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Assessment of different packaging structures in the stability of refrigerated fresh Brazilian Toscana sausage

[1]De Paula, R., [1]Soares , J. M., [1]Da Silva, P. F., [1]Backes, G.T., [1]Cansian, R. L., [2]Treichel, H. and [1*]Valduga, E.

[1]Department of Food Engineering, URI Erechim, Av. Sete de Setembro, 1621-Erechim-RS, 99700-000, Brazil
[2]Federal University of Fronteira Sul - Campus Erechim, Av. Dom João Hoffmann, 313, 99700-000, Erechim, RS, Brazil

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Abstract

This study aimed at evaluating different packaging structures (low-density polyethylene, nylon polyester and a formal copolymer of ethylene and vinyl alcohol), with vacuum, on the proteic and lipidic stability characteristics of fresh sausage stored at 8°C for 35 days. After 28 days of storage of the sample in the vacuum packaging, low-density polyethylene showed a slight to moderate rancid flavor, followed by nylon polyester vacuum packaging. A correlation was observed between the rancidity sensorially detected and that evaluated via the oxidation of lipids and protein.

Introduction

From a nutritional point of view, sausages are an important source of proteins of high biological value; however, these traditional meat products have negative characteristics as a consequence of their animal fat content (Beriain *et al.*, 2000; Milani *et al.*, 2003). The unsaturated fatty acid content is significantin regulating the shelf-life of meat or meat products (Sheard *et al.*, 2000; Boselli *et al.*, 2005). Lipid oxidation can damage sensory properties of food products because fat contributes to flavor, texture, mouth feel, juiciness and the overall sensation of lubricity of the product (Navarro *et al.*, 1997; Muguerza *et al.*, 2002; Qiu *et al.*, 2013). Additionally, oxidation can affect the nutritional value of food by the decomposition of vitamins or unsaturated essential fatty acids or the generation of toxic compounds (Ansorena and Astiasarán, 2004).

Fresh sausages are among the most common processed meat products worldwide. These products have very high water activity and microbial load, which leads to a short shelf life (Georgantelis *et al.*, 2007) and new techniques should be developed to mitigate this issue. For fresh pork sausages, it was reported that they could only be kept for 8 to 12 h without refrigeration (Savic, 1985) while chilled sausage could be maintained for 1 to 5 weeks (Soultos *et al.*, 2008; Silveira *et al.*, 2014). Packaging in modified atmospheres (vacuum and gas packaging) has been introduced as a commercial packaging for the fresh sausages. These packaging methods increase the shelf life, while also enabling attractive commercial formats such as sliced meat products, retail of sausages and other.

Flexible plastic packages are widely used in industry to wrap and store refrigerated meat and meat products. The advantages of its application are the flexibility of adaptation in production lines and different types of product, ease of handling, effective transport and protection of food, and especially the preservation of the sensory characteristics of the product, including preventing or retarding microbiological deterioration, keeping the desired color and retarding the loss of moisture and fat oxidation (Ansorena and Astiasarán, 2004; Rubio *et al.*, 2008). In order to incorporate all of these features in a polymer packaging, it is often necessary to use multilayer structures. Among polymers widely used for this purpose are low-density polyethylene, nylon polyester and a formal copolymer of ethylene and vinyl alcohol.

Authors such as Wang (2000), Summo *et al.* (2006), Fernández *et al.* (2007) and De Paula *et al.* (2011) have studied the effect of vacuum packaging on microbiological, physicochemical and

*Corresponding author.
Email: *veunice@uricer.edu.br*
Tel: +55-54-35209000 Fax: +55-54-35209090

organoleptic aspects of different types of sausage. The effect of packaging type has also been studied in fresh pork sausage (Liaros *et al*., 2009), Chinese-type sausage (Wang *et al*., 1995), Greektaverna sausage (Samelis *et al*., 2000), Milano-type sausage (Zanardi *et al*., 2002), poultry sausage (Al-Nehlawi *et al*., 2014: Lerasle *et al*., 2014), Galicianchorizo sausage (Fernández *et al*., 2007) and frozen Brazilian Toscana sausage (De Paula *et al*., 2011).

However, there is a lack of literature on the effect of the packing procedure in the lipid and protein oxidation of meat products and the correlation between oxidation and sensory properties of the product. Only a few works on this subject have been presented in recent open literature.

