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Differentiation of *Bicep femoris* and *Semimembranosus* muscles of smoked dry-cured ham by quality parameters

Nives Marušić Radovčić[1*], Ana Mikulić[1], Martina Turk[1], Helga Medić[1]

[1]*University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia*

*Corresponding author: nmarusic@pbf.hr

Abstract

*The aim of this work was to determine the effect of fat and protein oxidation on the colour and texture of Biceps femoris (BF) and Semimembranosus (SM) muscles of smoked dry-cured ham Dalmatinski pršut. Fat and protein oxidation was determined by spectrophotometer methods of which fat oxidation by 2-thiobarbituric acid reactive substances method (TBARS) and protein oxidation by 2,4-dinitrophenylhydrazine (DNPH) method. Colour parameters (L*a*b* values) and texture profile analysis (TPA test) were also analysed. There was no statistical significant difference (P>0.05) between BF and SM in fat and protein oxidation. SM is an external muscle and is more exposed to oxygen than internal muscle BF. As a result of that SM showed slightly higher TBARS values than BF. Slightly higher values of carbonyls in BF can be explained by the higher water content in this internal muscle and thus by the stronger proteolytic activity. BF had higher L*, a* and b* values than SM. Higher values of adhesive force, cohesiveness and stringiness and lower values of hardness, adhesiveness, gumminess, chewiness and fracture were found for BF than for SM.*

Keywords: *dry-cured ham; TBARS; carbonyls; texture; colour*

Introduction

Dalmatinski pršut is an autochthonous top quality product produced in southern part of Croatia–Dalmatia. It is labelled with Protected Geographical Indications (PGI), based on EU legislation and is processed with pelvic bones, skin and subcutaneous fatty tissue (Petričević et al, 2018). *Dalmatinski pršut* is prepared according to the traditional processing procedures without any additives such as nitrites or ascorbic acid. Production process involves following stages: dry salting with sea salt, pressing, smoking, drying and ripening. The ripening period (before consumption) is minimum 12 months. During last decades, there were studies that have examined the effects of production process on physico-chemical, aromatic and sensorial traits of dry-cured hams in different types of dry-cured ham: Italian Parma, San Daniele (Laureati et al, 2014) and Toscano (Pugliese et al, 2009), Spanish Iberian (Andrés et al, 2004) and Celta ham (Bermúdez et al, 2014), French Bayonne (Monin et al, 1997), Slovenian *Kraški pršut* (Pugliese et al, 2015) and Croatian types of dry-cured hams (Petričević et al, 2018). Studies reported that production process has significant effect on biochemical and textural changes, but with different intensity in different muscles. The anatomic location of muscles inside the dry-cured ham, whether external *Semimembranosus* (SM), or internal *Biceps femoris* (BF) are not subjected to the same conditions during processing and have different time course of proteolysis (Harkouss, 2015). SM is exposed to faster dehydration and salt uptake in the early processing stages than BF. BF has higher water content throughout the process and has higher proteolytic activity than SM. Proteolysis also impacts the final texture (hardness, cohesiveness) of the product as well as aromatic profile (Pugilese et al, 2015) and it is considered to be an important parameter for obtaining good sensory characteristics of the final product. So, the aim of this work was to determine the effect of fat and protein oxidation on the colour and texture of BF and SM muscles of smoked dry-cured ham *Dalmatinski pršut*.

Materials and methods

Dry-cured ham samples

Samples of BF and SM of smoked dry-cured ham *Dalmatinski pršut* were obtained from 3 different manufacturers at local store (N=9). Dry-cured hams were produced according to their PGI specification and were ripened for 18 months. Samples from each manufacturer were analyzed for fat and protein oxidation and texture and colour parameters.

