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EFFECT OF EXTENDED AGING UPON TEXTURAL ASPECTS OF TRADITIONAL BULGARIAN DRY-CURED HAM

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Abstract

The objective of this study was to determine the effect of prolong ripening and drying on quality aspects of traditional Bulgarian dry cured ham. After 8, 18 and 36 months of aging comparative textural profiling of dry cured ham were established. Ham sensory profile as well as physicochemical, morphological characteristics, proteolysis index and Warner-Bratzler shear force (WBSF) were investigated. Excessive aging up to 36 months of dry-cured ham lead to decreased hardness, but lower sensory values, characterizing overall textural perception tenderness of the samples. Hams of higher ripening and drying had statistically significant increased proteolysis index and lower values of WBSF. Morphological analysis revealed significant qualitative and quantitative differences between sample groups. Thus, miofibrilar fragments increased remarkably in samples with higher proteolysis index scores. In addition, longest aging hams showed increased degradation for a total myofibrillar structure. Warner-Bratzler shear force (WBS) had a significant relationship with the sensory tenderness variables, such as softness, chewiness, and rate of breakdown in mouth.

Key words: *dry-cured ham, excessive ripening, texture, morphological changes, Warner-Bratzler Shear Force, proteolytic index*

INTRODUCTION

Traditional dry-cured meat products from whole muscle meat are highly fascinating foods, both in social and market aspects (Talon et al., 2007; Laranjo et al., 2017; Rantsiou & Cocolin, 2008). They have a clear function in the global gastronomic heritage (Iaccarino et al., 2006) and are often associated with a certain organoleptic superiority (Panagou et al., 2013). These products can be categorized on the basis of different factors such as the specificity of the used raw materials and/or the applied technology (fermentation, salting, smoking), country of origin, degree of drying, maturation, etc. (Zdolec, 2017; Lücke, 1994; Lücke, 2000). Their production technology is closely tied to the local identity of the country or ethnicity concerned. Historically, the reasons for the wide variety of dry-cured meat products are related to the climatic and resource conditions of local production (Zdolec, 2017) and to the influence and nutritional practices of the various civilizations that have settled in the area Stearns, 2010; Gagaoua & Boudechicha, 2018). The sensory, physicochemical, microbiological and textural characteristics of the different European-produced dry ham vary greatly depending on the specifics of the technological process adopted by the different producers (Leroy et al., 2013; Toldrá, 2014, Zeng, et al., 2016; Rather et al., 2016). In their traditional production technology, the following general technological stages have been adopted: salting, post salting or process of achieving equilibrium salt concentration, drying and maturing. The continuance of drying and ripening varies from 2-3 months to 2-3 years in the highest quality dry ham, such as 48 months in some Spanish species such as Seraño ham (Petrova et al., 2016). Increasing the aging time provides for a more significant degree of enzyme digestion, which contributes to the production of a higher quality product (Toldra, 2002; 2004).

Numerous studies have been conducted on the physicochemical and sensory properties of a number of Italian and Spanish ham and variations of these properties depending on the technology used (Andrés et al., 2004a,b, Flores et al., 2006; Gou et al. 2008; Huang, & Huang, 2010; Ruiz-Ramirez, et al., 2006; Serra et al., 2005). However, in existing literature sources, no attempt has been made to combine two important aspects of the production of this group of dry-cured meat products: the biochemical and morphological changes in the protein fraction as a result of the long maturation and drying period over 1 year and their effect on the texture of meat products. Also, almost no data is available about Balkan traditional dry-cured meat products from whole muscle meat, despite the fact that traditional technologies exist for these products (Gagaoua & Boudechicha, 2018; Gök, Obuz, & Akkaya, 2012, Rajkovic, 2012), though not as popular and exported worldwide as Italian and Spanish ham.

During socialism, with the establishment of the amalgamated Economic Union in the Republic of Bulgaria, called "Rodopa", industrial and mass production occurred in meat processing, which gradually oppressed traditional meat crafts and replaced them with unified so called now "classical" meat technologies. Nevertheless, even today, in a variety of regions of Bulgaria there are preserved and produced even a few traditional meat products. Therefore, the aim of the present work is focused on physicochemical, morphological and textural changes occurring in the traditional Bulgarian dry-cured meat product "Elena ham" during a long period of aging of this product in natural drying chamber.