Ansorena and Astiasarán (2004) studied the effect of packaging on dry fermented sausages manufactured with a partial substitution of pork backfat with pre-emulsified olive oil. Valencia *et al.* (2006) evaluated the effect of different storage conditions on the lipid fraction in dry fermented sausages manufactured with a partial substitution of pork backfat with linseed oil and antioxidants. De Paula *et al.* (2011) evaluated the effect of different packaging conditions (low-density polyethylene, nylon polyester and a formal copolymer of ethylene and vinyl alcohol, with and without vacuum) on the physicochemical, microbiological and sensory characteristics of fresh sausage stored for a long period (150 days) underfreezing conditions, mainly correlating lipid oxidation with the sensory analysis of the product.

The main contribution of this work is the evaluation of the effect of different packaging conditions (low-density polyethylene, nylon polyester and a formal copolymer of ethylene and vinyl alcohol, with vacuum) on the physicochemical (TBARS - thiobarbituric acid reactive substances, index peroxide, hexanal, fatty acid composition, carbonyl and sulfhydryl groups) and sensory characteristics of fresh Brazilian Toscana sausage stored under refrigeration (8°C) for a period of 35 days.

Material and Methods

Sample preparation

Product was prepared using as ingredients pork meat, animal fats, water, sugars and additives, according to formulation of fresh Toscana sausage manufactured by a local industry. The maxima values of moisture, fats, and calcium established by the legislation (Brasil 2000) are 70%, 30%, and 0.1%, respectively. The minimum protein content should be 12%. The fresh sausage formulated here presented a nitrite and salt concentration of 100 and 1.8 mg/kg, respectively. pH and water activity (a_w) of 6.0 and 0.97 were obtained, respectively. The samples (approximately 500 g) were collected and packed in packaging structures with different oxygen permeabilities. Six sets of samples were produced in triplicate with different packaging structures. They were evaluated with the presence of vacuum, and all samples were stored at 8ºC until the analysis.

The packages used in this work were as follows: (A) LDPE (low-density polyethylene) with vacuum and an oxygen permeability of 500 cm^3O_2 m^{-2} day^{-1} atm^{-1} at 23ºC; (B) Nylon polyester with vacuum and a permeability of 100 cm^3O_2 m^{-2} day^{-1} atm^{-1} at 23ºC; and (C) EVOH (formal copolymer of ethylene and vinylalcohol) with vacuum and an oxygen permeability of 5 cm^3O_2 m^{-2} day^{-1} atm^{-1} at 23ºC.

The packages were sealed with vacuum (Selovac, Steravec 750, SP, Brazil) at 5 bar and 1.36 s. The sausage stability was evaluated by physicochemical analysis (TBARS, index peroxide, hexanal, fatty acid composition, carbonyl and sulfhydryl groups) and sensory evaluation (oxidative rancidity) at 0, 7, 14, 21, 28 and 35 days of storage under refrigeration (8ºC). For sensory evaluation, the sausage was firstly thawed in a refrigerated environment (4°C) for 12 h and baked at 90°C for 40 min on an electric grill (Fischer, Brusque, SC, Brazil).

Physicochemical analysis

The 2-thiobarbituric acid reactive substances (TBARS) test was used to determine the extent of oxidative rancidity over the storage time. The TBARS test was performed according to Raharjo *et al.* (1992), modified by Wang *et al*. (2002). The measurements were carried out at 530 nm (Agilent 8533 spectrophotometer, Santa Clara, CA, USA).

The peroxide test was performed according to the methodology described by AOAC (2002). Initially, a fat sample was extracted with a cold solvent mixture using the Bligh-Dyer methodology (Bligh and Dyer, 1959). The peroxide value of the fat was determined by dissolving a weight of fat in a chloroform solution of acetic acid, adding potassium iodide and titrating the liberated iodine (I^{-1} to I_2 is oxidized by the peroxide sample) using a standard solution of sodium thiosulfate with starch as the indicator. The result was expressed in milliequivalents of peroxide per kg of sample.

