International Journal of Advanced Engineering and Management Research

Vol. 5, No. 02; 2020

ISSN: 2456-3676

EFFECT OF MODIFIED ATMOSPHERE PACKAGING AND STARTER CULTURES ON THE QUALITY AND SHELF LIFE OF HORSE MEAT SAUSAGE

Sabrine EL ADAB*[1], Souad GOIEID[1], Asma TEKIKI[1], Olfa BEN MOUSSA[1], Mouna BOULARES[1], Saida BELGAIED[2], Mnasser HASSOUNA[1]

[1] Research Unity "Bio-Preservation and Valorization of Agricultural Products–UR 13 AGR02", High Food Industries School of Tunisia, 58 Avenue Alain Savary, Tunis El Khadra 1003, University of Carthage, Tunisia

[2] Technical Center of Food Packaging, Street Ferdjani Belhadj Ammar BP 64, Tunis El Khadra 1003, Tunisia

ABSTRACT

The effect of modified atmosphere packaging and mixed starter cultures on the quality and shelf-life of sausage were examined. Sausages were packaged under varying modified atmosphere conditions (60% CO2/40% N2; 40% CO2/60% N2) and stored at 4 °C for 28 days. The results showed that storage time affected all parameters whereas no significant differences were observed among packaging conditions and inoculated sausages. Inhibition effect of CO2 concentration on the evolution of harmful microorganisms was seen. The shelf life of control sausage was 21 days, while for the other type of sausages; this shelf life would be more than 28 days.

Keywords: Horse meat; Fermented sausage; Starter cultures; Quality; Modified atmosphere packaging; Shelf-life.

1. Introduction

The production of horse meat in Tunisia is not important enough. Horse meat is rich in protein source of essential amino acids, iron and vitamins (B3, B6 and B12). Moreover, Horse meat has not only a low lipid content (2-4%) but its composition in different fatty acids is very interesting: it has 60 to 70% unsaturated fatty acids, some of which could not be synthesized by the body (linoleic acid and alpha-linolenic acid) and which seem to play a role in the prevention of cardiovascular diseases (Lorenzo et al., 2010; Franco et al., 2011). In Tunisia, Horse meat sausage is made from a mixture of lean horse (75%), fat (25%), salt and different spices.

The determination of the shelf life and its validation are very important for the microbiological safety of food products. Extension of the shelf-life of meat products is one of the technology needs to meet the demands of consumers. In this respect, increasing attention is put on packaging techniques and the use of mixed starter cultures. Modified atmosphere packaging (MAP) using different combinations of carbon dioxide, nitrogen, and/or oxygen is recent innovation that have been gaining importance as preservation technique to improve the shelf life of meat and meat products (Özogul et al., 2004). Many studies have reported that carbon dioxide-enriched atmospheres inhibit the growth of undesirable microorganisms; an oxygen-enriched atmosphere promotes a red surface color in the meat, and nitrogen, while inert to meat products, is used as a

Vol. 5, No. 02; 2020

ISSN: 2456-3676

filler to reduce concentrations of more active gases (Fernandez- Fernandez et al., 2002; Rubio et al., 2008).

Moreover, the use of starter cultures with appropriate metabolic performances has increasingly gained the interest of researchers and food processors as preservation technique to extend the shelf life of meat products. In fact, these biopreservatives have been shown to possess antibacterial and antifungal activities against several microorganisms associated with meat, including gram-negative and gram-positive bacteria (El Adab et al., 2015).

Thus, the target of this work was to determine the shelf life of horse meat sausages inoculated with mixed starter cultures and preserved in modified atmosphere packaging during storage at 4 °C. Microbial quality, pH, water activity, color parameters, oxidative stability of lipids and sensory assessment of odor, color and appearance were determined at 0, 7, 14, 21 and 28 days of storage.

2. Materials and methods

2.1. Sausage preparation

The sausage formulation included horse lean meat (75%), horse fat (25%), salt (2.4%), black pepper (0.12%), paprika (0.12%), glucose (0.6%) and potassium nitrate (0.006%). After chopping and mixing the ingredients, the mixture was divided into two batches: batch 1, inoculated with *L. sakei* and *S. carnosus* (BFL-F06, CHR HANSEN, Nienburg, Germany) and batch 2, control. The sausages were stuffed into artificial casings and then placed in a fermentation chamber (BCR, CF 1 B, Antony, France) as described by El Adab et al. (2015).

2.2. Packaging of samples

Immediately after 28 days of ripening, some slices (4 cm thick) were aseptically removed from control horse meat sausages and packed in trays 50 PAPE µm thick which is suitable for the preservation of food products. Sausage samples were packed under modified atmospheres with the following gas mixtures: Atm 1: 40% CO_2/60% N_2 and Atm 2: 60% CO_2/40% N_2. The packages were gas flushed and sealed using a MULTIVAC packaging machine (TSM 95, Multivac, Wolfertschwenden, Germany). During storage, samples of dry fermented sausages (control, inoculated and packaged under modified atmospheres) were kept at 4 °C for 28 days. Twelve samples were taken after 0, 7, 14, 21 and 28 days of storage for physicochemical, microbiological and sensorial analysis.

2.3. Physicochemical analysis

The pH and water activity (a_w) were determined as described by El Adab et al. (2015).

Color parameters L*, a*, b*, h* and C* were determined as described by Cachaldora et al. (2013). Hue (h*) and chroma (C*) were calculated from the a* and b* values according to the formula: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$ and $h^* = acr\tan\frac{b^*}{a^*}$

Lipid stability was assessed by measuring thiobarbituric acid reactive substances (TBARS) during the storage period. This analysis was performed according to the method of Genot (1996) as described by El Adab et al. (2015).

2.4. Microbiological analysis

Ten grams of horse meat sausage was homogenized with 90 mL of sterile peptone water (Biolife, Milan, Italy). For each sample, serial decimal dilutions were prepared. Total viable counts (TVC)

Vol. 5, No. 02; 2020

ISSN: 2456-3676

were enumerated on Plate Count Agar (Biolife) at 30 °C for 48 h. Enterobacteriaceae were determined on Violet Red Bile Glucose (VRBG) (Biokar, Beauvais, France) at 37 °C for 24 h. Lactic acid bacteria (LAB) were enumerated on MRS (de Man, Rogosa and Sharpe) agar (Biolife) after 48 h of incubation at 30 °C. Yeasts and molds were enumerated on Sabouraud Dextrose Agar (Biokar) at 28 °C for 4 days. Psychrotrophic microbiota (PVC) was determined on Plate Count Agar (Biolife), and the plates were incubated at 4°C for 10 days. Fecal coliforms (*E. coli*) were determined on desoxycholate (0.1%) lactose agar (Biokar) at 44 °C for 24 h.

