



Effect of the fruit aqueous extract of balloon pepper (*Capsicum baccatum* var. *Pendulum*) on lipid oxidation, microbiological quality and consumer acceptance of fresh pork sausage and smoked

Natália de Oliveira CABRAL¹, Rodrigo Fortunato de OLIVEIRA^{1*} , Fabio da Costa HENRY¹, Daniela Barros de OLIVEIRA¹, Alexandre Cristiano do SANTOS JUNIOR², Jonhny de Azevedo MAIA JUNIOR¹, Meire Lelis Leal MARTINS¹

Abstract

This study assessed the effect of different concentrations of (*Capsicum baccatum* var. *pendulum*) on lipid oxidation, microbiological and sensorial characteristics of four different formulations of fresh pork sausage and smoked (control, F1, F2, F3), with the substitution of industrial antioxidant for the natural one. Lipid oxidation values (TBARS) did not display significant differences ($P>0.05$) among the treatments, and at different times of analysis (storage). The only significant differences were between the different types of sausages (fresh and smoked). The enumeration of all the microorganisms searched was below the standard required by Brazilian laws. F2 formula received the highest scores for acceptance but did not display significant difference ($P>0.05$) as it refers to taste, with the remaining formulations. The substitution of the synthetic antioxidant by the natural one displayed similar results, demonstrating that the natural extract from the balloon pepper does not affect the technological and sensorial properties of the sausages, which proves the viability of the product.

Keywords: food safety; frozen storage; meat products; peroxides; rancification.

Practical Application: The data represent alternatives of inclusion *Capsicum baccatum* var. *pendulum* without any prejudice to the quality of the fresh pork sausage.

1 Introduction

The reduction of the quality of foods usually associates with the deterioration of their compounds. Lipid oxidation is the main reason for this deterioration (Georgantelis et al., 2007). The phospholipids, polyunsaturated fatty acid together with the oxygen, heme pigments, and some metallic ions influence the deterioration process of processed foods either directly, or indirectly (Devatkal & Naveena, 2010).

Oxidation processes affect the nutritional, sensorial values of the products, and the level of thiobarbituric acid reacting substances (TBARS), which is the main parameter to assess the lipid oxidation (Almeida et al., 2015). Lipid peroxidation or lipoperoxidation (LPO) is the leading cause of chemical deterioration of food, a process that generates characteristic flavors and odors known as rancid (Campbell, 2015). The reaction mechanism involves the generation of free radicals (FR) that are highly reactive to atmospheric oxygen and form peroxy radicals (OOR), activating a chain reaction that culminates in the formation of compounds called reactive oxygen species (ROS). ROS are highly unstable oxidizing substances, as they have one or more unpaired electrons (Campbell, 2015). ROS are the bases for the production of end products of lipid oxidation, which comprise the derivatives of the decomposition of hydroperoxides, such as alcohols, aldehydes, ketones, esters, and other hydrocarbons. High temperatures and

small concentrations of pro-oxidant substances, such as metal ions, accelerate the LPO process (Campbell, 2015).

Brazilian legislation provides limits to the use of synthetic antioxidants, and, even the efficiency in food preservation, several studies demonstrate their risks for human health (Lima et al., 2010; Silva et al., 2010).

The carcinogenic potential associated with the use of synthetic antioxidants and other potential damages to health increased the interest in the use of natural antioxidants (Fратиanni et al., 2010; Franco et al., 2012; Radha Krishnan et al., 2014) significantly. The literature also suggests the reduction of the use of natural antioxidants to preserve the sensorial features of foods (Rosa et al., 2013).

Meat products are particularly complex food systems, due to their composition, displaying high levels of protein, lipids, water, in addition to the presence of minerals and other constituents (Weiss et al., 2010). The complexity of this food matrix and the variability among the different meat products justifies the need for studies on its extract. Studies involving the effect of time and storage conditions of meat products are essential factors in meat processing since problems related to storage stability are common (Kozáčins et al., 2012).

Received 18 Feb, 2021

Accepted 04 Mar, 2021

¹ Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF, Campos dos Goytacazes, RJ, Brasil

² Instituto Federal do Espírito Santo – IFES, Alegre, ES, Brasil

*Corresponding author: fortunatorodrigo@gmail.com

The present study evaluated the shelf life of frozen fresh pork sausage and smoked prepared with aqueous extract of balloon pepper fruits (*Capsicum baccatum* var. *pendulum*) as a natural ingredient with antioxidant properties in foods.

2 Materials and methods

Food processing experiments were performed in the Laboratory of Meat Processing at the Federal Institute of the state of Espírito Santo (IFES - Campus Alegre) in the municipality of Alegre, state of Espírito Santo, Brazil.

The male pigs were castrated surgically at seven days of age; all pigs started the experiment with 69 days of age and an average weight of 26.24 ± 5.44 kg.

Pork meat derived from hybrid (crossbred Landrace \times Large White \times Pietran) breeds of swine males, bred in confinement (feedlots) in the swine-breeding sector of the IFES- Campus Alegre in the municipality of Alegre, Espírito Santo, Brazil. The animals were slaughtered at 145 days of age and an average weight of 100.24 ± 5.44 kg. The longissimus dorsi muscles of 30 male pigs were sampled in six animals per treatment. Veterinarian food inspectors of the State Inspection System (SIE/IDAF) inspected and certified the meat from the slaughtered animals.

After slaughter, the meat was stored at -18 °C until the beginning of the sausage preparation process (end of rigor mortis) 24 hours after slaughter, according to Dokmanovic et al. (2015). The natural antioxidant from the balloon pepper was prepared at the sector of Food Chemistry of the Laboratory of Food Technology of the State University of Northern Rio de Janeiro (UENF: Universidade Estadual Norte Fluminense), after planting and harvesting of the fruits. The commercial synthetic additives (Sugar, Salt (NaCl), Sodium nitrite, Sodium Erythorbate) derived from commercial enterprises specialized in chemical additives for the production of meat products.

