PPAE was frozen (-70 ºC ± 10 ºC) for 24 hours in an ultra freezer (ColdLab, CL 600-80) for lyophilization (Terroni, model LS3000) for 26 hours, and the resultant product was the pomegranate peel lyophilized extracts (PPLE). PPAE and PPLE were stored at -20 °C in a hermetically sealed container protected from light.

*Characterization of the extracts*

Total phenols of PPAE and PPLE were quantified by the Folin-Ciocalteau's method (ROESLER et al., 2007), expressed as mg of gallic acid equivalent (GAE)/g of extract. Absorbance was measured by a spectrophotometer (Shimadzu, model UV-1800) at 760 nm. Total flavonoids were quantified by the $AlCl_3$- complexation method (ZHISHEN et al., 1999). Results were expressed as catechin equivalents (mg of CATE/g) and absorbance was read at 510 nm. Antioxidant activity was assessed by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical sequestering ability (ROESLER et al., 2007) and results were expressed as µmol of Trolox equivalent/ mg of sample. The samples were read at 510 nm.

*Manufacturing process of Tuscan sausages containing pomegranate peel extracts* (Table 1)

The manufacture of Tuscan sausages followed the Technical Regulation on Identity and Quality established by Normative Rule n°4 (BRASIL, 2000). Raw materials were obtained from pigs slaughtered at a locally inspected abattoir, in compliance with hygiene and sanitation standards and with the recommended slaughtering technology. After chilling at 7 ºC (±1 ºC) for 12 hours, the carcasses were deboned and the pork shoulder, leg, and belly were removed for the production of meat mixes. Three PPLE concentrations (0.025, 0.05, and 0.1%) and SE (0.025%) were used as antioxidant for the treatments. The sausages were vacuum packed into portions (± 120 grams) and stored at 5±1 ºC under an artificial white light source with a luminous flux of 415 lumens, controlled by a programmable analog timer, for 12 hours/day, simulating a gondola shelf.

*Physicochemical analysis*

The centesimal composition of elaborated sausages was evaluated according to Lutz (2008) and the formulated products were in accordance with the Normative Instruction (BRASIL, 2000). Samples were assessed at 1, 15, and 30 days, in triplicate, as to pH, color, peroxide levels, and thiobarbituric acid reactive substances (TBARS). The pH was measured by drilling in different points of the sausage, performed in triplicate and with the aid of calibrated pHmetro (Metrohm - 826 mobile). Color was evaluated by reflectance using a colorimeter (Minolta, model CR-400) and luminosity $L^*$, $a^*$ (red-green), and $b^*$ (yellow-blue). Peroxide levels were determined as proposed by

Table 1 - Tuscan sausage formulations at 1000 g yield, containing three freeze-dried pomegranate peel extracts (PPLE) at different concentrations (PPLE1 - 0.025%; PPLE2 - 0.05%; PPLE3 - 0.1%), compared to control formulation that has only sodium erythorbate (SE).

| Ingredients (g) | ----------Treatments---------- | | | |
|---|---|---|---|---|
| | PPLE1 (0.025%) | PPLE2 (0.05%) | PPLE3 (0.1%) | SE (0.025%) |
| Pork (g) | 850 | 850 | 850 | 850 |
| Ham (g) | 150 | 150 | 150 | 150 |
| NaCl (g) | 25 | 25 | 25 | 25 |
| Ice-cold water (mL) | 30 | 30 | 30 | 30 |
| Sugar (g) | 5 | 5 | 5 | 5 |
| Black pepper (g) | 1 | 1 | 1 | 1 |
| PPLE (g) | 0.25 | 0.5 | 1 | - |
| SE (g) | - | - | - | 0.25 |
| Total | 1000 | 1000 | 1000 | 1000 |

INSTITUTO ADOLFO LUTZ (2008) and the results were expressed as mEq/kg of sample. The TBARS was estimated by the spectrophotometric method described by VYNCKE (1970) and adapted by MARANGONI & MOURA (2011), and the results were expressed as mg of malonaldehyde/kg of sample.

*Consumer testing*

The PPLE3 (0.1%) and sodium erythorbate (SE 0.025%) treatments were subjected to sensory analysis at 28 days after sausage manufacture using the triangle and affective sensory tests applied to 53 panelists (taste testers). The panelists were previously given instructions on how to conduct the analyses and signed a free informed consent form, meeting the principles of the Research Ethics Committee of Universidade de Passo Fundo (UPF), process no. 63579517.6.0000.5342.