MATERIAL AND METHODS

Materials

The research was carried out with dry-cured traditional meat product "Elena ham" for the production of which fresh chilled pork green ham of cross-breed pigs up to 12 months old and live weight over 90 kg were bought by Biltrans Ltd, Elena town, and the preparation and production of studied hams was carried out in the same company, producing this traditional meat product. The shaping of the hams is done by separating them from the carcass by cutting between the last lumbar and the first cross vertebrae and cutting through the hock joint. The pelvic bone was gently released without cutting the muscles, leaving only a small part of the pelvic wing (2-3 cm), the inner surface of the thigh was formed by removing the fat, as the outer surface had a perfectly preserved skin on the surface and without damage. The skin was cut out of the outside and the fat, giving the ovoid shape to the buttocks, and the shaped hams were salted with table salt in special chambers with temperature from 3 to 5 °C and approx. humidity 85-90%. Salting was done manually by scrubbing and massaging the entire surface. The time for salting in these conditions lasted about 45 days and depended on the weight of the individual hams (approximately 5-6 days per kilogram), during this time hams were salted 3-4 times by sprinkling and massaging. After salting the hams were cleaned from the surface adhering salt mixture, washed with clean drinking water, drained and hung for ripening and drying in special natural drying rooms – at the specific mountain climate of town Elena, Bulgaria. This have been done in the months of February, March and early April when the air is cool and dry and the temperature varies between 2 - 5 ° C to plus 10-12 ° C. After about 20 days of initial drying, the hams are covered with a thin, but dense layer of specially prepared leaf fat and rice flour, especially to the areas without skin and around the joints and bones. After this manipulation, the hams were aged under the above conditions until they reached the organoleptic characteristics of the final product, but not shorter time than 6 months.

The research was carried out with samples of the traditional Bulgarian dry-cured "Elena ham", aged for 8, 18 and 36 months and in some analysis with raw meat. The samples taken included the following muscle groups: m.Semimembranosus, m. Semitendinosus and m. Biceps femoris. Samples were analysed to monitor changes during the ripening process in water content, water activity, pH, degree of hydrolysis of the protein fraction, morphological and texture profile of muscle tissue.

Methods

To determine moisture, drying of a homogenous mixture of the samples at 104 ± 1°C was performed using an electronic moisture analyser model KERN MLS-A (Kern & Sohn GmbH, Germany).

Water activity (aw) was measured using a LabSwift-aw system (Novasina AG, Switzerland) at 25°C. Three independent measurements were made for each sample.

The degree of hydrolysis of the protein fraction was determined as the ratio of the amount of free alpha-amine nitrogen to the total amount of nitrogen in the sample determined by the Lowry method. Extraction of the muscle proteins was performed with phosphate buffer with pH 7.3 and ion strength 0.55 M. From an average sample, 2.5 g were weighted, transferred to a 50 cm3 measuring flask and made up to the mark with phosphate buffer. The extraction was carried out with periodic stirring at 4 ± 2 ° C for 24 hours. After extraction, the homogenate was centrifuged at 10,000 g for 20 min, after which the above clear layer was separated and used to determine the content of protein by the Lowry method (1951) and the amount of α-amine nitrogen by the ninhydrin method described by Moore & Stein (1954).

For Warner–Bratzler (W–B) analyses, samples were cut into 30 × 30 × 30 mm chops (thickness × length × width) and cooled overnight at 4°C. After cooling, three slices (15 mm thick) without fat or connective tissue, parallel to the longitudinal orientation of the muscle fibres, were taken from each sample chop. Warner–Bratzler Shear Force (WBSF) was determined using a texture analyser TA-XT.Plus (Stable Micro Systems, Surrey, Great Britain) with a Warner-Bratzler stainless cutter blade. Samples were sheared perpendicular to the long axis of the core at a speed of test – 2 mm.s^{-1}, and WBSF was taken to be the peak force of the curve (Honikel, 1998; Iseya et al., 1996). Prior to analysis samples chop were left for 30 min at room temperature. Determinations were repeated six times per sample and were averaged.

Sensory analysis was focused on hardness, firmness and overall texture perception (Larmond, 1976) for the samples of “Elena” ham with different aging period. The overall consistency assessment involved the evaluation of three aspects: the easiness of initial dental penetration in the meat cut; the easiness with which the meat was broken into fragments, and the amount of residue left after chewing (Choe et al., 2016; Lawrie and Ledward, 2006;). The analysis was conducted according to the method described by Iseya et al., (1996) with some modifications. Hardness and firmness was evaluated by chewing a testing sample with molars and by biting off with front teeth the sample. Sensory analysis was performed by 7 trained assessors. The panel evaluated each characteristics according to a 5-point category scale (1=least, 5 = highly). Before analysis, the panel members had attended a preliminary training session where they were examined samples that varied in the evaluated attributes and the meaning of hardness, firmness and overall textural perception had been also discussed. The preliminary session was concluded when individual scores did not vary more than 1 unit for the mean scores, and all members understood and could use the scoring system.

