PAPER

PHYSICOCHEMICAL, MICROBIOLOGICAL AND COLOUR ATTRIBUTES OF HORSE SALAMI ESTABLISHED DURING THE RIPENING PERIOD

D. KOVAČEVIĆ[a], K. MASTANJEVIĆ[a] *, J. PLEADIN[b] and J. FRECE[c]
[a]Department of Food Technology, Faculty of Food Technology University of Osijek,
F. Kuhača 20, HR-31000 Osijek, Croatia
[b]Laboratory for Analytical Chemistry, Croatian Veterinary Institute,
Savska 143, HR-10000 Zagreb, Croatia
[c]Department of Biochemical Engineering, Faculty of Food and Biotechnology University of Zagreb,
Pierrotijeva 6, HR-10000 Zagreb, Croatia
*Corresponding author: Tel. +385 31 224 298 Fax +385 31 207 115,
email: kresimir.Mastanjevic@ptfos.hr

ABSTRACT

Changes in physicochemical, colour, textural, microbiological and sensory attributes occurring during the processing of Horse Salami and established on manufacturing days 0, 7, 14, 21, 28, 42, 60, 90 were studied. Significant changes ($P<0.05$) in physicochemical parameters attributable to moisture loss, as well as changes in colour and textural properties were observed during the fermentation and ripening stage. Proteolysis and lipolysis, coming as a result of endogenous enzymatic activity and high lactic acid bacteria and staphylococci counts, contributed to specific organoleptic properties of the final product. Sensorial profiling showed a significant ($P<0.05$) acid taste, lactic acid odour and flavour intensity, and low fat/lean ratio and smokiness and saltiness values. Final Horse Salami products were microbiologically safe, the dominant microbial population thereby being *Lactobacillus plantarum*, *Lactococcus lactis* ssp. *lactis*, *Enterococcus faecium* and *Staphylococcus xylosus*.

- Keywords: CIE L*, a*, b*, horse Salami, microbiological aspect, physicochemical properties, Sensory attributes, texture profile analysis -

INTRODUCTION

Horse Salami, an indigenous Croatian meat product, is a dry fermented sausage made of horse meat supplemented with pork fatback, salt and spices. In Croatia, the tradition of Horse Salami production is kept by the Italian minority populating the eastern part of the Country (in specific, the western Slavonian region). In the past, this product had been known as "the dish of the poor"; nowadays, it represents a highly appreciated autochthonous Croatian meat product having a great potential to become a PGI (*Protected Geographical Indications*) & PDO (*Protected Designation of Origin*). Although horse meat has a high nutrition, as well as a high mineral value (due to its vitamin B and iron content, respectively) (BADIANI *et al.*, 1997; FRANCO *et al.*,, 2011), human consumption is negligible in comparison with other conventional types of meat like pork, beef or chicken (LOMBARDI *et al.*, 2005). Horse meat used for the production of Horse Salami is obtained from horses slaughtered at the end of their (5-year or longer) lifecycle. The meat has no appreciable organoleptic qualities. Its original colour is deep red larded with yellow fat, while the meat is tough to chew due to the connective tissue maturation (LITWINCZUK *et al.*, 2008; TATEO *et al.*, 2008).

Horse Salami has specific sensorial properties (smell and taste) attributable mainly to drying and smoking, but also to ripening, as well as to enzymatic, lactic acid bacteria and mould activity. The recipe is 130 years old and the sole difference in final products coming from various producers boils down to the difference in mass fraction of fatback used in the Salami preparation (ranging from 12 to 15%). The production of the traditional Horse Salami mainly takes place on small farms; we are therefore talking a small-scale production seasonal in its nature, fluctuating on a year-by-year basis dependent on weather conditions. In light of the foregoing, standardization of the Horse Salami production becomes imperative. Dry sausages produced in various European countries, mainly Spain and Italy, have been extensively studied for their physicochemical composition, colour and textural properties (CASIRAGHI, *et al.*, 1996; GIMENO *et al.*, 2000; BRUNA *et al.*, 2001; SPAZIANI *et al.*, 2009). However, scientific information on this Croatian indigenous dry sausage, which would efficiently contribute to its characterization and production standardisation, is virtually non-existent.

Therefore, the aim of this study was to investigate, for the first time ever, physicochemical composition, microbiological and sensorial attributes of the dry-fermented sausage known as Horse Salami and the changes occurring during 90 days of its manufacturing. Investigations also included instrumental measurements of colour and texture of the studied Salami on certain processing days, as well as the isolation and identification of autochthonous microbial population and gathering of other data needed for microbiological safety evaluation of the final product.

MATERIAL AND METHODS

The manufacturing process

Samples of traditional Horse Salami (24 units) were manufactured in a small-scale facility in the western Slavonian region (the Eastern Croatia). All samples were prepared using traditional procedures that made no use of additives such as starter cultures supplemented with nitrites, nitrates or ascorbic acid (namely, the production of traditional Croatian meat products does not involve the use of additives). Such a traditional production takes about 3 months (90 days). Horse Salami is made of meat of older (5+ years), worn-out horses, mainly of the Hrvatski Posavac breed. After slaughtering, fat and connective tissue are carefully removed from the horse meat. This is especially important when it comes to fat, because horse fat has a particularly unpleasant smell and taste. The meat is then grinded using a grinding plate having holes measuring 6 mm in their diameter and left to rest overnight (12 hours at the minimum) in a special container equipped with a decantation hole. Grinded horse meat is then mixed with pig fatback represented in the amount of 12%. Before its mixing with the horse meat, the fatback is grinded using a grinding plate having holes measuring 10 mm in their diameter. The mixture of meat and fat is then mixed with salt added in the amount of 2.2%, red paprika powder added in the amount of 0.2%, hot red paprika powder added in the amount of 0.3%, garlic added in the amount of 0.2%, and black pepper added in the amount of 0.3%. In the subsequent course, the mixture gets to be stuffed into a horse small intestine (roughly 50 cm long and 50 mm wide in diameter) or into collagen casings (of the same dimensions). Thereafter, the Horse Salami is smoked on a dry hard wood (hornbeam, beech and its sawdust) every few days (for 2-3 hours) for the total of four weeks. At this stage, the temperature and relative humidity should be kept at 18 to 20°C and 70 to 90%, respectively. After smoking, the Horse Salami is left to ripen. This stage is the longest and should take about two months, throughout which period the Salami should be kept in a dark room at the temperature ranging from 14° to 17°C, with the relative humidity ranging from 70 to 80%. After that, Horse Salami is ready for consumption. Within this study frame, samples of Horse Salami were taken on the processing days 0, 7, 14, 21, 28, 42, 60 and 90. In total, 24 samples were produced; at each processing stage, three samples were taken for the analyses.