The hexanal was extracted from the sample by the technique of solid phase microextraction (SPME - headspace method), according to the methodology described by Fernando *et al.* (2003)

with some modifications. For testing solid phase microextraction, the headspace method using an 85 μm Carboxen/PDMSfiber (Supelco Inc., Bellefonte, PA, USA) and 10 mL glass jars, sealed with aluminum seals and rubber with Teflon was used. After extraction (concentration) of the sample analyte, the fiber was retracted into the syringe and was exposed inside the injector of a gas chromatograph coupled to a mass spectrometer (GC/MSD Shimadzu GC17A, QP5050A, Kyoto, Japan) for 10 min of thermal desorption. A quantitative estimate of the concentration of hexanal was obtained using an external hexanal standard (0 to 2,442 ppm).

The lipid extraction of the sample was performed according to the methodology of Bligh and Dyer (1959). For the analysis of fatty acids, an aliquot of the lipid extract containing approximately 200 mg of lipids was dried in a rotary evaporator processed according to the method of Hartman and Lago (1973) using an ammonia solution of acid chloride sulfuric acid in methanol as an esterifying agent.

Fatty acids were determined by gas chromatography coupled to a mass spectrometer (GC/MSD, GC17A Shimadzu QP 5050A, Kyoto, Japan). The chromatographic conditions were initially preset; carrier gas: helium at a flow rate of 1 mL min-1 in the electronic impact mode of 70 eV and split mode (flow rate 20 mL min^{-1}), temperature of injector and detector at 250°C, and injection volume of 1 μL. The column temperature was programmed to 120ºC for 2 min, increased at 10°C min^{-1} at 180°C for 3 min, and increased at 5°C min^{-1} at 230°C for 2 min. Identification of fatty acids was performed by comparing the retention times of the fatty acids of the samples with known standards (methyl oleate, methyl stearate, methyl linolenate, methyl palmitate and methyl linoleate) - Sigma Aldrich Chemical Company, St. Louis, MO, USA. Results were expressed in gram of fatty acid per kg of sample.

Protein oxidation was measured by assessing carbonyl groups formed during the experiment using the methodology proposed by Levine *et al.* (1990) with slight modifications. The concentration of protein was measured at 280 nm (Agilent 8533 spectrophotometer, Santa Clara, CA, USA) in the chloride acid (HCl) control using bovine serum albumin (BSA) in 6 M guanidine as a standard. The carbonyl concentration in the treated sample was measured with 2,4-dinitrophenylhydrazine (DNPH) incorporated on the basis of a molar absorption coefficient of 21 nM cm^{-1} at 370 nm of protein hydrazones. Results were expressed as nanomoles of DNPH per milligram of protein.

The determination of sulfhydryl groups (thiol concentration) was performed according to the methodology described by Sover and Draper (2010) with some modifications. Sulfhydryl groups are determined by reaction with 5,5-dithiobis (2-nitrobenzoic acid) (DTNB). One gram of sample was homogenized with 50 mL of distilled water. The protein concentration of the homogenate was diluted to 2 mg mL^{-1} with 0.1 M phosphate buffer (pH 7.4), and the protein content was determined using the Biuret method (AOAC 2002), where 0.5 mL of the homogenate was transferred to a tube and dissolved in urea buffer (1:1). After adding 0.5 mL of DTNB and incubating at room temperature for 15 min, the reading was taken by a spectrophotometer (UV- visible Agitent 8453E, Santa Clara, CA, USA) at 412 nm. The sulfhydryl content was calculated using a molar extinction coefficient of 11,400 M cm^{-1} for 5,5-dithiobis at this wavelength. Results were expressed as nmol per mg total protein free sulfhydryl groups.

Sensory evaluation

Sensory evaluation was carried out by an experienced ten member trained panel. The performance of the assessors was checked with the following tests. Three representative samples of each descriptor were presented to the panel, and each assessor evaluated three repetitions of the three samples. The individual results of the assessors and of the descriptor (rancidity flavor) were statistically analyzed by analysis of variance (ANOVA) and Tukey's test at the 5 % level of significance. Analysis of variance of three factors (sample, repetition and panel consensus) was performed for each assessor with respect to the attribute, and the assessors were selected according to values of significant Fsample ($p<0.30$, or $p<30$ %) and non-significant Frepetition ($p>0.05$ or $p>5$ %). The assessors were then selected based on their ability to discriminate among different samples, repeatability and agreement with the group (Meilgaard *et al*., 2006).

