A study of chemical profiles and appearance of white crystals in Istrian dry-cured ham: effect of desalting

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ABSTRACT

In order to find out the effect on physical-chemical profile of Istrian dry-cured ham with particular regard to the appearance of white crystals in the muscles, raw salted legs were desalted by soaking the legs in cold water for 24 hours. The 20 raw hams taken from 10 slaughtered hogs (Swedish Landrace breed) and processed in the traditional Istrian manner were used. After salting and pressing, the raw hams were divided into two groups: 1) the 10 left legs of each hog were desalted (D); 2) the 10 right legs were not desalted (N). Following this, both groups of legs were subjected to continuous processing. Samples for chemical analysis and counting of white spots were taken from the 20 legs (10 N and 10 D) from 10 hogs, each weighing 12 to 15 kg. Chemical analysis of muscle tissue showed a highly significant difference (P<0.0001) in the salt quantity in the N (6.85%) and D (5.31%) dry-cured hams, as expected. Desalting affected the level of calcium which was higher (P=0.0124) in the D hams (0.27 g) than in the N hams (0.22 g). Desalting did not affect the free amino acid content, with the exception of methionine which was lower (P=0.0041) in D (0.14 g) than in N hams (0.17 g). Desalting affected the level of two free fatty acids as follows: heptadecanoic acid was higher (P=0.0203) in N (0.18%) than in D hams (0.24%) and DPA was higher (P=0.0373) in N (0.49%) than in D hams (0.39%). By counting the white precipitates, it was established that the regularity of appearance of the precipitate was noted on both the D and N hams, such that where there was no precipitate on the right N ham, nor was their any on the left D ham of the same hog. However, desalting only lead to a slight decrease in the appearance of precipitates (average of 0.7 points), but it is certain that desalting reduces the salt content in the legs, which affects some physical-chemical changes in the ham tissues during processing.

Key words: Istrian dry-cured ham, Desalting, White spots, Chemical properties.
RIASSUNTO

STUDIO DEL PROFILO CHIMICO E DELLA COMPARSA DI CRISTALLI BIANCHI NEL PROSCIUTTO ISTRIANO: EFFETTI DELLA DESALATURA

Al fine di trovare gli effetti della desalatura sul profilo fisico chimico nel prosciutto istriano, con particolare attenzione alla presenza di cristalli bianchi nei muscoli, le cosce fresche salate sono state desalate attraverso immersione in acqua fredda per 24 ore. Sono stati usati 20 prosciutti provenienti dalla macellazione di 10 suini (Swedish Landrace) lavorati con metodologia tradizionale istriana. Dopo salatura e pressatura le cosce sono state suddivise in due gruppi: 1) le 10 cosce sinistre di ogni suino sono state desalate (D); 2) le 10 cosce destre non sono state desalate (N). In seguito le cosce di entrambi i gruppi sono state avviate alla lavorazione. Sono stati effettuati analisi chimiche e conteggio delle macchie bianche nei campioni prelevati dalle cosce, con peso compreso tra i 12 e i 15 Kg, provenienti dai 10 suini esaminati (10 N e 10 D).

Le analisi chimiche sui tessuti muscolari hanno evidenziato differenze altamente significative ($P<0,0001$) nella quantità di sale presente nei prosciutti N (6,85%) e D (5,31%), come atteso. La desalatura ha influenzato la concentrazione di calcio, maggiore ($P=0,0124$) nei prosciutti del gruppo D (0,27 g) rispetto a quelli del gruppo N (0,22 g), mentre non ha modificato il contenuto di aminocidi liberi con eccezione della metionina, il cui valore è stato minore ($P=0,0041$) nel gruppo D (0,14 g) rispetto al gruppo N (0,17 g). La desalatura ha influenzato il livello di due acidi grassi liberi: la concentrazione di acido eptadecanico è stata maggiore ($P=0,0203$) nel gruppo N (0,18%) rispetto al gruppo D (0,24%) e il contenuto di DPA ha evidenziato valori maggiori ($P=0,0373$) nel gruppo N (0,49%) rispetto al gruppo D (0,39%). La comparsa di precipitati bianchi è stata rilevata su entrambi i gruppi; la loro mancata presenza, quando registrata, ha interessato entrambe le cosce dello stesso suino. Tuttavia la desalatura ha apportato una leggera diminuzione nella comparsa di precipitati (0,7 punti in media) e una riduzione di sale nelle cosce, con conseguente modificazione delle caratteristiche fisico chimiche dei prosciutti stagionati.