ANALYTICAL METHODS

Physicochemical parameters

Before the analysis, the Sausage samples were homogenised using a knife mill Gridomix GM 200 (Retsh, Germany) and prepared according to ISO 3100-1:1975.

Water content was determined gravimetrically (ISO 1442:1997) at 103°C (Epsa 2000 Bari, Croatia), while the ash content was established according to ISO 936:1998, by virtue of burning the samples at 550 °C (LV9/11/P320 Nobertherm, Germany). Total protein content was determined using the Kjeldahl method (ISO 937:1978) that made use of an Unit 8 Basic digestion block (Foss, Sweden) and a Kjeltec 8400 automated distillation & titration device (Foss, Sweden). The total fat content was determined using the Soxhlet method (ISO 1443:1973), which involves digestion of a sample in acidic environment followed by fat extraction with petroleum ether using a Soxtherm 2000 Automatic device (Gerhardt, Germany). The determination of collagen content was performed through the analysis of hydroxyproline according to ISO 3496:1994 that made use of a spectrophotometer (Hach DR/4000U, Germany). Sodium chloride content was determined using the internal titration method (TRAJKOVIĆ *et al.*, 1983). In this analysis, 2 g of each sample were homogenized with sand and 3 mL of water. The content was transferred into a 100 mL-volumetric flask, stirred and placed for 15 min into a water bath at 100 °C. After cooling, the flask was filled with water up to the mark and filtered. An aliquot (25 mL) of the filtrate was transferred into an Erlenmeyer flask containing a few drops of K_2CrO_4 indicator (62 g/100 mL of water) and titrated with 0.1 M-$AgNO_3$ until a persistent reddish colour was obtained. Sodium chloride content was calculated based on the expenditure of titration reagent and its concentration.

pH values were determined in a homogenate diluted with distilled water (1:10, p/v) using pH/Ion 510 – Bench pH/Ion/mV Meter (Eutech Instruments Pte Ltd/ Oakton Instruments, USA) according to the pH/Ion 510 Instruction Manual. Water activity (a_w) was determined at the room temperature (20°±2°C) using a Rotronic Hygrolab 3 (Rotronic AG, Bassersdorf, Switzerland). All chemicals used for analyses of physicochemical parameters were of an analytical grade. For each sample, three independent measurements were made.

Instrumental determination of colour

Instrumental colour measurements (those of L*, a*, and b* values) were performed using a Hunter-Lab Mini ScanXE (A60-1010-615 Model Colorimeter, Hunter-Lab, Reston, VA, USA). The instrument was standardized on each occasion using a white ceramic plate (L_0 = 93.01, a_0 = -1.11, and b_0 = 1.30). The CIELAB space values (L*, a* and b*) (CIE, 1976) correspond to lightness, greenness (-a*), redness (a*), blueness (-b*) or yellowness (b*). The colour measurements performed on the Horse Salami took place at the room temperature (20°±2°C). Each sample was cut in slices and colour-measured at ten different spots.

Texture Profile Analysis

Texture Profile Analysis (TPA) was performed using a TA.XT2i SMS Stable Micro Systems Texture Analyzer (Stable Microsystems Ltd, Surrey, England) equipped with a P/75 aluminium cylindrical probe. This involved cutting the samples into 1.5 cm-thick slices and their double compression so as to downsize them to 40% of their original thickness. Force-time curves were recorded at the across-head speed of 5 mms^{-1} and at the same recording speed. The following parameters were quantified (BOURNE, 1978): hardness (kg), i.e. the maximum force required to compress the sample; springiness (ratio), i.e. the ability of the sample to recover its original form after the cessation of the deforming force; cohesiveness (ratio), i.e. the extent to which the sample could be deformed prior to rupture; chewiness (kg), i.e. labour required to masticate the sample before swallowing, which represents the product of hardness multiplied by cohesiveness and springiness; and finally resilience (ratio), so as to determine how well the product "fights to regain its original position". These parameters were obtained using the Texture Expert for Windows (Version 1.0) Stable Micro Systems. With each sample, eight determinations of texture parameters were made.

Microbiological analysis

After aseptically removing and discarding the casing, 10 g of the product were recovered in an aseptic manner, homogenized in 90 ml of the sterile 0.5%-saline solution and serially diluted before their planting on a non-selective (peptone yeast extract glucose agar, Biolife, Milano, Italy), PCA-agar (standard plate count agar) (Biolife, Milano, Italy) and the following selective media: MRS-agar (Biolife, Milano, Italy) intended for lactic acid bacteria growth and Baird-Parker agar (Merck, Darmstadt, Germany) intended for staphylococci growth. The plates were incubated under conditions specified in Table 1.

Isolation and identification of microbial population in the final product

Classical microbiological and biochemical (API) methods (Table 1) were used for the isolation and identification of the natural microbial population in the traditionally produced Horse

Table 1 - Classical microbiological and biochemical (API) methods of isolation and identification of microbial population applied in the Horse Salami analyses.

Microorganism	Nutrient media	Incubation conditions	API test
Salmonella sp.	RP-broth, XLD (Biolife, Italy)	37°C 24-48 h	API 20 E V4.1
Enterobacteriaceae	VRBG (Biolife, Italy)	37°C 24 h	API 20 E V4.1
Staphylococcus aureus	BP (Biolife, Italy)	37°C 48 h	API Staph V4.1
Coagulase negative staphylococci (CNS)	BP (Biolife, Italy)	37°C 48 h	API Staph V4.1
Sulphite reducing clostridia	Sulphite agar (Biolife, Italy)	37°C 72 h	-
Listeria monocytogenes	Fraser broth Palcam agar (Biolife, Italy)	37°C 24 h	API Listeria V1.2
Lactic acid bacteria	MRS agar (Biolife, Italy)	30°C 48-72 h	API 50 CHL V5.1 API 20 STREP V7.0
Yeasts	Sabouraud agar (Biolife, Italy)	25°C 48-72 h	API 20 C AUX V4.0 Yeasts

salami (i.e. in the final product obtained after 90 production days). Ten grams of the sample were homogenized in 90 mL of sterile 0.5% saline solution and serially diluted before planting on a non-selective medium (peptone yeast extract glucose agar, Biolife, Milano, Italy) and selective media under conditions specified in Table 1. Colonies randomly taken from selected plates were identified on the basis of their morphology, Gram-staining, cell morphology and catalase reaction. The identity of bacteria species was further confirmed using the API identification kits (BioMérieux, France).