The genotype used was the access 1613 of *Capsicum baccatum* of the germplasm collection of the Center for Agricultural Sciences and Technologies of the UENF. The ripened fruits were collected from the plant, stored in duly identified paper bags, and transported for the analyses to the Food Technology Laboratory (LTA) of the Center for Agricultural Sciences and Technologies (CCTA). The vegetal samples were deposited at the University herbarium under the number HUENF 9607.

The extraction method was adapted from Zimmer et al. (2012), choosing for the production of oleoresins. The oleoresin is an extract obtained by the use of an organic solvent that has substances that are responsive for the pungency, and that may contain substances that may act as antioxidants, besides being stable during the storage. The application of these oleoresins to the foods is justified for adding flavor and increasing the oxidative stability of the lipids, extending the shelf-life of these foods.

In the beginning, the fruits were cut to extract the seeds and washed with tap water, weighed, and placed on an air-circulation oven at 40 °C, until reaching a constant mass. The ethanol was the solvent used for the preparation of the extraction. After the drying, fruits were minced and submitted to extraction in

the Soxhlet equipment, using ethanol 70% (Fruit-solvent ratio: 1:10 weight/volume) for four hours.

A rotary evaporator evaporated the extracts at 79 °C. After this process, the antioxidant extract of the balloon pepper was obtained. The antioxidant extract of the balloon pepper was the one used in the present work due to the antioxidant activity and concentrations of total phenol, chlorogenic acid, caffeic acid, quercetin and rutin (Table 1).

The evaluation of the antioxidant activity was carried out by the photo-colorimetric method of stable free radical DPPH (2,2-Diphenyl-1-picrylhydrazyl). The technique consists of adding 1 mL of the ethanol extract at concentrations ranging from 10 to $1000 \mu\text{g mL}^{-1}$. 1 mL of a methanol solution of DPPH (0.1 mmol L^{-1}) was added to this, followed by the reaction which occurred at room temperature for 1 hour. The absorption of DPPH was immediately checked in a UV-Vis spectrophotometer at 515 nm.

The ability to scavenge free radicals was expressed as a percentage of radical oxidation inhibition and it is calculated as follows (Zimmer et al., 2012; Equation 1):

$$\% \text{ Inhibition} = (\text{ADPPH} - \text{ASAMPLE}) / \text{ADPPH} \times 100 \quad (1)$$

ADPPH - absorbance of DPPH solution; ASAMPLE - absorbance of the sample solution.

The total phenolic content was determined using the FolinCiocalteu method as described Singleton et al. (1999) which was based on the reduction of the phosphotungstic ($\text{H}_3\text{PW}_{12}\text{O}_{40}$) and phosphomolybdic ($\text{H}_3\text{PMo}_{12}\text{O}_{40}$) acids. To prepare the samples samples, 1 mg was weighed and solubilized with 1 mL of methanol, 30 μL of these solutions were drawn transferred to a 96 well plate. After that, they were added 50 μL of Folin-Ciocalteu reagent (1N) and added 100 μL of sodium carbonate (Na_2CO_3) 7.5% after 10 min. The absorbance was obtained at 760 nm after 2 h of incubation at room temperature. The total phenols' results were expressed as gallic acid equivalents using a gallic acid standard curve (5.0 to $200 \mu\text{g mL}^{-1}$).

Standards (phenolic and flavonoid acids) and the samples extracts were analyzed on Shimadzu Model LC-20A, with LC20AD

Table 1. Data obtained in the analysis of Antioxidant Activity and concentrations of total phenol, chlorogenic acid, caffeic acid, quercetin and rutin of aqueous extract of balloon pepper fruits (*Capsicum baccatum* var. *pendulum*).

Analyze	Aqueous extract of balloon pepper fruits (<i>Capsicum baccatum</i> var. <i>pendulum</i>)
Antioxidant activity (1000 $\mu\text{g}/\mu\text{L}$) (%)	100
Antioxidant activity (100 $\mu\text{g}/\mu\text{L}$) (%)	64
Total phenol (mg EAG/g ext.)	274.35
Chlorogenic acid (mg/g)	2.40
Caffeic acid (mg/g)	0.74
Quercetin (mg/g)	0.34
Rutin (mg/g)	0.82

pumps. The chromatograms were monitored at a wavelength of 254 and 350 nm for phenolic compounds (detector spectrum scanned by ultraviolet photodiode array SPD-M20A) and an injection volume of 20 µL. Reverse phase column RP-18 from Macherey-Nagel (5 µm, 4.0 × 250 mm) was used. To prepare the samples to be injected, 10 mg of the extract was weighed and solubilized in 1 mL of mobile phase: 500 µL was pipetted of mobile phase contained in the pump A and 500 µL of mobile phase contained in the pump B, following this procedure for the two gradients. The extract was filtered using of microfilters (Sartorius®) and syringes (BD Plastipak®) were then applied three times to each method, in the liquid chromatograph. Chlorogenic acid (Sigma-Aldrich), caffeic acid (Tedia Brazil), rutin (Merck) and quercetin (Merck) standards were used to measure the standard curves. To identify and quantify the compounds in samples, comparisons were made of the retention times of the samples with retention times of pure commercial standards. After this preliminary analysis were made co-injections of pure standards and samples were made for further analysis of their retention times. Comparisons between the UV spectrum of the compounds and the commercial standards were also carried out.