2.5. Sensory Evaluation

The sensory analysis was performed by a trained panel composed of 10 professional staff. A slice of each sample batch (5 mm thick approximately) was served to the assessors. The attributes which have been studied are the following: red color, off-odor and general appearance acceptability using a nine point hedonic rating scale as described by Lorenzo et al. (2012).

2.6. Statistical Analysis

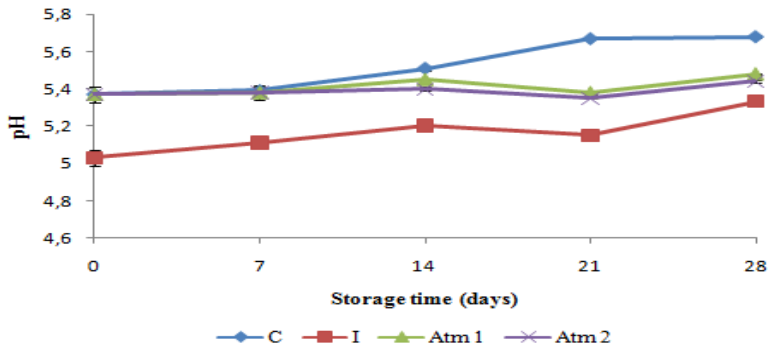
Statistical determinations on sausages were performed by analysis of variance (ANOVA). Significant differences ($p < 0.05$) between the mean values were detected with Tukey's test. Principal Components Analyses (PCA) were also carried out using XLSTAT professional.

3. Results and discussion

3.1. Physicochemical analysis

3.1.1. pH and a_w values

The initial pH of control and inoculated sausages tested were 5.37 and 5.03, respectively. The pH increased ($p > 0.05$) during storage to reach at the end of storage period values of 5.68, 5.48, 5.44 and 5.33 respectively, for control samples, sausages packaged under modified atmospheres (Atm1, Atm 2) and inoculated sausages (Fig. 1), which could be explained by the reduction in the number of lactic acid bacteria. Results showed that there were no significant differences ($p > 0.05$) in pH values between packaging conditions. The decrease observed in pH at day 21 of storage in sausages packaged under modified atmospheres has been related to the concentration of carbon dioxide (CO_2). Our results are in agreement with those of Juncher et al. (2001) and Jakobsen and Bertelsen (2004) who showed that a drop in pH coincides with a high concentration of CO_2 and this for dry fermented sausages made from pork meat. Gokoglu et al. (2010) explained the decrease in pH by the absorption of carbon dioxide by meat and the formation of carbonic acid.



Vol. 5, No. 02; 2020

ISSN: 2456-3676

Figure 1- Evolution of pH during storage at 4 °C of dry fermented horse meat sausages: C (control sausage), I (sausage inoculated with mixed starter cultures), Atm 1 (Sausage packaged under modified atmosphere 60% N_2/40% CO_2), Atm 2 (Sausage packaged under modified atmosphere 40% N_2/60% CO_2)

The initial water activity values of control and inoculated sausages were 0.822 ± 0.02 and 0.792 ± 0.01, respectively (Fig. 2). The values of water activity remained practically constant during storage of control samples, inoculated sausages and sausages packaged under MAP. Our results match with those reported by Rubio et al. (2007) and Zanardi et al. (2002) in salchichón and Milano-type sausages, respectively. The addition of starter cultures and packaging method had no significant effect ($p > 0.05$) on a_w values. Similar results were found by Rubio et al. (2007) and Cachaldora et al. (2013).

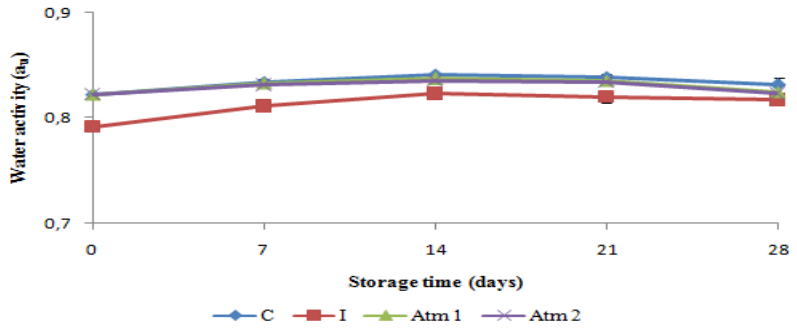


Figure 2- Evolution of water activity (a_w) during storage at 4 °C of dry fermented horse meat sausages: C (control sausage), I (sausage inoculated with mixed starter cultures), Atm 1 (Sausage packaged under modified atmosphere 60% N_2/40% CO_2), Atm 2 (Sausage packaged under modified atmosphere 40% N_2/60% CO_2)

3.1.2. Color evaluation

Meat color is one of the most important parameters by which consumers evaluate meat and meat products quality (Gøtterup et al., 2008). The color parameters, lightness (L*), redness (a*), yellowness (b*), chroma (C*) and hue (h*) are shown in Table 1.

Table 1- Evolution of color parameters during storage at 4°C of dry fermented horse meat sausages: C (control sausage), I (sausage inoculated with mixed starter cultures), Atm 1 (Sausage packaged under modified atmosphere 60% N_2/40% CO_2), Atm 2 (Sausage packaged under modified atmosphere 40% N_2/60% CO_2)

Data are means ± standard deviation.
Different letters in the same row indicate significant differences ($p < 0.05$).