Sensorial analysis

The final Horse Salami product (obtained after 90 days) was subjected to a quantitative descriptive analysis performed by a panel of seven (3 male and 4 female) trained experts according to ISO 6658:2005 standard. The panellists had completed a preliminary three session-training in order to familiarize themselves with the samples under investigation. Fourteen attributes were examined and rated on a 5-point scale, "1" thereby standing for "poorly perceived or absent" and "5" standing for "intensely perceived". During these three training sessions, the descriptors to be targeted by the analysis were agreed upon. The latter included as follows: 2 external attributes (appearance, hardness), 4 attributes descriptive of a slice (fat/lean ratio, easy peeling capability, colour intensity, sliceability), 5 attributes descriptive of perceptions during mastication (flavour intensity, juiciness, smokiness, acid taste, saltiness) and 3 attributes descriptive of the product smell (spice odour, lactic acid odour, mould odour). The Sausage samples were coded using a three-digit code and presented in form of oblique slices approximately 0.4 cm thick. Water was provided to clean the panellists' palate between analyses.

Data analysis

Differences between the average values of the same physicochemical, colour, texture, microbiological and sensory parameters were analyzed using the analysis of variance (ANOVA) and the Fisher's least significant difference test (LSD), with statistical significance being set at $P<0.05$. Moisture, fat, protein, collagen and NaCl content, pH, a_w, colour and textural parameters were subjected to correlation analysis (Pearson´s correlation test) so as to determine their possible statistically meaningful relationships. Statistical analysis was carried out using Statistica Ver. 8.0 StatSoft Inc. Tulsa, OK, USA.

RESULTS AND DISCUSSION

Physicochemical parameters

Basic chemical composition, salt (NaCl) content, pH values and water activity (a_w) of the Horse Salami, established at various processing stages, are given in Table 2. The average initial moisture content of the Horse Salami found to be 61.91% had significantly decreased (P<0.05) as the processing went on due to smoking and dry-ripening typical of dry fermented sausages (LIZASO *et al.*, 1999; PEREZ-ALVAREZ *et al.*, 1999; SALGADO *et al.*, 2005; SALGADO *et al.*, 2006; LORENZO *et al.*, 2012). Higher moisture losses were observed in the first 21 processing days and on day 28, which is characteristic for this type of product (< 40%) and dry sausages in general (PLEADIN *et al.*, 2014). Further ripening leads to additional moisture content reduction, so that the lowest value (28.51%) was determined on manufacturing day 90. In 2012, LORENZO and co-workers reported higher initial and final moisture values for the foal salchichon. This can be explained by the fact that horse meat has a lower water content as compared to foal meat (LITWINCZUK *et al.*, 2008; LANZA *et al.*, 2009; TATEO *et al.*, 2008), as well as by the longer ripening period of the Horse Salami. The final moisture content was also lower than in similar dry sausages coming from Spain (GIMENO *et al.*, 2000; RUBIO *et al.*, 2007; LORENZO *et al.*, 2012), which can also be attributed to a longer ripening period of the Horse Salami.

The highest amount of proteins (30.53%) was determined on day 90. The results are consistent with the published literature data, which show that due to prolonged drying and ripening (weight loss of up to 50%) and a high share of lean meat used in stuffing preparation, moisture and protein content in ripened dry-fermented sausages tend to be similar (30-40%), indicating a high nutritional value of the final product (PLEADIN *et al.*, 2014).

The average fat content of the Horse Salami had increased significantly (P<0.05) from day 1 to day 90 (from 13.84 to 28.54%), in proportion to the duration of the Horse Salami ripening process and dehydration, i.e. the continuous reduction of water content in the product; the same goes for the protein and collagen content (Table 2). Fat as a substantial component of fermented sausages has multiple functions; it represents a concentrated energy source (9 kcal/g) and the source of essential fatty acids and fat-soluble vitamins (MELA, 1990). Furthermore, it is contributing to the fullness of flavour, texture and softness of the product, all of the aforementioned being relevant for the quality and acceptability of the product in question (OLIVARES *et al.*, 2010). Hydrolysis and oxidation of fatty acids that occur during the ripening process largely contribute to the taste of fermented sausages (ORDONEZ *et al.*, 1999). The final fat content was lower, while the final protein content turned out to be higher than in Spanish and Italian dry fermented sausages (DELLAGLIO *et al.*, 1996; RUBIO *et al.*, 2008).

The average initial ash content was 3.13% and had increased significantly (P<0.05), reaching the ultimate value of 5.72%, whereas water activity (a_w) (Table 2) had decreased significantly (P<0.05) during the smoking and dry-ripening period (from 0.96 to 0.78). Changes in mass fraction of individual basic constituents and water activity decrease seen after 90 days of Horse Salami production (Table 2) are mostly caused by the drying process, i.e. the loss of water occurring during ripening.

Changes in pH values seen during the processing of the Horse Salami are presented in Table 2. pH value had decreased during the first 21 days of processing (from 5.58 to 4.71), possibly as a result of the presence of organic acid produced by bacteria (LUCKE, 1994). This pH drop is typical of most dry fermented sausage (PEREZ-ALVAREZ *et al.*, 1999; GIMENO *et al.*, 2000; LIZASO *et al.*, 1999; MUGUERZA *et al.*, 2002; BOZKURT and BAYRAM, 2006; VAN SCHALKWYK *et al.*, 2011). At the final processing stage, pH values increased to 4.94, possibly due to the liberation of peptides, amino acid and ammonia re-

Table 2 - Basic chemical composition, salt content, a_w and pH of the Horse Salami established during the manufacturing process.