Samples of sausage (approximately 2 cm^2) were distributed in plastic dishes coded with random numbers of 3 digits. Water and unsalted crackers were provided to clean the palate between samples. The sensory experiment was conducted according to a randomized design with a reference sample in each block (Meilgaard *et al*., 2006). Each experienced taster sampled and noted when the sample differed from the reference sample. The opinion was expressed on a 9 - point scale (0 - not rancid, 2 - slightly rancid, 4 - moderately rancid, 6 - very rancid, 8 - extremely rancid).

Statistical analysis

The results obtained in each analysis were subjected to an analysis of variance followed by Tukey's post-hoc test, using the software Statistica 6.1 (Statsoft, Tulsa, OK, USA). All analyses were performed considering a 95 % level of confidence ($p<0.05$).

Results and Discussion

Table 1 presents the values of the peroxide index of the Toscana sausage. As noted, the values gradually increased throughout the storage period, showing no tendency towards stabilization, independent of packaging used. On the seventh day of storage, the sample stored in packaging B (Nylon polyester) differed significantly ($p<0.05$) from the other packagings and presented on the 35th day a maximum value of 19.4 mEq kg^{-1}, although this period was not significantly different ($p<0.05$) from the other packagings.

The peroxide value is a very sensitive indicator in the initial stage of oxidation, as shown below, and its presence is evidence that the deterioration of taste and odoris due to its incipient instability (Sevanian and Hochstein, 1985; Kranner, 1994). In the literature, there are few papers expressing the peroxide values of fresh pork sausages as a function of storage time and type of packaging. Bezerra *et al.* (2012) found peroxide values of 9.7, 9.6 and 10.8 mEq kg^{-1} in samples of pork sausage packed in styrofoam trays covered with plastic wrap, in airtight packaging, and in vacuum packaging cooled to 5ºC, respectively.

Table 1 shows the TBARS values of samples from fresh Toscana sausage stored for 35 days under refrigeration (8°C) in various packaging structures (under vacuum). It appears that there was a significant progressive increase ($p<0.05$) in lipid oxidation. The TBARS of cooled Toscana sausage remained practically constant over the first 21 day period. In terms of packaging, LDPE (A) showed the highest values of lipid oxidation, differing significantly ($p<0.05$) from the other packaging, and the maximum level was observed at 28 days (0.72 mg MDA kg^{-1}). This difference can be attributed to the higher oxygen permeability (500 cm^3O_2 m^{-2} day^{-1} atm^{-1} to 23ºC). Packages of nylon polyester (B) and EVOH (C) showed similar lipid oxidation behavior. However, due to inhomogeneity in the fat percentage, there were some slight variations during the study period. Similar behavior has been reported in the literature (Almeida, 2005). Several authors have suggested that fluctuations in the TBARS values observed during the storage time are likely associated with increased concentrations of highly polar products resulting from the polymerization of secondary oxidation products. It was reported that malonaldehyde (MDA) reacts with a wide range of compounds and may form diene or triene MDA, which reduces the amount of MDA available to react with thiobarbituric acid, there by reducing measured TBARS values (Grau *et al*., 2001; Gatellier *et al.*, 2007).

The values found in this study were not high because of product refrigeration and the presence of sodium nitrite, which prevents lipid oxidation. Higher values have been reported, but there is still no consensus nor legal limit on the amount of TBARS. Jin *et al.* (2007) studied pork sausage, and reported TBARS values of 0.90-1.10 mg MDA kg^{-1} when stored at 0ºC for 2 to 4 weeks. Botes *et al.* (2005) suggested that the development of lipid oxidation in pork meat and meat products may vary significantly, sometimes reaching values of 1.5 to 2.0 mg MDA kg^{-1} in a few days and sometimes occurring so slowly that it is not considered a problem. Authors such as Maggioni *et al. (*2008) reported significantly higher values in the range of 1-6 mg MDA kg^{-1} when working with fresh meat stored under similar conditions. Ahmad and Srivastava (2007) reported studies in which samples of meat with TBARS numbers between 0.5 and 1.0 mg kg^{-1} showed a rancid odor.

