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ORIGINAL RESEARCH PAPER

QUALITY AND SAFETY ASPECTS OF TRADITIONALLY AIR-DRIED AND NITRITE-FREE MEAT PRODUCT WITH REDUCED SALT LEVEL

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Abstract

This paper studies the effect of reduced sodium chloride amounts (3%, 5% and 7%) on the quality aspects and safety elements of traditionally salted ham without any nitrates and nitrites added, dried and ripened under natural air-drying conditions. The physicochemical and textural properties, the degree of proteolysis, the microbiological characteristics and sensory profile of dry-cured ham were analysed at different production stages. A salting degree above 3% in the manufacture of this traditional dried product had a key role in the development of the desired microbiological and sensory characteristics in the absence of nitrites. Salting of the pork hams with 5% and 7% sodium chloride for 45 days at a temperature of 2±2 °C ensured a salt concentration and water activity that would guarantee the product safety with regard to the microbiological risks during the subsequent drying and ripening under natural air-drying conditions. The Elena ham samples salted with 5% sodium chloride and ripened for a period of 12 months in natural air-drying chambers were characterized as having the most balanced flavor.

Keywords: ham, sensory analysis, texture, proteolytic index, microbiology

Introduction

In-depth studies of traditional technological processes, their features and the effect they have on the quality and safety of meat products are important research topics of current relevance related to nutrition and health. Meat products, dried and ripened under natural conditions, are among the most authentic and highly valued sustainable food products manufactured through the interweaving of experience, science, culture, geography and history (Leroy *et al.*, 2015; Mediani *et al.*, 2022).

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Dry-cured pork hams are considered a typically Mediterranean product, although they are also produced outside southern Europe (Zahariev, 2008). Their characteristic high salt content results from the technology used. For instance, this content is about 6.5% in Iberian ham (Toldrá, 2002; Andrés *et al.*, 2004), 7.7% in Bayonna ham (Monin *et al.*, 1997), 6.3 to 7.4% in Istarski Prust Slovenian ham (Marušić *et al.*, 2014), and 4.2 – 6.2% in Parma ham (Protected Destination of Origin. Prosciutto di Parma). Other types of ham, such as Tuscan ham, Jinhua ham and Dalmatinski ham, are characterised by an even higher sodium chloride quantity, i.e., 8.3% (Prosciutto Toscano D.O.P.), 8 – 15% (Zhou and Zhao, 2007) and 7.5 – 9.8% (Marušić *et al.*, 2016), respectively. These high levels of sodium chloride are in conflict with the recommendations concerning sodium chloride reduction in foods because of its potential to cause hypertension, cardiovascular disease, and stroke (WHO, 2012; Grillo *et al.*, 2019; Lucarini *et al.*, 2021). However, the possibilities of reducing the salt content in dried meat products are limited from a microbiological and sensory perspective (Schivazappa and Virgili, 2020; Fraqueza *et al.*, 2021; Muñoz-Rosique *et al.*, 2022; Patarata *et al.*, 2022). The penetration of salt in 2 to 4% concentrations into the muscles plays an important technological role both in the inhibition of spoilage-causing microbial growth and in the occurrence of controlled protein hydrolysis (Candek-Potokar and Skrlep, 2012; Laureati *et al.*, 2014; Ockerman and Basu, 2014). The salt content and degree of dehydration in the pork hams determines the activity of proteolytic enzymes, thereby contributing greatly to the formation of the sensory quality of dry-cured products. This is mainly due to the fact that a low salt content may lead to excessive proteolysis, which is considered undesirable in the manufacture of dried ham due to the sensory flaws associated with it, such as soft consistency and risk of microbial spoilage of the product (Ruiz-Ramirez *et al.*, 2006; Morales *et al.*, 2007).

The effect of nitrites/nitrates in the manufacture of dried meat products is mainly manifested during the ripening and drying period. Depending on their presence and the amounts added, changes in the color and oxidative characteristics of the meat proteins (Feng *et al.*, 2016) and lipids (Zanardi *et al.*, 2004; Berardo *et al.*, 2016) are observed during this period, along with selection of the microbial species contributing to the formation of the quality and safety of these products (Hospital *et al.*, 2015).

This paper presents a study of the effect of reduced sodium chloride amounts on the quality aspects and safety elements of traditionally salted ham without any nitrates or nitrites added, ripened and dried under natural air-drying conditions.

Materials and methods

Raw and processed materials

The analyses were conducted using Elena ham, a meat product traditional for the Elena region of the Balkan Mountains (Bulgaria) and made from pork hind leg, according to a technology schematically presented in Figure 1.

