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Physicochemical, microbiological and sensory changes during storage in "Paio do Alentejo", a traditional Portuguese Iberian sausage

By Miguel Elias and Alfonso V. Carrascosa

The present work studied the characteristics of "Paio do Alentejo", a traditional Iberian dry cured sausage, produced in Alentejo region, in two selected industrial plants, that use different methods; one of them used smoking and the other one did not use it. Both factories did not use starter cultures. In this research work the evolution of that product during its storage at 25 °C, vacuum and modified atmosphere packaged was studied. Changes on the main physicochemical parameters (pH, a_w, chlorides, TVBN, Titratable acidity, L*, a*, b*), microbiological counts (mesophilic aerobic bacteria, psychotropic, moulds, yeasts, *Lactobacillus* spp., lactic acid bacteria, Micrococcaceae, Enterobacteriaceae, and Enterococci) and sensory evaluation (colour intensity, off colour, aroma intensity, off aroma, taste intensity, off taste, salt content and global evaluation) were studied in the final product and also at 3, 6 and 9 months of storage. A multifactor ANOVA/MANOVA was made, considering "factory", "storage package" and "storage time" factors and their interaction. This research work evidences the importance of smoking in order to maintain physicochemical, microbiological and sensory characteristics of a high quality dry cured sausage from the Alentejano pig breed, using vacuum packaging and modified atmosphere packaging, at 25 °C, without energy consumption, during 9 month, at least .

Keywords

- Storage
- Iberian sausage
- Paio
- Fermented meat sausages
- Physicochemical characteristics
- Microbiological parameters
- Sensory evaluation.

Alentejano pig breed is a Portuguese autochthonous breed produced in Alentejo region, south east of Portugal, similar to the Iberian pig breed. Alentejano dry-cured sausages are high-value products, manufactured with traditional ingredients and technologies, mixing ingredients, casing, smoking, curing and ripening, generally in small factories, without starter cultures. In Mediterranean countries the consumption of dry-cured sausages is widespread and in Portugal the most popular dry-cured sausages are "Paio" and "Linguiça". Hermetic packaging has been popular for cured meats conservation and maintains its popularity nowadays (Gök et al., 2008). Vacuum packaging has been used in the meat industry for quite a long time and has been accepted by consumers. Vacuum packaged flexible plastic film bags have low gas and water vapour permeability and adhere closely to the product.

However, vacuum packaging is reported to cause liquid exudation and product deformation (Santos et al., 2005). An alternative packaging method to overcome this problem is modified atmosphere packaging, using several mixtures of gases. The most frequently used gases are oxygen (usually for fresh meats), nitrogen and carbon dioxide. This one has been reported to be extensively used in raw, cooked and cured products because of its strong inhibitory effect on microorganisms. Several researchers reported the efficacy of carbon dioxide (20–30%) against aerobic microorganisms (Sørheim et al., 2004). Nitrogen does not have a strong inhibitory effect on microorganisms, and it is generally used in modified atmosphere packaging as a filler gas.

Although some physicochemical and microbiological characteristics of Spanish Iberian sausages like "chorizo" and "salchichón" has been described (Santos et al., 2003; Martín et al., 2007; Benito et al., 2007; Andrade et al., 2010), and to characterise Portuguese Iberian sausages (Elias and Carrascosa, 2010), no attention has been paid to the influence of vacuum or modified atmosphere packaged technology on this kind of sausages.

The aim of the present study was to investigate the technological process effect (not smoked or smoked), packaging method (vacuum or modified atmosphere 20:80 CO_2/N_2) and storage time on the physicochemical, sensorial and microbiological characteristics of "Paio do Alentejo". This is an up to date study for this Portuguese product, Alentejano pig breed sausage, considering that one of the most widely used methods for the conservation of Portuguese sausages is vacuum and 20 : 80 CO_2/N_2 atmosphere packing.

Materials and methods

Sausage technology and sampling procedures

Traditionally "Paio do Alentejo" has a cylindrical shape, variable diameter (4–5 cm), and a length of 25–30 cm. It is made with 70–80% of lean pork meat and 20–30% of pork back fat from Alentejano pig breed.