Figure 1 shows the correlation of lipid oxidation (Peroxide and TBARS) of samples of Toscana sausages stored under refrigeration (8°C) in different packaging structures (LDPE, EVOH and nylon polyester with vacuum). It can be seen that TBARS values and peroxide index increased with time of storage. Specifically the samples stored in the LDPE packaging is observed higher TBARS values and consequently lower levels of peroxides, demonstrating that primary compounds (hydroperoxide) were oxidised in malonaldehyde and also after 35 days of storage are already detected hexanal in this packet.

The fatty acids were determined, and the results are shown in Table 2. The saturated fatty acids found were palmitic and stearic acids and oleic and linolenic acids were the unsaturated acids. The composition in fatty acids of the fat (28 %, wt) used to prepare fresh Brazilian Toscana sausage was 10.1 % palmitic acid, 24.6 % stearic acid, 63.6 % oleic acid and 1.76 % linolenic acid.

Regarding the composition of palmitic acid (Table 2), it is seen that on the maximum 35^{th} day, palmitic acid content was 34.5 g kg^{-1} in packaging B (nylon polyester), differing significantly ($p<0.05$) from the other packagings. This corresponds to 15.9 % of total fatty acids. The stearic acid showed no significant difference ($p>0.05$) for packaging studied

Table 1. Values of peroxide index (meq kg^{-1}) and TBARS (mg malondialdehyde kg^{-1}) of fresh Toscana sausage under refrigeration (8°C) in different packaging structures for 35 days

Packaging	Peroxide Index (mEq kg^{-1})					
	0 day	7 day	14 day	21 day	28 day	35 day
A	1.81^{Ae} (±0.68)	4.93^{Bd} (±1.07)	7.99^{Bc} (±0.99)	9.82^{Abc} (±1.23)	11.04^{Ab} (±0.048)	16.14^{Aa} (±1.36)
B	1.60^{Ad} (±0.010)	10.03^{Abc} (±1.08)	11.83^{Aba} (±0.96)	10.99^{Aba} (±2.11)	12.62^{Aac} (±2.54)	19.45^{Aa} (±2.07)
C	1.70^{Ad} (±0.010)	4.72^{Bc} (±0.68)	8.42^{Bbc} (±0.66)	10.94^{Ab} (±2.10)	11.75^{Ab} (±2.53)	17.21^{Aa} (±1.92)
	TBARS (mg malondialdehyde kg^{-1}) *					
A	0.084^{Ae} (±0.008)	0.330^{Ad} (±0.068)	0.421^{Acd} (±0.007)	0.534^{Ab} (±0.010)	0.721^{Aa} (±0.048)	0.481^{Abc} (±0.041)
B	0.064^{Ac} (±0.009)	0.229^{Ab} (±0.023)	0.264^{Bb} (±0.018)	0.332^{Ca} (±0.024)	0.344^{Ba} (±0.035)	0.366^{Ba} (±0.008)
C	0.065^{Ac} (±0.009)	0.335^{Aab} (±0.054)	0.268^{Bb} (±0.044)	0.429^{Ba} (±0.022)	0.424^{Ba} (±0.042)	0.394^{Ba} (±0.001)

*Mean ± (standard deviation) followed by lower case/upper case letters in the same row/column do not differ statistically at the level of 5 % (Tukey test); A - LDPE with vacuum, B - Nylon Poly with vacuum, C - EVOH with vacuum.

Table 2. Content of unsaturated and saturated fatty acids (g kg^{-1}) in fresh toscana sausage in refrigerator (8°C) in different packaging structures during 35 days