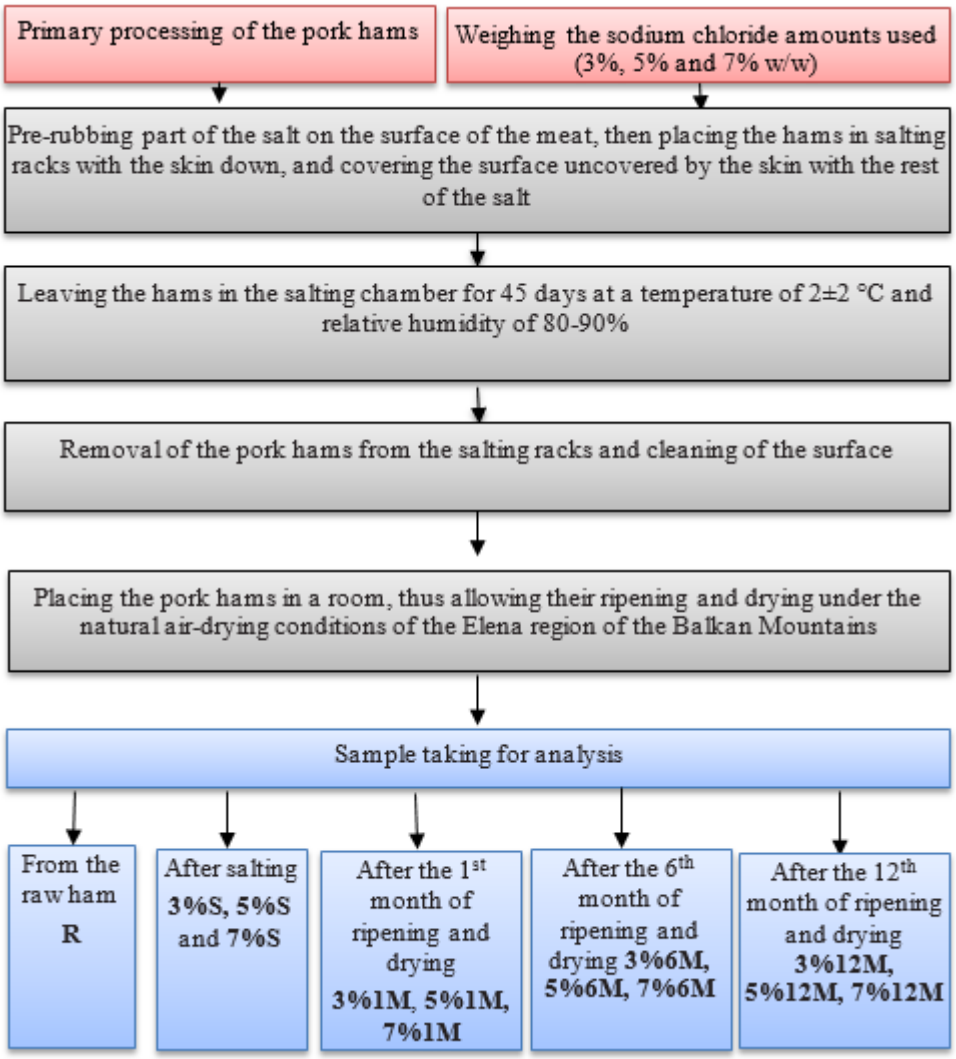

Figure 1. Diagram of the main technological steps in the manufacture of dry-cured Elena ham and stages of sample taking for analysis.

Ten pork hams obtained from pigs slaughtered at 12 months of age were used for the experiment. After the cooling period (24 hours at 4°C), the pork hind legs were separated through a cut between the last lumbar vertebra and the first sacral vertebra and a cut through the tarsometatarsal joint. The shaped hams averaged 10.673 ± 0.094 kg. The raw hams were salted with sodium chloride (Figure 1), in 3% and 5% concentrations, respectively, relative to the ham weight, and control samples were prepared in parallel using the traditionally applied concentration of 7% salt. The salting was carried out in chambers at a temperature of 2 ± 2°C and relative air humidity of 80 – 90% for a period of 45 days. After salting, the hams were cleaned of the excess salt mixture, washed with water, drained and hung for ripening and

drying in natural drying chambers in the specific Balkan climate of the town of Elena. This took place at the end of March, when the local climate is characterised by cool and dry air and average daily temperatures ranging from 2 – 5°C to 10 – 12°C. To carry out the research, samples were taken from the raw hams during shaping (R), after the end of salting (S), after 1 month of drying and ripening (1M), after 6 months of drying and ripening (6M) and after 12 months of drying and ripening (12M).

For the physicochemical analyses, two laboratory samples were prepared from each ham: from the outer surface and from the inside of the hams. The samples were transported to the laboratories of the UFT in Plovdiv, Bulgaria, in vacuum packaging and under refrigerated conditions (< 4°C), then analyzed directly or frozen (- 8°C) until the day of analysis, but for no more than one week.

Methods of analysis

Physical-chemical analysis

The moisture content was determined by drying the sample at 104 ± 1°C to constant weight using a KERN MLS-A moisture analyzer (Kern & Sohn GmbH, Germany). The pH value was measured in an aqueous extract of the product (1:9 w/v) using an MS 2004 pH meter (Microsyst, Bulgaria). The LabSwift-aw system (Novasina AG, Switzerland) was used for determination of the water activity, and the sodium chloride content was measured through determination of the chloride ion concentration by titration, or Mohr's Method.

The proteolytic index was determined as the percentage of the N-terminal α-amino groups (Moore and Stein, 1954), corresponding to the peptides and amino acids, in respect to the total protein content, established using the Lowry method (Lowry *et al.*, 1951).

Texture analysis

The texture profile analysis (TPA) of the finished product was performed using a TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, GB), following the method described by Bourne *et al.* (1978), with some modifications (Mitreva *et al.*, 2019). The TPA components have been presented as follows: hardness, elasticity, adhesiveness, cohesiveness and chewiness.

Microbiological analysis

The samples were prepared for the microbiological studies according to ISO 6887. The following values were determined: the total number of mesophilic aerobic and facultative anaerobic microorganisms according to ISO 4833; the number of coliforms through inoculations in a 1 cm^3 quantity of ten-fold dilutions on HiChrome Coliform Agar w/SLS (HiMedia), incubated at 37°C and 44°C for 48 hours: a method validated according to ISO 7251; lactic acid bacteria (LAB) according to ISO 15214, on MRS agar; enterococci through inoculations in a 1 cm^3 quantity of pre-made ten-fold dilutions on Bile Aesculin Azide agar (HiMedia) after 48 h at 30°C; quantification of staphylococci (non-pathogenic and pathogenic) and micrococci through inoculations in a 1 cm^3 quantity of pre-made ten-fold dilutions

on Manitol salt agar (HiMedia) at 30°C for 48 h; and moulds and yeasts through surface inoculations on a selective medium for moulds and yeasts cultivated at 20°C for 3/7 days; *Salmonella spp*. and *Listeria monocytogenes* according to ISO 6579 and ISO 11290-1.

Sensory analysis

The sensory analysis was carried out after the 6th and the 12th month of the drying and ripening of the Elena ham according to the methodology described below. The evaluation was carried out by a nine-member tasting panel including experts in the field of meat. The main evaluation indicators were divided into 4 categories: 1. appearance and cut surface; 2. color and color stability 3. consistency; 4. smell and taste. The product was given points from 1 to 5, the maximum score for each criterion being 5. Depending on the criterion, the score was multiplied by a weighting factor: ×1 for appearance and cut surface; ×3 for color and color stability; ×2 for consistency; ×4 for smell and taste. The reduction in the score for a given indicator was decided based on the common flaws for this group of meat products indicated in the tasting sheet (Table 1).

Table 1. Sensory Evaluation Sheet including sensory flaws most often observed in dry-cured Elena ham.