One-way ANOVA was applied to the assessment of the effect of aging time (Factor I) on the water content, proteolytic index, WBSF and sensory evaluations of the examined hams. Duncan’s test was applied for multiple comparisons between all mean value pairs. All calculations were made at confidence level α = 0.05. The experiments were carried out with three replications of three samples of one aged ham or green ham. The data in tables and figures are shown as average ± standard deviation (SD). The statistical procedures were performed using the Microsoft Excel 5.0 software and the Statgraphics 16 programme.

RESULTS AND DISCUSSION

Physicochemical and biochemical characteristics

To evaluate the influence of the time of ripening in natural drying chamber of the traditional dry cured meat product “Elena ham” changes in their physicochemical parameters listed in Table 1 are tracked.

Table 1. Change in moisture content, aw – value and proteolytic index of testing sample during extended aging.

Sample	Indicator			
	moisture content, $g.kg^{-1}$	a_w	proteolytic Index	pH
8 months	467,72±18,00c	0,895±0,003c	24,81±8,88a	6,10±0,02c
18 months	422,6±7,69b	0,845±0,005b	35,04±13,57a,b	6,04±0,04b
36 months	226,96±27,46a	0,813±0,006a	53,27±16,51b	5,85±0,06a

Means within each column having different letters are significantly different according to Duncan's test at p<0,05.

For all three samples, statistically significant differences were found for the results for pH, water content and water activity ($p<0.05$) (Tab. 1). The reason for significant differences in water content of the samples at 36 months compared to the other two samples of ham can be explained not only with the longer drying and ripening stage (36 months) in the natural climate chamber, but also with the lower average pH values measured for these samples (5.85 ± 0.06) (Tab. 1). At a lower pH, the resulting coagulation changes of meat proteins speed up drying and favour the loss of moisture from the product (Ockerman & Basu, 2008). The established decrease in pH with increasing maturation is in some contradiction with the increase in pH as a result of the accumulation of biogenic amines or ammonia due to deeper muscle proteolysis during the maturation process observed by other authors (Toldra, 2002). Cause for these results may also be related to the more intense hydrolysis reactions of the meat lipids (data not shown) and the subsequent chemical transformations of the protein and lipid degradation products accompanying the long maturation period in the ham aged 36 months.

For the values obtained for the water activity of the samples, statistically significant differences were recorded, as in the samples with higher water content, higher values for a_w were also measured. Comparing the a_w values measured for "Elena" ham samples with those reported from other authors that investigating more popular European dry cured ham, makes it clear that our results are significantly lower, with the exception of a_w values received for 8-month samples. For example, the reported water activity at the end of the production of Italian hams, aging for 12 ÷ 16 months, varied between 0.94 ÷ 0.87, for Spanish hams with a production cycle duration of 12 ÷ 48 months - 0.899 ÷ 0.860 (Martínez- Martínez-Onandi et al., 2016; Petrova et al., 2016), and for the French hams with production of about 9 months or over - 0.89 (Petrova et al., 2016; Parafita et al., 2015). Enzymatic degradation of proteins plays an important role in the formation of the aroma and taste of dry-cured hams. An important result associated also with proteolysis is the formation of ham consistency, which is mainly due to the breakdown of myofibrillar structures (Chandek-Potokar & Škrlep, 2012; Mora, Sentandreu, & Toldra, 2011; López-Pedrouso et al., 2019). The constant increase in the proteolytic index found in the studies is a direct indication for the extent of proteolytic changes in Elena ham, as maturation and drying progresses until the end of the investigated period (Tab. 1). These results are in agreement with data reported by other authors (Garcıa-Garrido et al., 1999; Pugliese et al., 2015, Zhao et al., 2008). In Spanish dry-cured ham, proteolysis index (PI) reflecting good quality could be considered between 33 and 36, while in Italian dry-cured ham between 22 and 30 (Careri et al., 1993; Pérez-Santaescolástica et al., 2018). In the present work we observed values between 24.81 and 53.27 in dry-cured hams, which can explain the fact that the 18-month aging sample was evaluated as most perceptional and wanted in sensory aspect. An increase in proteolysis during dry-cured ham processing has been associated with negative effects on the taste and aroma of the dry-cured ham (Pérez-Santaescolástica et al., 2018) which, as a result, may lead to consumer rejection of the product. However, the proteolysis index well explains the established relationship between water content and WBSF values of samples of dry-cured meat products and can serve as a marker for their consistency at different extent of drying and ripening (Ruiz- Ramirez et al., 2006; Pérez-Santaescolástic et al., 2018).