After thawing, all fat in muscle was removed, and the meat was diced and stored at approximately 4 °C. Pork fat was also thawed, diced, and stored at the same temperature. Next, *longissimus dorsi* muscles were ground in a meat grinder (20, Talleres Ramon). Pork fat was ground in the same equipment. All animal raw material was placed in a tray when seasoning and additive were added and hand-mixed to meat and fat.

After adding a mixture of curing salts and aqueous extract of balloon pepper fruits (*Capsicum baccatum* var. pendulum), fresh sausage material was placed in other trays, labeled, and refrigerated for 1 h at 2 °C to 4 °C. Sausages were manually encased using 36-mm natural sheep casing. The sausages prepared were vacuum-packaged (TM720, TecMaq) in polyethylene bags, labeled, stored at -18 °C, and thawed at 4 °C before analyses.

Four different formulations of fresh pork sausage and smoked sausage were produced for the study of lipid oxidation: Control, F1, F2 and F3. The formulations promoted the substitution of the synthetic antioxidant with the natural one. A control using sodium erythorbate was included in the experiment (Table 2).

After preparation, a portion of the samples were taken to the smoker, where they remained for approximately 3 hours until they reached an internal temperature of 75 °C. They were removed from the smoker and placed in a protected place for a period of 24 h for natural cooling. Then, the samples were vacuum packed and identified. Finally, stored in a cold room at -18 °C, until the moment of analysis.

Samples from different formulations were grounded and homogenized. Moisture (direct drying in stove at 105° C), dry ash (residue after incineration in muffle at 550° C), proteins (classic Kjeldahl's method) and crude ethereal extract (direct extraction in Soxhlet) were determined according to the described by Cechi (1999) and Association of Official Analytical Chemists (2000) (Table 3).

The microbiological analyses of the samples were performed at the microbiology laboratory of the IFES Campus Alegre. The sensorial analysis was performed at the Food Technology Laboratory, at the UENF University.

Preliminary tests were performed to verify the concordance with the Brazilian legislation on the standard for the production of fresh sausage as refers to the percentage of fresh material, and feed additives. Table 2 displays the percentage of fresh matter and additives of the different formulas.

The analyses described next were performed on non-stored samples [on the fabrication day; n=20 for each formulation and control group (total n=100)] and after 15, 30, 45 and 60 days of storage [n=20 for each formulation and control group (total n=100), for each period].

Table 2. Percentage of fresh matter and additives in the formulas of fresh pork sausage with the use of the natural antioxidant balloon pepper.

Fresh matter	Control	F1	F2	F3
Pork meat [†]	71.66	71.66	71.66	71.66
Pork fat	20.00	20.00	20.00	20.00
Salt (NaCl) [‡]	2.00	2.00	2.00	2.00
Sugar [§]	0.10	0.10	0.10	0.10
Water	6.21	5.73	5.23	4.73
Natural Antioxidant	0.00	0.500	1.000	1.50
Sodium Nitrite [¶]	0.02	0.02	0.02	0.02
Sodium Erythorbate [*]	0.03	0.000	0.000	0.000

[†]Pork loin (*Longissimus dorsi*); [‡]Salt "Cisne"; [§]Brown Sugar "União"; [¶]Curing Salt Kura K007 - "Doremus"; ^{*}Antioxidant - "Griffith".

Table 3. Percentage of composition of the formulations of fresh swine sausage and smoked with the natural antioxidant from balloon pepper (*Capsicum baccatum* var. pendulum).

Composition (%)	Control	F1	F2	F3
Moisture	65.28	64.08	63.82	63.24
Protein	18.01	17.05	17.15	14.82
Fat	24.13	23.19	23.53	22.40
Dry ash	2.82	2.99	2.96	2.94
Ratio Moisture/protein	3.62	3.76	3.72	4.27

2.1 Lipid oxidation

Five grams of fresh and smoked pork sausages were homogenized and placed in a 30 × 200 mm glass tube, without border, and with a rounded bottom. 30 mL of 5% trichloroacetic acid (TCA) and 1 mL of 0.15% synthetic antioxidant butylated hydroxytoluene (BHT) were added. The material was homogenized in an Ultra-Turrax for 60 seconds. Later the sample was filtered using qualitative filter paper (12.5 mm) to a 50 mL volumetric flask. The volume was completed with 5% TCA solution. Five mL of the flask content were withdrawn, transferred to a sample tube, and added with 5 mL of 0.08 M thiobarbituric acid. Tubes were incubated in a boiling water bath for 40 minutes, to allow the formation of the colored complex. After this period, the tubes were placed in running water until reaching room temperature, and the solution was measured in a Kasuaki (UV-5100 model) spectrophotometer at 532 nm wavelength. The results were subtracted from the results of the blank sample.

Readings were made against a concentration (x):absorbance (y) curve of 1,1,3,3-tetraethoxypropane (TEP), used as standard. TBARS levels were expressed as content of malondialdehyde (MDA) (mg MDA/kg). Analyses were carried out in duplicate and four repeats on days 0, 15, 30, 45, and 60 into storage.

2.2 Microbiological analysis

Microbiological analysis of the samples was performed just after thawing the sausage, according to Silva (2010). The results were compared with the standards recommended in the RDC 12 (Brasil, 2001), for coliforms at 45 °C (Most Probable Number method), Coagulase-positive Staphylococcus (Presence or absence), and Salmonella spp. (presence or absence).