"Paio do Alentejo" sausages were prepared in two local meat factories located in the Alentejo region. The first one (named as A) was a factory at the village of Barrancos (south of "Alentejo") that produces 200 t of sausage annually, without using smoking; the second one (named as B) was in the village of Estremoz (north of "Alentejo") and produces 600 t annually, with smoking; both factories produce sausages using traditional techniques and did not use microbial starter cultures.

In factory A, meat and fat were mechanically minced in cubic portions with 25 mm per side. After mincing, pork was mixed with "pimiento" paste (3%), garlic paste (2.2%), water (3.5%), salt (2%), 0,02% of disodium diphosphate and 0,02% of pentasodium triphosphate, 0,039% of $NaNO_3$, 0.008% of KNO_3 and 0.0076% of KNO_2. The mix was kept in controlled storage rooms at 6 °C and 80% of relative humidity for 96 hours, for maturing. After that, the mix was stuffed into natural pig casings, with 50–60 mm of diameter, and dried in controlled chambers at 10–12 °C and 75–80% of relative humidity, for 30 days, and in natural storage rooms at 19.5 °C and 65.7% of relative humidity on average, for 35 days. In factory B, meat and fat were also minced in cubic portions with 25 mm per side. After mincing, pig ingredients were mixed with "pimiento" paste (4%), garlic paste (3.5%), water (4%), salt (4%), 0.04% of P_2O_5, 0.039% of $NaNO_3$,

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0.008% of KNO_3 and 0.0076% of KNO_2. The mix was kept in controlled storage rooms at 6 °C and 85% of relative humidity for 48 hours, for maturing. After maturing, the mix was stuffed into natural casings from pig large intestine, with 50–60 mm of diameter, and smoked for 12 days at 16–27 °C in a smoking chamber using holm oak wood. After smoking, drying was carried out in controlled storage rooms at 15–17 °C and a relative humidity between 65–80%, for 21 days.

Dry cured sausages were used to perform physicochemical, microbiological and sensory analysis. At the same time, dry cured sausages from the same batches were individually packed under vacuum (V) and under modified atmosphere (MAP), with 20% CO_2 and 80% N_2, using a multilayer film composed by polyethylene, ethylvinylalcohol and polyamide, with 35 $cm^3/m^2/24h/bar$ of oxygen permeability, 150 $cm^3/m^2/24h/bar$ of CO_2 permeability and 20 g/m^2/24h of steam permeability.

Packed end products were stored at room temperature (25 °C). Samples of those products were analysed at 0 (before packaging), 3, 6 and 9 months of storage (T0, T3, T6 and T9). Five samples from each factory at each storage time were analysed.

Physico-chemical analysis

After removing the sausage casings, physicochemical parameters were determined (pH, a_w, chlorides, water content, total protein, fat, total ash, TVBN and colour). The colour determination was carried out immediately after sample laboratory arrival, without mixing or homogenising, with a Minolta CR-300 colorimeter. Colour CIE L*a*b* parameters (L*: lightness, a*: redness, b*: yellowness) were measured in flesh meat (avoiding fat) with a Minolta CR-200 colorimeter (ARTIGAS, GIL and FELIPE, 1985).

pH was measured with a pH-meter (Crison 507, Barcelona, Spain); water activity (a_w) with a hygrometer (Hygroskop Rotronic DT, Zurich, Switzerland) with WA-40 probe, at 25 °C; chlorides (AOAC, 1990) giving all the results as percentages; water content (ISO, 1997) as percentage.

Total volatile basic nitrogen (TVBN) was determined following the Conway's cells method (PEARSON, 1976), the results were given in mg NH_3 per 100g of the product. Titratable acidity was measured according the method of AOAC (2000) and expressed as % oleic acid based on dry weight.

Microbiological analysis

Sampling procedures were carried out aseptically in the factories and in the laboratory. Samples were taken aseptically in a laminar air flow cabinet (Telstar AV-100, Barcelona, Spain), after removing the casing. Counts of mesophilic aerobic bacteria, psychrotrophic, moulds, yeasts, *Lactobacillus* spp., lactic acid bacteria, Micrococcaceae, Enterobacteriaceae, and Enterococci were carried out.