TBARS test

To measure lipid oxidation, the 2- thiobarbituric acid reactive substances method (TBARS method) described by Bruna et al (2001) with slight modifications was carried out. For that, 5 g of dry-cured ham samples were homogenised in 15 mL of 0.38 M $HClO_4$ for 3 min in an ice bath. To avoid further oxidation 0.5 mL of a 0.19 M butylated hydroxytoluene (BHT) ethanolic solution was added. The homogenate was centrifuged (3000 rpm, 5 min, 5°C) and filtered through Whatman No. 54. An aliquot of 0.7 mL was mixed with 0.7 mL of a 0.02 M TBA solution and heated at 100°C for 30 min. After cooling, the mixture was centrifuged at 3000 g for 15 min at 5°C. Finally, the absorbance was measured at 532 nm. Results were expressed as mg malonaldehyde (MDA)/kg sample.

DNPH method

Protein oxidation, as measured by the total carbonyl content, was evaluated by derivatisation with 2,4-dini-

trophenylhydrazine (DNPH) according to the method described by Armenteros et al (2009). Dry-cured ham samples were minced and then homogenized 1:10 (w/v) in pyrophosphate buffer (pH 7.4) (PB) consisting of 2.0 mM $Na_4P_2O_7$, 10 mM tris–maleate, 100 mM KCl, 2.0 mM $MgCl_2$ and 2.0 mM ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) using an ultraturrax homogenizer for 30 s. The homogenates were divided in two equal aliquots of 0.1 mL. Then, proteins were precipitated in both aliquots by adding 1 mL of 10% TCA and centrifuged for 5 min at 5000 rpm. Finally, the supernatants were removed and one pellet was treated with 1 mL 2 N HCl (for quantifying protein concentration) and the other one with an equal volume of 0.2% (w/v) DNPH in 2 N HCl (for carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature (shaken every 15 min). After that, samples were precipitated with 1 mL of 10% trichloroacetic acid (TCA) and washed twice with 1 mL of 1:1 ethanol/ethyl acetate (v/v), shaken, and centrifuged for 5 min at 10 000 rpm. The pellets were then dissolved in 1.5 mL of 20 mM sodium phosphate buffer pH 6.5 containing 6 M guanidine hydrochloride, stirred and centrifuged for 2 min at 5000 rpm to remove insoluble fragments. Protein concentration was calculated from absorption at 280 nm using bovine serum albumin (BSA) as standard. The amount of carbonyls was measured at 370 nm and expressed as nmol of carbonyl per mg of protein using the adsorption coefficient for the protein hydrazones (21.0 $mM^{-1}\,cm^{-1}$).

Colour instrumental measurement

Colour measurements were carried out with a Minolta CM-700d (Osaka, Japan) spectrophotometer (illuminant D65, 10° standard observer, 8 mm aperture, with open cone). The L* (lightness), a* (redness), and b* (yellowness) colour was measured (CIE, Commission Internationale de l'Eclairage, 1976). Each sample was analysed in ten replicates, avoiding regions with excess fat to achieve representative measurements of the lean colour.

Texture profile analysis (TPA) test

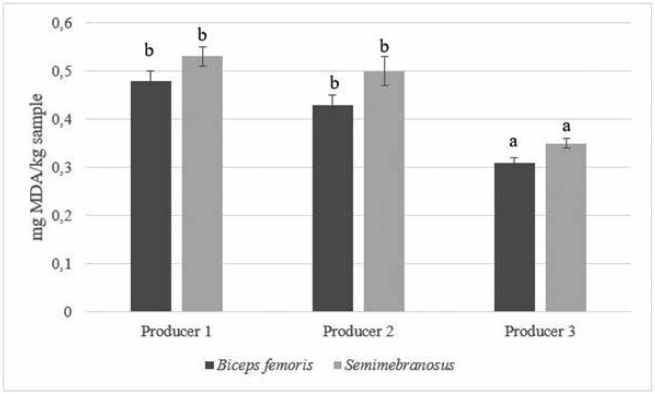
Texture measurements in the form of texture profile analysis (TPA; Bourne, 1978) of the dry-cured ham were performed at room temperature using a texture analyser (Ametek Lloyd Instruments Ltd., UK) with a 50 kg load cell supported by the software NexygenPlus. Duplicate cubes 10 x 10 x 10 mm were analysed. Samples were conditioned at 20 °C for 2 h before analysis. The sample was compressed twice to 50% deformation at a crosshead speed of 1 mm/s (resting time between cycles was 5 s), and the following parameters were obtained from force–distance curves: hardness (N), adhesive force (N), cohesiveness, adhesiveness (Nmm), gumminess (N), springiness (mm), chewiness (Nmm), resilience, fracture (N) and stringiness (mm).

