Research Article

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Lipid degradation and sensory characteristics of ripened sausages packed in modified atmosphere at different carbon dioxide concentrations

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Abstract

BACKGROUND: Conflicting results about the effect of modified atmosphere packaging (MAP) rich in CO_2 on the quality of different kinds of meat products are present in the literature. In this study, the degree of lipid degradation and the sensory characteristics of ripened sausages packed in modified atmosphere at three different carbon dioxide (CO_2) concentrations were evaluated during 5 months of storage.

RESULTS: The degree of hydrolytic degradation of the lipid fraction was found to decrease with increasing CO_2 concentration. Similarly, oxidative phenomena occurred at a lower rate when the CO_2 concentration increased. The variations in CO_2 concentration influenced the perception of rancid flavor in the examined sausages.

CONCLUSION: An increase in CO_2 concentration in MAP slowed down the evolution of lipid oxidation owing to the minor extent of hydrolytic degradation, whose products have pro-oxidant activity. This effect was more evident in the first 2 months of storage.

Keywords: carbon dioxide; lipid oxidation; modified atmosphere packaging; ripened sausages; sensory evaluation

INTRODUCTION

The quality of dry-cured meat products is basically due to the development of good aroma with absence of off-flavors. The development of the characteristic aroma of sausages is the result of complex combinations of volatile (aldehydes, ketones, esters and alcohols) and non-volatile (amines, amino acids and small peptides) compounds. The majority of these substances are formed during ripening as a result of enzymatic processes (glycolysis and proteolysis) or chemical reactions (lipid oxidation and Maillard reaction).[1]

On the other hand, some microbial activities (carbohydrate fermentation and amino acid catabolism) and chemical reactions may take place also during storage, with the consequent development of volatile compounds able to confer off-flavors to dry-cured meat products. For example, nonanal, hexanoic acid and nonanoic acid, derived from lipid autoxidation, are related to the development of rancid, sour and woody off-flavors and can thus compromise product acceptability.[2] In contrast, volatile compounds such as 3-methylbutanal and diacetyl, derived from microbial activities, confer buttery and sweet odors with very low sensory thresholds.[3]

To prolong the shelf life of dry-cured meat products, various solutions have been proposed, such as the use of vacuum packaging or modified atmosphere packaging (MAP). Vacuum packaging involves a reduction in air total pressure without replacing it by another gas. Its positive effect on shelf life is related to the reduction in oxygen (O_2) concentration, which prevents the development of aerobic microorganisms as well as chemical oxidation of the lipid fraction. MAP consists in using a gaseous mixture different from that of natural air. An increase in carbon dioxide (CO_2) partial pressure and a decrease in O_2 partial pressure are usually applied in the case of meat-based products. These conditions are known to have both antimicrobial[4,5] and antioxidant[6,7] effects.

Several studies regarding the effect of modified atmosphere rich in CO_2 on the evolution of the oxidative degradation of different kinds of meat products have already been carried out, with conflicting results.[4,7–12] The reported effects of CO_2 require its prior dissolution into meat.[13] Both intrinsic (pH, water activity, fat content, presence and nature of skin) and extrinsic (CO_2 partial pressure, meat/headspace ratio, storage temperature) factors influence the dissolution of CO_2 into meat.[13] These variables could explain the above-cited discordance among the results of different studies. As regards dry-cured meat products, studies have focused on the comparison of vacuum packaging and MAP, the latter with

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and without CO_2 in the gaseous mixture. The effect of different CO_2 concentrations in MAP has been marginally investigated and only for some typical products such as the Spanish blood sausage 'Morcilla de Burgos',[14] ostrich steaks[11] and fresh pork sausages.[4] Regarding ripened sausages, we previously carried out research[15] on the influence of different CO_2 concentrations in MAP on the evolution of volatile compounds during storage. Our results highlighted that the amounts of some volatile compounds originating from lipid autoxidation were influenced by the packaging atmosphere applied.[15]

In order to better understand the influence of different packaging atmospheres on the degradation phenomena of the lipid fraction of ripened sausages, various analytical approaches, both instrumental and sensory, were adopted to evaluate the effects of three different CO_2 concentrations on the evolution of the lipid fraction degradation.

EXPERIMENTAL

Manufacture of sausages

Sausages were prepared at a local factory according to the industrial processing technology described in a previous paper.[16] The ingredients used in the formulation were sodium chloride (25 g kg^{-1}), potassium nitrate (120 mg kg^{-1}), sodium L-ascorbate (1 g kg^{-1}) (as antioxidant), milk powder (15 g kg^{-1}), black pepper (6 g kg^{-1}), white wine (2.5 mL kg^{-1}), vinegar (2.5 mL kg^{-1}) and fresh garlic (1.3 g kg^{-1}). Lyophilized lactic bacteria (*Lactobacillus sakei* and *Staphylococcus xylosus*, Teck-Al, Parma, Italy) at a cellular density of 7 log colony-forming units (CFU) g^{-1} were added as starters. At the end of the ripening process, the mean chemical composition was 322.4 g moisture, 276.5 g fat, 327.5 g protein and 57.5 g ash kg^{-1}.