Ten grams of each sample were homogenised with 90 ml of a sterile solution of tryptone (0.3%) and NaCl (0.85%) for 2 min in a Stomacher 400 Lab Blender (Seward Medical, London, UK). Tenfold dilutions were made in the same diluent. Mesophilic aerobic bacteria and psychrotrophic counts were made in Tryptone-Glucose-Extract Agar (Merck), incubating respectively at 30 °C during 48 h and at 6.5 °C for 10 days; yeasts and moulds in yeast extract Agar (Merck), with 0.5% of cyclohexamide, incubating at 25 °C for 5 days; *Lactobacillus* spp. and lactic acid bacteria in agar MRS (Man, Rogosa and Sharpe, Oxoid) at pH 5.7 and 6.2 respectively, incubating for 72 h at 30 °C in GasPak jar (Oxoid); Micrococcaceae in MSA (Manitol Salt Agar, Oxoid), incubating for 72 h at 30 °C, taking care to avoid counting non characteristic colonies and manitol+ colonies for being presumptive bacilli; Enterobacteriaceae in VRBG agar (Oxoid), incubating at 37 °C for 48 h and counting characteristic colonies; and Enterococci in KAA Agar (Oxoid), incubating at 37 °C during 48 h, and counting characteristic colonies, expressing the results as means± standard deviation of the log cfu/g.

Sensory evaluation

A panel of 14 trained judges aged from 29 to 65 evaluated five sausages samples per factory at each storage time (T0, T3, T6 and T9). Sensory evaluation was made between 10 pm and 12 pm in a room according to the one described by COSTELL and DURÁN (1981 a,b,c,d). Packed whole sausages were removed from their packages 2 hours before sensory evaluation and sliced (3 mm thick) 30 minutes before this analysis. A group of five sausages was presented per session in a randomised order. For each dry cured sausage three slices were presented on a small white plate carrying a three digit random number. The descriptive quantitative method was adopted, using a structured scale using a 100 point intensity scale ranging from 0 (sensation not perceived) to 100 (maximum of the sensation).

Tab. 1: Mean ± standard deviation of the physico-chemical properties of the Portuguese Iberian sausage Paio do Alentejo, considering the factors: factories (A and B), storage method (vacuum and modified atmosphere) and storage time (0, 3, 6 and 9 months).

	Storage package	Storage time	pH	a_w	Chlorides (%NaCl)	TVBN ($mgNH_3$/100g product)	Titratable acidity (% oleic acid based on dry weight)	L*	a*	b*
Factory A	Vacuum	0 m	5.43±0.43	0.820±0.063	5.02±0.41	101.23±6.81	6.55±1.02	40.42±4.21	17.81±5.11	13.77±5.91
		3 m	5.84±0.25	0.777±0.073	4.83±0.44	176.35±9.79	14.35±0.40	39.19±7.54	15.29±5.78	14.21±9.57
		6 m	5.76±0.04	0.783±0.015	4.63±0.13	237.20±9.13	23.30±1.79	46.40±1.79	14.77±1.73	12.29±0.60
		9 m	5.27±0.08	0.725±0.024	4.21±0.07	203.60±4.72	41.67±4.93	51.72±1.97	25.78±0.45	31.53±1.29
	MA	0 m	5.43±0.43	0.820±0.063	5.02±0.41	101.23±6.81	6.55±1.02	40.42±4.21	17.81±5.11	13.77±5.91
		3 m	5.91±0.24	0.786±0.058	4.51±0.41	195.55±18.02	14.65±0.94	42.31±8.12	17.02±3.32	14.90±4.55
		6 m	5.67±0.14	0.758±0.038	4.24±0.14	158.63±6.16	22.60±0.89	48.87±3.72	20.62±1.69	22.83±1.21
		9 m	5.50±0.22	0.738±0.041	4.41±0.18	219.47±18.93	41.00±4.73	48.86±1.88	24.22±2.32	27.90±4.46
Factory B	Vacuum	0 m	5.76±0.26	0.828±0.068	5.97±0.49	97.25±19.32	5.64±1.67	43.12±6.53	16.39±3.97	12.83±7.68
		3 m	5.70±0.17	0.803±0.076	5.81±0.01	140.10±49.96	10.37±0.54	37.10±3.19	15.47±2.91	10.08±3.16
		6 m	5.64±0.13	0.803±0.046	5.72±0.29	139.14±34.49	16.93±5.66	49.68±8.55	17.41±2.85	17.38±7.30
		9 m	5.70±0.09	0.762±0.039	5.80±0.04	155.10±22.23	22.50±4.88	45.83±5.34	17.52±2.97	14.07±3.94
	MA	0 m	5.76.±0.26	0.828±0.068	5.97±0.49	97.25±19.32	5.64±1.67	43.12±6.53	16.39±3.97	12.83±7.68
		3 m	5.80±0.19	0.814±0.031	5.81±0.01	133.97±35.84	11.57±0.63	39.14±8.34	11.78±3.16	8.48±3.76
		6 m	5.78±0.14	0.771±0.033	5.57±0.53	128.51±30.94	17.54±4.09	46.78±7.16	13.98±2.60	9.79±0.98
		9 m	5.67±0.10	0.777±0.044	5.62±0.28	184.90±35.43	21.77±3.37	52.77±2.82	19.68±1.45	20.40±3.91