Oleic (C18:1)	Unsaturated Fatty Acids (g kg^{-1})					
	0 day	7 day	14 days	21 day	28 day	35 day
A	123.70^{Ab} (±0.005)	120.70^{Ab} (±0.02)	140.90^{Ab} (±2.33)	141.40^{Aa} (±0.02)	129.40^{Ab} (±0.04)	144.30^{Ab} (±0.01)
B	131.90^{Aa} (±0.11)	125.10^{Aa} (±0.19)	133.10^{Aa} (±0.01)	126.90^{Bb} (±0.04)	132.10^{Aa} (±0.08)	121.10^{Bc} (±0.07)
C	127.70^{Ab} (±0.05)	127.30^{Ab} (±0.24)	125.90^{Ab} (±0.05)	127.30^{Bb} (±0.03)	132.30^{Aa} (±0.01)	128.00^{Bb} (±0.02)
Linolenic (C18:3)						
A	3.70^{Ad} (±0.005)	3.40^{Ad} (±0.005)	4.90^{Aac} (±0.10)	4.90^{Aa} (±0.005)	4.70^{Aab} (±0.005)	4.10^{Abcd} (±0.005)
B	3.20^{Ac} (±0.005)	3.50^{Adc} (±0.011)	4.10^{Ac} (±0.005)	4.70^{Aa} (±0.023)	4.40^{Ab} (±0.005)	4.50^{Acd} (±0.005)
C	3.40^{Ac} (±0.004)	3.60^{Ad} (±0.005)	5.00^{Aa} (±0.005)	4.80^{Ab} (±0.001)	4.50^{Ac} (±0.002)	4.60^{Ac} (±0.005)
Palmitic (C16:0)	Saturated fatty acids (g kg^{-1})					
A	27.40^{Ac} (±0.010)	37.50^{Aa} (±0.005)	22.40^{Ac} (±0.16)	24.70^{Ad} (±0.011)	14.10^{Af} (±0.025)	20.30^{Ab} (±0.075)
B	25.30^{Bc} (±0.21)	28.50^{Bb} (±0.43)	21.20^{Ac} (±0.034)	19.80^{Ac} (±0.064)	16.00^{Ac} (±0.088)	34.50^{Ba} (±0.005)
C	24.40^{Aa} (±0.16)	24.00^{Ba} (±0.40)	21.10^{Aa} (±0.051)	20.00^{Aba} (±0.034)	15.50^{Ab} (±0.015)	20.60^{Aa} (±0.001)
Estearic (C18:0)						
A	52.50^{Aad} (±0.017)	46.70^{Abcd} (±0.023)	55.60^{Aab} (±0.91)	60.00^{Aa} (±0.005)	54.30^{Aac} (±0.017)	43.80^{Abcd} (±0.011)
B	47.50^{Acd} (±0.049)	49.40^{Ab} (±0.098)	46.60^{Bc} (±0.005)	52.20^{Ba} (±0.040)	48.50^{Abd} (±0.058)	48.60^{Abc} (±0.034)
C	46.80^{Ad} (±0.070)	49.60^{Abc} (±0.046)	52.60^{ABa} (±0.030)	49.90^{Bb} (±0.011)	48.10^{Acd} (±0.102)	49.90^{Ab} (±0.011)

*Mean ± (standard deviation) followed by lowercase/uppercase letters in the same row/column do not differ statistically at the level of 5 % (Tukey test); A - LDPE vacuum, B -Nylon Poly vacuum, C -with EVOH vacuum.

Table 3. Oxidation of protein - carbonyl (nmol carbonyl mg^{-1} protein) and sulfhydryl group of frescal Toscana sausage in different packaging structures for 35 days

Packaging	Oxidation of protein- Carbonyl group (nmol carbonyl mg^{-1} protein) *					
	0 day	7°day	14°day	2° day	28°day	35°day
A	7.11 ^{Aa}	16.61 ^{Bc}	19.51 ^{Dc}	21.28 ^{De}	22.95 ^{Ee}	24.51 ^{Et}
	(±0.05)	(±0.04)	(±0.07)	(±0.04)	(±0.05)	(±0.04)
B	7.35 ^{Aa}	12.30 ^{Cb}	14.24 ^{Cb}	18.85 ^{Dc}	17.78 ^{Dc}	20.31 ^{Dc}
	(±0.06)	(±0.09)	(±0.02)	(±0.07)	(±0.04)	(±0.22)
C	7.04 ^{Aa}	13.87 ^{Cb}	19.03 ^{Dc}	20.43 ^{Dc}	19.67 ^{Dc}	19.21 ^{Dc}
	(±0.02)	(±0.07)	(±0.06)	(±0.10)	(±0.10)	(±0.10)
	Oxidation of protein - Sulfhydryl group (µmoles sulfhydryl mg^{-1} protein)					
A	42.43 ^{Aa}	23.64 ^{Ab}	17.46 ^{Ac}	17.45 ^{Ac}	15.81 ^{Ac}	13.90 ^{Ac}
	(±2.86)	(±1.71)	(±1.15)	(±1.14)	(±0.53)	(±0.82)
B	41.52 ^{Aa}	29.65 ^{Ab}	21.81 ^{Abc}	21.30 ^{Ac}	16.66 ^{Ac}	16.47 ^{Ac}
	(±1.37)	(±6.48)	(±2.26)	(±1.84)	(±1.31)	(±0.72)
C	42.57 ^{Aa}	26.89 ^{Ab}	21.68 ^{Ac}	18.18 ^{Acd}	15.24 ^{Ad}	15.23 ^{Ad}
	(±2.34)	(±0.97)	(±1.86)	(±0.46)	(±2.11)	(±1.59)