*Statistical analysis*

Results of the physicochemical analysis of sausages were analyzed using the mean and standard deviation of triplicates and statistically treated by analysis of variance ANOVA (with normality test) and Tukey test at 5% significance (P>0, 05) using the SPSS Statistics software (version 23). For the statistical representation of the results of the sensory analysis the nonparametric Mann-Whitney test was used.

## RESULTS AND DISCUSSION

*Yield and characterization of extracts*

PPLE had a yield of 0.78% compared to the initial fruit mass and of 18.96% compared to the crude peel powder. PPAE contained 66.14 mg of GAE/g of extract of total polyphenols and 6.84 mg of CATE/g of extract of total flavonoids. The rate of inhibition of the DPPH radical was 91.70% for the extract at the concentration of 100 mg/mL, equivalent to 3.39 µmol of Trolox equivalent/mg of sample. PPLE contained 89.52 mg of GAE/g and 12.05 mg of CATE/g of extract of polyphenols and flavonoids; respectively, and the rate of inhibition in the DPPH assay was 90.21% for the extract at the concentration of 100 mg/mL, equivalent to 3.34 µmol of Trolox equivalent/mg of sample.

No difference was observed in antioxidant potential between PPAE and PPLE. However, PPLE showed higher total polyphenol (35.35%) and total flavonoid (76.16%) levels than PPAE, indicating that this processing technique may increase the concentrations of active compounds, also showing the potential use of pomegranate peel extract as a source of bioactive compounds and natural antioxidants. Other authors have reported values of total polyphenol content which ranged between 42.16 and 320.26 mg GAE/g in pomegranate peel (DIAMANTI et al. , 2017; ELFALLEH et al., 2009; GALAZ et al., 2017; HASNAOUI et al., 2014; KAZEMI et al., 2016; LI et al., 2006; MORSY et al., 2018). In processed goat meat products, the antioxidant effect of pomegranate peel extract was higher than those reported in citric fruit peel extracts and of seed powder described by DEVATKAL et al. (2010).

The antioxidant capacity of pomegranate peel was described by FISCHER et al. (2011), who mention that variations in such capacity could be related to extraction methods, solvents used,

and time and temperature of protocols. Water, as extracting solvent, precludes the need for later purifications and possible risks associated with the use of the extracts in foods. Factors such as fruit cultivar, fertilization, soil, climate, sun exposure, maturation stage, among others, can also be held accountable for the differences in results.

*Characterization of Tuscan sausages containing PPLE and SE*

The influence of PPLE on pH of Tuscan sausages during refrigerated storage is shown in table 2. The results obtained depended on the storage time, mean pH values ranged from 5.66 to 6.16". Treatments were similar in terms of pH (P>0.05). However, when compared on the 1st day of storage, statistical significance of PPLE3 was higher (P<0.05) than those reported in the control (SE), which occurred again on the 15th day. The pH in the 6.2-5.7 range were observed by SALEH et al. (2017) when used pomegranate peel powder in their meat sausages.

The gradual decrease in pH can be attributed to storage temperature (5 ºC ± 1) and reduction of oxygen content, brought about by vacuum packing, leading to slower glycogen degradation and lactic acid formation, as well as to homogenization of meat mixes and standardization, which renders sausages less susceptible to pH changes. The pH changes are more commonly observed in cured and aged emulsified meat products, affecting their quality and shelf-life.

In emulsified products with addition of pomegranate peel powder, pH ranged from 6.27 and 5.73 (SALEH et al., 2017), with no significant differences in pH (P>0.05) during storage (EL-NASHI et al., 2015). QIN et al. (2013) observed a decrease in pH from 5.88 to 5.61 after addition of pomegranate peel powder in pork, but no significant difference in pH in breaded chicken cutlets with addition of pomegranate juice extract (NAVEENA et al., 2008).

In color analysis, PPLE treatments showed difference (P<0.05) in luminosity $L^*$ when compared with each other and with the control at the beginning of the storage period (Figure 1). PPLE2 and PPLE3 treatments did not reveal any differences (P>0.05) at 15 and 30 days, whereas PPLE1 was similar to the control at 30 days. ESTÉVEZ & CAVA (2004) reported that the $L^*$ value increased in sausages stored for 60 days without addition of rosemary extracts. ESTÉVEZ et al., 2005) used rosemary essential oil to verify the protein oxidation of meat products, proving that the addition of antioxidants (300 and 600 ppm) in the product reduces color changes when compared to the control product.

The stability exhibited by PPLE2 and PPLE3 for $a^*$ were the same at 30 days, without however maintaining the levels of the SE treatment for the red color of sausages. Color of emulsified meat products is maintained, in many cases, by the use of dyes. No dyes were used in the present study, since the aim was to assess nothing but the effects of SE and PPLE on color attributes. In color parameter $b^*$, treatments had different behaviors during shelf life, with no differences (P>0.05) between PPLE2 and PPLE3 at 15 and 30 days.