Source: ELIAS and CARRASCOSA FLEISCHWIRTSCHAFT International 2/2013

The attributes colour intensity, off colour, aroma intensity, off aroma, taste intensity, off taste, salt content and global evaluation were considered. For the salt content attribute, the optimum value considered was 50. Values below 50 indicate a low salted product and above 50 salty sausages. Judges rinsed their mouth with neutral water and a melba toast between samples.

Statistical analysis

An analysis of variance (ANOVA/MANOVA) was performed using "factory", "storage time" and "packaging system" as factors. "Factory x storage time", "factory x package procedure" and "storage time x package procedure" interactions and post-hoc contrast (HSD Turkey) were performed using a STATISTICA software package v.5.1.

Results and discussion

Physicochemical results

According to ANOVA/MANOVA results, physicochemical parameters were different between factories (excepting L*), were not influenced by the package type (vacuum or modified atmosphere) and exhibit significant differences ($p<0{,}001$) among the storage period.

In Factory B the pH values were stable during the storage period (Tab. 1), probably in consequence of microorganisms' inhibition by smoke. The pH variation in factory A was probably due to proteolysis, during the first tree months of storage, producing free amino acids resulting in an increase of the pH values. Several pH results were reported for Spanish sausages with long ripening times and with no initial fermentation period at high temperatures (LORENZO et al., 2000; MARTÍN at al., 2007) and Italian sausages (MAURIELLO et al., 2004; CENCI-GOGA et al., 2008). After this period pH values decreased continuously (Tab. 2), maybe due to lactic acid bacteria activity. A similar opinion was formulated by GÖK et al. (2008). The multilayer film used as package material was permeable to water and consequently a_w decreased during storage time. Chlorides were higher in factory B due to a more salt addition in sausage preparation and a higher water loss during the curing period in this factory. Curiously, during the storage period chlorides decreased. One possible reason for this fact was the accumulation of water in the casing with the origin in meat used for the sausage. The chlorides perhaps were migrated with water and consequently their level decrease in meat sausage. TNBV increased during storage period, with higher values in sausages from factory A. The role of enzymes with peptidase activity from microorganisms were related with high TNBV levels, as well as showed by FADDA et al. (1999) for *Lactobacillus* spp. activity, and SELGAS et al. (1993) and BOVER-CID et al. (1999a,b) for Micrococcaceae. That activity could be related with a more intensive catabolic metabolism of peptides from bacteria in order to obtain energy, once sausages are poor in carbohydrates (MARTÍN-SÁNCHEZ et al., 2011). In our study, the presence of smoke and the lower counts in lactic acid bacteria in factory B sausages explains, probably, the lower TNBV levels. In the present work, titratable acidity always increased during storage time. Despite microaerobic conditions inside the packages, in consequence of the reduced oxygen permeability of the package material, lipolytic activity persisted, more intensively in factory A, where products are not smoked. Among the studied microorganisms, probably yeasts and Micrococcaceae were the most lipolytic. However, yeasts decreased significantly during storage and Micrococcaceae were higher in products from factory B, where titratable acidity was lower. Considering these facts lipolysis increase during storage, could be due to a meat endogenous lipases, namely acid lipase, active at pH values usually found in fermented sausages (ORDOÑEZ, 1999). L* a* b* values increased during storage time. Oxidative processes result in fat yellowness and consequently higher b* values. In the present study chlorides decreased during storage time and in the literature it is reported that greater NaCl availability induces both, an increase in the red intensity and a reduction in the yellow intensity (PÉREZ-ALRVAREZ et al., 1999; SUMMO et al., 2006).