Vol. 5, No. 02; 2020

ISSN: 2456-3676

Treatment	Days	L	a	b	C*	h*
C						
	0	27.07 ± 1.49^{a}	2.86 ± 0.51^{a}	10.81 ± 0.9^{a}	11.18 ± 0.8^{b}	1.31 ± 0.01^{a}
	7	29.46 ± 0.29$_{ab}$	3.16 ± 0.37^{a}	9.67 ± 0.87^{a}	10.17 ± 0.82^{a}	1.25 ± 0.01^{a}
	14	30.54 ± 1.84^{ab}	3.65 ± 0.74^{a}	11.35 ± 1.59^{a}	11.92 ± 1.55^{c}	1.25 ± 0.01^{a}
	21	33.79 ± 1.89^{b}	2.37 ± 0.47^{a}	15.09 ± 1.56^{b}	15.27 ± 1.45^{e}	1.41 ± 0.01^{b}
	28	31.56 ± 1.82^{ab}	3.92 ± 0.85^{a}	12.75 ± 0.31^{ab}	13.33 ± 0.42^{d}	1.27 ± 0.01^{a}
I						
	0	29.91 ± 2.23^{a}	7.85 ± 0.82^{c}	13.48 ± 2.01^{b}	15.59 ± 1.98^{c}	1.04 ± 0.02^{a}
	7	28.73 ± 0.28^{a}	2.13 ± 0.41^{a}	8.24 ± 0.28^{a}	8.51 ± 0.25^{a}	1.31 ± 0.01^{b}
	14	32.61 ± 0.58^{a}	5.72 ± 0.25^{bc}	13.11 ± 0.2^{b}	14.30 ± 0.14^{b}	1.16 ± 0.02^{b}
	21	30.9 ± 3.06^{a}	5.58 ± 0.98^{ab}	18.08 ± 0.74^{c}	18.92 ± 0.71^{d}	1.27 ± 0.02^{b}
	28	31.04 ± 1.89^{a}	5.26 ± 1.76^{bc}	14.55 ± 0.35^{b}	15.47 ± 0.29^{c}	1.22 ± 0.02^{b}
Atm 1						
	0	27.07 ± 1.49^{ab}	2.86 ± 0.51^{a}	10.81 ± 0.9^{b}	11.18 ± 0.71^{b}	1.31 ± 0.01^{d}
	7	29.35 ± 1.5b^{c}	3.47 ± 0.31^{a}	8.01 ± 0.42^{a}	8.72 ± 0.35^{a}	1.16 ± 0.01^{b}
	14	31.73 ± 0.89^{c}	5.93 ± 0.29^{b}	10.28 ± 0.58^{b}	11.86 ± 0.45^{c}	1.04 ± 0.02^{a}
	21	28.61 ± 1.78^{bc}	4.68 ± 0.46^{b}	14.94 ± 1.26^{b}	15.65 ± 0.8^{d}	1.27 ± 0.01^{c}
	28	24.13 ± 0.77^{a}	9.81 ± 0.47^{c}	15.88 ± 0.33^{c}	18.66 ± 0.26^{e}	1.02 ± 0.02^{a}
Atm 2						
	0	27.07 ± 1.49^{a}	2.86 ± 0.51^{a}	10.81 ± 0.9^{a}	11.18 ± 0.86^{a}	1.31 ± 0.01^{c}
	7	28.15 ± 1.98^{a}	5.91 ± 0.1^{a}	9.37 ± 0.8^{a}	11.07 ± 0.75^{a}	1.01 ± 0.01^{a}
	14	26.54 ± 1.81^{a}	4.91 ± 0.32^{b}	17.08 ± 0.68^{b}	17.77 ± 0.49^{d}	1.29 ± 0.01 ^{c}
	21	29.42 ± 3.78^{a}	3.92 ± 0.59^{a}	14.28 ± 1.92^{b}	14.80 ± 1.22^{b}	1.30 ± 0.02^{c}
	28	24.13 ± 1.09^{a}	7.85 ± 0.71^{c}	14.98 ± 1.07^{c}	16.29 ± 0.88^{c}	1.07 ± 0.01^{b}

Vol. 5, No. 02; 2020

ISSN: 2456-3676

As can be seen, lightness increased ($p > 0.05$) during the two first weeks of storage of sausages and then decreased ($p > 0.05$) (Table 1). Lightness in food is related with many factors, including the water content and occluded air content (Roland et al., 1999). Our results show that packaging method and the addition of starter cultures have significant effect on lightness of sausages ($p < 0.05$). Moreover, no significant differences ($p > 0.05$) were observed in L* values among sausages packaged under Atm 1 and Atm 2.

With respect to a* values, the initial mean value in the inoculated sausages (7.85 ± 0.82) was higher ($p < 0.05$) than that found in control sausages (2.86 ± 0.51) (Table 1). This could be related to the nitrate reductase activity of coagulase negative staphylococci (CNS) (Essid and Hassouna, 2013). Horse meat is rich in myoglobin. Higher oxygen concentrations improve the bright red color of fresh meat, whereas low oxygen levels accelerate the oxidation of red myoglobin to brown metmyoglobin. Studies have shown that in meat products handled by nitrites, the color quickly turns brown when oxygen and light are present (Cachaldora et al., 2013). Table 1 shows a decrease in a* values during the storage of inoculated sausages. However, an increase was observed in a* values during the storage of sausages packaged under modified atmospheres. The lowest values are noted on control samples. Cachaldora et al. (2013) found that Blood sausage "*morcilla*" packed under $O_2/N_2/CO_2$ combinations had lower a* values than those packaged under vacuum and N_2/CO_2 combinations. Redness of sausages was significantly affected by the storage time, packaging conditions and the addition of starter cultures ($p < 0.05$). Our results don't match with those of Ruiz-Capillas and Jiménez-Colmenero (2010) who reported that a* values remain constant during the storage of meat products packaged under modified atmosphere. The evolution of redness in sausages was related to many factors such as the composition, the technology used and surface water availability (Young and Sang, 2004; Geun et al., 2004).

Table 1 shows that b* values increased during the storage period of the different samples, which could be due to the use of paprika for the preparation of sausages. Similar results were found by Cachaldora et al. (2013). This result is not in agreement with that found by Ruiz-Capillas and Jiménez-Colmenero (2010) who reported a constant b* values during the storage of sausages under modified atmospheres. Moreover, packaging conditions and the use of starter cultures had no significant effect on the evolution of yellowness.

In regards to the vividness of the color, the chroma (C*) value increased during the storage of the different sausages. No significant differences were found between sausages packaged under Atm 1 and Atm2. Our results show that sausages packaged under modified atmospheres had the highest value of C*. Finally, the hue (h*) value remained practically constant during storage of sausages.

3.1.3. Lipid oxidation

The initial sr-TBA values, which were 0.93 mg MDA/kg and 0.7 mg MDA/kg, increased ($p < 0.05$) to 0.93 mg MDA/kg and 0.7 mg MDA/kg, respectively for the control and starter-mediated sausages, after 28 days of storage (Fig. 3). Significant difference ($p < 0.05$) was found between the different samples during the storage period. Lipid oxidation is affected by many factors such

Vol. 5, No. 02; 2020

ISSN: 2456-3676

as the composition of meat, light, and access to oxygen (Ahn and Fernando, 2002). The TBARS values are lower ($p > 0.05$) in sausages inoculated with mixed starter cultures than those measured on control samples. Several studies reported the antioxidant activities of *S. xylosus* and *S. carnosus* due to their catalase and superoxide dismutase enzymes. Similar results were found by Kargozari et al. (2014) and Mejri et al. (2017).