*Mean ± (standard deviation) followed by lowercase/uppercase letters in the same row/ column do not differ statistically at the level of 5 % (Tukey test); A - LDPE vacuum, B -Nylon Poly vacuum, C -with EVOH vacuum.

during the storage period. On the 21st day, there was an increase in stearic acid values for the samples stored in the packaging of LDPE (A), corresponding to 60 g kg^{-1}. Pardi *et al.* (2001) reported that these fatty acids are the primary glyceride component of pigs, the main raw material used in the formulations of the present study.

The profile of the oleic acid (Table 2) of the Toscana sausage samples, after 28 days of storage, was not significantly affected ($p>0.05$) by the structure of packaging studied. The amounts of oleic acid in the samples varied between 124 and 144.3 g kg^{-1}. The linolenic acid was found to have a content of 5.0 g kg^{-1} for the sample stored in packaging EVOH on the 14th day of storage. The composition of linolenic acid experienced minor variations throughout the study period, and on the 35th day of storage, none of the selected packages differed significantly ($p<0.05$).

Baggio *et al*. (2004) reported finding approximately 132 g kg^{-1} of unsaturated fatty acids insamples of chilled Toscana sausage, values similar to those observed in the present study, where the average content of unsaturated fatty acids were 124 g kg^{-1}. Larkeson *et al*. (2000) obtained the same unsaturated fatty acids in meatballs and burgers as that of the present work.

Although the fatty acid composition is not the most important parameter to verify lipid oxidation, changes in fatty acid oxidation may occur to produce hexanal and pentane (Fenaille *et al*., 2003; Goodridge *et al*., 2003). However in the samples was not detected the presence of linoleic acid. It was observed in this study that hexanal was not detected during the first 28 days of storage, but on the 35th day traces were detected (<0.01 ppm) in the samples stored in the LDPE. In the literature, it is reported that the presence of hexanal is related to the oxidation of linoleic acid (Nelf *et al*., 1992; Araujo, 2009), as it is the main aldehyde formed by the decomposition of such fatty acids. The lower the linoleic acid concentration, the greater the resistance to oxidation and consequently, the formation of hexanal (Gerd, 2007).

The evolution of protein oxidation, by measuring the carbonyl and sulfhydryl groups of samples of Toscana sausage packed in containers of different structures for 35 days of storage are shown in Figure 2 (Table 3). The formation of carbonyl compounds (aldehydes and ketones) are one of the most prominent changes in oxidized proteins, serving as an indicator of protein oxidation (Levine *et al.* , 1990). There was a significant increase ($p<0.05$) in the oxidation of proteins starting from the 7th day, which continued to increase over the rest of the study period, especially for the samples stored in packaging A (LDPE), which reached the highest value of 24.5 nmol carbonyl mg^{-1} protein at the end of 35 days, significantly differing from the content of the samples stored in packagings B (Nylon polyester) and C (EVOH). It is noteworthy that the samples stored in polyethylene bags showed the highest content of carbonyl groups throughout the storage period. However, the sulfhydryl groups decreased over the studied period, with a reduction of approximately 40 % at time zero in relation to the 7th day of storage. The LDPE packaging showed the greatest reduction in relation to two other packaging structures.