Sensory Attribute	Sensory Flaws
Appearance and Cut Surface	*Appearance* - atypical shape, fat and skin thickness over 3 cm, undesirable mould, other atypical surface deposits, non-uniform surface colouration, external greying, uncharacteristic colouration, pink colouration of the fat, yellow colouration of the fat, fatty surface, dark red colour of the meat, low marbling, high marbling, cracks and ruptures on the surface, other flaws *Cut surface* - too compact outer layer, too hard surface, too soft surface, low marbling, high marbling, oily surface, muscle surface gloss, other flaws
Colour and Colour Stability	Pale colouration, too dark red colour, uneven colouration, grey-green colouration, reddish adipose tissue, yellowish adipose tissue, rapid discolouration of the cut surface, iridescent areas, blood spots, other flaws
Consistency	Too soft, too hard, too dry, too wet, too greasy, hard to chew, tough, pasty, lack of juiciness, gritty, crumbly, other
Smell and Taste	*Smell* - absence of ripe meat smell, faint ripe smell, stale smell, rotten ammonia smell, rancid smell, mouldy smell, uncharacteristic smell *Taste* - too salty, too sweet, atypical of the product, very intense taste, tasteless, sour, bitter, rancid, greasy, metallic, buttery, fishy, soapy, other smell and taste flaws

The minimum score required to cover the minimum criteria for each of the evaluated categories was 3. The sums of the multiplied scores for the individual indicators were

divided by 10. The value obtained in this way represented the overall sensory score of the product tested.

Statistical analysis

The data obtained were statistically processed using the Statgrafics XVI software. A two-factor analysis of variance was applied for evaluation of the effect of the sodium chloride concentration used (Factor 1) and the manufacturing stage (Factor 2) of the Elena ham samples studied. The calculations for both experiments were performed at a confidence level of $\alpha = 0.05$. Duncan's multiple range test was used to compare the sample means. The analyses were performed in 5-fold replicates. Statistically significant differences between the mean values were found at probability lower than 0.05.

Results and discussion

Changes in the physical-chemical parameters and proteolytic index

The mean values for the moisture of Elena ham after the 6th month, when it was considered ready for consumption, were 54.52% for the samples with 3% salting, 52.01% for 5%, and 51.11% for 7%, respectively (Table 2). With the extension of the period of ripening under natural air-drying conditions, the moisture content continued to decrease, reaching values below 50% in the finished products after the 12th month. Such moisture values have been reported for most Spanish hams (Pérez-Santaescolástica *et al.*, 2018) obtained over a similar period of manufacture, as well as for the traditional Croatian Istrian ham (Marušić *et al.*, 2014), produced without skin but with an even layer of fatty tissue on the surface.

A statistically significant increase in the pH values of the hams compared to the initial pH of the raw ham (R) (5.78) was observed after the 6th month of ripening and drying, a trend that was maintained with the extension of the manufacturing process (up to the 12th month) (Table 2). The highest pH values were measured after the 12th month of ripening in the samples salted with 3% sodium chloride (6.09 ± 0.02) for the external sample), these values being on average 0.31 units higher than the initial pH value regardless of the long production period. The reasons for the different rate of the pH changes in the inside and on the surface part of the ham could be seen in the different degree of salt penetration and the moisture release process which affect both the proteolytic activity and the conditions for microbial growth. Evidence of this can also be found in the changes in the proteolytic index of the samples (Table 2) and the results of the microbiological studies (Table 4).

The water activity showed a most significant decrease after the end of salting, from 0.974 to 0.928, respectively, for the samples with the highest percentage of salt used (Table 2).

Table 2. Changes in the physicochemical parameters and the proteolytic index depending on the degree of salting (3%, 5% and 7% (w/w)) of the Elena ham samples after shaping (R), after the end of salting (S), after 1 month of drying and ripening (1M), after 6 months of drying and ripening (6M), and after 12 months of drying and ripening (12M) under natural air-drying conditions.

Indicator	**Moisture, %**	**pH value**		**a_w value**		**NaCl, %**		**PI, %**
Sample		***outer***	***inner***	***outer***	***inner***	***outer***	***inner***	
R	70.68 ± 1.65^{a}	5.78 ± 0.06^{a}		0.974 ± 0.005^{a}		-		1.4 ± 0.6^{a}
3%S	$65.83\pm0.95^{b,x}$	$5.91\pm0.08^{a,x}$		$0.945\pm0.005^{b,x,j}$	$0.968\pm0.010^{b,x,h}$	$2.99\pm0.50^{a,x,j}$	$2.13\pm0.22^{a,x,h}$	$12.2\pm2.0^{b,x}$
5%S	$63.99\pm1.03^{b,x,y}$	$5.74\pm0.04^{a,y}$		$0.932\pm0.002^{b,y,j}$	$0.955\pm0.001^{b,y,h}$	$4.28\pm0.42^{a,y,j}$	$2.28\pm0.22^{a,y,h}$	$9.8\pm0.2^{b,y}$
7%S	$62.88\pm1.22^{b,y}$	$5.89\pm0.09^{a,x}$		$0.928\pm0.001^{b,z,j}$	$0.950\pm0.002^{b,z,h}$	$6.31\pm0.80^{a,z,j}$	$2.84\pm0.20^{a,z,h}$	$8.8\pm0.8^{b,y}$
3%1M	$59.13\pm0.80^{c,x}$	$5.90\pm0.04^{a,x,j}$	$5.80\pm0.03^{a,x,h}$	$0.926\pm0.002^{c,x,j}$	$0.948\pm0.004^{c,x,h}$	$3.04\pm0.18^{a,x,j}$	$2.90\pm0.35^{b,x,j}$	$15.3\pm1.4^{b,x}$
5%1M	$57.77\pm1.30^{c,x,y}$	$5.84\pm0.04^{a,xy,j}$	$5.88\pm0.02^{a,y,j}$	$0.900\pm0.001^{c,y,j}$	$0.932\pm0.005^{c,y,h}$	$5.95\pm0.23^{b,y,j}$	$4.97\pm0.58^{b,y,h}$	$12.7\pm0.6^{c,y}$
7%1M	$56.33\pm0.77^{c,y}$	$5.78\pm0.02^{a,y,j}$	$5.92\pm0.00^{a,y,j}$	$0.895\pm0.003^{c,z,j}$	$0.917\pm0.010^{c,z,h}$	$6.12\pm0.10^{a,y,j}$	$5.88\pm0.10^{b,z,h}$	$10.6\pm1.5^{b,y}$
3%6M	$54.52\pm0.45^{d,x}$	$6.01\pm0.04^{b,x,j}$	$6.09\pm0.08^{b,x,h}$	$0.901\pm0.006^{d,x,j}$	$0.928\pm0.006^{d,x,h}$	$3.33\pm0.12^{a,x,j}$	$3.12\pm0.36^{b,x,j}$	$28.2\pm1.0^{c,x}$
5%6M	$52.01\pm0.86^{d,y}$	$5.90\pm0.00^{b,y,j}$	$5.94\pm0.04^{b,y,j}$	$0.890\pm0.005^{c,x,j}$	$0.900\pm0.010^{d,y,j}$	$6.16\pm0.15^{b,y,j}$	$5.88\pm0.33^{b,y,j}$	$24.9\pm2.0^{d,y}$
7%6M	$51.11\pm1.11^{d,y}$	$5.90\pm0.01^{b\,y,j}$	$5.97\pm0.04^{b,xy,h}$	$0.880\pm0.004^{d,y,j}$	$0.897\pm0.003^{d,y,j}$	$6.78\pm0.18^{b,z,j}$	$6.61\pm0.25^{c,z,j}$	$20.3\pm1.5^{c\,z}$
3%12M	$46.77\pm0.98^{e,x}$	$6.09\pm0.02^{c,x,j}$	$6.08\pm0.00^{b,x,j}$	$0.870\pm0.006^{e,x,j}$	$0.880\pm0.002^{e,x,h}$	$4.27\pm0.16^{b,x,j}$	$4.08\pm0.14^{c,x,j}$	$35.6\pm1.1^{d,x}$
5%12M	$45.61\pm0.80^{e,x}$	$6.00\pm0.03^{c,y,j}$	$5.97\pm0.02^{b,y,j}$	$0.852\pm0.004^{e,y,j}$	$0.855\pm0.005^{e,y,j}$	$6.88\pm0.24^{c,y,j}$	$6.72\pm0.27^{c,y,j}$	29.4 ± 2.0^{dy}
7%12M	$43.22\pm1.00^{e,y}$	$6.01\pm0.02^{c,y,j}$	$5.98\pm0.04^{b,y,j}$	$0.850\pm0.006^{e,y,j}$	$0.852\pm0.001^{e,y,j}$	$8.75\pm0.73^{c,z,j}$	$8.51\pm0.48^{d,z,j}$	25.5 ± 1.0^{dz}