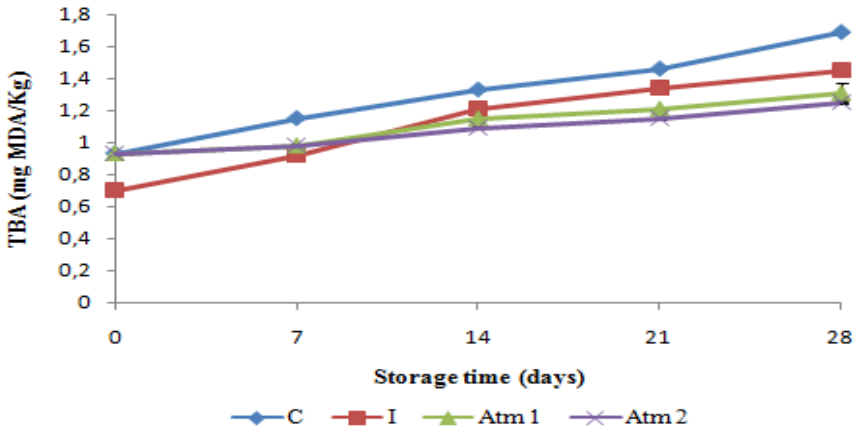


Figure 3- Changes in thiobarbituric acid (TBA) values during storage at 4 °C of dry fermented horse meat sausages: C (control sausage), I (sausage inoculated with mixed starter cultures), Atm 1 (Sausage packaged under modified atmosphere 60% N_2/40% CO_2), Atm 2 (Sausage packaged under modified atmosphere 40% N_2/60% CO_2)

Moreover, results show that sausages packaged under modified atmospheres (Atm1 and Atm 2) had the lowest sr-TBA values compared to the control and inoculated samples. In fact, the sr-TBA values increased ($p < 0.05$) from 0.93 mg MDA/kg, to reach at the end of the storage period average values of 1.31 mg MDA/kg and 1.25 mg MDA/kg, respectively in sausages packaged under Atm 1 and Atm 2. These findings could be explained by the inhibitory effect of carbon dioxide on the lipid oxidation (Cachaldora et al., 2013). Gokoglu et al. (2010) found that a concentrations of $CO_2 > 70\%$ in frankfurter-type sausages could limit the oxidation.

3.2. Microbiological analysis

The number of LAB decreased during storage period to reach at day 28 values of 4.3, 5.41, 4.83 and 4.85 log CFU/g, respectively in control, inoculated samples and sausages packaged under Atm 1 and Atm 2 (Fig. 4). Our results match with those found by other studies showing that the use of modified atmosphere packaging does not delay the growth of LAB during storage (Kant-Muermans et al., 1997; Pexara et al., 2002). Cachaldora et al. (2013) explained the dominance of lactic acid bacteria by their ability to resist to stress.

Vol. 5, No. 02; 2020

ISSN: 2456-3676

Initial total viable counts (TVC) were 7.78 and 8.78 log CFU/g respectively, for the control sausages and those that were inoculated with a mixture of strains (Fig. 4). (Essid and Hassouna, 2013; Mejri et al., 2017). The higher TVC was observed in control. In fact, they reached at the end of storage value of 7.08 log CFU/g (Fig. 4). However, the number of TVC for samples packaged under MAP decreased during storage which could be due to the inhibition effect of CO_2.

In control and inoculated sausages, the initial numbers of *E. coli* were 1.48 and 1 log CFU/g, respectively (Fig. 4). The higher number of *E. coli* counts was observed in control. They reached at the end of storage value of 2.93 log CFU/g. The evolution of *E. coli* in MAP packages was similar ($p > 0.05$). In fact, their number increased during the first seven day of storage and then decreased to reach at day 28 of storage values of 1.67 and 1.6 log CFU/g, respectively in the sausages packaged under Atm1 and Atm2 (Fig. 4). However, the lowest counts of *E. coli* were found in inoculated sausages. The reduction of the number of harmful germs is attributed first of all to the antimicrobial and the acidification activities of *L. sakei* (Deumier and Collignan, 2003) and second to the inhibitory effect of carbon dioxide injected which has been found to have a bacteriostatic effect on gram negative spoilage organisms. In fact, Ho et al. (2003) reported that concentrations as low as 10-20% of carbon dioxide could inhibit effectively the growth of pathogenic microorganisms. In fact, CO_2 is able to produce rapid acidification of the cells thus influencing metabolic activities. Moreover, CO_2 appears to have an effect on certain enzyme systems (Ho et al., 2003). According European Committee legislation (Regulation EC 2073/05, DOUE L338/1, 2005) the limit established in dry fermented sausages for *E. coli* counts is $5\ 10^2$ CFU/g. Therefore, the shelf life of control horse meat sausage would be 21 days, while for inoculated and MAP samples, this shelf life would be more than 28 days of storage. Djenane et al. (2001) and Lorenzo and Gomez (2012) found a shelf life of 14 to 21 days for beef meat and foal meat packaged under different modified atmospheres.

As hygiene indicator, Enterobacteriaceae showed at the beginning of the storage values of 2.39 and 1.55 log CFU/g, respectively in control and inoculated sausages (Fig. 4). These values increased during the storage for inoculated samples ($p > 0.05$), whereas this increase was more pronounced for the control ones. However, Enterobacteriaceae counts decreased significantly ($p < 0.05$) in sausages packaged under modified atmospheres, during storage (Fig. 4). No significant differences were found between packaging conditions ($p > 0.05$). Our results are in agreement with many other authors who reported a similar behavior in MAP packages (Esmer et al., 2011; Karabagias et al., 2011). However, Seydim et al. (2006) found that the Enterobacteriaceae counts increased during all the storage time and the type of packaging had no effect on the evolution of this germ.