DEVATKAL et al. (2010) evaluated the use of pomegranate and bergamot antioxidants in goat meat hamburgers during storage (4 ± 1 °C) in different packaging methods. The vacuum and 1% concentration of pomegranate peel extract resulted in greater color stability when compared to treatments such as modified atmosphere.

DEVATKAL & NAVEENA, (2010), conducted a five-treatment study evaluating the color and oxidative stability of raw ground goat meat stored at 4 ± 1 °C. The comparison between:

Table 2 - pH of Tuscan sausages according to storage time and treatments.

| Treatment | ----------pH*---------- | | |
|---|---|---|---|
| | 1st day | 15th day | 30th day |
| PPLE 0.025%, | 6.03±0.01 $^{abA}$ | 5.56±0.01 $^{abB}$ | 5.21±0.02 $^{Ac}$ |
| PPLE 0.05%, | 6.03±0.05 $^{abcA}$ | 5.59±0.01 $^{acB}$ | 5.21±0.00 $^{Ac}$ |
| PPLE3 0.1%, | 6.05±0.05 $^{bA}$ | 5.60±0.01 $^{cB}$ | 5.20±0.01 $^{Ac}$ |
| SE 0.025%. | 6.03±0.05 $^{aA}$ | 5.57±0.05 $^{aB}$ | 5.21±0.01 $^{Ac}$ |

*Means ± (standard deviation) followed by the same lower-case letters in the rows and the same upper-case letters on the lines are not statistically different, according to Tukey's test (p>0.05). PPLE: Pomegranate peel lyophilized extracts; SE: sodium erythorbate.

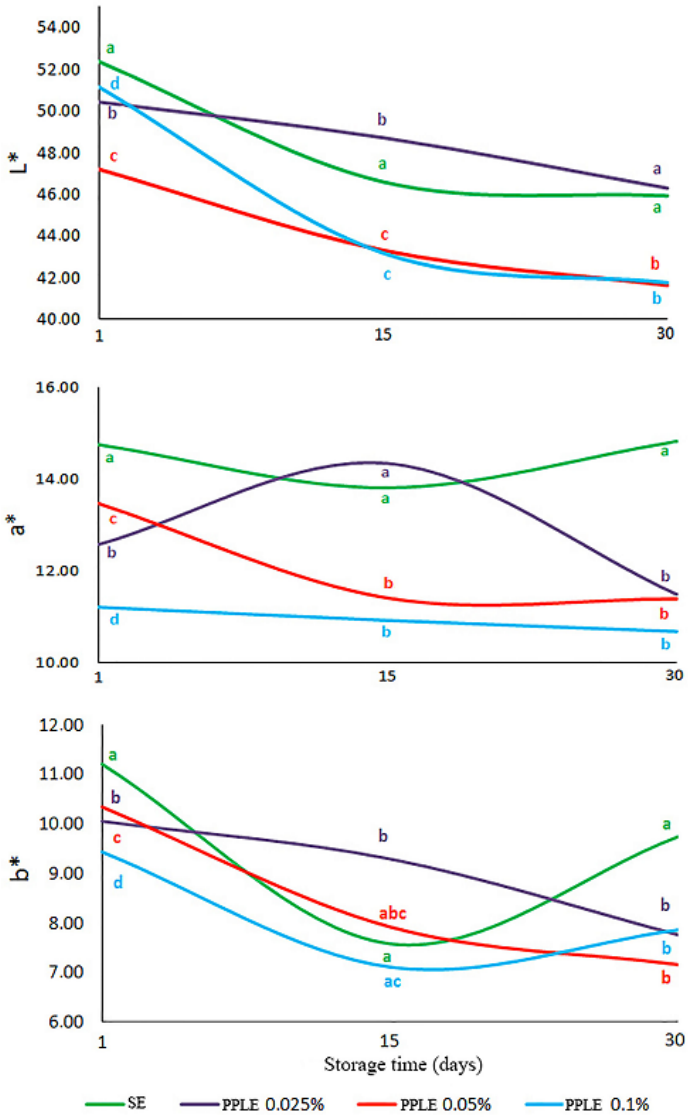


Figure 1 - Behavior of color parameters during the storage of Tuscan sausages.