The results obtained have been presented as mean values (n = 5); *The values in the columns and rows bearing different superscripts showed statistically significant differences ($p < 0.05$, Duncan's test). The following superscripts have been used in the columns: *x, y, z* in relation to the salt concentration, and *a, b, c, d* and *e* in relation to the production stage; in the rows, the letters *j* and *h* have been used to indicate significant differences ($p < 0.05$) with respect to the place in the ham where the sample analysed was taken.

To prevent the growth of psychrotrophic pathogens, such as the non-proteolytic strains of *Clostridium botulinum* inside the meat, the temperature during salting should be below 5°C; in addition, the salting process must also be sufficient in order to ensure water activity below 0.96 (Cassens, 1994; Hospital *et al.*, 2016). These critical water activity values were reached for all samples as early as the 45th day of salting, except for the 3%S samples from the inside of the hams.

A considerable increase in the proteolytic index (PI) compared to the mean value in the raw pork hams (1.42 ± 0.56) was already observed during the salting process. This increase was strongly influenced by the degree of salting of the samples, those salted with 3% (w/w) sodium chloride showing the highest PI value (Table 2). This faster growth trend was maintained throughout the period studied. In contrast, the samples prepared with 7% (w/w) sodium chloride demonstrated the lowest proteolytic index values, which could have been due to the inhibited progress of the proteolytic reactions initiated by both the tissue proteases and, possibly, the bacterial enzymes. Harkouss *et al.* (2012; 2014) regarded salt content above 4% and moisture content below 50% as such inhibiting conditions.

Changes in the texture profile

The hardness, chewing force and adhesiveness of the Elena ham samples were significantly affected by the processing stage ($p < 0.05$), (Table 3). These changes were directly related to the changes in the moisture content and the changes in the protein fraction (Monin *et al.*, 1997), and corresponded well to the results obtained by Lorenzo and Purrinos (2013) on changes in the textural parameters of Lacón dry-cured shoulders. Until the 6th month of ripening and drying, the cohesiveness values for Elena ham did not show statistically significant differences ($p > 0.05$); however, cohesiveness increased after this period, albeit slightly, but to a statistically significant level ($p < 0.05$) (Table 3). A probable reason for this increase in cohesiveness was both the compaction of the meat structure due to the more significant degree of dehydration in the samples and the deeper protein changes in them, as also indicated by the data obtained on the proteolytic index after the 6th and the 12th month of drying and ripening. The loss of moisture during the production process contributes to an increase in hardness, which, when adhesiveness decreases, leads to the improved cutting of the samples (Pérez-Santaescolástica *et al.*, 2018). Such a decrease in adhesiveness along with an increase in hardness that improved the slicing of the Elena ham was observed in the 12-month samples.

As regards the degree of salting, lower and statistically significant hardness values were measured, along with higher values for the adhesiveness, cohesiveness and chewiness of the samples made with 3% (w/w) sodium chloride compared to the other hams. The results obtained also explained the lower scores for the consistency indicator given to these samples during the sensory analysis (Figure 2).

Table 3. Changes in the texture characteristics of dry-cured Elena ham depending on the degree of salting (3%, 5% and 7% (w/w)) and during its production stage: after shaping (R), after the end of salting (S), after 1 month of drying and ripening (1M), after 6 months of drying and ripening (6M), and after 12 months of drying and ripening (12M) under natural air-drying conditions.