Data analysis

Statistical analysis was carried out using analysis of variance (ANOVA) of Statistica V.10 software (Statsoft Inc.,Tulsa, USA). Tukey's HSD test was used as comparison test when samples were significantly different after ANOVA ($P<0.05$).

Results and discussion

Lipid oxidation is one of the cause of deterioration of meat and meat products. Lipid oxidation effects fatty acids, especially polyunsaturated fatty acids which results in forming various products that change the quality of the final product (changes in color, aroma, taste, texture, and even nutritional value). Primary products of autoxidation are hydroperoxides. Their degradation generates secondary products such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (MDA). 2-thiobarbituric acid reactive substances method (TBARS test) is used to detect oxidation of unsaturated fatty acids and fats. It depends on the development of red pigment that is generated by TBA reaction with MDA. Results of TBARS test are shown in Figure 1. A statistically significant difference ($P<0.05$) of TBARS values was found between different producers of smoked *Dalmatinski pršut*, but type of the muscle (BF and SM) did not show statistical significant differences ($P>0.05$). TBARS in BF had values 0.31-0.48 mg MDA/kg of sample, while in SM ranged from 0.35-0.53 mg MDA/kg of sample. SM had slightly higher values than BF and this can be a result of more exposure of SM to oxygen and therefore slightly higher fat oxidation. Obtained values were in accordance with the authors in other types of dry-cured hams (Andrés et al, 2004; Cilla et al, 2006; Fuentes et al, 2014; Marušić Radovčić et al, 2016).



*Different letters (a,b) mean statistical significant difference (P<0.05)

Figure 1. *Lipid oxidation in samples of Dalmatinski pršut from three different producers*

As a major component of muscle tissue, proteins play a decisive role in meat products regarding sensory, nutritional and technological aspects (Lawrie, 1998). Protein oxidation causes physico-chemical changes in proteins including amino acid destruction, decreases in protein solubility due to protein polymerization, loss of enzyme activity and impaired protein digestibility. The DNPH method is a routine procedure that enables the quantification of the total amount of carbonyls from a protein sample. The results are widely used as a general index of protein oxidation in meat and meat products (Estevez, 2011). The method is based on the reaction between the DNPH with protein carbonyl compounds to form a 2,4-dinitrophenyl (DNP) hydrazone product which displays a maximum absorbance peak at around 370 nm. The concentration of DNP hydrazones is calculated by measuring reacted DNPH spectrophotometrically on the basis of an absorption of 22,000 $M^{-1}\,cm^{-1}$ at 370 nm while concentration of protein is deter-