	Processing time (days)							
	0	**7**	**14**	**21**	**28**	**42**	**60**	**90**
Moisture (%)	61.91a±0.06	55.67b±0.01	48.92c±0.09	43.22d±0.06	37.57e±0.01	35.16f±0.04	31.61g±0.06	28.51h±0.02
Fat (%)	13.84h±0.03	15.83f±0.03	17.71e±0.01	18.55d±0.02	18.59d±0.04	20.59c±0.02	25.45b±0.06	28.54a±0.12
Protein (%)	17.05h±0.04	22.48g±0.01	23.73f±0.01	24.36e±0.08	27.62d±0.04	27.95c±0.05	29.34b±0.23	30.53a±0.05
Collagen (%)	0.63e±0.11	1.19d ±0.11	1.56cd±0.07	2.05c±0.21	2.06c±0.09	2.82b±0.15	2.84b±0.12	3.93a±0.10
Ash (%)	3.13g±0.02	3.73f±0.06	4.56e±0.04	4.87d±0.01	4.94d±0.01	5.36c±0.05	5.45b±0.06	5.72a±0.01
Salt (NaCl) (%)	2.30g±0.04	2.71f±0.05	3.29e±0.03	3.63d±0.05	3.75c±0.05	3.81c±0.05	4.24b±0.02	4.51a±0.03
aw	0.96a±0.01	0.93ab±0.02	0.91b±0.01	0.88c±0.01	0.87c±0.03	0.86c±0.04	0.86c±0.01	0.78d±0.01
pH	5.58a±0.03	4.99b±0.05	4.74f±0.10	4.71g±0.16	4.72fg±0.08	4.76f±0.06	4.81d±0.10	4.93c±0.07

Values are means ±SD obtained with three measurements. Values displayed in the same row and tagged with different letters (a-h) are significantly different (P<0.05).

sulting from a proteolityc reaction (SPAZIANI *et al*, 2009). The final pH was lower than in most dry fermented sausages (5.2 to 5.8) (BOVER-CID *et al.*, 2001; RUBIO *et al.*, 2007; ROSERIO *et al.*, 2010), which can be explained by horse meat properties in terms of higher glycogen content as compared to pork, beef and foal meat (LAWRIE and LEDWARD, 2006).

The salt content of the Horse Salami had significantly increased during processing (P<0.05) (Table 2). Literature sources have reported the average mass fraction of salt in dry sausage stuffing to range from 2.0% to 2.6%, and that in final products to range from 3.3% to 4.3% (OCKERMAN and BASU, 2007; STAHNKE and TJENER, 2007). In this study, mass fraction of salt (NaCl) established during the Horse Salami manufacturing process ranged from 2.31% to 4.51%.

Instrumental colour properties

The CIELAB space (L*, a* and b*) values of the Horse Salami were significantly affected (P<0.05) by the length of smoking and ripening period (Table 3). Lower lightness L* values seen with an increased length of processing are probably related to the dark colour of the Horse Salami coming as a consequence of browning. A similar decrease in L* values during ripening was reported by BOZKURT and BAYRAM (2006) for Turkish sucuk, and by LORENZO *et al.* (2012) for foal salchichon.

Redness (a*) had significantly (P<0.05) decreased at all processing stages. Similar lower a* values were seen during the ripening of Spanish pork dry sausages and foal salchichon, as reported by PEREZ-ALVAREZ *et al.* (1999) and LORENZO *et al.* (2012). Lowering of a* values can possibly be explained by total or partial denaturation of nitrosomyoglobin coming as a result of lactic acid production. L* and a* values lower than those reported by LORENZO *et al.* (2012) can probably be related to the nature of horse meat, which is darker and redder than foal (TATEO *et al.*, 2008)

Yellowness (b*) had decreased from 20.32 to 9.11 and had varied significantly (P<0.05) during the production process. The decrease in b* values seen with the prolongation of the processing time was also reported by other authors (PEREZ-ALVAREZ *et al.*, 1999; LORENZO *et al.*, 2012) and explained by the decrease in concentration of oxymyoglobin coming as a result of oxygen consumption executed by microorganisms.

Texture Profile Analysis

Texture Profile Analysis (TPA) parameters of the Horse Salami established during the smoking and dry ripening period are presented in Table 4. Average hardness values had significantly increased (P<0.05) from 0.32 to 20.54 kg as the processing went by. This can be related to the coagulation of muscle protein coming as a result of low pH values and sausage drying (BOZKURT and BAYRAM, 2006).

Springiness and cohesiveness had significantly decreased (P<0.05) during the processing (from 0.76 to 0.64 and from 0.67 to 0.43, respectively). Springiness is related to elastic proper-

Table 3 - Colour parameters of the Horse Salami established during the manufacturing process.

	Processing time (days)							
	0	7	14	21	28	42	60	90
L*	46.67a±1.08	43.01b±1.05	41.81c±0.37	40.54d±0.30	38.75e±0.35	33.77f±1.27	33.28f±0.92	31.28g±0.89
a*	17.71ab±0.40	17.29ab±2.03	18.54a±3.44	16.16ab±2.65	15.49b±0.39	12.07c±0.62	10.86cd±01.06	8.15d±0.69
b*	20.32a±1.49	18.01bc±2.52	17.98bc±2.63	16.51c±2.22	13.39d±0.43	13.14d±0.80	12.14d±2.04	9.11e±0.58

Values are means ±SD obtained with ten measurements. Values displayed in the same row and tagged with different letters (a-g) are significantly different (P<0.05).

Table 4 - Parameters obtained by virtue of Textural Profile Analysis (TPA) of the Horse Salami during the manufacturing process.

	Processing time (days)							
	0	7	14	21	28	42	60	90
Hardness (kg)	0.32h±0.01	3.67g±0.19	4.61f±0.09	6.19e±0.21	9.91d±0.11	14.94c±0.25	17.58b±0.81	20.54a±0.92
Springiness	0.76a±0.03	0.62def±0.03	0.68bc±0.01	0.73ab±0.02	0.58f±0.01	0.61ef±0.02	0.66cd±0.01	0.64cde±0.03
Cohesiveness	0.67a±0.04	0.51c±0.04	0.48cd±0.01	0.65ab±0.12	0.46cd±0.01	0.46cd±0.01	0.43d±0.03	0.43d±0.03
Gumminess (kg)	0.26f±0.01	1.87e ±0.15	2.26e±0.07	4.02d±0.50	4.56d±0.06	6.87c±0.13	7.73b±0.50	8.83a±0.54
Chewiness (kg)	0.20g±0.02	1.16f±0.09	1.54e±0.04	2.94d±0.40	2.64d±0.04	4.19c±0.10	5.10b±0.49	5.65a±0.55
Resilience	0.19a±0.04	0.15bcd±0.02	0.16bc±0.03	0.18ab±0.05	0.15bcd±0.01	0.12e±0.01	0.13de±0.02	0.14cde±0.01

Values are means ±SD obtained with eight measurements. Values displayed in the same row and tagged with different letters (a-h) are significantly different (P<0.05).