Considering "factory x storage time" significant interaction for TNBV, in both factories values increased significantly between T0 and T9. Values of proteolysis always were significantly higher in factory A. ROSEIRO et al. (2010) had studied a proteolysis of one type of Portuguese traditional sausage similar to those produced in factory B and concluded that processing environmental conditions applied during the initial stages, drying and smoking phases, had remarkable influence on the accumulation of free amino acids in the final product. In the same work, those authors evidenced the increase of free amino acids during vacuum storage at room temperature. Titratable acidity results always increased in both factories and were significantly higher in factory A. The colour coordinates b* from products of factory A were always higher than in factory B and at T9 significantly higher than in factory B. These results occurred in consequence of high titratable acidity in factory A, consequence of higher lipolysis in intramuscular fat resulting in yellowness. "Storage process x storage time" interaction for TNBV revealed differences

Tab. 2: Mean ± standard deviation of microbiological results of the Portuguese Iberian sausage Paio do Alentejo, considering the factors: factories (A and B), storage method (vacuum and modified atmosphere) and storage time (0, 3, 6 and 9 months).

	Storage Package	Storage time	Mesophilic aerobic bacteria	Psychro-trophic	Moulds	Yeasts	*Lacto-bacillus* spp	Lactic acid bacteria	Micro coccaceae	Enterobac-teriaceae (log cfu/g)	Entero-cocci (log cfu/g)
Factory A	Vacuum	0 m	6.60±0.65	5.73±0.80	1.36±1.80	5.05±1.31	7.82±0.70	7.68±0.70	5.98±0.76	0.14±0.53	0.82±1.02
		3 m	4.83±0.52	0.76±1.69	0.00±0.00	2.82±0.75	4.47±0.59	4.66±0.13	4.62±0.48	0.00±0.00	2.70±1.15
		6 m	5.38±0.07	0.54±1.21	0.00±0.00	0.00±0.00	3.65±0.42	3.48±0.54	4.41±0.19	0.00±0.00	0.26±0.58
		9 m	7.56±0.33	1.58±2.21	0.00±0.00	5.36±0.51	6.41±0.93	6.19±0.25	5.55±0.40	3.81±0.78	0.00±0.00
	MA	0 m	6.60±0.65	5.73±0.80	1.36±1.80	5.05±1.31	7.82±0.70	7.68±0.70	5.98±0.76	0.14±0.53	0.82±1.02
		3 m	5.05±0.79	0.00±0.00	1.66±1.61	3.22±1.39	4.86±0.23	4.75±0.43	5.04±0.73	0.00±0.00	1.33±1.88
		6 m	4.69±0.17	0.40±0.89	0.00±0.00	0.00±0.00	3.51±0.31	2.93±0.09	4.57±0.19	0.00±0.00	0.00±0.00
		9 m	6.94±1.44	0.70±1.56	0.00±0.00	5.08±0.56	5.04±0.48	5.96±0.44	5.22±0.44	3.27±0.74	0.00±0.00
Factory B	Vacuum	0 m	5.86±0.35	4.41±0.69	0.76±1.39	3.68±0.59	6.32±0.51	6.18±0.60	6.12±0.58	0.47±1.11	0.31±1.09
		3 m	4.01±1.10	0.60±1.34	0.00±0.00	0.40±0.89	5.25±0.69	3.54±0.51	6.04±0.87	0.00±0.00	0.86±1.18
		6 m	4.75±1.44	0.00±0.00	0.00±0.00	2.45±3.36	3.06±0.24	2.94±0.15	6.29±0.40	0.00±0.00	0.34±0.76
		9 m	2.80±0.13	0.00±0.00	0.80±1.12	0.76±1.69	3.50±1.07	0.00±0.00	6.56±0.19	0.00±0.00	0.2±0.45
	MA	0 m	5.86±0.35	4.41±0.69	0.76±1.39	3.68±0.59	6.32±0.51	6.18±0.60	6.12±0.58	0.47±1.11	0.31±1.09
		3 m	3.58±0.83	0.00±0.00	0.00±0.00	0.84±1.15	5.27±1.59	3.67±0.61	6.08±1.30	0.00±0.00	0.46±1.03
		6 m	3.33±1.00	0.00±0.00	0.00±0.00	2.91±2.78	2.53±0.20	2.31±0.24	5.42±0.28	0.00±0.00	0.52±1.16
		9 m	3.64±1.24	0.00±0.00	0.00±0.00	1.09±2.44	3.66±1.68	0.00±0.00	6.64±0.22	0.00±0.00	0.00±0.00