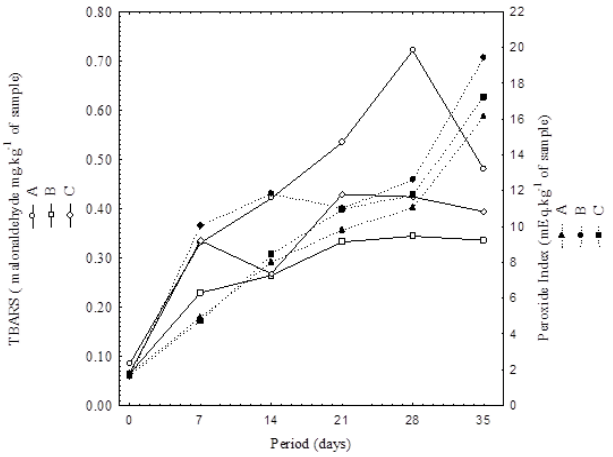

Figure 1. Correlation between the of peroxide and TBARS values of samples toscana sausage stored in different packaging structures (A - LDPE vacuum, B -Nylon Poly vacuum, C -with EVOH vacuum)

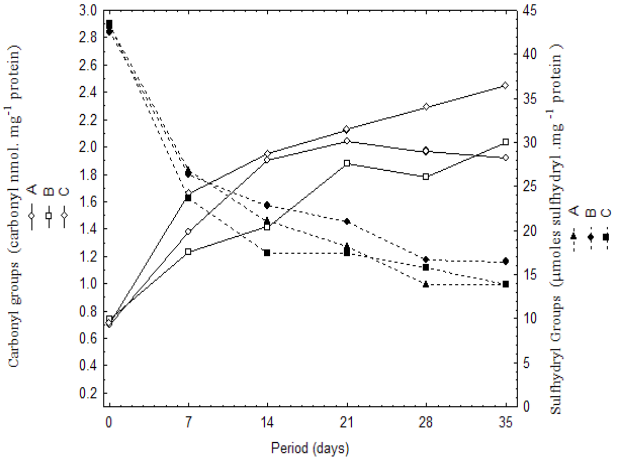

Figure 2. Correlation between the carbonyl and sulfhydryl groups of samples of toscana sausage stored in different packaging structures (A - LDPE vacuum, B -Nylon Poly vacuum, C -with EVOH vacuum)

Howell *et al*. (2001) and Gerrard (2002) showed that the oxidation of proteins is linked to the oxidation of lipids in meat products. Purchas *et al.* (2004) and Estévez and Cava (2004) reported a significant correlation between non-heme iron and the increase in oxidative processes, which may be a possible cause of the increased oxidation of the protein in Toscana sausage found in this study.

An important aspect from the point of view of the consumer is the possibility of detection of lipid and protein oxidation by sensory analysis. In this sense, the results suggest a relationship between the lipid and protein oxidation and possible changes in the sensory characteristics of the final product. It appears that over 28 days of storage, the samples packed in LDPE and nylon polyester showed a slightly rancid taste. One can see that from 28 days of storage, the sample stored in LDPE packaging showed a slight to moderate rancidity flavor (value of 2.8 - point scale), followed by the packaging of nylon polyester (value of 1.58 - point scale). After this time, there was a correlation between the rancidity flavor detected and that evaluated via the oxidation of lipids (Table 1 and their correlations in Figure 1) and protein (Table 3 and their correlations in Figure 2), and the sample packaging LDPE was characterized as being slightly to moderately rancid (value 3.06 - point scale).

As previously mentioned, some authors (Ahmad and Srivastava, 2007; Terra *et al*., 2008) reported correlations between perceived sensory rancidity and that determined by analysis of lipid oxidation, stating that low TBARS values are not sensorially perceived. However, at concentrations of 0.4 to 0.5 mg MDA kg^{-1}, slight to moderate rancidity was detected.

In summary, the results showed similar behavior of the samples stored in Nylon polyester and EVOH packaging. Although the samples stored in LDPE after 28 days of storage showed small changes in the TBARS values and oxidation of proteins - Carbonyl group was detected sensorially moderate flavor rancidity. In addition to these factors, the choice of the type of packaging will depend on the conditions of storage and transportation as well as the cost of packaging. In this aspect, the packaging LDPE has a value 1.56 is 1.44 times lower compared to the EVOH and Nylon polyester, respectively.

Conclusion

Fresh sausages present a reduced shelf-life, and new techniques should be developed to improve this negative aspect. Packaging in modified atmospheres is being introduced as a commercial way for the selling of sausages. Based on the results obtained in this work, we can conclude that after 28 days of storage of the sample in the vacuum packaging, low-density polyethylene showed a slight to moderate ($p<0.05$) rancid flavor, followed by nylon polyester vacuum packaging. A correlation was observed between the rancidity sensorially detected and that evaluated via the oxidation of lipids and protein.

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