	Hardness, N	**Elasticity, %**	**Chewiness, N**	**Adhesiveness, N.mm**	**Cohesiveness, -**
R	8.04±0.13 ^{a}	0.53±0.0 ^{a}	2.95± 0.11 ^{a}	-0.69±0.09 ^{a}	0.64±0.02 ^{a}
3%S	30.00±2.73 ^{b,x}	0.51±0.05 ^{a,x}	9.69±4.58 ^{b,x}	-1.53±0.55 ^{b,x}	0.64±0.01 ^{a,x}
5%S	42.25±2.22 ^{b,y}	0.60±0.04 ^{a,x}	16.37±2.21 ^{b,y}	-0.95±0.32 ^{a,xy}	0.68±0.02 ^{a,x}
7%S	48.40±0.96 ^{b,z}	0.52±0.04 ^{a,x}	17.44±0.44 ^{b,y}	-0.88±0.03 ^{b,y}	0.70±0.06^{a,x}
3%1M	41.40±4.43 ^{c,x}	0.50±0.03 ^{a,x}	15.47±2.55^{bc,x}	-2.05±0.55^{b,x}	0.68±0.03 ^{a,x}
5%1M	58.20±4.38 ^{c,y}	0.56±0.02 ^{a,x}	20.75±4.73^{b,xy}	-1.68±0.22 ^{b,x}	0.65±0.02 ^{a,x}
7%1M	69.03±1.99 ^{c,z}	0.55±0.02 ^{a,x}	23.62±2.00 ^{c,y}	-1.86±0.50 ^{c,x}	0.62± 0.04 ^{a,x}
3%6M	49.66±1.86 ^{d,x}	0.56±0.03 ^{a,x}	19.90±3.42 ^{c,x}	-2.37±0.62 ^{b,x}	0.78±0.02 ^{b,x}
5%6M	65.25±1.19 ^{d,y}	0.55±0.02 ^{a,x}	20.83±1.20 ^{b,x}	-1.50±0.40 ^{b,x}	0.64±0.02 ^{a,y}
7%6M	76.88±4.14 ^{d,z}	0.62±0.02 ^{a,y}	30.54±3.95 ^{d,y}	-1.27±0.67 ^{b,x}	0.67±0.02 ^{a,y}
3%12M	87.90±6.27 ^{e,x}	0.57±0.03 ^{a,x}	41.19±4.95 ^{d,x}	-1.90±0.20 ^{b,x}	0.88±0.02 ^{c,y}
5%12M	100.35±4.30^{e,y}	0.61±0.02 ^{a,x}	48.15±2.52 ^{c,x}	-0.95±0.45^{b,y}	0.72±0.03 ^{b,x}
7%12M	118.50±9.18^{e,z}	0.58±0.02 ^{a,x}	57.35±5.50 ^{e,y}	-0.98±0.09 ^{b,y}	0.75±0.01 ^{b,x}

The results obtained have been presented as mean values (n = 5); *The values in the rows bearing different superscripts showed statistically significant differences ($p < 0.05$, Duncan's test): *x, y, z* in relation to the salt concentration, and *a, b, c, d, e* in relation to the production stage.

Changes in the microbiological characteristics

With the salt penetration and the progress of dehydration, a characteristic microbiota was selected in the Elena ham, mainly dominated by salt-tolerant and xerophilic species such as micrococci and staphylococci and a certain amount of lactic acid bacteria (Table 4). This is consistent with the microbiological features of dry-cured ham reviewed by Wang (2008). Staphylococci, micrococci and molds are the most often isolated microbial species from dry-cured hams, and these dominant species are considered to largely define the microbial community and influence the flavor and quality of the ham (Chen *et al.*, 2021). Even during salting, the total number of bacteria increased significantly ($p < 0.05$), and apart from an increase in micrococci and staphylococci, a significant increase in the number of enterococci, yeasts and the coliforms cultivated at 37°C was observed (Table 4). Interaction of salt penetration and concentration, pH and a_w decline of hams as well as temperatures of process lead to a similar behavior of microbial groups increasing their numbers along the first stages of process and then decreasing during the last stages (Reynolds *et al.*, 2001, Martínez-Onandi *et al.*, 2019). In the least salted sample, the quantitative changes in the microflora differed considerably. They demonstrated a sharp increase in the microbial load, the qualitative composition of which differed from that found in the 5%S and 7%S samples.

Table 4. Microbiological profile of dry-cured Elena ham depending on the degree of salting (3%, 5% and 7% (w/w)) and during its production stage: after shaping (R), after the end of salting (S), after 1 month of drying and ripening (1M), after 6 months (6M), and after 12 months of drying and ripening (12M) under natural air-drying conditions.