PPLE: Pomegranate peel lyophilized extracts; SE: sodium erythorbate.
*Mean ± (standard deviation) was performed in the formulations with respect to storage time. Each line has three times, when the letters of the same line are different, there was significance.
*Tukey test (P>0.05) was performed between formulations (each line color represents one formulation). When the letter is different between the formulations, there was significance.

control sample (meat only), salted meat, salted meat and pomegranate peel powder, salted meat and pomegranate peel, salted meat and pomegranate seeds, with pomegranate added samples, those with the highest antioxidant effect and the best color.

The discoloration in meat products increased when the products are displayed in illuminated counters at the point of sale, where this study was simulated, this is due to the dissociation of heme nitric oxide.

There was no significant difference (P>0.05) in peroxide levels between treatments on the 1st day (Figure 2). However, there was some difference at 15 days in PPLE3 (P<0.05) when

compared to the other treatments, with the lowest peroxide levels (4.91±0.45), whereas PPLE1 had the highest levels (10.16±0.21) at 30 days. At the end of the shelf life, PPLE2 and PPLE3 showed no differences (P>0.05) when compared to the control (Figure 2). There was a significant increase (P<0.05) in all treatments at 15 days, with the maximum of 10.16 mEq/kg in the PPLE1 at 30 days. This increase is believed to be more associated with the quick formation of peroxides during storage than with the degradation of peroxides into secondary oxidation products (BAZARGANI-GILANI et al., 2015).

The TBARS is also indicated to assess lipid oxidation, as this analysis quantifies the levels of malonaldehyde (MDA), one of the main by-products of the degradation of poly-unsaturated fatty acid hydroperoxides, formed during oxidation. No significant difference (P>0.05) was observed in TBARS levels in PPLE2 and PPLE3 on the first day of storage (Figure 2). The increase in PPLE concentrations (P<0.05) might have improved the capacity to eliminate radicals involved in lipid peroxidation, when compared to SE, as suggested by authors like NAVEENA et al. (2008) and NEGI et al. (2003). On the first day, TBARS levels were equal or close to 0.1 mg of MDA/kg in all treatments, which was observed by ALMEIDA et al. (2015) and KIM et al. (2014) in meat products. These levels often go up when the storage of meat products is extended, reaching a maximum value and decreasing thereafter due to additional reactions of MDA with amine groups (CHANNON & TROUT, 2002).

Inhibitory effects of PPLE are attributed to its phenolic compounds, which participate in the

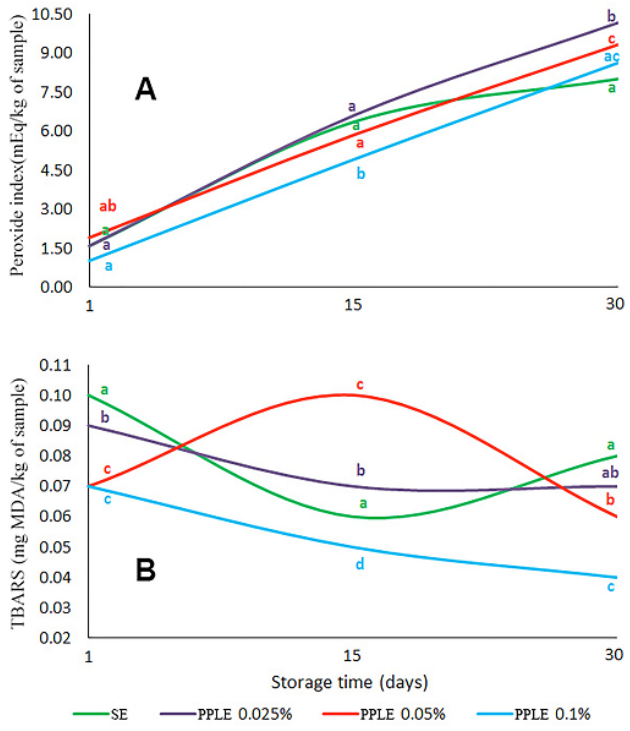


Figure 2 - Peroxide (A) and T BARS (B) levels during the storage of Tuscan sausages. PPLE: Pomegranate peel lyophilized extracts; SE: sodium erythorbate.

*Mean ± (standard deviation) was performed in the formulations with respect to storage time. Each line has three times, when the letters of the same line are different, there was significance.
*Tukey test (p>0.05) was performed between formulations (each line color represents one formulation). When the letter is different between the formulations, there was significance.

oxidation process by blocking the free radical chain reactions (NEGI et al., 2003). There was a decrease in TBARS probably because the action of antioxidants lasts more than 30 days of shelf life in fresh sausages manufactured and stored under the conditions used in this study. The level of perception of rancid odors by trained and untrained panelists ranges from 0.5 to 2.0 mg of MDA/kg of sample (ALMEIDA et al., 2015; GATELLIER et al., 2017; TRINDADE et al., 2009). The values reported for Tuscan sausages with PPLE and SE were lower than 0.5 mg of MDA/kg and; therefore, imperceptible when stored under refrigeration (5±1 ºC) for 30 days. QIN et al. (2013) described TBARS levels between 0.41 and 1.07 mg of MDA/kg up to 12 days of storage using pomegranate juice and peel powder in ground pork. NAVEENA et al. (2008) considered pomegranate peel extract to be an efficient antioxidant for chicken meat, delaying oxidative rancidity.