Sampling

At the end of the ripening period, three batches of 48 sausages were sampled, three of which were immediately analyzed (T0). The remaining 45 were packed by applying different MAP conditions to each batch: MAP1 (70:30 nitrogen (N_2)/CO_2), MAP2 (80:20 N_2/CO_2) and MAP3 (95:5 N_2/CO_2). All packed (unsliced) sausages were stored in the dark at 4 °C for 5 months. Three sausages from each type of MAP were sampled after one (T1), two (T2), three (T3), four (T4) and five (T5) months of storage and submitted to analyses.

Packaging conditions

The ripened sausages were packed in plastic films (Gamma-Pack, Ravenna, Italy) made of polyethylene and ethylene vinyl alcohol (thickness 60 μm, weight 59.89 g m^{-2}) with O_2, CO_2 and N_2 transmission rates of <2, <10 and <1 cm^3 m^{-2} day^{-1} respectively and a water vapor transmission rate of <1.5 g m^{-2} day^{-1} at 23 °C and 0% relative humidity.

Analytical determinations

The pH was determined by blending 15 g of sample with 150 mL of deionized water for 2 min. The pH of the resultant suspension was measured by means of a Crison Basic 20 pH meter (Barcelona, Spain) equipped with a Crison 50 10 T electrode. The lipid fraction was cold extracted with methanol/chloroform (1:2 v/v) following the method proposed by Folch *et al.*[17]

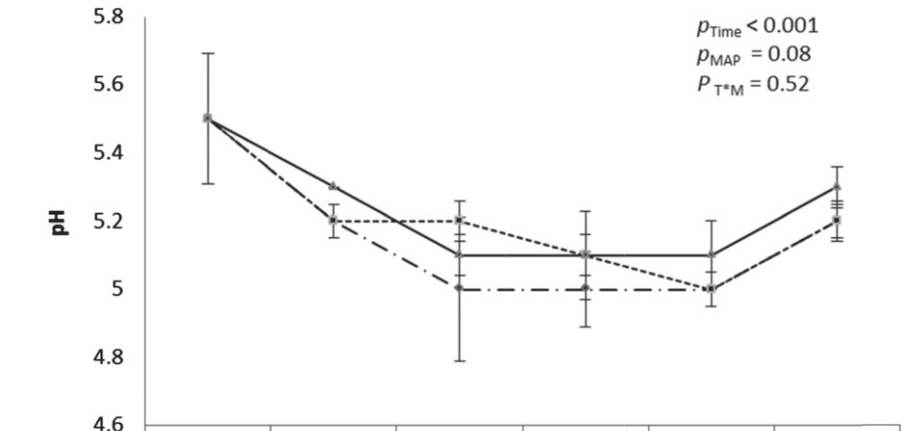
The cold extracted lipid fraction was submitted to silica gel column chromatography according to AOAC method 982.27[18] in order to separate the polar compounds. They were subsequently analyzed by high-performance size exclusion chromatography (HPSEC) in order to separate and quantify the triacylglycerol oligopolymers (TAGP), oxidized triacylglycerols (ox-TAG) and diacylglycerols (DAG). The HPSEC system consisted of a Series 200 pump (Perkin-Elmer, Norwalk, CT, USA) with Rheodyne injector, a 50 μL loop, a PL-gel guard column of 5 cm length × 7.5 mm i.d. and a series of two PL-gel columns (Perkin-Elmer, Beaconsfield, UK) of 30 cm length × 7.5 mm i.d. each, with particles of 5 μm and a pore diameter of 500 Å. The analysis was conducted using tetrahydrofuran as eluent at a flow rate of 1 mL min^{-1} and a Series 200A refractive index detector (Perkin-Elmer, Norwalk, CT, USA). Peaks on the chromatograms were identified and quantified as reported in a previous paper.[19]

The volatile compounds were determined by solid phase microextraction (SPME) coupled with gas chromatography/mass spectrometry (GC/MS). The sum of the peak areas of hexanal, octanal, nonanal, hexanoic acid and nonanoic acid was considered as indicator of secondary volatile oxidation products ox-VoC. Samples of minced sausages (2 g) were weighed into 10 mL headspace vials and sealed with polytetrafluoroethylene/rubber septa. The vials were kept in a forced air oven at 40 °C to equilibrate the headspace. The extraction was carried out using a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane SPME fiber assembly (Supelco, Bellefonte, PA, USA). The SPME fiber was exposed to the headspace while maintaining the vials at 40 °C for 30 min. An Agilent 6850 gas chromatograph (Milan, Italy) equipped with an Agilent 5975 mass spectrometer was used for GC analysis. Compounds were resolved on a Supelco HP-Innowax capillary column (60 m × 0.25 mm × 0.25 μm) under the following conditions: injection port temperature 230 °C; helium pressure 30 kPa; oven temperature 40 °C for 8 min, then 4 °C min^{-1} to 210 °C and final isothermal for 10 min.

The MS detector was set at the following conditions: detector voltage 500 V; interface temperature 230 °C; source temperature 230 °C; ionization energy 70 eV; emission 200 A; scan range *m/z* 33–270. Peak identification was performed by comparing the retention times with those of standards purchased from Sigma-Aldrich (Buchs, Switzerland). To compensate for the variation in the performance of the SPME fiber and the MS detector during the 5 month storage period, all detector responses of duplicated samples were standardized to the response of an external standard. The results were expressed as area units resulting from counting the total ion chromatogram. The analysis of each sample were carried out in triplicate.