Sample	*Total Plate Count, log cfu/g*	*LAB, log cfu/g*	*Enterococci, log cfu/g*	*Staphylococci and micrococci, log cfu/g*	*Coliforms at 37 °C, log cfu/g*	*E.coli at 37 °C, log cfu/g*	*Coliforms at 44 °C, log cfu/g*	*E.coli at 44 °C, log cfu/g*	*Moulds and yeasts*
R	4.77 ± 0.10 ^{a}	1.52 ± 0.10 ^{a}	3.22 ± 0.05 ^{a}	2.76 ± 0.05 ^{a}	2.64 ± 0.13 ^{a}	2.33 ± 0.17 ^{a}	2.14 ± 0.13 ^{a}	1.83 ± 0.05 ^{a}	1.19 ± 0.00 ^{a}
3%S	7.53 ± 0.19 ^{b,z}	2.14± 0.36 ^{b,x}	4.85± 0.45 ^{b,y}	3.95± 0.10 ^{b,x}	5.22 ± 0.10 ^{b,y}	5.07 ± 0.03 ^{b,y}	2.96± 0.26 ^{b,y}	2.67 ± 0.24 ^{b,y}	2.69 ± 0.10 ^{b,x}
5%S	6.52 ± 0.16 ^{b,y}	2.85 ± 0.40 ^{b,x}	4.44 ± 0.23 ^{b,xy}	4.62 ± 0.15 ^{b,y}	4.12 ± 0.21 ^{b,x}	3.90 ± 0.16 ^{b,x}	2.27 ± 0.12 ^{a,x}	1.99 ± 0.09 ^{a,x}	3.07 ± 0.00 ^{b,z}
7%S	4.98 ± 0.05 ^{b,x}	2.74 ± 0.33 ^{b,x}	3.96 ± 0.33 ^{b,x}	4.72 ± 0.20 ^{b,y}	4.51 ± 0.45 ^{b,x}	4.58 ± 0.05 ^{b,y}	2.18 ± 0.24 ^{a,x}	1.89 ± 0.14 ^{a,x}	2.90 ± 0.00 ^{b,y}
3%1M	7.56 ± 0.36 ^{b,y}	2.97 ± 0.19 ^{c,x}	5.00 ± 0.03 ^{b,y}	4.36 ± 0.08 ^{c,x}	5.74 ± 0.63 ^{b,y}	5.60 ± 0.19 ^{c,y}	2.67 ± 0.68 ^{ab,x}	2.78 ± 0.20 ^{b,y}	2.50 ± 0.25 ^{b,x}
5%1M	6.05 ± 0.25 ^{b,x}	4.12 ± 0.20 ^{c,y}	4.65 ± 0.16 ^{b,x}	5.38 ± 0.05 ^{c,y}	4.22 ± 0.25 ^{b,x}	4.61 ± 0.22 ^{c,x}	1.00 ± 0.28 ^{b,y}	1.50 ± 0.09 ^{a,x}	3.00 ± 0.13 ^{b,x}
7%1M	6.04 ± 0.48 ^{c,x}	3.86 ± 0.13 ^{c,y}	4.40 ± 0.21 ^{b,x}	5.47 ± 0.23 ^{c,y}	4.55 ± 0.13 ^{b,x}	4.85 ± 0.35 ^{b,x}	2.03 ± 0.13 ^{a,x}	1.56 ± 0.21 ^{a,x}	2.78 ± 0.13 ^{b,x}
3%6M	6.53 ± 0.23 ^{c,y}	4.08 ± 0.95 ^{d,x}	5.00 ± 0.19 ^{b,y}	4.84 ± 0.06 ^{d,x}	4.94 ± 0.05 ^{c,y}	5.14 ± 0.06 ^{b,z}	2.32 ± 0.11 ^{a,y}	2.25 ± 0.06 ^{c,y}	1.69 ± 0.19 ^{c,x}
5%6M	5.81 ± 0.26 ^{b,x}	4.17 ± 0.16 ^{c,x}	4.20 ± 0.43 ^{b,x}	5.59 ± 0.22 ^{c,y}	3.60 ± 0.17 ^{c,x}	3.85 ± 0.13 ^{b,y}	1.20 ± 0.08 ^{b,x}	1.42 ± 0.05 ^{a,x}	2.32 ± 0.10 ^{a,y}
7%6M	6.08 ± 0.34 ^{c,xy}	4.19 ± 0.65 ^{c,x}	3.98 ± 0.30 ^{b,x}	5.49 ± 0.26 ^{c,y}	3.77 ± 0.15 ^{c,x}	3.25 ± 0.17 ^{c,x}	1.44 ± 0.30 ^{b,x}	1.47 ± 0.11 ^{a,x}	1.60 ± 0.32 ^{c,x}
3%12M	5.12 ± 0.10 ^{d,y}	2.75 ± 0.46 ^{c,x}	4.18 ± 0.26 ^{c,y}	4.72 ± 0.18 ^{d,x}	4.05 ± 0.15 ^{d,x}	4.05 ± 0.10 ^{d,y}	1.00 ± 0.00 ^{c}	1.00 ± 0.00 ^{d}	1.50 ± 0.40 ^{c}
5%12M	4.83 ± 0.15 ^{d,x}	3.00 ± 0.15 ^{b,x}	3.41 ± 0.15 ^{a,x}	4.50 ± 0.26 ^{b,x}	2.40 ± 0.05 ^{d,y}	2.40 ± 0.12 ^{d,x}	*nd*	*nd*	*nd*
7%12M	4.95 ± 0.05 ^{d,x}	3.03 ± 0.17 ^{b,x}	3.47 ± 0.26 ^{a,x}	4.44 ± 0.32 ^{b,x}	2.05 ± 0.40 ^{d,y}	2.15 ± 0.66 ^{d,x}	*nd*	*nd*	*nd*

The results obtained have been presented as mean values (n = 5); *The values in the rows bearing different superscripts showed statistically significant differences ($p<0.05$, Duncan's test): *x, y, z* in relation to the salt concentration, and *a, b, c, d* in relation to the production stage. *nd*: not determined.

The amounts of coliforms and enterococci reported for the 3%S sample were statistically significantly higher compared to the other two samples, and the growth rate of staphylococci and micrococci was lower ($p < 0.05$), (Table 4). These results corresponded to the finding of Blesa *et al.* (2008) that with lower salted hams, a longer time after salting is needed to reach water activity values that leading to a decrease in microbial counts.

From the group of lactose-positive bacteria, *E. coli* was the dominant microbial species that survived well during the salting period. The decrease in their number was only observed 6 months after the drying and ripening of the Elena ham

The number of enteropathogenic coliforms (incubated at 44°C) in the samples with a higher degree of salting remained approximately constant until the 6th month of drying and ripening, after which it began to decrease, whereas in the samples with 3% salt, the coliforms and *E.coli* incubated at 44°C increased their numbers, albeit slightly, during salting and were subsequently detected even during the 12th month of drying and ripening (Table 4). *Salmonella* bacteria and *L. monocytogenes* in 25 g of the finished product were not detected in any of the Elena ham samples examined.

It is interesting to note the more significant increase in the lactic acid population in the period of drying and ripening of the Elena ham, with the highest values of about 4 logarithmic units measured in the sixth month. Subsequently, the number of lactic acid bacteria decreased to values of 2.75, 3.00 and 3.03 log CFU/g, respectively, in samples 3%12M, 5%12M and 7%12M (Table 4). In fact, the number of lactic acid bacteria already began to increase at the stage of salting. Their greater number during the first month of drying and ripening (1M) could mainly be attributed to the placement of the hams in the natural drying chambers where they probably formed part of the natural production microflora. Their lower participation, however, in the general microbiological picture of the samples could have been related to the salt concentration and the dehydration, which did not provide a microbiological niche for their more significant growth.

Sensory analysis

Extending the ripening from 6 to 12 months under natural air-drying conditions resulted in higher and statistically discernible smell and taste scores for the samples salted with 5% and 7% salt (w/w) compared to those obtained during the 6th month. In contrast, the samples with 3% (w/w) salting demonstrated a decrease in these values with the extension of the ripening up to the 12th month. The main flaws that led to a lower score were bitterer and less salty taste reported for these samples. In the 7%12M samples, the reason for their slightly lower scores compared to 5%12M was the saltier taste and slightly rancid smell. The Elena ham samples prepared with 5% salt and aged for 12 months in natural drying chambers were characterised as the most balanced ones in terms of smell and taste (Figure 2). A softer and paste-like consistency was reported for the 3%6M samples, leading to their significantly lower scores. The improvement in the consistency scores for the 7%12M samples compared to the 7%6M samples, despite their lower moisture content, indicated that the proteolytic activity in the dry-cured Elena ham was preserved even after 6 months of ripening in the natural drying chambers.