2.3 Sensory analysis

An acceptance test with hedonic scale was used for the sensorial evaluation, using a scale of 9 points (9- liked immensely; 8- liked a lot; 7- liked moderately; 6- liked lightly; 5- indifferent; 4- disliked lightly; 3- disliked moderately; 2- disliked a lot and 1- disliked extremely). The sausage were analyzed for sensory attributes by 51 untrained participants recruited among students

and employees of the UENF University, at the Campus dos Goytacazes (RJ-Brazil) Campus. Among them, 53% were women, 47%, men. The age ranged from 18 to 45 years. Briefly, 1.0 cm-thick slices were cut starting from the middle of the sausage cooked electric oven PHILCO PFO32L at 180 °C for 10 minutes. One slice of cooked sausage of each formulation were placed in labeled plates and randomly presented to participants. Water and unsalted crackers were provided for cleansing the palate between evaluations. Participants were given an evaluation form with the nine-point hedonic scale to assess color, aroma, taste, texture and general impression. The evaluations were carried out in individual booths.

For the attributes associated to the will to buy, the evaluation sheet had five points (5- definitely, I would buy; 4- probably I would buy; 3- maybe I would buy, maybe not; 2- probably I would not buy; 1- definitely I would not buy). In these sheets, the judges expressed numerical values according to their preferences.

Sensory analysis was performed after the microbiological analysis. All samples satisfied the Brazilian microbiological requirements as RDC 12/2001 (Brasil, 2001). The ethical in health committee endorsed this project under the statement 2,066,829.

2.4 Statistical analysis

The results were submitted to Analysis of Variance (ANOVA) at 5% probability. The treatments were compared by the test Student-Newman-Keuls.

Sensory analysis results were submitted to statistical analysis by the analysis of variance (ANOVA) and Tukey test at 5% probability. All statistical analyses were performed using the statistical program SAS Institute (2009) version 9.3.

3 Results and discussion

The natural antioxidant (the extract of balloon pepper) was added at different concentrations in the different formulations in order to delay lipid oxidation and extend the shelf life of the fresh sausage. Table 4 present the values of TBARS obtained (Table 4).

TBARS values in the fresh sausages did not display significant differences ($P > 0.05$) among the treatments, and at different times

Table 4. Average values of TBARS expressed as MDA/kg found by lipid oxidation in fresh and smoked pork sausage formulations with the use of the balloon pepper.

	TBARS (days) ¹				
	Fresh				
	0	15	30	45	60
Control	0.004 ± 0.003 ^a	0.015 ± 0.009 ^a	0.026 ± 0.007 ^a	0.018 ± 0.001 ^a	0.038 ± 0.026 ^a
F1	0.030 ± 0.007 ^a	0.020 ± 0.019 ^a	0.024 ± 0.012 ^a	0.028 ± 0.011 ^a	0.057 ± 0.023 ^a
F2	0.026 ± 0.016 ^a	0.024 ± 0.013 ^a	0.028 ± 0.008 ^a	0.019 ± 0.005 ^a	0.065 ± 0.012 ^a
F3	0.036 ± 0.003 ^a	0.017 ± 0.018 ^a	0.024 ± 0.011 ^a	0.029 ± 0.013 ^a	0.062 ± 0.015 ^a
	Smoked				
Control	0.058 ± 0.018 ^a	0.026 ± 0.026 ^a	0.06 ± 0.044 ^a	0.033 ± 0.009 ^a	0.046 ± 0.018 ^a
F1	0.066 ± 0.021 ^a	0.016 ± 0.015 ^a	0.035 ± 0.022 ^a	0.043 ± 0.009 ^a	0.038 ± 0.016 ^a
F2	0.064 ± 0.015 ^a	0.013 ± 0.005 ^a	0.069 ± 0.078 ^a	0.052 ± 0.031 ^a	0.042 ± 0.021 ^a
F3	0.042 ± 0.023 ^a	0.019 ± 0.018 ^a	0.047 ± 0.029 ^a	0.038 ± 0.013 ^a	0.064 ± 0.021 ^a

¹Averages on the same line and column, followed by lowercase letters, not differ by SNK test ($p < 0.05$).

of analysis (storage), but varied along the experimental period. TBARS values ranged between 0.015 mg MDA/kg of sample and 0.065 mg MDA/kg of the sample, respectively at 15, and 60 days of storage. The data of TBARS point out the similarity between the action of the natural antioxidant derived from the balloon pepper, and the synthetic sodium erythorbate. The lack of differences along the storage time might associate with the vacuum-storage. In these conditions, the degradation of TBARS might have produced other substances, but TBARS.

Smoked sausage samples also did not display significant differences ($P > 0.05$) associated neither to the treatment nor the storage time. TBARS values ranged between 0.013 and 0.069 mg MDA/kg of sample along the experimental period. The results of TBARS of smoked sausage samples may be associated with MDA degradation due to the action of phenolic compounds produced during the smoking process.

Neither the TBARS values of fresh nor smoked sausage displayed a significant increase ($P > 0.05$) during the experimental period. The results of TBARS during the experimental period highlighted the satisfying antioxidant action of the pepper extract since no differences with the control treatment could be observed along the experimental time. Even the same, it is possible to affirm that the storage by freezing and vacuum packaging contributed positively, as comparing the treatments with antioxidants (both synthetic and natural). These observations highlighted the delay in the lipid oxidation of the products along the 60 days of the experiment.

During processing and storage of the sausages, physical and chemical alterations caused the production of free radicals, which caused the oxidation of the unsaturated fatty acids of meat (Falowo et al., 2014; Hygreeva et al., 2014).

The analysis of lipid oxidation through the TBARS assay quantified the malonaldehyde compound (MDA), which is one of the leading products of decomposition of hydroperoxides of the fatty acids produced during the oxidation process. According to Gray & Pearson (1987), lipid oxidation in meat products begins as TBARS levels are between 0.5 and 2.0 mg MDA/kg of the sample.