Vol. 5, No. 02; 2020

ISSN: 2456-3676

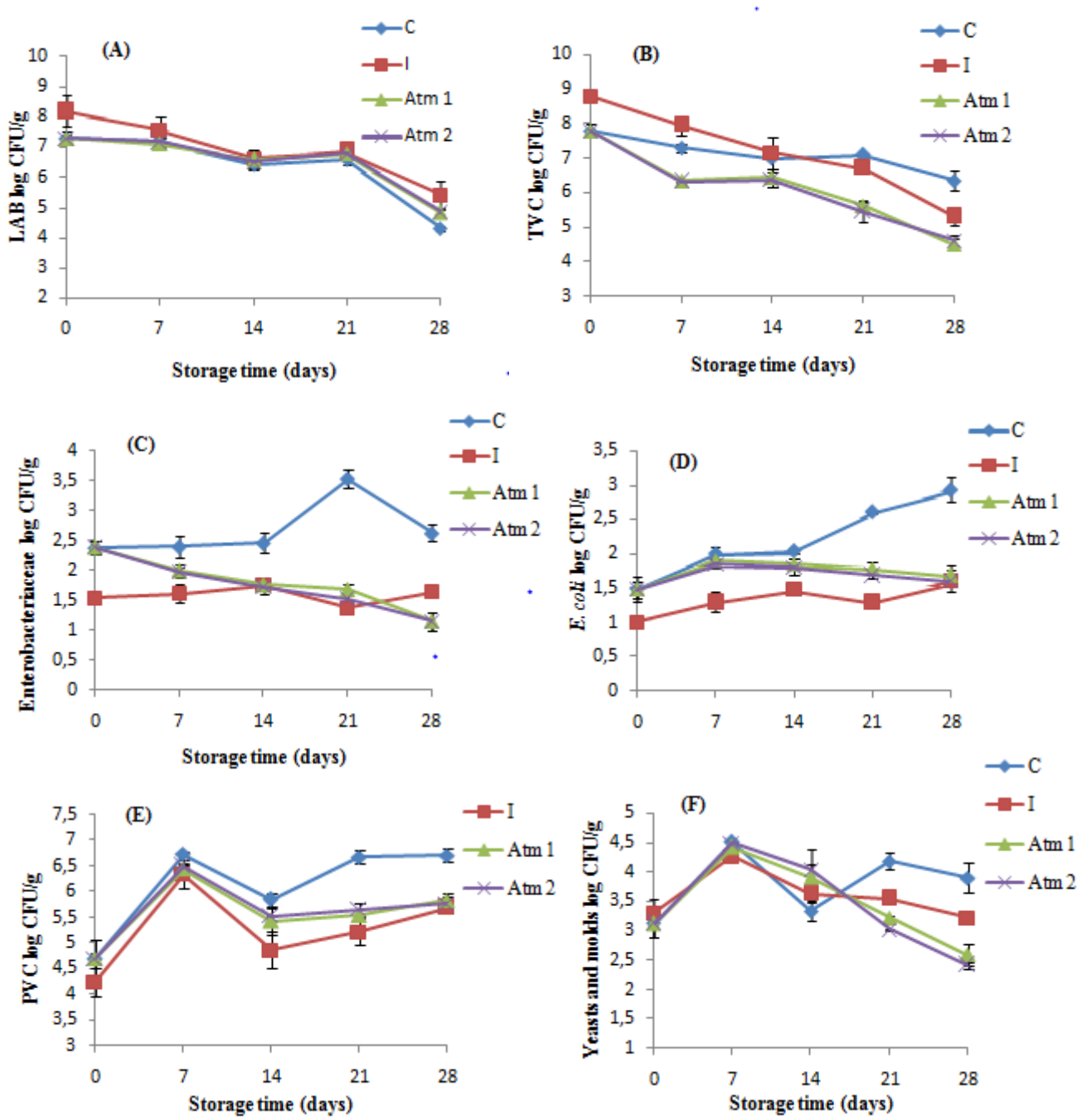


Figure 4- Evolution of LAB (A), TVC (B), Enterobacteriaceae (C), *E. coli* (D), PVC (E) and moulds and yeasts (F) during storage at 4 °C of dry fermented horse meat sausages: C (control sausage), I (sausage inoculated with mixed starter cultures), Atm 1 (Sausage packaged under

Vol. 5, No. 02; 2020

ISSN: 2456-3676

modified atmosphere 60% N_2/40% CO_2), Atm 2 (Sausage packaged under modified atmosphere 40% N_2/60% CO_2)

The numbers of psychrotrophic bacteria increased in the different types of sausages, during storage (Fig. 4). The highest numbers are those of control sausages. In fact, the number of Psychrotrophic bacteria in the control samples increased during storage period to reach at 28th day mean value of 6.71 log (CFU/g). Similar results were found by other studies (Diez et al., 2009; Ruiz-Capillas and Jiménez, 2010). Results show that the evolution of psychrotrophic bacteria was similar ($p > 0.05$) in the inoculated sausages and sausages packaged under modified atmospheres, there are an increase in the number of this germ. Our results are in agreement with those found by Fernández-López et al. (2008) and Cachaldora et al. (2013).

For yeasts and molds, the average values measured on the 28th day of storage, respectively in control samples and inoculated sausages, were 3.9 log (CFU /g) and 3.22 log CFU/g (Fig. 4). Moreover, in sausages packaged under modified atmospheres, the number of yeasts and molds decreased significantly ($p < 0.05$) during storage to reach values of 2.59 and 2.45 log CFU/g after four weeks of storage, respectively, in sausages packaged under Atm 1 and Atm 2 (Fig. 4). These results are in agreement with those found by Cachaldora et al. (2013). The decrease in the numbers of yeasts and molds is explained by the anaerobic conditions created by the packaging under modified atmospheres, which represents an unfavorable medium to the growth of aerobic germs. Sørheim et al. (2004) have shown that a dose of 20 to 30% of CO_2 can preserve the growth of aerobic bacteria.

3.3. Sensory evaluation

The effect of mixed starter cultures and modified atmosphere packaging on sensory attributes during storage is shown in Table 2. Sausages packaged under MAP conditions and sausages inoculated with starter cultures had high values between 7.6 and 8.2 after 7 days of storage, while control samples showed a lower score (4.4). The limit of acceptability (score of 5) was reached after 4 weeks of storage for inoculated sausages and sausages packaged under MAP. Our results were similar to those found for color evaluation. Cachaldora et al. (2013) found that Blood sausage "*morcilla*" packed under O_2/N_2/CO_2 was less acceptable after 8 weeks of storage than those packaged under vacuum or with N_2/CO_2 combinations.