After the 6th month of ripening, the following flaws in the colour of the dry-cured Elena ham were observed: in the 3% salted samples, the colour was too pale, with iridescence, and in the 7% salted samples, it was too dark and uneven, with grey-brown areas and yellow colouration of the adipose tissue. In contrast, the samples salted with 5% sodium chloride again received the highest and statistically discernible score for this indicator ($p = 0.003$).

On the basis of the comparison of the scores obtained for their overall sensory quality, the Elena ham samples could be arranged in the following order: 5%12M > 7%12M > 5%6M > 7%6M > 3%6M > 3%12M.

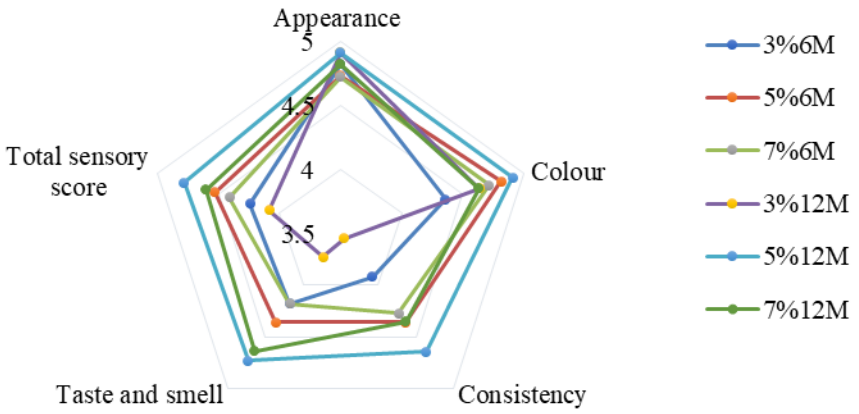

Figure 2. Sensory evaluation of Elena ham samples with different degree of salting (3%, 5% and 7% (w/w)) after 6 months of drying and ripening (6M), and after 12 months of drying and ripening (12M) under natural air-drying conditions

Conclusions

In summary, none of the sodium chloride concentrations used in the experiments was found to lead to significant inhibition of the proteolytic activity of the tissues, considering the increasing proteolytic index and pH values until the end of the drying and ripening period studied. A degree of salting above 3% in the manufacture of this traditional dry-cured product played a key role in the development of its desired microbiological characteristics. This is essential due to the absence of nitrites or nitrates in the salting mixture. The complex analysis of the results obtained showed that the use of 5% (w/w) sodium chloride for salting the pork hams presented a scientifically based possibility of avoiding the use of high salt concentrations in the traditional production of dry-cured ham delicacies with a view to improving the health profile of this group of meat products.

References

Andrés, A.I., Cava, R., Ventanas, J., Thovar, V., Ruiz, J. 2004. Sensory characteristics of Iberian ham: Influence of salt content and processing conditions. *Meat Science*, **68**(1), 45-51.

Berardo, A., De Maere, H., Stavropoulou, D.A., Rysman, T., Leroy, F., De Smet, S. 2016. Effect of sodium ascorbate and sodium nitrite on protein and lipid oxidation in dry fermented sausages. *Meat Science*, **121**, 359-364.

Blesa, E., Aliño, M., Barat, J. M., Grau, R., Toldrá, F., Pagán, M. J. 2008. Microbiology and physico-chemical changes of dry-cured ham during the post-salting stage as affected by partial replacement of NaCl by other salts. *Meat Science*, **78**(1), 135-142.

Bourne, M.C., Kenny, J.F., Barnard, J.A. 1978. Computer-assisted readout of data from texture profile analysis curves. *Journal of Texture Studies*, **9**(4), 481-494.

Candek-Potokar, M., Skrlep, M. 2012. Factors in pig production that impact the quality of dry-cured ham: A review. *Animal*, **6**(2), 327-338.

Cassens, R.G. 1994. *Meat preservation, preventing losses and assuring safety, food and nutrition*. CRC Press, Trumbull, Connecticut, USA.

Chen, L., Wang, Z., Ji, L., Zhang, J., Zhao, Z., Zhang, R., Bai, T., Hou, B., Wang, W. 2021. Flavor composition and microbial community structure of Mianning ham. *Frontiers in Microbiology*, **11**:62377.

Feng, X., Li, C., Jia, X., Guo, Y., Lei, N., Hackman, R.M, Chen, L., Zhou, G. 2016. Influence of sodium nitrite on protein oxidation and nitrosation of sausages subjected to processing and storage. *Meat Science*, **116**, 260-267.

Fraqueza, M.J., Laranjo, M., Elias, M., Patarata, L. 2021. Microbiological hazards associated with salt and nitrite reduction in cured meat products: control strategies based on antimicrobial effect of natural ingredients and protective microbiota. *Current Opinion in Food Science*, **38**, 32-39.

Grillo, A., Salvi, L., Coruzzi, P., Salvi, P., Parati, G. 2019. Sodium intake and hypertension. *Nutrients*, **11**(9), 1970.

Harkouss, R., Astruc, T., Lebert, A., Gatellier, P., Loison, O., Safa, H., Portanguen, S., Parafta, E, Mirade, P.S. 2014. Quantitative study of the relationships among proteolysis, lipid oxidation, structure and texture throughout the dry-cured ham process, *Food chemistry*, **166**, 522-530.

Harkouss, R., Mirande, P.-S., Gatellier, P. 2012. Development of rapid, specific and efficient procedure for the determination of proteolytic activity in dry-cured ham: Defenition of new proteolytic index. *Meat Science*, **92**, 84-88.

Hospital, X.F., Hierro, E., Stringer, S., Fernández, M. 2016. A study on the toxigenesis by *Clostridium botulinum* in nitrate and nitrite-reduced dry fermented sausages. *International Journal of Food Microbiology*, **218**, 66-70.

Hospital, X.F., Carballo, J., Fernandez, M., Arnau, J., Gratacos, M., Hierro, E. 2015. Technological implications of reducing nitrate and nitrite levels in dry-fermented sausages: Typical microbiota, residual nitrate and nitrite and volatile profile. *Food Control*, **57**, 275e281.

ISO 11290-1. 2017. Microbiology of the food chain. Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. Part 1: Detection method.