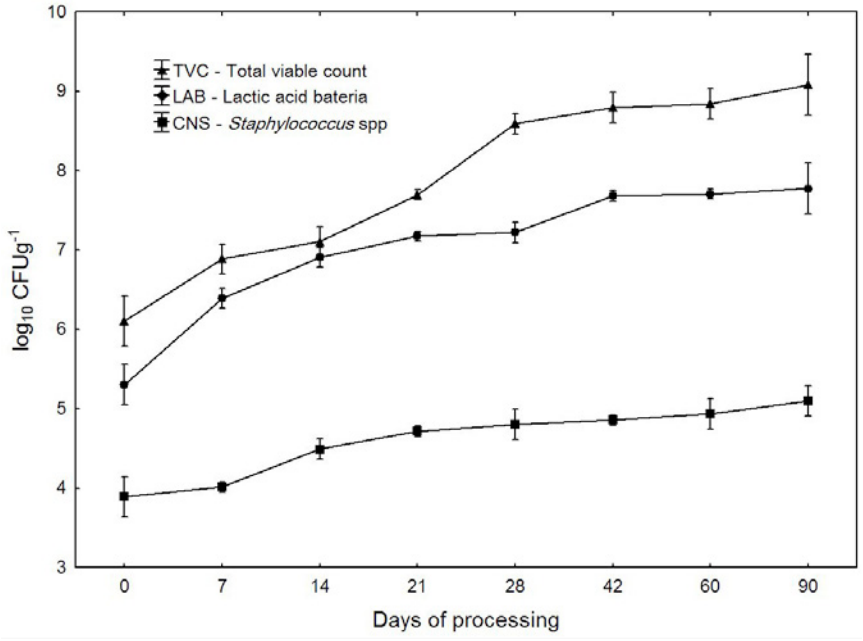

Fig. 1 - Changes in microbial counts seen during the processing of the Horse Salami (mean±standard deviation obtained with three samples).

ties, so that the decrease in this textural property of the Horse Salami is most likely to be related to the removal of water (BOZKURT and BAYRAM, 2006).

Increases in gumminess and chewiness values (from 0.26 to 8.83 and from 0.20 to 5.65, respectively) seen during the Horse Salami processing were statistically significant ($P<0.05$). Increase in chewiness values indicates that the Horse Salami becomes tougher during the ripening period (SZCZESNIAK, 2002), possibly due to moisture loss.

Resilience values established at the beginning and at the end of the processing were 0.19 and 0.14, respectively. Significant changes in resilience during smoking and ripening failed to be observed ($P>0.05$) (Table 4).

Microbial counts

Microbial flora changes seen during manufacturing are shown in Fig. 1. The initial bacterial counts were 6.09 log CFU g^{-1} for total viable count (TVC), 5.29 log CFU g^{-1} for lactic bacteria (LAB), and 3.88 log CFU g^{-1} for *Staphylococcus* spp, respectively. Relatively low bacterial counts in the Salami stuffing indicate a good hygienic quality of the raw materials. TVC, LAB and *Staphylococcus* spp counts had significantly increased during the ripening period ($P<0.05$). This increase in bacterial count is typical of most naturally dry fermented European sausages (KOZAČINSKI *et al.*, 2008). At the end of the Horse Salami production process, the mean values were 9.10, 7.79 and 5.10 log CFU g^{-1}, respectively.

As reported by many studies, microorganisms most represented during the ripening of cured sausages and meat products are LAB (LIZASO *et al.*, 1999; SAMELIS and GEORGIADOU, 2000), whose counts tend to remain stable throughout the ripening period. Within the frame of this study, high LAB counts had been found during the first 28 ripening days, which can be related to a substantial pH drop witnessed during that period (Table 2). LAB inhibit the growth of pathogenic and spoilage bacteria by virtue of formation of lactic acid, acetic acid and possibly bacteriocins (LUCKE, 2000).

Isolation and identification of microbial population

Native sausage products are of a higher quality than those obtained by virtue of controlled fermentation with the addition of industrial starters (LEBERT *et al.*, 2007). Many authors support the view that indigenous microflora or microorganisms present in traditional sausages originate from raw materials or the manufacturing environment (MAURIELLO *et al.*, 2004; RANTSIOU *et al.*, 2005). This microbiota is commonly referred to as "the house flora" (GARCIA-VARONA *et al.*, 2000).

Therefore, in this study, the isolation and identification of autochthonous microbial population inhabiting the Horse Salami was performed. The results of a microbiological analysis (Table 5) showed the dominant microflora to be the lactic acid bacteria strain termed *Lactobacillus plantarum, Lactococcus lactis* ssp. *lactis*, and *Enterococcus faecium* while the most represented coagulase-negative staphylococci strain was *S. xylosus*. The yeast *Candida famata/Debaryomyces hansenii* was found as well, which is in agreement with the results of NIELSEN *et al.* (2008), who stated that halophilic yeasts most frequently isolated from fermented meat prod-

Table 5 - Biochemical (API) results of the final product obtained after 90 days of manufacturing.

Microorganism	Values log CFU g^{-1}±SD	API test
Salmonella sp.	-	-
Enterobacteriaceae	-	-
Staphylococcus aureus	-	-
CNS (Coagulase negative staphylococci)	5.10±1.5	*S. xylosus*
Sulphite reducing clostridia	-	-
Listeria sp.	-	*Listeria grayi*
Lactic acid bacteria	7.79±1.3	L. *lactis* ssp. *lactis, Lactobacillus plantarum, Enterococcus faecium*
Yeasts	3.25±1.2	*Candida famata/Debaryomyces hansenii*

ucts are *Debaromyces hansenii*, *Candida famata*, *Candida zeylanoides*, *Trichosporon* sp., *Cryptococcus* sp. and *Rhodotorula* sp. Yeasts also play an important role in the maturation of sausages, since their lipolytic and proteolytic activity contributes to the development of sensory characteristics of fermented sausages (KOVAČEVIĆ, 2001; ALAGIĆ *et al.*, 2008).

In the Horse Salami samples, bacteria of the *Salmonella* genus, *Enterobacteriaceae*, sulphite-reducing Clostridia, *L. monocytogenes* or *S. aureus* were not found; however, API biochemical tests uncovered the presence the *Listeria grayi* bacterium which is non-pathogenic (Table 5). Issues sometimes emerging with this type of fermented meat product are short shelf-life and poor hygienic surroundings, but the sausages produced in this investigation were proven to be microbiologically safe. It should be pointed out that biochemical (API) tests gave very good results (identification of one species with ID > 98,2-99.9 %).