Textural analysis

The Warner-Bratzler device is known as a tool for tenderness measurement of livestock meat and in some cases for fish (Iseya et al., 1996; Kemp et al., 1968). Shear force values were similar in samples aged 18 and 36 months, but generally favoured the hams in trials 36 months (Tab. 2). These mechanical parameter were attenuated as the time of ripening and drying of ham increased, as a marked change in this textural parameter occurred in samples aged over 8 month ($p < 0.05$) (Fig. 1).

Table 2. Changes in Warner-Bratzler Shear force of testing sample during extended aging.

Sample	cutting force, N/mm	coefficient of variation
raw meat	$7{,}53 \pm 0{,}63^{c}$	8,380%
"Elena ham"- 8 months	$2{,}26 \pm 0{,}456^{b}$	20,202%
"Elena ham"-18 months	$1{,}\ 57 \pm 0{,}640^{a}$	40,983 %
"Elena ham"- 36 months	$1{,}414 \pm 0{,}410^{a}$	28,955%

Means within each column having different letters are significantly different at p<0,05

Coefficient of variations were 8.38% for the shear force of raw meat and ranged between 20,20 – 40,98 % for hams. Other studies have shown similar high coefficient of variation for WBS testing (Caine et al., 2003). There were significant differences in the cutting force between 8 month and 18 month as well as between 8 and 36 month ($p<0.05$). The texture profile is related to water activity and water content by observing a negative non-linear relationship between ham hardness and measured water content and water activity (Ruiz-Ramírez et al., 2005, Andrés et al., 2005). However, the increasing rate of shear force in the Warner-Bratzler shear test for samples, that have been aged 18 and 36 months and have lower moisture content, was higher than that received for the ham sample aged for 8 months and having higher moisture content.

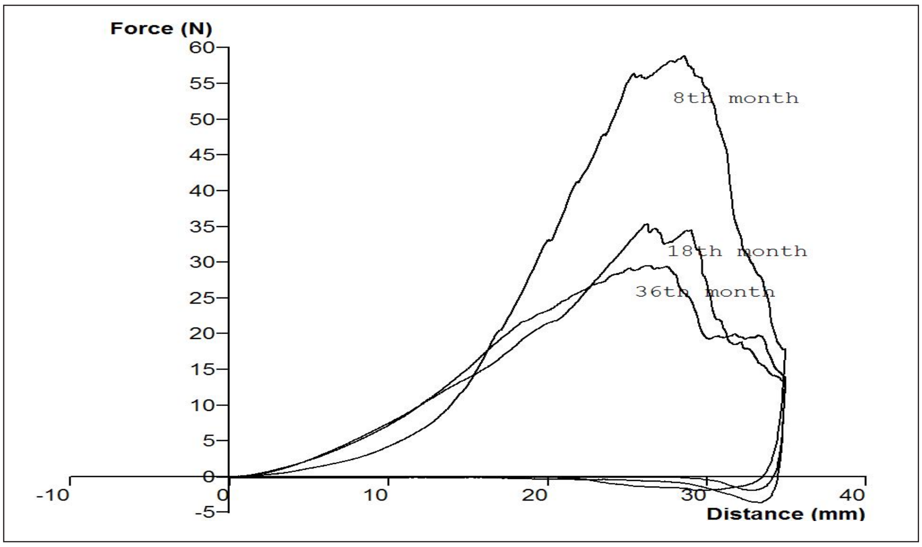

Figure 1. WBSF of "Elena" hams aged for 8, 18 and 36 months.

Morphological analysis

Group of cytoskeletal proteins consisting mainly myofibrillar proteins, and proteins of the stroma are of great importance in respect of the meat texture (Damodaran et al., 2008, Petrova, 2016). At the microstructural level, it has been shown that myofibrillar proteins are mainly proteins that undergo substantial proteolytic changes during the production of raw-dry-cured meat products (Larrea et al., 2007).