ISO 15214. 1998. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of mesophilic lactic acid bacteria. Colony-count technique at 30 degrees C.

ISO 4833-2. 2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms. Part 2: Colony count at 30 degrees C by the surface plating technique.

ISO 6579-1. 2017. Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of *Salmonella*. Part 1: Detection of *Salmonella* spp.

ISO 6887-2. 2004. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 2: Specific rules for the preparation of meat and meat products.

ISO 7251. 2005. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of presumptive *Escherichia coli*. Most probable number technique.

Laureati, M., Buratti, S., Giovanelli, G., Corazzin, M., Lo Fiego, D.P., Pagliarini, E. 2014. Characterization and differentiation of Italian Parma, San Daniele and Toscano dry-cured hams: A multi-disciplinary approach. *Meat Science*, **96**(1), 288-294.

Leroy, F., Scholliers, P., Amilien, V. 2015. Elements of innovation and tradition in meat fermentation: Conflicts and synergies. *International Journal of Food Microbiology*, **212**, 2-8.

Lorenzo, J.M., Purrinos, L. 2013. Changes on physico-chemical, textural, proteolysis, lipolysis and volatile compounds during the manufacture of dry-cured "Lacón" from Celta pig breed. *Journal of Biological Sciences*, **13**, 168-182.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*,**193**(1), 265- 275.

Lucarini, M., Durazzo, A., Sette, S., Lombardi-Boccia, G., Santini, A., Strazzullo, P. 2021. Sodium intake and related diseases. *International Journal of Molecular Sciences*, **22**(14), 7608.

Martínez-Onandi, N., Sánchez, C., Nuñez,M., Picon, A. 2019. Microbiota of Iberian dry-cured ham as influenced by chemical composition, high pressure processing and prolonged refrigerated storage. *Food Microbiology*, **80**, 62-69.

Marušić, N., Vidaček, S., Janči, T., Petrak, T., Medić, H. 2014. Determination of volatile compounds and quality parameters of traditional Istrian dry-cured ham. *Meat Science*, **96**, 1409-1416.

Marušić, N.R., Vidaček, S, Janči, T, Medić, H. 2016. Characterization of volatile compounds, physico-chemical and sensory characteristics of smoked dry-cured ham. *Journal of Food Science and Technology*, **53**(11), 4093-4105.

Mediani, A., Hamezah, H.S., Jam, F.A., Mahadi, N.F., Chan, S.X.Y., Rohani, E.R., Che Lah, N.H., Azlan, U.K., Khairul Annuar, N.A., Azman, N.A.F., Bunawan, H., Sarian, M.N., Kamal, N., Abas, F. 2022. A comprehensive review of drying meat products and the associated effects and changes. *Frontiers in Nutrition*, **9**, 1057366.

Mitreva, D., Gradinarska-Ivanova D., Valkova-Yorgova, K. 2019. The gradual development of quality complex of Bulgarian dry-cured ham during aging under natural climatic conditions, *Scientific Works of University of Food Technologies*, **66**(1), 16-24.

Monin, G., Marinova, P., Talmant, A., Martin, J.F., Cornet, M., Lanore, D., Grasso, F. 1997. Chemical and structural changes in dry-cured hams (Bayonne hams) during processing and effects of the dehairing technique. *Meat Science*, **47**(**1-2**), 29-46.

Moore, S., Stein, W.H. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *Journal of Biological Chemistry*, **211**(2), 907–913.

Morales, R., Serra, X., Guerrero, L., Gou, P. 2007. Softness in dry-cured porcine biceps femoris muscles in relation to meat quality characteristics and processing conditions. *Meat Science*, **77**(4), 662-669.

Muñoz-Rosique, B., Salazar, E., Tapiador, J., Peinado, B., Tejada, L. 2022. Effect of Salt Reduction on the quality of boneless dry-cured ham from Iberian and white commercially crossed pigs. *Foods*, **11**(6), 812.

Ockerman, H.W., Basu, L. 2014. Production and consumption of fermented meat products. In: *Handbook of fermented meat and poultry*. Toldrá, F., Hui, Y.H., Astiarán, I., Sebranek, J.G., Talon, R. Willey Blackwell: West Sussex, UK, pp. 7–11.

Patarata, L., Fernandes, L., Silva, J.A., Fraqueza, M.J. 2022. The risk of salt reduction in dry-cured sausage assessed by the influence on water activity and the survival of *Salmonella*. *Foods*, **11**(3), 444.

Pérez-Santaescolástica, C., Carballo, J., Fulladosa, E., Garcia-Perez, J.V., Benedito, J., Lorenzo, J.M. 2018. Effect of proteolysis index level on instrumental adhesiveness, free amino acids content and volatile compounds profile of dry-cured ham. *Food Research International*, **107**, 559-566.

Reynolds, A.E. Harrison, M.A., Rose-Morrow, R., Lyon C.E. 2001. Validation of dry cured ham process for control of pathogens. *Journal of Food Science*, **66**(9), 1373-1379.

Ruiz-Ramirez, J., Arnau, J., Serra, X., Gou, P. 2006. Effect of pH(24), NaCl content and proteolysis index on the relationship between water content and texture parameters in biceps femoris and semimembranosus muscles in dry-cured ham. *Meat Science*, **72**(2), 185-194.

Schivazappa, C., Virgili, R. 2020. Impact of salt levels on the sensory profile and consumer acceptance of Italian dry-cured ham. *Journal of the Science of Food and Agriculture*, **100**, 3370-7.

Toldrá, F. 2002. *Dry-cured meat products*. Trumbull, CT: Food & Nutrition Press Inc., USA.

Wang, L. 2008. Reviews on effects of microorganisms in dry-cured ham. *Meat Research*, **22**(10), 16–18.

WHO. 2012. Guideline: Sodium intake for adults and children. World Health Organization (WHO), Geneva.

Zahariev, I. 2008. *Handbook for a technologist in a meat processing enterprise*. pp.129-130. Association of Meat Processors in Bulgaria, Sofia.

Zanardi, E., Ghidini, S., Battaglia A., Chizzolini R. 2004. Lipolysis and lipid oxidation in fermented sausages depending on different processing conditions and different antioxidants. *Meat Science*, **66**(2), 415-423.

Zhou, G.H., Zhao, G.M. 2007. Biochemical changes during processing of traditional Jinhua ham. *Meat Science*, **77**(1), 114-120.