Sensory evaluation

Quantitative descriptive analysis was performed by eight trained panelists from 25 to 46 years old (four females and four males) selected from among researchers, research laboratory technicians and PhD students. All panel members had previously attended courses on the sensory evaluation of some foods and beverages such as oil and wine. Four 60 min training sessions were conducted to identify the attributes linked to the sensory defects of sausages. The identification of the descriptors was carried out by considering those usually reported in the literature to evaluate the sensory defects of sausages and dry-cured meat products,[20,21] as well as through the tasting of sausages stored for a long time (more than a year) at ambient temperature. Seven descriptors, three for odor (*rancid*, *pungent* and *moldy*), three for flavor (*rancid*, *pungent* and *moldy*) and one for taste (*acid*), were detected in the sausages stored for a long time by all panelists and were selected for the analysis. An unstructured 10 cm linear scale was



	T0	T1	T2	T3	T4	T5
MAP1	a	abcd	cd	d	d	bcd
MAP2	a	abcd	bcd	bcd	cd	ab
MAP3	a	abc	bcd	bcd	bcd	abcd

Figure 1. Evolution of pH in ripened sausages packed in different modified atmospheres during storage. MAP1, 70:30 N_2/CO_2; MAP2, 80:20 N_2/CO_2; MAP3, 95:5 N_2/CO_2; T0, ripened sausages; T1 – T5, ripened sausages stored for 1, 2, 3, 4 and 5 months respectively. Results obtained by means of two-way ANOVA with first-order interaction are also shown. Different letters indicate significant differences at $P < 0.05$.

used to score each attribute, considering the left end of the scale as corresponding to the lowest intensity (value 0) and the right end as corresponding to the maximum intensity. For each batch and at each time of sampling, a panel session was performed. In each session, two whole slices (0.3 cm thick) from each sausage, coded by three-digit numbers, were distributed to all panel members at room temperature. All samples were presented randomly to avoid any order effect. All sensory evaluation sessions were performed in an air-conditioned room free of external aromas, as usually employed for the sensory evaluation of foods.

Color measurement

Color measurement was carried out using a Minolta Chromameter 2 reflectance colorimeter (Tokyo, Japan) equipped with the measurement head CR 300 and CIE Standard Illuminant D65. Results were expressed as L^* (lightness), a^* (red index) and b^* (yellow index). Two slices were cut from each sample and submitted to three measurements.

Statistical analysis

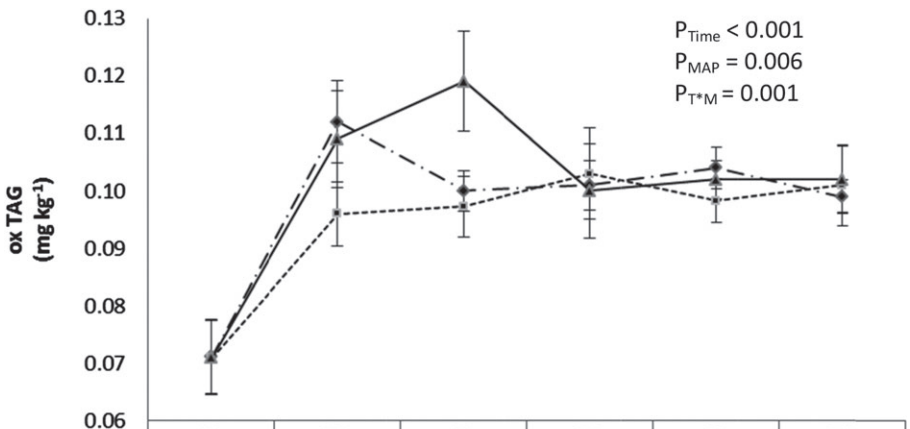
All analyses were carried out in triplicate. Two-way analysis of variance (ANOVA) followed by Tukey's HSD test for multiple comparisons, considering the time of storage and the atmosphere composition as independent variables, was carried out on the experimental data using XLStat software (Addinsoft, New York, NY, USA).

RESULTS AND DISCUSSION

Figure 1 shows the evolution of pH during the storage of sausages as a function of different MAP. After 2 months, a significant decrease in pH was observed, related to the activity of lactic bacteria, as already verified by other authors.[22,23] Successively, the pH tended to increase, though significantly only for MAP3. The increase in pH in long-term stored sausages could be related to the production of amines as a consequence of proteolysis phenomena.[24] The different MAP slightly influenced the pH of the sausages ($P = 0.08$), with values that were generally lower when atmospheres rich in CO_2 were applied. Several authors observed a significant decrease in pH when atmospheres rich in CO_2 were applied, as a consequence of the solubilization of CO_2 in water and lipid fraction of the meat until saturation or equilibration is reached,[13] with the formation of carbonic acid.[4] Rubio *et al.*[22] did not observe significant differences among sausages packed at different CO_2 concentrations, indicating a low extent of CO_2 solubilization in the examined sausages.