Source: ELIAS and CARRASCOSA

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between vacuum package and modified atmosphere only in T6, vacuum packed sausages having higher values. Considering interaction "factory x storage process", for sausages packaged in modified atmosphere redness (a*) was significantly higher in those produced in factory A. SOBKO et al. (2005) referred to a strong correlation between bacterial acid production and ($pH \leq 5,5$) and NO formation in products with addition of nitrate/nitrite and the red colour intensity could be due to a MbFe^{II}NO concentration. RUBIO et al. (2008) have found significantly higher values for the redness value (a*), which is used as an indicator of colour stability in meat and meat products, in "salsichón" packed in modified atmosphere (20%/80% CO_2/N_2) than "salsichón" packed in vacuum packages. In this study L* and a* were higher ($p<0.05$) at 210 days of storage than values found at 0 days.

Microbiological results

Considering microbiological results, ANOVA/MANOVA evidenced that the conservation process (vacuum or modified atmosphere) did not interfere with these results, despite the fact that previous research (DEVLIEGHERE and DEBEVERE, 2000) demonstrated that the concentration of dissolved CO_2 in the water phase of a food determined the growth inhibition of microorganisms in a modified atmosphere. The sausage's origin (factory A or B) and storage time influenced significantly microbiological population. "Factory x storage process" interaction was not significantly for any variable, the "factory x storage time" interaction was not significantly only for psychotropics and the "storage process x storage time" interaction was significantly only for mesophilic aerobic bacteria counts. Generally sausages from factory A had a high number of microorganisms but Micrococcaceae and *Clostridia* sulphite reducers were significantly higher in products from factory B.

Mesophilic aerobic bacteria microorganisms in sausages from factory A decreased between T0 and T6 and then increased significantly until T9 (Tab. 2). In this last period yeasts increased notoriously, too, probably anaerobic facultative yeasts, whose development may contribute to mesophilic aerobic bacteria increase. Lactic acid bacteria increased, too, in factory A, from T6 to T9, and contributed for the increase in mesophilic aerobic bacteria. Usually, proteolysis and lipolysis improve yeast's growth. Sausages from factory A exhibited an increase of *Lactobacillus* spp., lactic acid bacteria and Micrococcaceae during storage time and these could contribute to sausage proteolysis which generally promote microbial growth. The origin of lactic acid bacteria and Micrococcaceae in sausages is related with their presence in fresh meat. In sausages from factory B microorganisms decreased during storage time but Micrococcaceae counts remained constant during the storage period. In several traditional fermented sausages lactic acid bacteria constituted the major microflora of the sausages (LIZASO et al., 1999; GONZÁLEZ and DÍEZ, 2002; COMI et al., 2005; DROSINOS et al., 2005; RUBIO et al., 2007). Despite these facts, due to good adaptation of lactic acid bacteria to the meat environment and their faster growth rates during fermentation and sausage ripening, they become the dominant microflora (DROSINOS et al., 2005). Enterobacteriaceae remained in low levels until T9, in factory B, and until T6 in factory A. In this one, Enterobacteriaceae increased from 0 log cfu/g in T6 up to 3 log cfu/g in T9. This increment could be related with substrate degradation which created the conditions for Enterobacteriaceae growth, which until then existed in a small number, undetectable from the analysis method used. In factory B Enterobacteriaceae were not detected and in both factories Enterococci decreased during storage time. In other studies (RUBIO et al., 2007) the Enterobacteria counts were subjected to a significant inhibition and values under the detection limit were found after 15 days of storage, probably due to astrong competitive effect of lactic acid bacteria on the rest of the endogenous flora.