Nam et al. (2001) observed that packaging raw meat under vacuum conditions was enough to protect the cholesterol and fatty acids from oxidation. In this study, all the samples used to study the maturation process were individually vacuum-packed and, possibly, this process protected cholesterol and fatty acids, especially the unsaturated ones, from adverse conditions that could have promoted oxidation. However, TBARS is a relatively stable compound. Ham et al. (2020) evaluated the efficacy of ascorbic acid on processing characteristics and lipid oxidation of pre-rigor salted chicken breasts during seven days of vacuum-packaged storage, and suggested that pre-rigor salting with ascorbic acid could be useful in improving lipid oxidation stability during refrigerated vacuum storage, without negative impacts on processing characteristics.

The treatments and the interactions treatment \times type did not display significant differences ($P > 0.05$). The only significant differences were between the different types of sausages: fresh and smoked, Table 5 describe.

Figures 1 and 2 display the average MDA/kg (expressed as mg) during the storage period in the samples of fresh and smoked sausage, respectively.

Fresh sausages displayed higher MDA values than the smoked ones. These higher values may have been due to the degradation of MDA, and other compounds during the vacuum storage and to the action of phenolic compounds produced during the smoking process when partial dehydration of the product occurs. Smoke components include halophilic and lipophilic substances that react differently in meat products. Among the preservative properties of the smoking processes can also be mentioned the antioxidant effect that the smoking compounds exert, mainly the monoglyd and dimethyl ether of pyrogallol capable of delaying the oxidative and hydrolytic rancification of fats (Pardi et al., 2007). These results corroborate those presented by Schwert et al. (2011, 2020) where they concluded that, the use of liquid and traditional smoke with temperature

Table 5. Average TBARS values expressed as MDA/kg due to lipid oxidation in fresh and smoked pork sausage produced using natural antioxidants derived from the balloon pepper.

Type	TBARS ¹
Fresh	0.1410 ^a
Smoked	0.0252 ^b

¹Averages on the same column, followed by lowercase letters, differ by SNK test ($p < 0.05$).

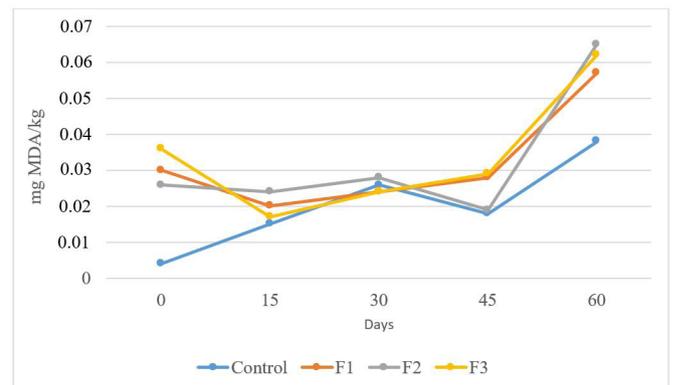


Figure 1. Averages mg MDA/kg of the four treatments during the storage period under chilling for the samples of fresh swine sausage.

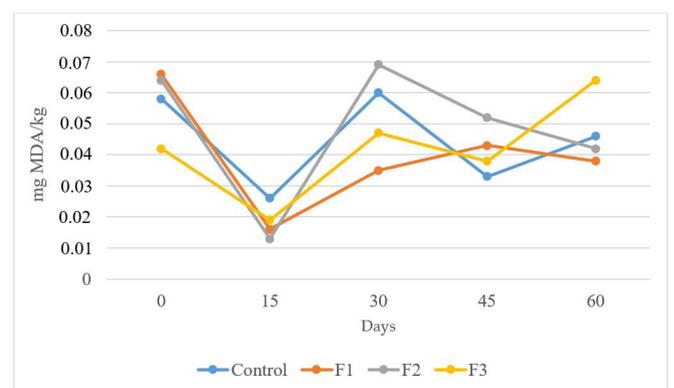


Figure 2. Averages mg MDA/kg of the four treatments during the storage period under chilling for the samples of smoked swine sausage.

control ~ 300 °C for a smoke generation are promising and safe approaches for obtaining low levels of benzo (a) pyrenes, lipids and protein oxidation in Sausage type Calabrese.

As observing the values found in this study, TBARS values are within quality values. Studies display that TBARS values up to 1.59 mg MDA/kg of the sample are considered low, are not perceived as rancidity by sensorial analyses, and do not cause any harm to the consumers' health (Torres & Okani, 1997). According to Ahmad & Srivastava (2007) no rancidity smell in meat is detectable as TBARS values span between 0.5 and 1.0 mg MDA/kg of the product. For this reason, the average values found are within standards of acceptability, defining that sausages may be appropriate for consumption.

As defining the effect of the addition of oregano and marjoram extracts in "Toscana" sausage, Bussata (2010) described 0.806 mg MDA/kg after 35 days of storage. Almeida et al. (2015) observed that TBARS values differed at the same storage time in sausages prepared with the extract of the peel of the Brazilian grape (*Plinia cauliflora*). According to the same authors, the lower lipid oxidations are due to the phenols of the *Plinia cauliflora* peel.

Abu Salem & Ibrahim (2010) assessed the addition of salvia oil extract at 0.05% and 0.025% in buffalo sausages and found values of 0.91 mg MDA/kg of product in sausages without natural antioxidant, and 0.51 and 0.46 mg MDA/kg in sausages treated with the salvia extract.

Jayawardana et al. (2011) used Adzuki bean at 0.2% in smoked and not smoked sausages. The authors described positive results in the control of lipid oxidation in both products and associated these results to the presence of the polyphenols in the extract of the Adzuki bean, which are rich in anthocyanins.

The antioxidant effect of the phenols derives from the hydroxyl group linked to the aromatic ring. The aromatic ring can donate electrons to hydrogen atoms, neutralizing the free radicals. According to Almeida et al. (2015), this mechanism blocks the degradation of the oxidative form, as observed for the MDA.