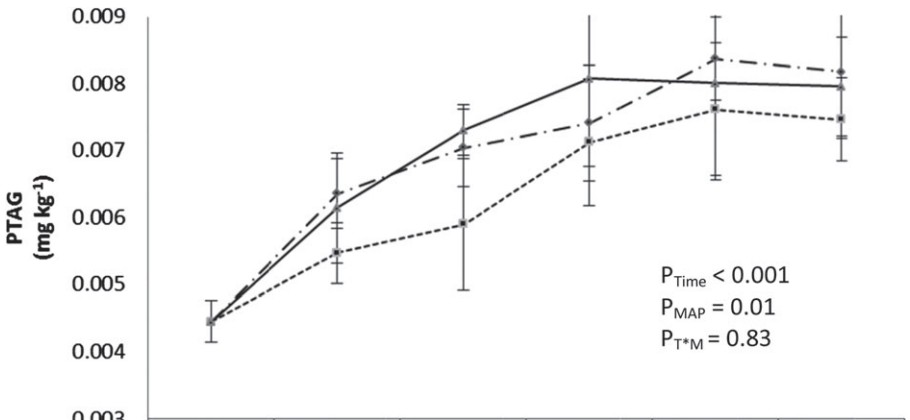
Figures 2 – 4 report the evolution of the parameters linked to the lipid fraction oxidation during storage, together with the results of statistical analysis. Different analytical approaches have been utilized to evaluate the oxidative phenomena. In particular, the HPSEC analysis of polar compounds allows us to quantify the ox-TAG, which include primary oxidation products as well as oxidized forms of triacylglycerols other than hydroperoxides, and the TAGP, products of triacylglycerol polymerization that represent a reliable index of secondary oxidative degradation.[19] The sum of volatile compounds linked to the lipid fraction oxidation (ox-VoC) has been used to determine the secondary oxidation compounds derived from the hydroperoxides breaking down, as well as from further oxidative processes, such as hexanal, octanal, nonanal, hexanoic acid and nonanoic acid deriving from the further oxidation of the corresponding aldehydes.[25]

Regarding the ox-TAG content (Fig. 2), a significant increase was observed after 1 month of storage in all sausages under investigation. A further increase in ox-TAG was observed for the MAP3 trial after 2 months, while it was not observed in the sausages packed in MAP1 and MAP2, richer in CO_2. During the remaining storage period, no significant variations were observed in the MAP1 and MAP2 trials, while a significant decrease was observed after T2 in the MAP3 trial. The evolution of the ox-TAG content during storage reflects the typical evolution of the oxidative phenomena affecting lipids: the unstable products due to primary oxidation, such as the hydroperoxides, evolve into volatile and non-volatile compounds due to secondary oxidation, such as the triacylglycerol oligopolymers. The increase in lipid oxidation phenomena during storage has already been verified for sausages stored in vacuum, both in our previous research[26] and by Fernández-Fernández *et al.*,[24] and



	T0	T1	T2	T3	T4	T5
MAP1	d	ab	bc	bc	abc	bc
MAP2	d	c	bc	abc	bc	bc
MAP3	d	abc	a	bc	bc	bc

Figure 2. Evolution of oxidized triacylglycerols (ox-TAG) in ripened sausages packed in different modified atmospheres during storage. MAP1, 70:30 N_2/CO_2; MAP2, 80:20 N_2/CO_2; MAP3, 95:5 N_2/CO_2; T0, ripened sausages; T1–T5, ripened sausages stored for 1, 2, 3, 4 and 5 months respectively. Results obtained by means of two-way ANOVA with first-order interaction are also shown. Different letters indicate significant differences at $P < 0.05$.



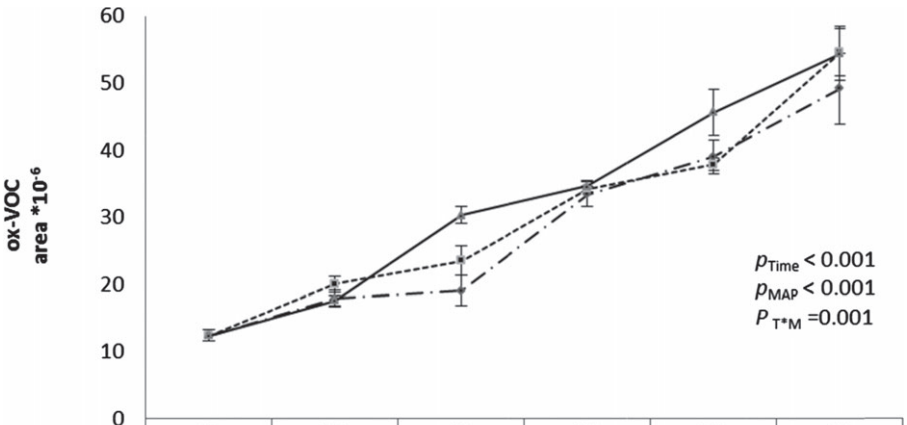
	T0	T1	T2	T3	T4	T5
MAP1	e	abcde	abcd	abcd	a	ab
MAP2	e	de	cde	abcd	abc	abcd
MAP3	e	bcde	abcd	ab	ab	abc