The interaction "factory x storage time" evidenced that the mesophilic aerobic bacteria were always higher in factory A and in this factory increased significantly between T6 and T9, probably due to substrate changes with aerobic facultative and microaerophilic microorganisms growth. On the other hand mesophilic bacteria counts in T0 were significantly higher than at other sampling dates. Yeast counts were significantly higher in factory A, except in T6 with values significantly higher in factory B. Micrococcaceae were significantly higher in factory B.

Sensory results

Considering the ANOVA/MANOVA results for the sensory evaluation, all attributes exhibited significant differences for the factory factor, for the packaging system factor only off taste did not exhibit differences and for the storage time factor only the salt intensity attribute reveals significantly differences. "Factory x method of conservation" and "method of conservation x storage time" interactions did not exhibit significant differences for any attribute. "Factory x storage time" interaction results were significant for off colour ($p<0.05$), aroma ($p<0.05$), off taste ($p<0.001$) and overall evaluation ($p<0.001$)

Tab. 3: Mean ± standard deviation of sensory evaluation results (values obtained in a scale from 0 to 100), of the Portuguese Iberian sausage Paio do Alentejo, considering the factors: factories (A and B), storage method (vacuum and modified atmosphere) and storage time (0, 3, 6 and 9 months).

	Storage method	Storage time	Colour intensity	Off colour	Aroma intensity	Off aroma	Taste intensity	Off taste	Salt content	Overall evaluation
Factory A	Vacuum	0 m	63.63±16.87	4.53±9.46	61.68±16.22	5.85±12.55	67.47±14.79	5.34±8.90	57.39±13.45	59.08±16.12
		3 m	61.11±15.94	4.10±8.60	56.24±15.74	3.95±8.18	63.16±16.32	10.45±18.88	59.30±13.05	51.44±18.02
		6 m	62.50±14.42	5.03±10.21	65.31±13.22	4.78±10.00	65.37±16.91	16.63±20.01	59.37±8.95	43.43±20.19
		9 m	60.53±11.92	10.58±13.78	56.47±18.11	6.63±11.94	68.77±16.67	20.84±21.03	59.16±8.20	37.83±16.53
	MA	0 m	63.63±16.87	4.53±9.46	61.68±16.22	5.85±12.55	67.47±14.79	5.34±8.90	57.39±13.45	59.08±16.12
		3 m	58.42±14.57	5.28±9.93	57.55±13.69	2.65±6.13	61.02±17.20	6.88±12.68	59.37±11.39	51.86±18.57
		6 m	61.28±13.97	3.09±6.63	62.02±11.20	4.72±10.36	63.55±16.48	11.02±16.23	58.19±8.18	49.25±21.34
		9 m	62.35±13.28	7.24±11.62	56.34±16.25	5.83±9.59	67.18±13.90	18.53±20.18	57.44±9.75	42.94±19.47
Factory B	Vacuum	0 m	70.40±13.24	1.26±3.88	66.76±13.99	2.22±5.78	68.68±14.88	3.33±7.36	61.18±13.22	64.21±15.80
		3 m	68.56±13.36	1.31±4.23	65.53±13.38	1.98±4.73	67.31±14.31	3.94±8.41	62.56±13.07	62.16±16.09
		6 m	69.08±13.21	0.99±4.18	64.54±13.73	2.05±5.09	65.42±14.86	4.95±13.32	62.72±11.53	60.25±17.57
		9 m	70.07±13.46	2.01±6.24	61.38±15.53	3.21±7.77	69.08±12.48	7.29±13.70	63.50±12.94	60.67±16.28
	MA	0 m	70.40±13.24	1.26±3.88	66.76±13.99	2.22±5.78	68.68±14.88	3.33±7.36	61.18±13.22	64.21±15.80
		3 m	68.35±13.90	0.99±2.97	60.78±15.92	2.02±5.04	67.65±12.95	3.42±7.51	62.23±12.22	61.53±16.11
		6 m	69.25±12.51	1.31±4.89	61.53±14.10	1.81±4.44	66.47±13.60	5.88±14.99	61.94±10.54	59.59±17.26
		9 m	64.26±15.03	3.26±8.36	60.75±15.17	2.35±7.11	69.38±12.86	5.36±12.69	62.21±11.76	63.26±14.87