Table 2- Sensory evaluation (red color, off-odor and general appearance) during storage at 4°C of dry fermented horse meat sausages: C (control sausage), I (sausage inoculated with mixed starter cultures), Atm 1 (Sausage packaged under modified atmosphere 60% N_2/40% CO_2), Atm 2 (Sausage packaged under modified atmosphere 40% N_2/60% CO_2)

Vol. 5, No. 02; 2020

ISSN: 2456-3676

Data are means ± standard deviation.
Different letters in the same row indicate significant differences ($p < 0.05$).

Moreover, Ravyts et al. (2010) reported that inoculated sausages get high score of red color than control ones. This could be due to nitrate reductase activity of CNS.
The "off-odor" was absent until the end of storage time respectively, in inoculated sausages and sausages packaged under Atm 1 and Atm 2, and no significant differences ($p > 0.05$) were observed between the different three treatments. In fact, the lipid oxidation has an effect on the "off-odor". Results show that "off-odor" was related ($p < 0.05$) to TBARS values with correlation coefficient of $r = - 0.845$, for control, inoculated sausages and sausages packaged respectively, under Atm 1 and Atm 2 (Table 3). Greene and Cumuze (1981) reported that levels of TBARS < 2.0 mg/kg didn't affect the odor of meat products.
The scores of "appearance" decreased in the different sausages (Table 2) during storage. No significant differences ($p > 0.05$) were observed between samples packaged under Atm 1 and Atm 2 along the whole storage period. Similar results were found by Bingol and Ergun (2011).

Tableau 3- Pearson's correlation coefficients between TBARS and sensorial data measured during the storage of the different samples of sausages alone or inoculated with various bacterial strains or packaged under modified atmospheres

Treatment	Days	Red color	Off-odor	Appearance
C	7	4.4 ± 0.29^{d}	5.16 ± 0.37^{d}	5.67 ± 0.37^{d}
	14	3.82 ± 0.34^{c}	4.65 ± 0.74^{c}	4.35 ± 0.59^{c}
	21	2.70 ± 0.59^{b}	3.97 ± 0.47^{b}	3.09 ± 0.56^{b}
	28	1.8 ± 0.4^{a}	3.72 ± 0.85^{a}	2.75 ± 0.31^{a}
I	7	8.2 ± 0.4^{d}	7.13 ± 0.41^{d}	7.54 ± 0.28^{d}
	14	7.8 ± 0.28^{c}	6.72 ± 0.25^{c}	6.5 ± 0.2^{c}
	21	6.66 ± 0.58^{b}	6.58 ± 0.98^{b}	5.42 ± 0.74^{b}
	28	5.76 ± 0.61^{a}	5.26 ± 1.76^{a}	4.88 ± 0.35^{a}
Atm 1	7	7.8 ± 0.49^{d}	6.97 ± 0.31^{d}	7.3 ± 0.42^{d}
	14	6.77 ± 0.5^{c}	6.33 ± 0.29^{c}	6.48 ± 0.58^{c}
	21	6.53 ± 0.39^{b}	5.68 ± 0.46^{a}	5.5 ± 0.26^{b}
	28	5.78 ± 0.28^{a}	5.81 ± 0.47^{b}	4.6 ± 0.33^{a}
Atm 2	7	7.6 ± 0.49^{d}	6.71 ± 0.1^{d}	7.1 ± 0.4^{d}
	14	6.56 ± 0.38^{c}	6.21 ± 0.32^{c}	6.12 ± 0.68^{c}
	21	6.23 ± 0.21^{b}	5.52 ± 0.59^{b}	5.2 ± 0.2^{b}
	28	5.40 ± 0.44^{a}	5.11 ± 0.71^{a}	4.3 ± 0.33^{a}

Vol. 5, No. 02; 2020

ISSN: 2456-3676

Variables	TBARS	Red color	Off-odor	Appearance
TBARS	1*	-0,786*	-0,845*	-0,904*
Red color	-0,786*	1*	0,953*	0,917*
Off-odor	-0,845*	0,953*	1*	0,949*
Appearance	-0,904*	0,917*	0,949*	1*

*P < 0.05.

LAB: Lactic acid bacteria; PVC: Psychrotrophic bacteria; TVC: Total viable counts; EB: Enterobacteriaceae; YM: Yests and molds

3.4. Principal components analysis

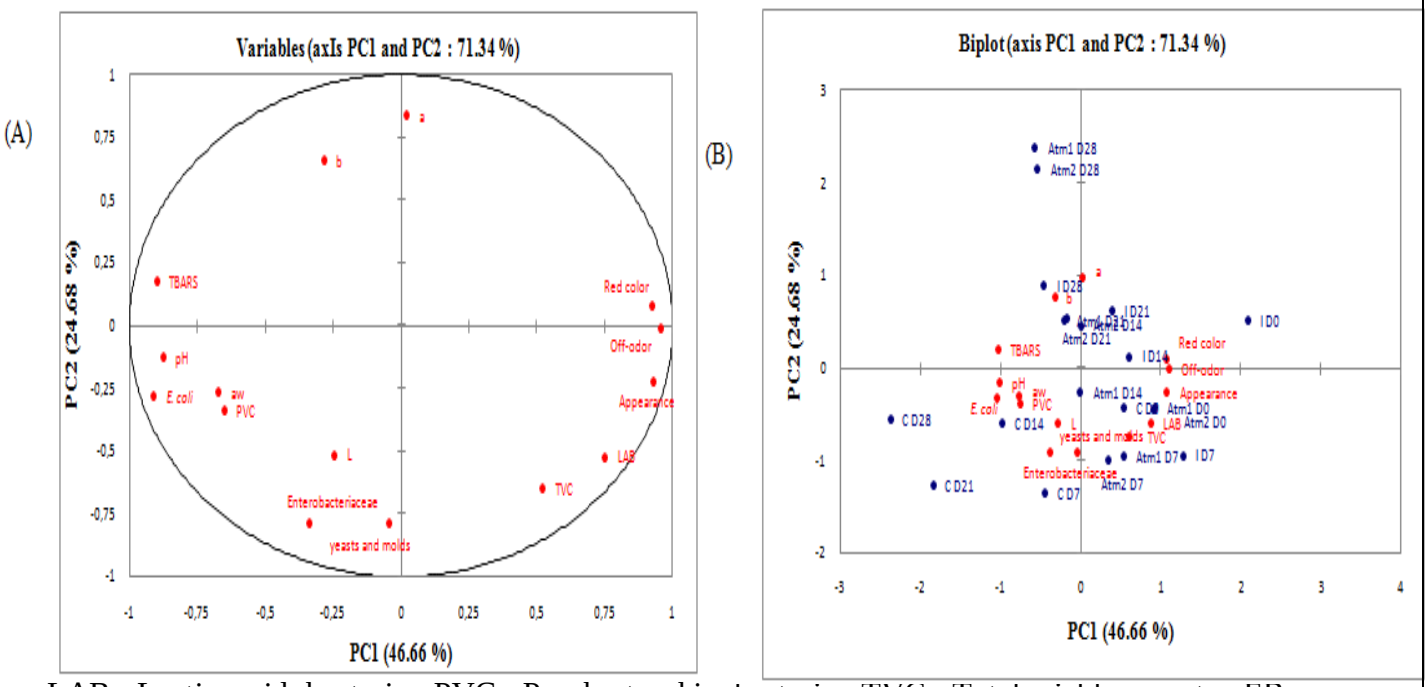
The results of this analysis are summarized in Fig. 5A and B. The aim of the use of PCA is to analyze the relationships between the microbiological, physicochemical and sensorial variables and to identify the proximities between the sausages samples studied. The PCA showed that 71.34 % of the variability was explained by two first principal components. The principal component 1 (PC1) was the most important variable in terms of differences among sausages studied as it represented 46.66 % of the total variability.