The morphological images (Fig.2) representing changes in transverse and longitudinal sections of muscle tissue, used for the preparations of the tested samples, shown that, as the drying and maturing progresses, deeper structural changes were observed, most pronounced at the sample of 36 months. These changes were mainly related to a decrease in the diameter of the muscle fibres as well as to the appearance of more enlightened areas in them due to the ongoing hydrolysis of muscle proteins (Fig.2).

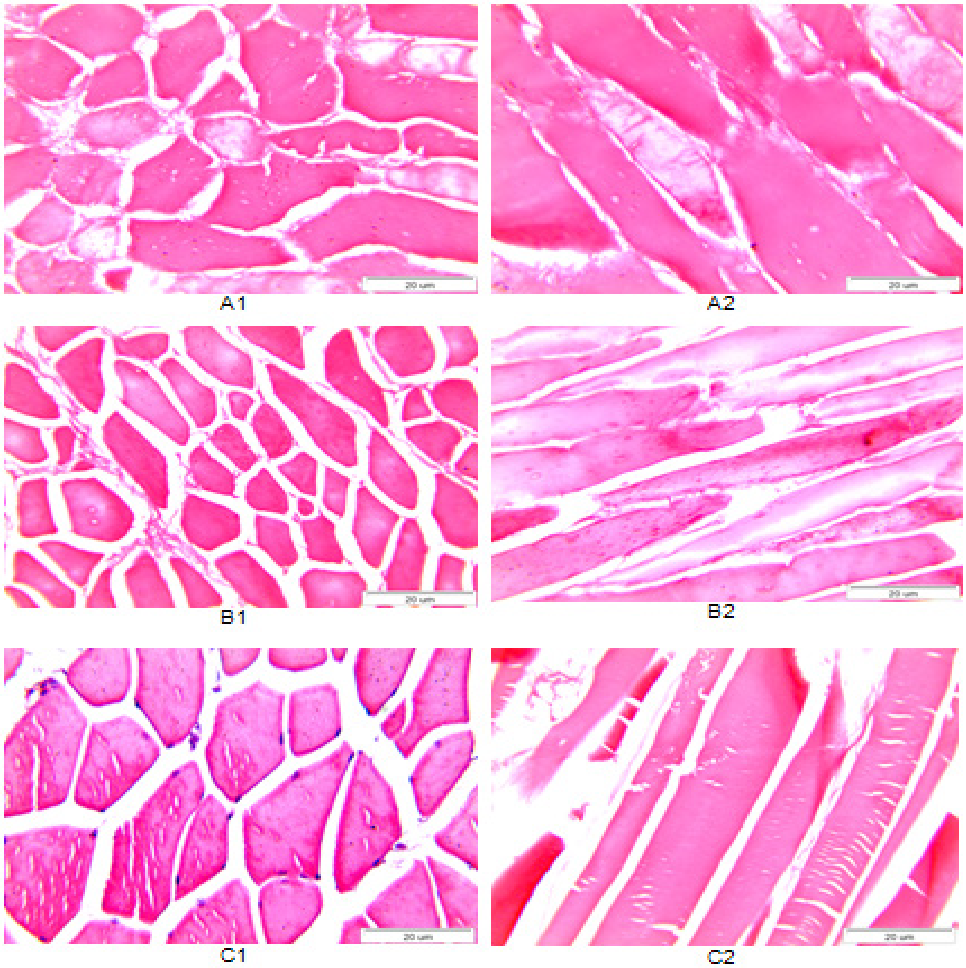

Figure 2. Light microscopic morphological images of samples A- Elena Ham at 8 months; B – "Elena ham" at 18 months; C – "Elena ham" at 36 months; haematoxylin stained; magnification 400×.

Morphological changes in the studied ham pointed the occurrence of proteolysis that caused destruction of the endomysial envelope and partial lysis of the structure of muscle fibres (Fig. 2). The presence of multiple light sections in both the muscle fibres and the intracellular space with a formed network of finely granulated protein mass and muscle nuclei were the consequence of the myolemma disruption. This significant changes in muscle fibre structure due to the ongoing hydrolysis of protein substances over the entire volume of muscle fragments was associated with a decrease in water retention capacity and correlates well with the water content of the tested samples. The greater degree of muscle fibres destruction leads to more significant degradation of myofibrillar proteins, as evidenced by the higher values for the proteolytic index and the sensory scores characterizing the consistency of the samples at 18 and 36 months received by the sensory analysis performed (Tab. 3).