mined in at 280 nm using BSA as standard. Protein oxidation is also noticed in dry-cured ham which have long process of production. Differences in physico-chemical characteristics such as water and salt contents between SM and BF muscles could affect protein oxidation at quantitative level so a more intense proteolysis would be expected in the internal muscle, BF (Gallego et al., 2018). Also in dry-cured hams, a higher concentration of carbonyl was determined then in fresh hams due to longer and more intensive drying and ripening period (Ventanas et al, 2007). The concentration of carbonyl in BF ranged from 10.57-16.84 nmol carbonyl/mg of protein, while in SM ranged from 9.31-16.47 nmol carbonyl/mg protein (Figure 2). Based on the obtained values, there was no statistically significant difference (P>0.05) of protein oxidation between these two muscles however slightly higher values of carbonyls were detected in BF. Also, higher protein oxidation was in BF than in SM muscle in Bayonne dry-cured hams (Harkouss et al., 2015). Toldra et al. (1997) and Ruiz-Ramirez et al. (2006) found similar findings, with higher protein oxidation in BF than in SM muscle. These authors indicated that this difference could be explained by a higher residual moisture content in BF muscle, which permits a higher enzymatic activity of the endogenous proteases (Harkouss et al., 2015). In a study by Cava et al (2009) in Spanish Iberian dry-cured ham, in BF, the carbonyl concentration ranged from 6.8 to 10.9 nmol carbonyl/mg of protein, which is lower than the carbonyl concentration obtained in this work. Similar results were obtained from Ventanas et al (2007) also in Iberian dry-cured ham, where the carbonyl concentration in BF ranged from 6.84 to 8.87 nmol carbonyl/mg protein. This difference is probably result of different process of production. Lower values of carbonyl content in Iberian dry-cured ham is probably due to addition of nitrates and nitrites in production process which can have antioxidant role.

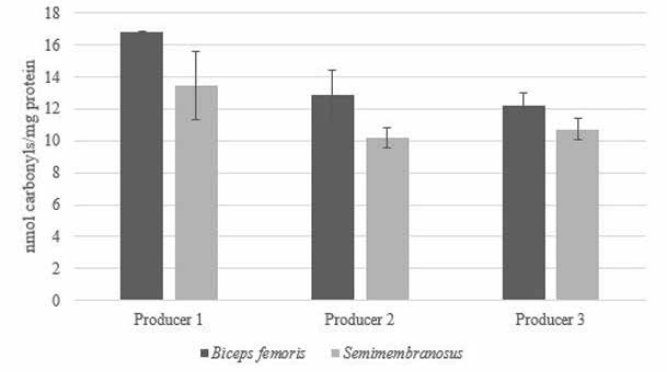


Figure 2. *Protein oxidation in samples of Dalmatinski pršut from three different producers*

The dry-cured ham colour depends mainly on features of natural meat pigments and muscle structure (Pérez-Alvarez et al, 1998). Colour parameters (L* a* b* values) of BF and SM muscles of *Dalmatinski pršut* from three producers are shown in table 1. L* values in BF ranged from 50.39-51.40 while SM had lower values (47.06-47.36). Differences in L* value between the two muscles, are related to the water content, pH, dehydration to the surface and the muscle structure itself (García-Esteban et al, 2003). SM is an external muscle and is subject to surface dehydration, while BF is covered with skin and subcutaneous fatty tissue. SM, as an external muscle, is the only one that comes into direct contact with the salt and it regulates the diffusion of the salt towards the BF (internal muscle). Then the water comes out of the BF and moves towards the surface (Pérez-Alvarez et al, 1998). L* values in BF of *Dalmatinski pršut* (50.39-51.40) showed higher values than BF in Spanish (34.8-38.8) (Pérez-Alvarez et al, 1998) and Italian (37.9-38.0) (Laureati et al, 2014) dry-cured hams. L* values in SM muscle was 47.06-47.36 which is higher than values obtained in Spanish Serrano (31.16-38.17) (García-Esteban et al, 2003) and Teruel (31.75) (Cilla et al, 2006) dry-cured ham. A statistically significant difference (P<0.05) of L* values was found between different producers of smoked *Dalmatinski pršut* only in BF and between both BF and SM muscle. a* values in BF in this research ranged from 4.37 to 5.46, which was lower than a* values determined in Spanish types of dry-cured ham (16.6-18.9) (Marušić Radovčić et al, 2016). Spanish dry-cured ham had intense red colour (higher a* values) than *Dalmatinski pršut* because of addition of nitrates and nitrites in these types of dry-cured hams. The use of nitrites seems to improve the colour: higher intensity of the red colour and brightness in the lean and lower dryness of the final product (Toldrá et al 2009). Similar was for a* values (2.99-3.99) obtained in SM muscle while b* values for *Dalmatinski pršut* were 4.35-5.72 in BF and 2.72-4.14 in SM which was similar to the values reported for Spanish and Italian dry-cured hams. A statistically significant difference (P<0.05) of a* and b* values was found between different producers as well as different type of the muscle. BF had higher a* and b* values compared to SM.