As can be seen in Fig. 5A, PC 1 was positively related with sensory parameters (red color, off-odor, appearance), LAB and TVC. In addition, PC1 was inversely related with psychrotrophic (PVC), *E. coli*, TBARS, pH and water activity (a_w). On the other hand, Enterobacteriaceae bacteria, yeasts and molds and color parameters (L*, a*, b*) variables are well represented on the PC 2 axis (24.68%) (Figure 5A).

The different samples of sausages C D0, Atm1 D0, Atm2 D0, Atm1 D7, Atm2 D7, I D7 are well placed on the positive PC1 axis, which testifies their proximity in the space translating their real resemblance in terms of hygienic and physicochemical quality (figure 5B). On the other hand, Fig. 5B shows that there was no significant difference ($p > 0.05$) between the different sausages packaged under modified atmospheres (Atm1 and Atm2) during the whole period of storage since they are very close in the space. However, there was a significant difference ($p < 0.05$) between the control and the other three treatments (inoculated sausages and sausages packaged under modified atmospheres).

Vol. 5, No. 02; 2020

ISSN: 2456-3676



LAB: Lactic acid bacteria; PVC: Psychrotrophic bacteria; TVC: Total viable counts; EB: Enterobacteriaceae; YM: Yests and molds; D: Day.

Figure 5- Relationships among the sausage samples (control (C), inoculated (I) and packaged under modified atmospheres (Atm1/Atm2)) and microbial counts, physicochemical and sensory properties obtained by PCA. (A) Projection of the variables in the plane defined by the first two principal components. (B) Projection of the variables and the sausage samples in the plane defined by the first two principal components

Results show that pH was positively and significantly correlated ($p < 0.05$) with the following variables: Enterobacteriaceae, *E. coli*, psychrotrophic bacteria (PVC), TBARS and water activity (a_w). Moreover, simple, negative and statistically significant linear correlations ($p < 0.05$) were noted between lipid oxidation index, total viable counts (TVC), LAB and sensory parameters (red color, off-odor and appearance). Enterobacteriaceae, *E. coli* and pH variables seem to be the determining factors of the hygienic quality of sausages (figure 5A).
In conclusion, PC1 differentiated control sausages from inoculated samlples and sausages packaged under modified atmospheres (Atm1 and Atm2). Control sausages were related to microbial analysis (*E.coli*, PVC), lipid oxidation (TBARS) and water activity (a_w) (Fig. 5B), which were more abundant in control samples and they contributed to decrease their shelf life.

4. Conclusion

Vol. 5, No. 02; 2020

ISSN: 2456-3676

The use of mixed starter cultures and MAP had the same effects on microbiological, physicochemical and sensorial parameters of dry fermented horse meat sausages compared to control ones. Based on the microbial results, the shelf life of control horse meat sausage was 21 days, while for inoculated samples and sausages packaged under modified atmospheres (Atm1 and Atm2); this shelf life would be more than 28 days of storage. It can be concluded that modified atmosphere packaging and the use of mixed starter cultures are two techniques that could be useful to extend the shelf life of meat products.

Acknowledgements

This study was sponsored by the «Tunisian Ministry of Higher Education and Scientific Research», Tunisia. The authors thank the Technical Center of Food Packaging (PACKTEC) (Tunis El Khadra, Tunisia) for providing part of the equipment used in this work.

References

Ahn J., Grün I.U. and Fernando L.N. 2002. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. J. Food Sci. 67: 1364-1369.

Bingol E.B. and Ergun O. 2011. Effects of modified atmosphere packaging (MAP) on the microbiological quality and shelf life of ostrich meat. Meat Sci. 88: 774-785.

Cachaldora A., García G., Lorenzo J.M. and García-Fontán M.C. 2013. Effect of modified atmosphere and vacuum packaging on some quality characteristics and the shelf-life of "morcilla", a typical cooked blood sausage. Meat Sci. 93: 220-225.

Deumier F. and Collignan A. 2003. The effects of sodium lactate and starter cultures on pH, lactic acid bacteria, Listeria monocytogenes and Salmonella spp. levels in pure chicken dry fermented sausage. Meat Sci. 65: 1165-1174.

Díez A.M., Jaime I. and Rovira J. 2009. The influence of different preservation methods on spoilage bacteria populations inoculated in morcilla de Burgos during anaerobic cold storage. Inter. J. Food Microbiol. 132: 91-99.

Djenane D., Sánchez-Escalante A., Beltrán J.A. and Roncalés P. 2001. Extension of the retail display life of fresh beef packaged in modified atmosphere by varying lighting conditions. J. Food Sci. 66: 181-186.

El Adab S., Essid I. and Hassouna M. 2015. Microbiological, biochemical and textural characteristics of a Tunisian dry fermented poultry meat sausage inoculated with selected starter cultures. J. Food Safety. 35: 75-85.

Esmer O.K., Irkin R., Degirmencioglu N. and Degirmencioglu A. 2011. The effects of modified atmosphere gas composition on microbiological criteria, color and oxidation values of minced beef meat. Meat Sci. 88: 221–226.