The results of our study corroborate those of the other works presented, as we did not observe an increase of lipid oxidation, keeping TBARS values relatively constant until the end of the experiment (60 days).

Table 6 describes the microbiological quality of the fresh sausage, as it refers to coliforms (MPN/g), coagulase-positive *Staphylococcus* (UFC/g), *Salmonella* sp., and sulfate-reducing *Clostridium* at 46 °C (UFC/g).

The storage process is directly associated with the development and survival of microorganisms. The microbiological stability is crucial to the products' quality. According to Fellows (2006), food preservation is due to antimicrobial action addicted to the product, the presence of lactic acid derived from the fermentation processes, and the control of pH.

The enumeration of all the microorganisms searched was below the standard required by Brazilian laws (Brasil, 2001). The microbiological quality was due to the quality of the raw materials, good manufacturing practices, vacuum packaging of the product, and use of additives, which prevented the growth of some species of microorganisms, and the use of natural antioxidant, with its antimicrobial action.

Table seven displays the average notes of the sensorial analysis for the attributes color, scent, taste, texture, and impression (Table 7).

Sensorial analysis displayed that the attribute "color" did not display significant differences ($p>0.05$) between control and F2, and between F1 and F3. There was a significant difference ($p<0.05$) between control and F1, control and F3 and between F1 and F2. The consumers observed a color reduction with the inclusion of 0.5% of balloon pepper extract, compared to the formulas with 1.0 and 1.5% addition. The red tone of the extract, influencing the final color of the sausages, can explain this reduction of the color. López-Vargas et al. (2014) observed this correlation in pork hamburger added with passion fruit flour. The product added with the lower concentrations displayed lighter colors than the remaining. Valle (2017) studied bovine restructured meat beef added with aqueous extract of pink

Table 6. Results of the microbiological analyses of fresh pork sausage added with natural antioxidants from the balloon pepper.

	Coliforms (MPN/g) ¹	coagulase positive <i>Staphylococcus</i> (UFC/g) ²	<i>Salmonella</i> sp.	sulphite-reducing <i>Clostridium</i> at 46 °C (UFC/g) ²
Control	<3.0	Negative	Negative	Negative
F1	<3.0	Negative	Negative	Negative
F2	<3.0	Negative	Negative	Negative
F3	<3.0	Negative	Negative	Negative
RDC 12/01 Standard	<3.0	Negative	Negative	Negative

¹Most Probable Number per gram; ²Colony Forming Unit per gram.

Table 7. Average values for each attribute assessed in the fresh pork sausage added with natural antioxidants derived from balloon pepper.

Treatment ¹	Color	Aroma	Taste	Texture	General impression
Control	6.8 ^a	6.8 ^a	7.1 ^a	7.0 ^{ab}	6.9 ^a
F1	5.4 ^c	6.0 ^b	6.6 ^a	6.4 ^b	6.2 ^b
F2	6.6 ^{ab}	6.9 ^a	7.3 ^a	7.0 ^{ab}	7.1 ^a
F3	5.9 ^{bc}	6.5 ^{ab}	7.3 ^a	5.9 ^{ab}	6.8 ^a

¹Averages followed by the same letter in the same column differ among each other by the Tukey test ($p<0.05$).

pepper (*Schinus terebinthifolius*, Raddi), and the results of the research disagree with those presented in the previous article.

The aroma did not show significant differences ($p > 0.05$) between the control, F2 and F3, and between F1 and F3. This datum suggests that taster perceive the inclusion of the extract at low (0.5%) or high (1.5%) concentrations, but do not detect the product at intermediate concentrations (1.0%).

This observation differs as there is the inclusion of different kinds of ingredients as antioxidants, as displayed by Valle (2017). Maia et al. (2020) also points out that the tasters did not perceive the inclusion of passion fruit meal, potassium chloride (KCl), and calcium chloride (CaCl₂) in smoked sheep meat sausage.

The attribute “taste” did not display any significant difference ($p > 0.05$) among the formulas. The texture did not differ significantly between the control, F2, and F3, and between F1, F2, and F3. The reduction of water use was a crucial parameter in the results obtained in this study, as there was no significant difference among the different formulas that received the extract of balloon pepper and between the formulas that received, and the one that did not receive the extract.

The general impression of the product displayed significant differences ($P < 0.05$) between F1 and the remaining treatments. This result is probably due to the higher perception of the tasters for the inclusion of the extract of the balloon pepper (*Capsicum baccatum* var. *pendulum*) at low concentrations (0.5%).

The results highlighted that the attributes color, aroma, texture, and general impression of the treatment F2 (addition of 1% of pepper extract to substitute the antioxidant) received higher acceptance by the consumers. The average scores received are closer to seven. According to Dutcosky (2011), the acceptability index of 7.0 is the minimum that a product should reach to be considered of positive acceptability in the different sensorial parameters assessed. As refers to the buying intention, scores 4 to 5, 3 to 2, and 1 were associated respectively to “acceptation,” “indifference,” and “rejection.”

The average scores of the buying intention of the F2 and F3 formulas were higher than the remaining, suggesting that consumers “definitely would buy” these products on the market. On the other side, F1 treatment received an average score of 1.9, indicating higher rejection. Consumers “definitely would not buy” this product.

4 Conclusions

The results point out that the balloon pepper extracts acted competently as antioxidants, preventing rancidity, and preserving the original qualities of the product during the 60 days of storage. The freezing and vacuum storage also contributed to delay the oxidative processes. The fresh sausages displayed higher lipid oxidative values as the smoked ones, but their responses after 60 days of storage were similar. The sensorial analysis pointed out the higher acceptability of the F2 formula by the consumers, and the higher willingness to buy. This paper clearly shows that the use of a 1% extract

of the balloon pepper demonstrates good acceptability among the consumers.