Table 1. *Colour parameters (L*a*b* values) in samples of Dalmatinski pršut*

Colour parameter	Producer 1	Producer 2	Producer 3
Biceps femoris			
L*	50.87 ± 0.22^{abA}	50.39 ± 0.37^{aA}	51.40 ± 0.18^{bA}
a*	5.46 ± 0.16^{bA}	5.40 ± 0.28^{bA}	4.37 ± 0.22^{aA}
b*	5.72 ± 0.26^{bA}	5.02 ± 0.34^{abA}	4.35 ± 0.16^{aA}
Semimembranosus			
L*	47.32 ± 0.16^{B}	47.16 ± 0.10^{B}	47.06 ± 0.18^{B}
a*	3.99 ± 0.17^{bB}	3.29 ± 0.14^{aB}	2.99 ± 0.10^{aB}
b*	4.14 ± 0.15^{cB}	3.18 ± 0.07^{bB}	2.72 ± 0.09^{aB}

* Different letters (a, b) in the same row are assigned for statistical difference between producers at P<0.05, while different letters (A, B) are assigned for statistical difference between muscles, P<0.05 (along the columns).

The TPA parameters of the BF and SM muscles of *Dalmatinski pršut* are shown in Table 2. A statistically significant difference (P<0.05) between different producers of smoked *Dalmatinski pršut* was found in BF for adhesive force and adhesiveness while in SM in all parameters except springiness. However, type of the muscle showed significant difference (P<0.05) in hardness, gumminess, chewiness and fracture which can be explained by the different water content in SM and BF. BF is muscle with higher water content so a harder texture (hardness) is expected in the SM muscle due to the lower water content. Also, BF and SM are subjected to different conditions during the dry-cured ham production. SM as an external muscle that has a high NaCl content in the first stages of production and rapidly reduces the water content while the inner muscle BF with lower NaCl content during the first stages of the process and with a higher water content during the process. This implies greater activity of proteolysis in BF muscle, which affects the texture. In contrast, in the SM muscle, a smaller amount of water can be reached on the surface, affecting the hardness of the product (Morales et al, 2007). Concussively, hardness in BF in *Dalmatinski pršut* was lower (27.42-42.82 N) than in SM (68.57-86.14 N).