Figure 3. Evolution of triacylglycerol oligopolymers (TAGP) in ripened sausages packed in different modified atmospheres during storage. MAP1, 70:30 N_2/CO_2; MAP2, 80:20 N_2/CO_2; MAP3, 95:5 N_2/CO_2; T0, ripened sausages; T1–T5, ripened sausages stored for 1, 2, 3, 4 and 5 months respectively. Results obtained by means of two-way ANOVA with first-order interaction are also shown. Different letters indicate significant differences at $P < 0.05$.

could be due to the residual oxygen present in the internal part of the product. Among the sausages packed in different atmospheres, significant differences were observed only during the first 2 months of storage, with higher levels of ox-TAG in MAP3 than in MAP2. Regarding the secondary oxidation products, the TAGP content (Fig. 3) increased during the whole period of storage, with differences that were significant after 2 months for MAP1 and MAP3 and after 3 months for MAP2. All different types of packaging atmosphere adopted induced an increase in TAGP, though to a lower extent in MAP2 than in MAP1 and MAP3 ($P < 0.01$).

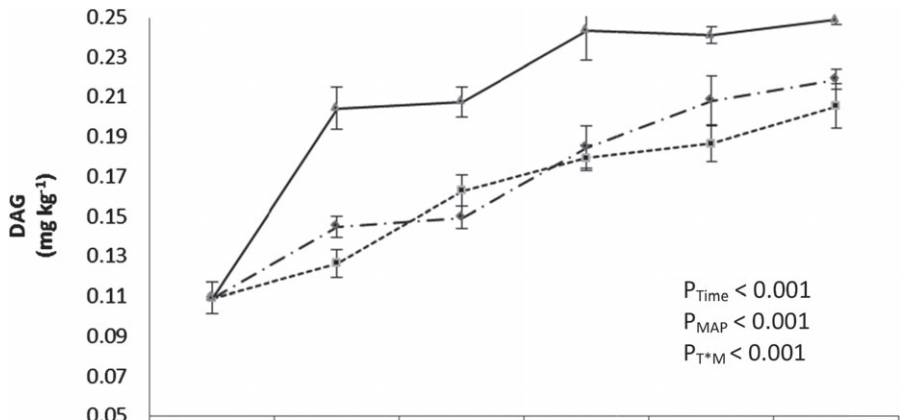
The ox-VoC compounds (sum of the areas of hexanal, octanal, nonanal, hexanoic acid and nonanoic acid in Fig. 4) showed a progressive increase during storage that became significant at T1 for MAP2, at T2 for MAP3 and at T3 for MAP1. With the exception of T1, the highest ox-VoC contents were observed in MAP3, with significant differences with respect to MAP1 at T2 and to MAP2 at T4. A significant correlation between ox-VoC and TAGP was also observed ($r = 0.841$).

On the whole, the different analytical approaches to measure the evolution of the lipid oxidation of sausages showed differences between the three MAP types considered in the initial period of storage (first 2 months), with lower oxidation levels when MAP was richer in CO_2 (MAP1 and MAP2). Subsequently, no substantial variations were observed. Several studies regarding the effect of MAP rich in CO_2 on the evolution of the oxidative degradation of various types of meat products have already been carried out, with conflicting results. Fernández-López *et al.*[11] observed no significant differences in the evolution of the lipid oxidation of ostrich steaks



	T0	T1	T2	T3	T4	T5
MAP1	h	gh	gh	de	cd	ab
MAP2	h	g	fg	de	d	a
MAP3	h	gh	ef	de	bc	a

Figure 4. Evolution of oxidation volatile compounds (ox-VoC = sum of areas of hexanal, octanal, nonanal, hexanoic acid and nonanoic acid) in ripened sausages packed in different modified atmospheres during storage. MAP1, 70:30 N_2/CO_2; MAP2, 80:20 N_2/CO_2; MAP3, 95:5 N_2/CO_2; T0, ripened sausages; T1 – T5, ripened sausages stored for 1, 2, 3, 4 and 5 months respectively. Results obtained by means of two-way ANOVA with first-order interaction are also shown. Different letters indicate significant differences at $P < 0.05$.



	T0	T1	T2	T3	T4	T5
MAP1	j	hi	h	efg	cd	bc
MAP2	j	ij	gh	fg	def	cde
MAP3	j	cde	cd	a	ab	a

Figure 5. Evolution of diacylglycerols (DAG) in ripened sausages packed in different modified atmospheres during storage. MAP1, 70:30 N_2/CO_2; MAP2, 80:20 N_2/CO_2; MAP3, 95:5 N_2/CO_2; T0, ripened sausages; T1 – T5, ripened sausages stored for 1, 2, 3, 4 and 5 months respectively. Results obtained by means of two-way ANOVA with first-order interaction are also shown. Different letters indicate significant differences at $P < 0.05$.

packed under vacuum or in various types of MAP during 18 days of storage, while Valencia *et al.*[9] observed the same trend in linseed oil sausages and in control sausages packed under vacuum and in MAP. In contrast, Cachaldora *et al.*[27] observed significantly lower thiobarbituric acid-reactive substance (TBARS) values in 'morcilla', a typical cooked blond sausage, packed in 60% CO_2/40% N_2 than in the same product packed under vacuum or in MAP with low levels of CO_2. The authors attributed their findings to the antioxidant effect of CO_2, also reported in other studies.[6,7] Martínez *et al.*[4] and Rubio *et al.*[28] observed instead an increase in the rate of lipid oxidation when using higher amounts of CO_2 in the packaging atmosphere of fresh meat and dry-fermented sausages respectively.