The isolated lactic acid bacteria *L. lactis* ssp. *lactis*. *L. plantarum and E. faecium* could be used as starter cultures for meat products. *L. plantarum* as an autochthonous meat microflora is widely spread in nature (SALAMA *et al.*, 1995; AYAD *et al.*, 2001), *L. lactis* ssp. *lactis* in fermented sausage has rarely been reported so far and therefore further studies must to include detail molecular identification of isolated strains because API identification is not 100% precisely. The interest in exploring the potential of new strains isolated from different natural ecosystems to the effect of aroma compounds production has recently increased (AYAD *et al.*, 2001; FRECE *et al.*, 2009; BABIĆ *et al.*, 2011, FRECE *et al.*, 2014). Metabolic properties of the *L. plantarum, E. faecium* and *L. lactis* species have both direct and indirect influence on organoleptic, nutritional and hygienic quality of fermented products. More and more research is focused on the isolation and identification of autochthonous functional starter cultures with the aim of developing new functional meat products that will be recognised and labelled as autochthonous to the region in which they are produced (BABIĆ *et al.*, 2011, FRECE *et al.*, 2014, FRECE *et al.*, 2014 a,b). Therefore, *L. plantarum, E. faecium, L. lactis* and *S. xylosus* as potential functional autochthonous starter cultures will be thoroughly investigated in the future. Further studies will be carried out to detail phenotypic, genotypic and physiological characterization of isolated strains of staphylococci and LAB.

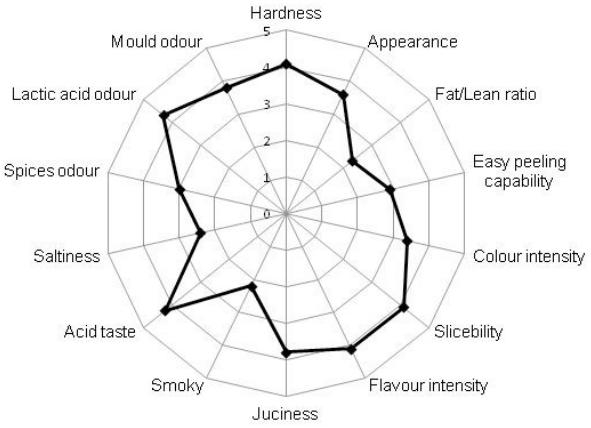

Fig. 2 - Mean values of sensory properties of the final Horse Salami.

Sensory characteristics

Complex interaction between physicochemical, biochemical and microbiological processes, playing a role in formation of chemical compounds, and the modification of molecules responsible for the texture and appearance of the final product also determine its sensory characteristics.

Average scores given by the panellists at the end of the Horse Salami manufacturing process are shown in Fig. 2. As for the external attributes, the Horse Salami scored highly when it comes to hardness (4.10±0.71) and low when it comes to appearance (3.60±0.43). It was highly rated for its sliceability, but low-rated when it comes to its colour intensity, fat/ lean ratio and easy peeling capacity. After slicing, the highest scores were obtained for the fat distribution (4.78±0.67), while the fat/lean ratio scored low (2.22±0.44).

Regarding the attributes that describe perceptions during mastication, Horse Salami was highly rated for its flavour intensity (4.10±0.44), juiciness (4.24±0.21) and acid taste (3.79±0.17), and low-rated for its saltiness and smokiness (2.41±0.31 and 2.20±0.19).

During the fermentation of dry sausages, LAB produce lactic acid (MATEO *et al.*, 1996) responsible for the sour taste (LOTONG *et al.*, 2000) and odour of the product, while mould odour is to be associated with 1-octen-3-ol, which spreads a typical mushroom odour (MEYNIER *et al.*, 1998). In the present study, all three attributes scored highly (lactic acid taste 4.24±0.18; lactic acid odour 4.31±0.22; mould odour 3.82±0.15).

As for the smell descriptors, lactic acid (4.3±0.22) and mould odour (3.8±0.15) were dominant, while the spice odour scored low (3.00±0.28).

Correlation between the parameters

Instrumental colour parameters of the Horse Salami, established during its processing, were significantly inversely correlated (P<0.05) to the protein, fat, ash, collagen and salt content. Moisture content and a_w values exhibited a significant direct correlation (P<0.05) to the instrumental colour parameters (Table 6). Relationships between the moisture, protein, fat, ash, collagen and salt content and a_w on one hand, and hardness, gumminess and chewiness on the other, were also significant (P<0.05) (that between moisture and a_w being an inverse one). Pearson's correlation coefficients indicated that springiness and resilience are not significantly (P>0.05) correlated to the basic chemical composition, salt content and a_w (Table 6).

CONCLUSIONS

This study investigated into the changes in physicochemical, colour, textural, microbiological and sensorial properties of the Horse Salami as an indigenous Croatian dry fermented sausage. During 90 days of manufacturing, major changes in physicochemical, colour and textural properties took place during the fermentation and ripening stage, pointing to proteolysis and lipolysis phenomena coming as a result of endogenous enzymatic activity, as well as to high lactic acid bacteria and staphylococci counts contributing to the specific organoleptic attributes of the final product. Sensorial profiling of the final Horse Salami showed a significant acid taste, lactic acid odour and flavour intensity, and low fat/lean ratio, smokiness and saltiness values. The final product was proven to be microbiologically safe, the dominant microbial population being *L. lactis* ssp. *lactis, L. plantarum, E. faecium* and *S. xylosus*.

REFERENCES

Alagić D., Kozačinski L., Filipović I., Zdolec N., Hadžiosmanović M., Njari B., Kozačinski Z. and Uhitil S. 2008. Microbiological changes during ripening of fermented sausages of horsemeat. Meso. 10: 200.

Ayad E.H.E., Verheul A., Engels W.J.M., Wouters J.T.M. and Smit G. 2001. Enhanced flavour formation by combination of selected lactococci from industrial and artisanal origin with focus on completion of a metabolic pathway. J. Appl. Microb. 90: 59.

Babić I., Markov K., Kovačević D., Trontel A., Slavica A., Đugum J., Čvek D., Svetec I. K., Posavec S. and Frece J. 2011. Identification and characterization of potential autochthonous starter cultures from a Croatian "brand" product "Slavonski kulen". Meat Sci. 88: 517.