References

- Ahmad, S., & Srivastava, P. K. (2007). Quality and shelf life evaluation of fermented sausages of buffalo meat with different levels of heart and fat. *Meat Science*, 75(4), 603-609. <http://dx.doi.org/10.1016/j.meatsci.2006.09.008>. PMID:22064024.
- Almeida, P. L., Lima, S. N., Costa, L. L., Oliveira, C. C., Damasceno, K. A., Santos, B. A., & Campagnol, P. C. B. (2015). Effect of jaboticaba peel extract on lipid oxidation, microbial stability and sensory properties of Bologna-type sausages during refrigerated storage. *Meat Science*, 110, 9-10. <http://dx.doi.org/10.1016/j.meatsci.2015.06.012>. PMID:26156583.
- Association of Official Analytical Chemists – AOAC. (2000). *Official methods of analysis of the AOAC International*. Virginia: AOAC International.
- Brasil, Agência Nacional de Vigilância Sanitária. (2001, January 10). Regulamento técnico sobre padrões microbiológicos para alimentos (RDC nº 12, de 2 de janeiro de 2001). *Diário Oficial [da] República Federativa do Brasil*. Retrieved from http://portal.anvisa.gov.br/documents/33880/2568070/RDC_12_2001.pdf/15ffddf6-3767-4527-bfac-740a0400829b
- Bussata, C. (2010). *Caracterização química e atividade antimicrobiana in vitro em alimentos dos extratos de oregano e manjerona* (Dissertação de mestrado). Universidade Regional Integrada, Erechim.
- Campbell, T. W. (2015). Clinical chemistry of birds. In M. A. Thrall, G. Weiser, R. W. Allison & T. W. Campbell (Eds.), *Veterinary hematology and clinical chemistry* (2nd ed., pp. 509-521). Rio de Janeiro: Guanabara Koogan.
- Cechi, H. M. (1999). *Fundamentos teóricos e práticos em análise de alimentos* (1. ed.). Campinas: Universidade Estadual de Campinas.
- Devatkal, S. K., & Naveena, B. M. (2010). Effect of salt, kinnow and pomegranate fruit by-product powders on color and oxidative stability of raw ground goat meat during refrigerated storage. *Meat Science*, 85(2), 306-311. <http://dx.doi.org/10.1016/j.meatsci.2010.01.019>. PMID:20374904.
- Dokmanovic, M., Baltic, M. Z., Duric, J., Ivanovic, J., Popovic, L., Todorovic, M., Markovic, M., & Pantic, S. (2015). Correlations among stress parameters, meat and carcass quality parameters in pigs. *Asian-Australasian Journal of Animal Sciences*, 28(3), 435-441. <http://dx.doi.org/10.5713/ajas.14.0322>. PMID:25656214.
- Dutcosky, S. D. (2011). *Análise sensorial de alimentos* (3. ed.). Curitiba: Editora Universitária Champagnat.
- Falowo, A. B., Fayemi, P. O., & Muchenje, V. (2014). Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: a review. *Food Research International*, 64, 171-181. <http://dx.doi.org/10.1016/j.foodres.2014.06.022>. PMID:30011637.
- Fellows, P. J. (2006). *Tecnologia do processamento de alimentos: princípios e práticas* (1. ed.). Porto Alegre: Artmed.
- Franco, D., González, L., Bispo, E., Latorre, A., Moreno, T., Sineiro, J., Sánchez, M., & Núñez, M. J. (2012). Effects of calf diet, antioxidants, packaging type and storage time on beef steak storage. *Meat Science*, 90(4), 871-880. <http://dx.doi.org/10.1016/j.meatsci.2011.10.008>. PMID:22193039.
- Fратиanni, F., De Martino, L., Melone, A., De Feo, V., Coppola, R., & Nazzaro, F. (2010). Preservation of chicken breast meat treated with thyme and balm essential oils. *Journal of Food Science*, 75(8),