The results obtained in our investigation highlighted that, in the experimental conditions applied, the higher the amount of CO_2 used as a component of the gas mixture in MAP, the lower the rate of oxidative phenomena occurring. Various studies reported the antioxidant effect of CO_2[6,27,29] without explaining the mechanism of such activity. In most cases the investigations carried out compared modified atmospheres by increasing CO_2 levels while decreasing O_2 levels; therefore the slowing down of oxidation processes could be attributed to lower O_2 pressures.

Figure 5 shows the evolution during storage of the content of DAG, derived from triacylglycerol hydrolysis, together with the results of statistical analysis. A significant increase, starting from T1 in MAP1 and MAP3 and from T2 in MAP2, was observed in the DAG content of all sausages over time. An increase in lipid hydrolysis in sausages during storage was already observed in vacuum-packed sausages.[19,30] The hydrolytic degradation of the

Table 1. Mean values (total area counts $\times 10^{-7}$) and their standard deviation (SD) for volatile compound composition of ripened sausages packed in different modified atmospheres during storage

Time	MAP		Volatile compounds from spice	Volatile compounds from wine and vinegar	Volatile compounds from carbohydrate fermentation	Volatile compounds from amino acid catabolism	Volatile compounds from lipid β-oxidation	Volatile compounds from smoking process
T0		Mean	79.80a	7.94e	11.73a	5.56cdef	0.62cde	2.07f
		± SD	5.54	1.79	1.35	0.62	0.18	0.17
T1	MAP1	Mean	62.23def	3.75f	10.03ab	5.14 fg	0.45defgh	2.10f
		± SD	2.96	0.78	1.95	0.74	0.07	0.39
	MAP2	Mean	67.19bcd	2.21f	10.79ab	5.64cdef	0.55cdefg	2.43def
		± SD	3.09	0.08	0.48	1.30	0.13	0.66
	MAP3	Mean	67.70b	2.04f	10.14bc	5.23efg	0.54cdefg	3.01abc
		± SD	2.15	0.28	1.03	0.23	0.07	0.10
T2	MAP1	Mean	64.39bcdef	7.63e	7.13d	7.75a	0.60cde	3.39a
		± SD	1.80	1.32	0.63	0.28	0.03	0.41
	MAP2	Mean	64.75bcdef	8.82de	6.96d	5.65cdef	0.53cdefgh	3.08ab
		± SD	3.66	1.27	1.12	0.81	0.05	0.41
	MAP3	Mean	63.32bcdef	7.50e	6.48d	6.26bcdef	0.64bcd	3.08ab
		± SD	2.10	0.82	0.90	0.31	0.02	0.25
T3	MAP1	Mean	65.62bcde	2.98f	9.63bc	4.37 g	0.64bc	2.22ef
		± SD	1.90	0.21	1.04	0.49	0.16	0.46
	MAP2	Mean	64.92bcde	3.47f	8.90c	7.09ab	0.82b	2.45cdef
		± SD	2.02	0.42	1.26	0.51	0.05	0.07
	MAP3	Mean	66.32bcde	3.35f	8.90c	5.70cdef	1.18a	2.54bcdef
		± SD	0.79	0.38	1.17	0.51	0.25	0.35
T4	MAP1	Mean	62.69cdef	11.92bc	3.23e	6.45bcd	0.57cdef	2.76bcde
		± SD	0.72	0.52	0.37	0.07	0.03	0.24
	MAP2	Mean	61.99ef	12.51ab	4.46e	6.40bcd	0.53cdefgh	2.32ef
		± SD	1.96	0.98	0.28	1.14	0.05	0.40
	MAP3	Mean	59.90f	13.84a	3.95e	6.30bcd	0.43efgh	2.94abcd
		± SD	2.60	0.68	0.66	0.42	0.05	0.10
T5	MAP1	Mean	66.79bcde	10.97bc	1.42f	6.64abc	0.35 h	2.54bcdef
		± SD	1.90	0.48	0.03	0.95	0.06	0.45
	MAP2	Mean	67.60bc	10.52 cd	1.23f	5.45cdef	0.37gh	2.29ef
		± SD	2.16	1.65	0.06	0.70	0.06	0.46
	MAP3	Mean	63.27bcdef	12.25abc	1.56f	6.53bcd	0.41fgh	2.56bcdef
		± SD	0.95	1.35	0.12	0.57	0.06	0.11

The volatile compounds were grouped on the basis of their most probable origin. MAP1, 70:30 N_2/CO_2; MAP2, 80:20 N_2/CO_2; MAP3, 95:5 N_2/CO_2; T0, ripened sausages; T1–T5, ripened sausages stored for 1, 2, 3, 4 and 5 months respectively. Results obtained by means of two-way ANOVA with first-order interaction are also shown. Different letters indicate significant differences at $P < 0.05$.

lipid fraction was greatly influenced by the packaging atmosphere. As seen in Fig. 5, the rise in DAG content was significantly greater in MAP3 where the concentration of CO_2 was the lowest. The differences observed could be imputable to the inhibiting effect of CO_2 on microbial growth. Gram-negative bacteria in particular are generally more sensitive to CO_2 than Gram-positive bacteria, since most Gram-positive bacteria are facultative or strict anaerobes.[31] Also, the growth rates of lactic bacteria and of yeasts and molds[31] were slowed down by increasing CO_2 concentration. In a study carried out by Sachindra *et al.*[32] on buffalo meat sausages, the authors observed inhibitory effects of CO_2 on the growth of yeasts and molds during storage, while these effects were not observed in meat stored under N_2. The differences in the evolution of microbial population, particularly of microorganisms involved in lipolysis,[33] could be the cause of the differences observed in the levels of lipid hydrolysis products.