Vol. 5, No. 02; 2020

ISSN: 2456-3676

Essid I. and Hassouna M. 2013. Effect of inoculation of selected *Staphylococcus xylosus* and *Lactobacillus plantarum* strains on biochemical, microbiological and textural characteristics of a Tunisian dry fermented sausage. Food Control. 32: 707-714.

Fernández-Fernández E., Vázquez-Odériz M.L. and Romero-Rodriguez M.A. 2002. Sensory characteristics of Galician chorizo sausage packed under vacuum and under modified atmospheres. Meat Sci. 62: 67-71.

Fernández-López J., Sayas-Barberá E., Muñoz T., Sendra E., Navarro C. and Pérez-Álvarez J. A. 2008. Effect of packaging conditions on shelf-life of ostrich steaks. Meat Sci. 78: 143-152.

Franco D., Rodríguez E., Purriños L., Crecente S., Bermúdez R. and Lorenzo J.M. 2011. Meat quality of "Galician Mountain" foals breed. Effect of sex, slaughter age and livestock production system. Meat Sci. 88: 292-298.

Genot C. 1996. Some factors influencing TBA test. Report of diet-ox project (AIRIII-CT-92-1577).

Geun P.H., Sung L. and Sang G.M. 2004. Effects of substituted level of added water for fat on the quality characteristics of spreadable liver sausage. Food Sci. Biotechnol. 13: 397-402.

Gokoglu N., Yerlikaya P., Uran H. and Topuz O.K. 2010. The effect of modified atmosphere packaging on the quality and shelf life of frankfurter type-sausages. J. Food Quality. 33: 367-380.

Gøtterup J., Olsen K., Knøchel S., Tjener K., Stahnke L.H. and Møller J.K.S. 2008. Colour formation in fermented sausages by meat-associated staphylococci with different nitrite- and nitrate-reductase activities. Meat Sci. 78: 492-501.

Greene B.E. and Cumuze T.H. 1981. Relationship between TBA numbers and inexperience panellists assessments of oxidized flavour in cooked beef. J. Food Sci. 47: 52-58.

Ho C.P., Huang N.Y. and Mcmillin K.W. 2003. Microflora and color of ground beef in gas exchange modified atmosphere packaging with abusive display temperatures. J. Food Sci. 68: 1771-1776.

Jakobsen M. and Bertelsen G. 2004. Predicting the amount of carbon dioxide absorbed in meat. Meat Sci. 68: 603-610.

Juncher D., Ronn B., Mortenssen E.T., Henckel P., Karlsson A. and Skibsted L.H. 2001. Effects of pre-slaughter physiological conditions on the oxidative stability of color and lipid oxidation during chill storage of pork. Meat Sci. 58: 347-357.

Karabagias I., Badeka A. and Kontominas M.G. 2011. Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. Meat Sci. 88: 109-116.

Kargozari M., Moini S., Basti A.A., Emam-Djomeh Z., Ghasemlou M., Martin I.R., Carbonell-Barrachina A.A. and Szumny A. 2014. Development of Turkish dry-fermented sausage (sucuk) reformulated with camel meat and hump fat and evaluation of physicochemical, textural, fatty acid and volatile compound profiles during ripening. LWT - Food Sci. Technol. 59: 849-858.

Kant-Muermans M.L.T., Stekelenburg F.K., Zwietering M.H. and Huis In't Veld J.H.J. 1997. Modeling the Shelf-life of Packed, Cooked Meat Products. World Congress on Food Hygiene, The Hague, pp. 53-57.

Vol. 5, No. 02; 2020

ISSN: 2456-3676

Lorenzo J.M., Fuciños C., Purriños L. and Franco D. 2010. Intramuscular fatty acid composition of "Galician Mountain" foals breed. Effect of sex, slaughtered age and livestock production system. Meat Sci. 86: 825-831.

Lorenzo J.M. and Gómez M. 2012. Shelf life of fresh foal meat under MAP, overwrap and vacuum packaging conditions. Meat Sci. 92: 610-618.

Mejri L., Ziadi A., El Adab S., Boulares M., Essid I. and Hassouna M. 2017. Effect of commercial starter cultures on physicochemical, microbiological and textural characteristics of a traditional dry fermented sausage reformulated with camel meat and hump fat. J. Food Meas. Charact. 11: 758-767.

Özogul F., Polat A. and Özogul Y. 2004. The effects of modified atmosphere packaging on chemical, sensory and microbial changes of Sardines (Sardina pilchardus). Food Chem. 85: 49-57.

Pexara E.S., Metaxopoulos J. and Drosinos E.H. 2002. Evaluation of shelf life of cured, cooked, sliced turkey fillets and cooked pork sausages "Piroski" stored under vacuum and modified atmospheres at +4 and +10 °C. Meat Sci. 62: 33-43.

Roland A.M., Phillips L.G. and Boor K.J. 1999. Effects of fat content on the sensory properties, melting, color, and hardness of ice cream. J. Dairy Sci. 82: 32–38.

Ravyts F., Steen L., Goemaere O., Paelinck H., De Vuyst L. and Leroy F. 2010. The application of staphylococci with flavor generating potential is affected by acidification in fermented dry sausages. Food Microbiol. 27: 945-954.

Rubio B., Martínez B., Sánchez M.J., García-Cachán M.D., Rovira J. and Jaime I. 2007. Study of the shelf life of a dry fermented sausage "salchichón" made from raw material enriched in monounsatured and polyunsatured fatty acids and stored under modified atmospheres. Meat Sci. 76: 128-137.

Ruiz-Capillas C. and Jiménez-Colmenero J. 2010. Effect of an argon-containing atmosphere on the quality of fresh pork sausages during refrigerated storage. Food Control. 21: 1331-1337.

Seydim A.C., Acton J.C., Hall M.A. and Dawson P.L. 2006. Effects of packaging atmospheres on shelf-life quality of ground ostrich meat. Meat Sci. 73: 503-510.

Sørheim O., Wahlgren M., Nilsen B.N. and Lea P. 2004. Effects of high oxygen packaging on tenderness and quality characteristics of beef longissimus muscles. Proceedings 50th International Congress of Meat Science and Technology, Helsinki, Finland, August 8–13.

Young T.L. and Sang S.K. 2004. Effect of elevated steeping temperature on the starch properties of stored milled rice. Food Sci. Biotechnol. 13: 332-336.

Zanardi E., Dorigoni V., Badiani A. and Chizzolini R. 2002. Lipid and color stability of Milano-type sausages: Effect of packing conditions. Meat Sci. 61: 7-14.