Badiani A., Nanni N., Gatta P., Tolomelli B. and Manfredini M. 1997. Nutrient profile of horsemeat. J. Food Compos. Anal. 10: 254.

Bourne M.C. 1978. Texture Profile Analysis. Food Technol.-Chicago. 32: 62.

Bover-Cid S., Izquierdo-Pulido M. and Vidal-Carou M.C. 2001. Effectiveness of a *Lactobacillus sakei* starter culture in the reduction of biogenic amine accumulation as a function of the raw material quality. J. Food Protect. 64: 367.

Bozkurt H. and Bayram M. 2006. Colour and textural attributes of sucuk during ripening. Meat Sci. 73: 344.

Bruna J.M., Ordonez J.A., Fernandez M., Herranz B. and De la Hoz L. 2001. Microbial and physico-chemical changes during the ripening of dry fermented sausages superficially inoculated with or having added an intracellular cell-free extract of *Penicillium aurantiogriseum*. Meat Sci. 59: 87.

Table 6 - Pearson´s correlation coefficients established between basic chemical composition, salt content, aw, texture and instrumental colour parameters.

	Hardness (kg)	Springiness	Cohesiveness	Gumminess (kg)	Chewiness (kg)	Resilience	L*	a*	b*
Moisture (%)	-0.95**	0.19	0.68**	-0.96**	-0.94**	0.48	0.95**	0.89**	0.95**
Fat (%)	0.95**	0.058	-0.64	0.94**	0.95**	-0.35	-0.91**	-0.95**	-0.93**
Protein (%)	0.96**	-0.28	-0.77**	0.95**	0.93**	-0.51	-0.95**	-0.90**	-0.97**
Collagen (%)	0.95**	-0.09	-0.59	0.95**	0.94**	-0.37	-0.94**	-0.96**	-0.96**
Ash (%)	0.92**	-0.10	-0.61	0.94**	0.93**	-0.44	-0.95**	-0.87**	-0.91**
Salt (NaCl) (%)	0.94**	-0.03	-0.62	0.94**	0.95**	-0.33	-0.92**	-0.90**	-0.95**
a_w	-0.86**	0.12	0.51	-0.86**	-0.84**	0.20	0.84**	0.89**	0.94**

Values marked with ** are statistically significant (P<0.05).

Casiraghi E., Pompei C., Dellaglio S., Parolari G. and Virgili R. 1996. Quality attributes of Milano salami, an Italian dry-cured sausage. J. Agr. Food Chem. 44: 1248.

CIE. 1976. "Colorimetry: official recommendations of the international commission on illumination". Comisión Internationale de l'Ëclairage [International Commission on Illumination], CIE No. 15 (E-1.3.1). Paris, FR.

Dellaglio S., Casiraghi E. and Pompei C. 1996. Chemical, physical and sensory attributes for the characterization of an Italian dry-cured sausage. Meat Sci. 42: 25.

Franco D., Rodríguez E., Purriños L., 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.

Frece J., Kovačević D. and Markov K. 2014.a National Patent P20130089A: "Formulation of bacterial starter cultures for the production of dry sausage and its use„ (15.8. 2014.) Hrvatski glasnik intelektualnog vlasništva 17/2014.

Frece J., Kovačević D. and Markov K. 2014.b National Patent P20130569A**:** "Use of probiotic bacterial cultures *Lactobacillus plantarum* 1K for the production of functional foods„ (9.10.2014.) Hrvatski glasnik intelektualnog vlasništva 1/2015. (2.1. 2015.)

Frece J., Kos B., Svetec I. K., Zgaga Z., Beganović J., Leboš A. and Šušković J. 2009. Synbiotic effect of *Lactobacillus helveticus* M92 and prebiotics on the intestinal microflora and immune system of mice. J. Dairy Res. 76: 98.

Frece J., Kovačević D., Kazazić S., Mrvčić J., Vahčić N., Delaš F., Ježek D., Hruškar M., Babić I. and Markov K. 2014. Comparison of sensory properties, shelf live and microbiological safety of industrial sausages produced with autochthonous and commercial starter cultures (Starter cultures for sausages production). Food Technol. Biotech. 52: 307.

Garcia-Varona M., Santos E. M., Jaime I. and Rovira J. 2000. Characterisation of *Micrococcaceae* isolated from different varieties of chorizo. Int. J. Food Microbiol. 54:189.

Gimeno O., Ansorena D., Astiasaran I. and Bello J. 2000. Characterization of chorizo de Pamplona: Instrumental measurements of colour and texture. Food Chem. 69:195.

ISO 3496:1994. Meat and meat products. Determination of hydroxyproline content.

ISO 1442:1997. Meat and meat products. Determination of moisture content.

ISO 1443:1973. Meat and meat products. Determination of total fat content.

ISO 3100-1:1975. Meat and meat products. Sampling and preparation of test samples. Part 1: Sampling.

ISO 936:1998. Meat and meat products. Determination of total ash.

ISO 937:1978. Meat and meat products. Determination of nitrogen content.

ISO 6658:2005. Sensory Analysis - Methodology - General guidance.

Kovačević D. 2001. "Chemistry and technology of meat and fish". Faculty of food technology, Josip Juraj Strossmayer University of Osijek, Grafika. Osijek. Croatia.

Kozačinski L., Drosinos E., Čaklovica F., Cocolin L., Gasparik-Reichardt J. and Vesković S. 2008. Investigation of microbial association of traditionally fermented sausages. Food Technol. Biotechnol. 46: 93.

Lanza M., Landi C., Scerra M., Galofaro V. and Pennisi P. 2009. Meat quality and intramuscular fatty acid composition of Sanfratellano and Haflinger foals. Meat Sci. 81: 142.

Lawrie R.A. and Ledward D.A. 2006. "Lawrie's meat science" (7th Ed.). CRC Press. Boca Raton, FL.

Lebert I., Leroy S., Giammarinaro P., Lebert A., Chacornac J. P., Bover-Čid S., Vidal-carouc M.C. and Talona R. 2007. Diversity of microorganisms in the environment and dry fermented sausages of small traditional French processing units. Meat Sci. 76: 112.

Litwinczuk A., Florek M., Skalecki P. and Litwinczuk Z. 2008. Chemical composition and physicochemical properties of horse meat from the longissimus lumborum and semitendinosus muscle. J. Musc. Food. 19: 223.

Lizaso G., Chasco J. and Beriain M.J. 1999. Microbiological and biochemical changes during ripening of salchichon, a Spanish dry cured sausage. Food Microbiol. 16: 219.