Several studies in the literature point out a close relation between hydrolytic and oxidative phenomena in meat products. In particular, fatty acids are more prone to oxidation when free, as a product of hydrolysis, than when esterified in triacylglycerols or phospholipids.[34,35] Therefore the greater extent of hydrolytic degradation in sausages stored in MAP3, especially in the first 3 months of storage, could have led to increased oxidation rates in MAP3 sausages in the same period, when the residual oxygen contained in the food matrix was presumably more abundant.

Table 1 reports the mean values, their standard deviations and the results of statistical analysis of the volatile compounds, grouped according to their most probable origin.[36] The volatile compounds deriving from lipid autoxidation are not included in the table, since they were reported in Fig. 4. On the whole, as found in our previous study, which considered the same packaging atmospheres for a shorter storage period,[15] the 'time' variable influenced the volatile compound composition of the sausages more than the different MAP applied. The volatile compounds deriving from the added spices (black pepper, garlic) represented the prevalent volatile compounds in the headspace of ripened

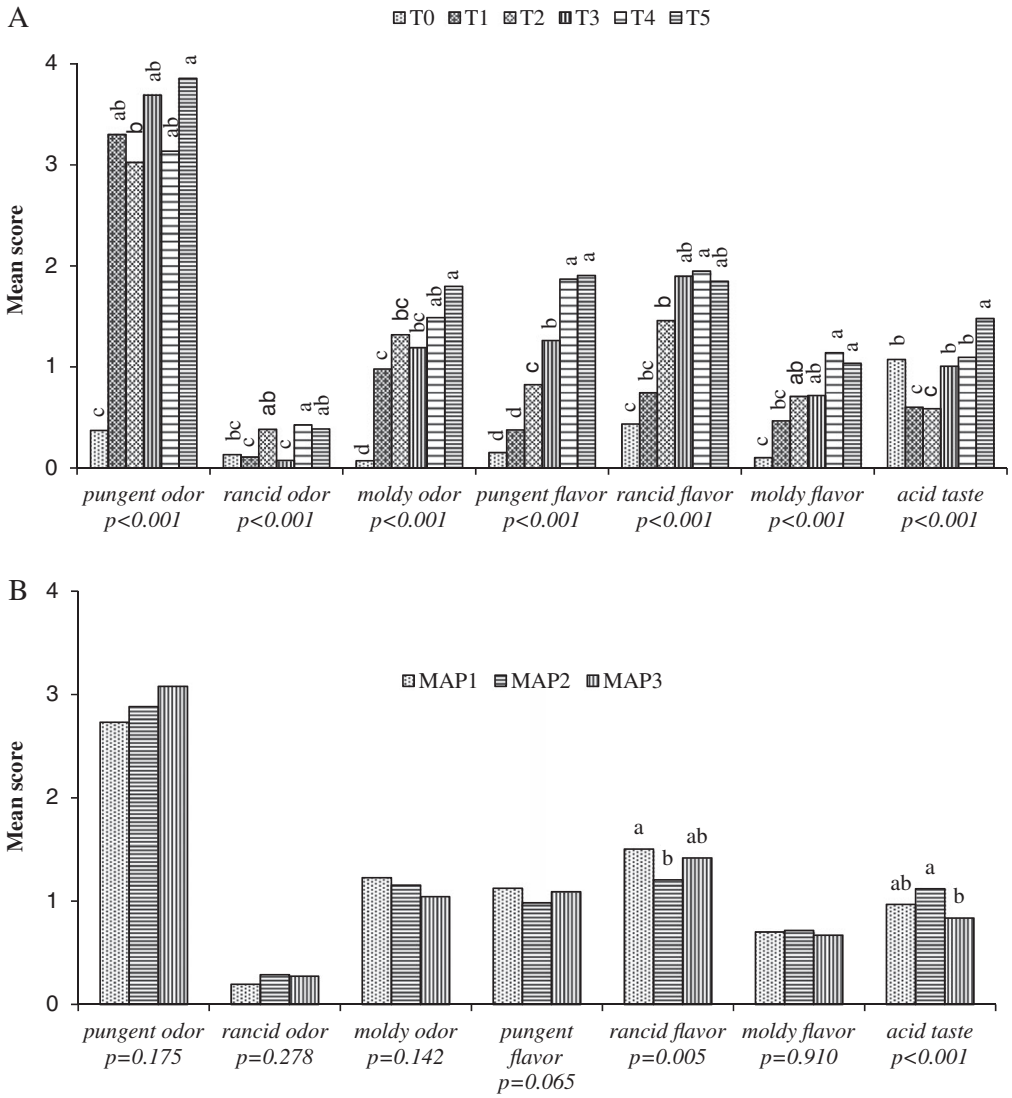


Figure 6. Mean scores of descriptors used in sensory evaluation of ripened sausages as a function of (A) time of storage and (B) modified atmosphere composition. MAP1, 70:30 N_2/CO_2; MAP2, 80:20 N_2/CO_2; MAP3, 95:5 N_2/CO_2; T0, ripened sausages; T1–T5, ripened sausages stored for 1, 2, 3, 4 and 5 months respectively. Results obtained by means of two-way ANOVA are also shown. Different letters indicate significant differences for the same descriptor at $P < 0.05$.