In some cases, in the absence of TBA, acid extracts can have peak absorbance at 532 nm, leading to overestimation of lipid extension by TBARS (GANHÃO et al., 2011). This was evident with the value obtained for PPLE2 at 15 days. These results should be viewed with caution, since the reaction to TBARS is not specific to MDA, given that other compounds can also react with the acid (FIGUEIRÊDO et al., 2014).

*Sensory analysis*

In the triangle sensory analysis, 56.6% of panelists did not detect differences (P>0.05) between the sausages containing control (SE) or PPLE3. The levels of acceptance did not differ (P>0.05) according to the Mann-Whitney test for the assessed parameters. It was not possible to detect unpleasant flavor, signs of rancidity, or astringent taste that could lead panelists to reject the samples. These values are represented on an interval between 7 (like moderately) and 8 (like very much), as shown in figure 3.

Sausages treated with PPLE3 and SE were equally accepted, in line with ALMEIDA et al. (2015) and DEVATKAL et al. (2010). Acceptability was satisfactory in terms of color, with mean scores of 7.56 and 7.26 for PPLE3 and SE, respectively. Regarding global acceptability, the highest mean was 7.56 for PPLE3 and 7.47 for ES, whose evaluation on the hedonic scale was between “like moderately” and “like very much” indicating that the addition of PPLE to Tuscan sausages presente good sensory parameters, when compared to the control, supporting its use as natural antioxidant in this meat product.

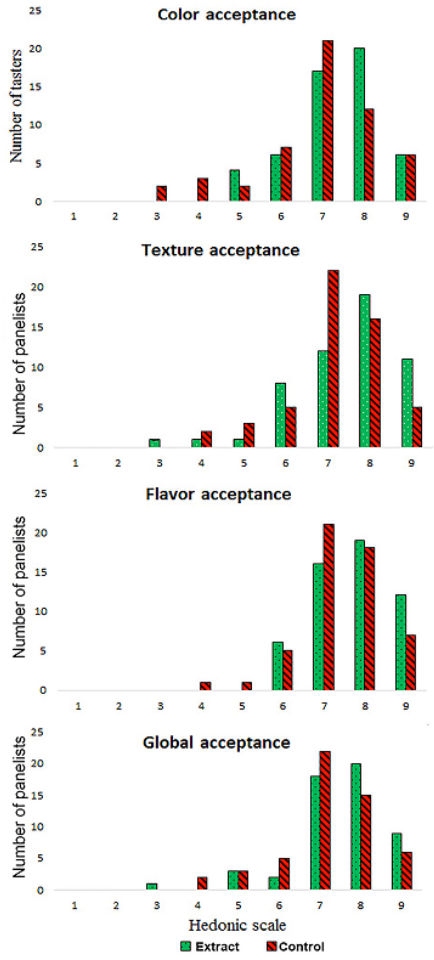


Figure 3 - Hedonic scale showing the acceptability test for fresh sausage containing PPLE and SE.

PPLE: Pomegranate peel lyophilized extracts; SE: sodium erythorbate.
The attributes color, texture, flavor and overall acceptability of the control formulation and the extract formulation were evaluated by sensory analysis, acceptability and classified by hedonic scale: 1-Dislike extremely; 2-Dislike very much; 3-Dislike moderately; 4-Dislike lightly; 5-Neither like or dislike; 6-Like slightly; 7- Like moderately; 8-Like very much; 9-Like extremely.
The figure shows the amount of test that obtained the hedonic scale grade in relation to the formulations.

## CONCLUSION

PPLE delayed lipid oxidation and proved to be better than or similar to SE. Lipid changes were not significant at 30 days, demonstrating stability of the manufactured Tuscan sausages. The extract was

able to decrease lipid oxidation, confirming global sustainability because of the use of an agroindustrial residue (pomegranate peel). Sausages prepared with the peel extract showed good acceptability.

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## DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTIONS

GRZ, ELT and LRS conceived and designed experiments. GRZ, DB and CDB performed the experiments, GB, DB and CPF carried out the lab analyses. GRZ, CDB and LRS performed statistical analyses of experimental data. GRZ, FMG and CPF prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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