Lombardi-Boccia G., Lanzi S. and Aguzzi A. 2005. Aspects of meat quality: trace elements and B vitamins in raw and cooked meats. J. Food Compos. Anal.18: 39.

Lorenzo J.M., Temperan S., Bermudez R., Cobas N. and Purrnios L. 2012. Changes in physico-chemical, microbiological, textural and sensory attributes during ripening of dry-cured foal salchichon. Meat Sci. 90: 194.

Lotong V., Chambers E. and Chambers D.H. 2000. Determination of the sensory attributes of wheat sourdough bread. J. Sens. Stud. 15: 309.

Lucke, F.K. 1994. Fermented Meat-Products. Food Res. Int. 27: 299.

Lucke, F.K. 2000. Utilization of microbes to process and preserve meat. Meat Sci. 56: 105.

Mateo J., Dominguez M.C., Aguirrezabal M.M. and Zumalacarregui J.M. 1996. Taste compounds in chorizo and their changes during ripening. Meat Sci. 44: 245.

Mauriello G., Casaburi A., Blaiotta G. and Villani F. 2004. Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy. Meat Sci. 67: 149.

Mela D.J. 1990. The basis of dietary preference. Trends Food Sci. Tech. 1: 55.

Meynier A., Genot C. and Gandemer G. 1998. Volatile compounds of oxidized pork phospholipids. J. Am. Oil Chem. Soc. 75: 1.

Muguerza E., Fista G., Ansorena D., Astiasaran I. and Bloukas J.G. 2002. Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages. Meat Sci. 61: 397.

Nielsen D.S., Jacobsen T., Jespersen L., Koch A.G. and Arneborg N. 2008. Occurence and growth of yeasts in processed meat products-Implications for potential spoilage. Meat Sci. 80: 919.

Ockerman H.W. and Basu L. 2007. Reduction and consumption of fermented meat products. Ch 2. "Handbook of fermented meat and poultry". F. Toldrá (Ed.), p. 9. Blackwell Publishing, Ames, IA.

Olivares A., Navarro J.L., Salvador A. and Flores M. 2010. Sensory acceptability of slow fermented sausages based on fat content and ripening time. Meat Sci. 86:251.

Ordonez J.A., Hierro E.M., Bruna J. and Hoz L. 1999. Changes in the components of dry-fermented sausages during ripening. Crit. Rev. Food Sci. 39: 329.

Perez-Alvarez J.A., Sayas-Barbera M.E., Fernandez-Lopez J. and Aranda-Catala V. 1999. Physicochemical characteristics of Spanish-type dry-cured sausage. Food Res. Int. 32: 599.

Pleadin J., Krešić G., Barbir T., Petrović M., Milinović I. and Kovačević D. 2014. Changes in basic nutrition and fatty acid composition during production of "Slavonski kulen". Meso. 16: 522.

Rantsiou K., Urso R., Iacumin L., Cantoni C., Cattaneo P. and Comi G. 2005. Culture-dependent and -independent methods to investigate the microbial ecology of Italian fermented sausages. Appl. Environ. Microb. 71: 1977.

Roseiro L.C., Gomes A., Gonçalves H., Sol M., Cercas R. and Santos C. 2010. Effect of processing on proteolysis and biogenic amines formation in a Portuguese traditional dry-fermented ripened sausage "Chouriço Grosso de Etremoz e Borba PGI". Meat Sci. 84: 172.

Rubio B., Martinez B., Sanchez M.J., Garcia-Cachan M.D., Rovira J. and Jaime I. 2007. Study of the shelf life of a dry fermented sausage "salchichon" made from raw material enriched in monounslaturated and polyunsaturated fatty acids and stored under modified atmospheres. Meat Sci. 76: 128.

Rubio B., Martinez B., Garcia-Cachan M.D., Rovira, J. and Jaime I. 2008. Effect of the packaging method and the storage time on lipid oxidation and colour stability on dry fermented sausage salchichon manufactured with raw

material with a high level of mono and polyunsaturated fatty acids. Meat Sci. 80: 1182.

Salama M.S., Musafija-Jekni, T., Sandine W.E. and Giovannoni S.J. 1995. An ecological study of lactic acid bacteria: isolation of new strains of Lactococcus including *Lactococcus lactis* subsp. *cremoris*. J. Dairy Sci. 78: 1004.

Salgado A., Fontan M.C.G., Franco I., Lopez M. and Carballo J. 2005. Biochemical changes during the ripening of Chorizo de cebolla, a Spanish traditional sausage. Effect of the system of manufacture (homemade or industrial). Food Chem. 92: 413.

Salgado A., Fontan M.C.G., Franco I., Lopez M. and Carballo J. 2006. Effect of the type of manufacture (homemade or industrial) on the biochemical characteristics of Chorizo de cebolla (a Spanish traditional sausage). Food Cont. 17: 213.

Samelis J. and Georgiadou K.G. 2000. The microbial association of Greek taverna sausage stored at 4 and 10 degrees C in air, vacuum or 100% carbon dioxide, and its spoilage potential. J. Appl. Microbiol. 88: 58.

Spaziani M., del Torre M. and Stecchini M.L. 2009. Changes of physicochemical, microbiological, and textural properties during ripening of Italian low-acid sausages. Proteolysis, sensory and volatile profiles. Meat Sci. 81: 77.

Stahnke L. H. and Tjener K. 2007. Influence of processing parameters on cultures performance. Ch 18. In "Handbook of fermented meat and poultry". F. Toldrá (Ed.), p. 187. Blackwell Publishing, Ames, IA.

Szczesniak A.S. 2002. Texture is a sensory property. Food Qual. Prefer. 13: 215.

Tateo A., De Palo P., Ceci E. and Centoducati P. 2008. Physicochemical properties of meat of Italian Heavy Draft horses slaughtered at the age of eleven months. J. Anim. Sci. 86: 1205.

Trajković J., Mirić M., Baras J. and Šiler S. 1983. Analize životnih namirnica. Tehnološko metalurški fakultet. Beograd, YU.

Van Schalkwyk D.L., McMillin K.W., Booyse M., Witthuhn R.C. and Hoffman L.C. 2011. Physico-chemical, microbiological, textural and sensory attributes of natured game salami produced from springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazella*), kudu (*Tragelaphus strepsiceros*) and zebra (*Equs burchelli*) harvested in Namibia. Meat Sci. 88: 36.

Paper Received November 4, 2015 Accepted April 22, 2015