sausages. A significant decrease during the first month of storage was observed in all trials, while no significant differences were observed as a function of the different MAP. This group of volatiles includes terpenic compounds derived from pepper (e.g. Δ3-carene and limonene) as well as allylmethylsulfide, 1-methylthio-1-propene and diallylsulfide derived from garlic. The decrease observed over time could be related to the antioxidant activities of these compounds, already reported in the literature.[37] Among the other volatile compounds detected, a significant decrease was observed at each storage time considered for those derived from carbohydrate fermentation, such as diacetyl and the two 2,3-butandiol isomers, probably due to the decrease in available sugars and the low stability of these compounds.

Figures 6A and 6B report the mean scores of the sensory descriptors related to defects as a function of storage time and MAP respectively. At T0, all sausages were characterized by low scores of the considered descriptors, evidencing the good starting sensory quality of the samples investigated. During storage (Fig. 6A), the scores of all descriptors significantly increased. In particular, the descriptors *pungent odor* and *moldy odor* significantly increased at T1; the descriptors *pungent flavor*, *rancid flavor* and *moldy flavor* increased after 2 months, *rancid odor* after 4 months and *acid taste* after 5 months. The greater perception of *rancid odor* and *rancid flavor* in sausages stored for longer times could be related to the increase in ox-VoC during storage (Fig. 4), while the increase in volatile compounds derived from the Maillard reaction could be responsible for the intensification of the descriptors *pungent odor* and *pungent flavor* during storage, as reported in our previous research.[19] Regarding the influence of packaging atmosphere (Fig. 6B), significant variations were observed for the descriptors *rancid flavor* (comparing MAP1 and MAP2) and *acid taste* (comparing MAP2 and MAP3). On the whole, the storage time influenced the sensory characteristics of ripened sausages more than the different MAP. Similarly, Fernández-Fernández *et al.*,[24] in Galician chorizo sausages packed under vacuum and in different MAP, and Rubio *et al.*,[22] in sausages enriched with mono- and polyunsaturated fatty acids and stored in different MAP, observed significant differences in the sensory profile of sausages during storage,

whereas no significant differences were evidenced as a function of MAP.

With regard to the colorimetric index (data not shown), significant variations only for the *L** value were observed during storage in the sausages under investigation, whereas no significant differences were observed for the intensity of red (*a**) and yellow (*b**) indices, as already reported in another study.[11] In particular, the *L** value significantly increased in the first phase of storage, reaching its maximum at T2. Subsequently, this index significantly decreased at T3 for MAP3 and at T5 for MAP1. Also, Rubio *et al.*,[38] in 'Cecina de Leòn', a dry-cured beef product, and García-Esteban *et al.*,[39] in dry-cured ham, observed an increase in the *L** value during storage in vacuum or in MAP, explaining the increase by the presence of a 'white film' on the surface. The evolution of the brightness during storage could be related also to the evolution of the pH value observed. When the pH of meat decreases, as occurred in the first 2 months of storage, proteins come near to their isoelectric point and therefore tend to release water. As a consequence, the surface of sausages was more wet and was characterized by high light scatter.[39] Regarding the effect of the atmosphere composition on the brightness of sausages, no significant differences among the samples were seen in the first 2 months of storage, whereas, starting from the third month of storage, the *L** values observed for MAP3 were significantly lower than those observed for MAP1 and MAP2. The lower pH observed in the sausages stored in MAP richer in CO_2 could explain these differences.

CONCLUSIONS

The obtained results pointed out the influence of storage time and packaging atmosphere on the quality characteristics of fermented sausages. Regarding the oxidation affecting the lipid fraction, the different analytical approaches utilized highlighted that the evolution of oxidative phenomena occurred at a lower rate as the amount of CO_2 in MAP increased. This phenomenon could be related to the smaller extent of hydrolytic degradation of the lipid fraction, imputable to the inhibiting effect of CO_2 on microbial growth. The differences in oxidative degradation were observed in the initial period of storage, when residual O_2 was likely available in sufficient amounts in the food matrix. Also, the sensory characteristics of ripened sausages were influenced by the different CO_2 concentrations applied in MAP. Lower scores of some defect descriptors (*acid taste* and *rancid flavor*) were observed when MAP was lower in CO_2. Regarding the colorimetric indices, starting from the third month of storage, the *L** values observed for MAP3 were significantly lower than those observed for MAP1 and MAP2. Further investigations should be carried out on the relationship between the hydrolytic and the oxidative degradation of the lipid fraction in ripened sausages, as well as the role of the microorganisms involved.

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