- M528-M535. <http://dx.doi.org/10.1111/j.1750-3841.2010.01791.x>. PMID:21535509.
- Georgantelis, D., Blekas, G., Katikou, P., Ambrosiadis, I., & Fletouris, D. J. (2007). Effect of Rosemary extract, chitosan and α -tocopherol on lipid oxidation and colour stability during frozen storage of beef burgers. *Meat Science*, 75(2), 256-264. <http://dx.doi.org/10.1016/j.meatsci.2006.07.018>. PMID:22063657.
- Gray, J. I., & Pearson, A. M. (1987). Rancidity and warmed-over flavor. In A. M. Pearson & T. R. Duston (Eds.), *Restructured meat and poultry products, advances in meat research* (pp. 221-269). New York: Van Nostrand Reinhold.
- Ham, Y. K., Song, D. H., Ha, J. H., Park, S. K., Kim, Y. R., Chin, K. B., & Kim, H. W. (2020). Efficacy of ascorbic acid on processing characteristics and lipid oxidation of pre-rigor salted chicken breasts during vacuum refrigerated storage. *LWT*, 118, 108691. <http://dx.doi.org/10.1016/j.lwt.2019.108691>.
- Hygreeva, D., Pandey, M. C., & Radhakrishna, K. (2014). Potential applications of plant based derivatives as fat replacers, antioxidants and antimicrobials in fresh and processed meat products. *Meat Science*, 98(1), 47-57. <http://dx.doi.org/10.1016/j.meatsci.2014.04.006>. PMID:24845336.
- Jayawardana, B. C., Hirano, T., Han, K. H., Ishii, H., Okada, T., Shibayama, S., Fukushima, M., Sekikawa, M., & Shimada, K. (2011). Utilization of adzuki bean extract as a natural antioxidant in cured and uncured cooked pork sausages. *Meat Science*, 89(2), 150-153. <http://dx.doi.org/10.1016/j.meatsci.2011.04.005>. PMID:21663804.
- Kozačins, L., Fleck, C., Filipović, I., Mitak, M., Bratulić, M., & Mikuš, T. (2012). Evaluation of shelf life of pre-packed cut poultry meat. *Veterinarski Arhiv*, 82(1), 47-58.
- Lima, A. R., Pereira, R. G. F. A., Abrahão, S. A., Duarte, S. M. S., & Paula, F. B. A. (2010). Compostos bioativos do café: atividade antioxidante in vitro do café verde e torrado antes e após a descafeinação. *Química Nova*, 33(1), 20-24. <http://dx.doi.org/10.1590/S0100-40422010000100004>.
- López-Vargas, J. H., Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2014). Quality characteristics of pork burger added with albedo-fiber powder obtained from yellow passion fruit (*Passiflora edulis* var. *flavicarpa*) co-products. *Meat Science*, 97(2), 270-276. <http://dx.doi.org/10.1016/j.meatsci.2014.02.010>. PMID:24607997.
- Maia, J. A. Jr., Henry, F. C., Della Lucia, S. M., Santos, A. C. Jr., Cabral, N. O., Quirino, C. R., & Valle, F. R. A. F. (2020). Sensory attributes and lipid oxidation of smoked lamb sausage formulated with passion fruit meal, potassium chloride and calcium chloride. *Food Science and Technology*, 40(2), 423-429. <http://dx.doi.org/10.1590/fst.02419>.
- Nam, K. C., Du, M., Jo, C., & Ahn, D. U. (2001). Cholesterol oxidation products in irradiated raw meat with different packaging and storage time. *Meat Science*, 58(4), 431-435. [http://dx.doi.org/10.1016/S0309-1740\(01\)00046-8](http://dx.doi.org/10.1016/S0309-1740(01)00046-8). PMID:22062435.
- Pardi, M. C., Santos, I. F., Souza, E. R., & Pardi, H. S. (2007). *Ciência, higiene e tecnologia da carne* (2. ed., Vol. II, pp. 784-792). Goiânia: CEGRAF-UFG.
- Radha Krishnan, K., Babuskin, S., Babu, P. A. S., Sasikala, M., Sabina, K., Archana, G., Sivarajan, M., & Sukumar, M. (2014). Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International Journal of Food Microbiology*, 171, 32-40. <http://dx.doi.org/10.1016/j.ijfoodmicro.2013.11.011>. PMID:24308943.
- Rosa, C. S., Kubota, E., Stein, M., Nogara, G. P., & Vizzotto, M. (2013). Avaliação do efeito de extrato de farinha de alfarroba (*Ceratonia siliqua* L.) na estabilidade oxidativa e cor de hambúrgueres congelados. *Semina: Ciências Agrárias*, 34(5), 93-98. <http://dx.doi.org/10.5433/1679-0359.2013v34n5p2277>.
- Abu Salem, F. M., & Ibrahim, H. M. (2010). Dry fermented buffalo sausage with sage oil extract: Safety and quality. *Grasas y Aceites*, 61(1), 76-85. <http://dx.doi.org/10.3989/gya.075109>.
- SAS Institute. (2009). *User's guide statistics*. Cary: SAS.
- Schwert, R., Verlindo, R., Cichoski, A. J., Oliveira, D., & Valduga, E. (2011). Comparative evaluation of liquid and traditional smoke on oxidative stability, color and sensory properties of Brazilian calabrese sausage. *CYTA: Journal of Food*, 9(2), 131-134. <http://dx.doi.org/10.1080/19476337.2010.491581>.
- Schwert, R., Verlindo, R., Soares, J. M., Silva, P. F., Cansian, R. L., Steffens, C., Treichel, H., & Valduga, E. (2020). Effect of Liquid Smoke Extract on the Oxidative Stability, Benzopyrene and Sensory Quality of Calabrese Sausage. *Current Nutrition and Food Science*, 16(3), 343-353. <http://dx.doi.org/10.2174/1573401315666190126120749>.
- Silva, M. L. C., Costa, R. S., Santana, A. S., & Koblitz, M. G. B. (2010). Compostos fenólicos, carotenóides e atividade antioxidante em produtos vegetais. *Semina: Ciências Agrárias*, 31(3), 669-682. <http://dx.doi.org/10.5433/1679-0359.2010v31n3p669>.
- Silva, N. (2010). *Manual de métodos de análise microbiológica de alimentos e água* (1. ed.). São Paulo: Varela.
- Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. In L. Packer (Ed.), *Oxidants and antioxidants, part A* (1st ed., Vol. 299, p. 152). New York: Academic Press.
- Torres, E. A. F. S., & Okani, E. T. (1997). Teste de TBA: ranço em alimentos. *Revista Nacional da Carne*, 243, 68-76.
- Valle, F. R. A. (2017). *Avaliação do efeito de extrato aquoso de aroeira (SCHINUS terebinthifolius, RADDI) adicionado à produtos cárneos* (Tese de doutorado). Centro de Ciências e Tecnologias, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes.
- Weiss, J., Gibis, M., Schuh, V., & Salminen, H. (2010). Advances in ingredient and processing systems for meat and meat products. *Meat Science*, 86(1), 196-213. <http://dx.doi.org/10.1016/j.meatsci.2010.05.008>. PMID:20619800.
- Zimmer, A. R., Leonardi, B., Miron, D., Schapoval, E., Oliveira, J. R., & Gosmann, G. (2012). Antioxidant and anti-inflammatory properties of *Capsicum baccatum*: from tradicional use to scientific approach. *Journal of Ethnopharmacology*, 139(1), 228-233. <http://dx.doi.org/10.1016/j.jep.2011.11.005>. PMID:22100562.