Table 2. *Texture parameters in samples of Dalmatinski pršut*

Texture parameter	Producer 1	Producer 2	Producer 3
Biceps femoris			
Hardness (N)	42.82 ± 7.48^{A}	32.93 ± 3.69^{A}	27.42 ± 2.14^{A}
Adhesive Force (N)	-0.87 ± 0.12^{a}	-0.77 ± 0.13^{ab}	-0.48 ± 0.05^{b}
Cohesiveness	0.54 ± 0.02	0.54 ± 0.02	0.53 ± 0.01
Adhesiveness (Nmm)	0.53 ± 0.05^{ab}	0.70 ± 0.10^{b}	0.33 ± 0.05^{a}
Gumminess (N)	23.56 ± 4.56^{A}	16.57 ± 2.93^{A}	14.59 ± 1.27^{A}
Springiness (mm)	-2.52 ± 0.14	-2.30 ± 0.22	-2.30 ± 0.12
Chewiness (Nmm)	20.83 ± 1.20^{A}	37.30 ± 8.59^{A}	33.88 ± 3.70^{A}
Resilience	0.42 ± 0.02	0.48 ± 0.03	0.44 ± 0.02
Fracture (N)	23.56 ± 7.40^{A}	35.46 ± 1.82^{A}	20.87 ± 3.61^{A}
Stringiness (mm)	4.71 ± 0.87	5.14 ± 0.80	3.93 ± 0.96
Semimembranosus			
Hardness (N)	86.14 ± 16.86^{bB}	64.58 ± 8.55^{bB}	68.57 ± 1.74^{aB}
Adhesive Force (N)	-1.39 ± 0.39^{a}	-1.00 ± 0.15^{ab}	-0.49 ± 0.07^{b}
Cohesiveness	0.47 ± 0.02^{a}	0.48 ± 0.03^{a}	0.56 ± 0.01^{b}
Adhesiveness (Nmm)	0.69 ± 0.08^{b}	0.38 ± 0.08^{a}	0.65 ± 0.10^{ab}
Gumminess (N)	41.98 ± 9.39^{bB}	29.81 ± 2.59^{abB}	16.17 ± 1.29^{aB}
Springiness (mm)	-2.78 ± 0.22	-2.53 ± 0.23	-2.34 ± 0.16
Chewiness (Nmm)	95.96 ± 26.32^{bB}	69.50 ± 3.13^{abB}	39.39 ± 4.55^{aB}
Resilience	0.35 ± 0.03^{a}	0.37 ± 0.04^{ab}	0.47 ± 0.03^{b}
Fracture (N)	72.53 ± 21.41^{bB}	44.27 ± 2.53^{abB}	21.31 ± 2.67^{aB}
Stringiness (mm)	2.44 ± 0.30^{a}	4.58 ± 0.92^{ab}	5.35 ± 0.94^{b}

* Different letters (a, b) in the same row are assigned for statistical difference between producers at P<0.05, while different letters (A, B) are assigned for statistical difference between muscles, P<0.05 (along the columns).

Hardness showed statistically significant difference (P<0.05) between two muscles. In BF hardness was in the range from 27.42-42.82 N. Similar values were obtained in Spanish Teruel (21.39 N) (Cilla et al, 2006) and Serrano (21.36-26.17 N) dry-cured ham (García-Esteban et al, 2004) while higher values (52,51 and 62 N) were obtained in Slovenian *Kraški pršut* (Pugliese et al, 2015, Andronikov et al, 2013). Samples of *Dalmatinski pršut* had higher values in SM muscle (28.57-86.14 N) than BF. Similar values had samples of Spanish dry-cured hams (84.48 N) (Cilla et al, 2006) while higher hardness had samples of Slovenian *Kraški pršut* (107.22 and 122 N) (Pugliese et al, 2015, Andronikov et al, 2013). Different type of the muscle did not show statistically significant difference (P>0.05) for adhesive force, cohesiveness and adhesiveness and were in the same range as other Mediterranean types of dry-cured ham. Type of the muscle also effected gumminess and chewiness (P<0.05). Gumminess in BF was in the range from 14.59-23.56 N while in SM 16.17-41.98 N while chewiness 20.83-37.30 Nmm (BF) and 20.83-37.30 Nmm (SM).

Generally speaking, a greater role of water content is observed in SM muscle than in BF muscle. This may be because SM muscle dries rapidly, quantifying many structural and textural parameters of this superficial ham muscle and requires taking into account water content. Also, the intense salt diffusion and water evaporation that occur in the SM muscle compared with the BF muscle, especially during the first stages of the process, leads to these differences between the two muscles, and showing again the importance of the geometrical location of the muscles (Harkouss et al., 2015).

Conclusions

There was no statistical significant difference (P>0.05) between BF and SM in fat and protein oxidation. SM is an external muscle and is more exposed to oxygen than internal muscle BF. As a result of that SM showed slightly higher TBARS values than BF. Slightly higher values of carbonyls in BF can be explained by the higher water content in this internal muscle and thus by the stronger proteolytic activity. BF had higher L*, a* and b* values than SM which is a result of different location of the muscle. In accordance with that higher values of adhesive force, cohesiveness and stringiness and lower values of hardness, adhesiveness, gumminess, chewiness and fracture were found for BF than for SM.

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