Source: ELIAS and CARRASCOSA

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attributes. Sensory characteristics were not influenced by the preservation method but different processes adopted in both factories and storage time were responsible for differences in sensory characteristics. In the sausages produced in factory A off colour, off aroma and off taste increased during storage time, mainly in vacuum packaged sausages (Tab. 3) and off colours and off tastes were significantly higher in the same factory. In consequence of these results overall evaluation decreased during storage time in factory A. In factory B the attributes classification punctuation were maintained stable during storage time and off colours, off aromas and off tastes classification punctuations were lower than those of factory A and without significant increase during storage time. In factory B, with lower a_w values than in factory A, aroma intensity was significantly higher and overall evaluation was significantly higher, too, more and more evident with the increase of the storage period. Lower a_w values limited lipolytic and proteolitic processes which altered the sausage's sensory characteristics. In factory A sausages, with higher a_w values, proteolisis were more intense (confirmed by ABVT values) resulting in changes in textural characteristics and in the production of off aromas and off tastes. According to Summo et al. (2010) the oxidative processes resulted to be more intense in the initial phases of storage in vacuum-packed ripened sausages, probably due to the higher availability of oxygen. On the other hand, hydrolytic phenomena affecting the lipid fraction continued with significant increasing of the amount of hydrolysis products during the whole storage period, supported by the high moisture level of the product. Despite the extreme importance of proteolysis for the development of the final texture, and for taste and flavour (Ordoñez et al., 1999; Roseiro et al., 2008; Toldrá, 2002; Verplaetse, 1994; Martín-Sánchez et al., 2011), excessive proteolysis results in texture, taste and flavour defects. Considering 50 as minimum limit in order to accept sausages based in global evaluation classification punctuation, in factory A vacuum packed sausages were acceptable only during 3 months of storage and during 6 months for modified atmosphere packed sausages. The products from factory B maintained acceptable during all the time of the study. The main reason for a low classification punctuation in the overall evaluation attribute in factory A was the off tastes detection by the panellist. Several studies of traditional sausages were made by various authors (Pérez-Cacho et al., 2005; Rason et al., 2007; Summo et al., 2010; Martín-Sánchez et al., 2011).

Conclusions

The physicochemical parameters were influenced by different methods used in both factories (with lower variation in factory B) and storage time, and were not influenced by the packaging system. The microbiological flora was modified by the sausage origin (factory A or B) and the storage time but there were no differences in the microbiological population when comparing the two packaging systems studied. The results of the sensory analysis, similarly to the microbiological results, were influenced by the origin of the sausages and the packaging system. In factory A vacuum packed sausages were acceptable from the sensory point of view only during the first three months of storage, and during six months for modified atmosphere packed sausages. However, sausages from factory B exhibited better microbiological and sensorial quality. The judges gave a higher score to the sausages from factory B, excluding off colour, off aroma and off taste attributes, and in this one overall evaluation attribute scores were maintained from T0 to T9, both in vacuum packed and modified atmosphere packed sausages.

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