Sensory Evaluation of Texture

Most sensory analyses presented in the literature point at consistency and flavour complex as the most important characteristics influencing the overall organoleptic quality of dry-cured ham (Laureati et al., 2014). Changes in the texture profile of the tested samples during aging were also evaluated by the sensory analysis and the results are presented in Table 3.

Table 3. Sensory changes in the texture of the testing sample during aging.

Sample	Hardness	Firmness	Overall texture perception
8 months	$4{,}60\pm0{,}50^{b}$	$4{,}65\pm0{,}35^{b}$	$4{,}10\pm0{,}65^{a}$
18 months	$4{,}50\pm0{,}40^{b}$	$4{,}25\pm0{,}20^{b}$	$4{,}75\pm0{,}45^{b}$
36 months	$3{,}50\pm0{,}50^{a}$	$3{,}90\pm0{,}10^{a}$	$4{,}40\pm0{,}50^{a,b}$

Means within each column having different letters are significantly different at p<0,05

Sensory evaluated hardness and firmness decreased significantly after the 18th month of the extended aging in the natural drying chamber. There were no significant differences between scores obtained by each panel member (p <0.01). As a result of multiple range test, there were significant differences in the sensory scores between tested samples. The significant increases in sensory evaluated tenderness were observed at 36 months of aging as assessors easily detected this textural change. Sensory assessments for the samples at 36 months, compared to the 18-month samples (Table 3), were expressed as a more undesirable solid consistency. The reason for this can be found in the established lower water content in sample of 36 months and respectively reduced water activity. This have a significant influence on the required strength to chew the product, despite deeper proteolytic changes in the muscle tissue of this ham (Andrés et al., 2005; Benedini, Parolari, Toscani, & Virgili, 2012; Ruiz- Ramirez et al., 2006). For example, Ruiz et al. (2002) and Laureati et al (2014) attribute this negative influence to the drier and fibrous structure obtained from both insoluble collagen and myofibrillar proteins aggregation as a result of dehydration during drying and maturation (Córdoba et al. 1994; Chizzolini et al., 1996). However, overall maturation of over 8 months leads to better texture perception as a result of improved sensory tenderness variables, such as softness, chewiness, and rate of breakdown in the mouth.

CONCLUDING REMARKS

As the aging process progressed, there were significant changes not only in the water content and the proteolytic index but also in the morphological and texture profile of the studied traditional dry-cured meat product. These changes were also compared with the data obtained by the textural sensory analysis and the period of ripening and drying of the samples at 18 months was most appreciated by the panellist. The dry-cured traditional meat product "Elena ham" differed from the most popular and offered on the European market raw-dried pork hams. The study on changes during the initial stages of the production of "Elena ham" until the 8th month will allow more detailed and clearer understanding of the ongoing transformations in this traditional dry meat product as well as the overall assessment of its qualitative characteristics as a function of drying and ripening time. Examination of microbiological safety in the course of its technology and antioxidant capacity will allow for the production of better quality and safer traditional meat products and will meet the increasing demands of modern food safety.

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ЕФЕКТ НА ЗГОЛЕМЕНО СТАРЕЕЊЕ НА ТЕКСТУРАЛНИТЕ АСПЕКТИ НА ТРАДИЦИОНАЛНАТА СУШЕНА БУГАРСКА САЛАМА

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Резиме

Целта на ова истражување е да се одреди ефектот од продолженото зреење и сушење на бугарската салама. По 8, 18, и 36 месеци стареење беа дадени параметрите на сушената салама. Профилот на саламата како и физичките, хемиските и морфолошките карактеристики со протеолитичкиот индекс на Ворнер-Брацлер беше истражуван. Продолженото стареење до 36 месеци доведе до зголемена тврдост но со мали сензорни вредности кои карактеризираа преголема мекост. Саламите со подолго зреење и сушење имаат статистички зголемен протеолитички индекс. Морфолошката анализа открива значајна квалитативна и квантитативна разлика помеѓу примероците. Притоа, се зголемија и миофибриларните фрагменти. Тоа доведе до зголемена деградација на целосната миофибрична структура. Силата на волната на Ворнер-Брацлер имала значајна врска со сензорната мекост како ровкост, џвакање и ниво на топење во устата.

Клучни зборови: *сушена салама, зголемено зреење, текстура, морфолошки промени, сила на волна, протеолитички индекс.*