Parole chiave: Prosciutto istriano, Desalatura, Macchie bianche, Proprietà chimiche.

Introduction

The basic characteristics of dry-cured ham production, regardless of the type, is salting the specifically processed hog leg at low temperatures (0 to 5°C) and drying and ripening at higher temperatures (12 to 18°C) for a specific period of time. Salting significantly affects the dry-cured ham quality due to the direct effect on the formation of taste as well as the conservation process. According to Flores et al. (1985), the salt concentration in various types of dry-cured hams ranges from 4 to 9%, depending on the type and size of dry-cured ham, the quantity and granulation of the added salt, thickness of fatty tissue and processing. The flavour and aroma of dry-cured ham are directly related to proteolysis and lipolysis. According to Martín et al. (1998a, 1998b), a higher salt concentration and low water activity values inhibit proteolysis. Therefore, salt levels significantly affect the level and types of compounds which arise during proteolysis in the processing of Iberian ham (Andrés et al., 2005). Precipitations of white crystals randomly distributed within muscle tissues of the ham are connected with protein breakdown, and it is presumed that the precipitates are the result of excessive proteolysis (Toldrá, 2002). The intensity of the precipitations depends on the concentration and solubility of tyrosine and other amino acids, and activity of the cathepsins and calpains (Arnau et al., 1996). White crystals are frequently found in frozen/thawed hams that are aged for a long period of time, where structural changes can affect nucleation and crystal growth (Bañón et al., 1999). Dense distribution of tiny tyrosine crystals
(0.5-2 mm) is particularly found in hams with high salt concentration (11%), sparse and larger crystals (5-5 mm) are found more often in hams with a lower salt concentration (6-9%), (Silla et al., 1985). According to Arnau et al. (1994), the incidence of hams with tyrosine crystals and white film was lower in those with high pH of the meat. All of these studies were conducted by using the lower salt percentage in the salting treatment. Instead, Krvavica and Đugum (2007) used the desalting treatment of the salted hams, but did not establish statistical differences in the effect of desalting on ham weight loss and pH of matured ham.

Material and methods

The desalting process contributes to decreasing salt quantity and a more uniform distribution of salt in the dry-cured ham by removing excess salt from the surface layers of the ham. As such, the objective of this study was to determine the extent to which salt concentration levels in the dry-cured ham can influence the quality of Istrian dry-cured ham and the most important chemical parameters, with a particular emphasis on the frequency of white precipitates in muscle tissue.

According to the objectives, 10 Swedish Landrace breed hogs were fattened in extended fattening to a final body mass of 190-200 kg. Then, the hogs were slaughtered, cut up, cooled and the raw hams trimmed. In order to avoid the PSE/RSE/RFN hams, only those hams with a pH1 ≥6.0 (45 minutes after slaughtering) and pH24 <6.0 (24 hours after slaughtering) were taken. Legs were trimmed in the traditional Istrian manner, medially and laterally without rind and the subcutaneous adipose tissue, with the aitch bones and without the foot. The weights of trimmed raw hams were 12 to 15 kg with an average weight of 13.38 kg.

Dry salting of hams was conducted using a mixture of course and fine sea salt in a ratio of 70:30, with an addition of the following herbs: 3% ground black pepper, 1% powdered garlic and 0.5% powdered bay leaf. Each ham was individually salted by hand, with a strong rubbing motion of the salt into all surfaces of the ham in all cavities and cuts, the open area of the cut in the tarsal joint and particularly in the area of the femur knob. After 7 days, the hams were re-salted, particularly in the area of the femur knob and left for another 7 days, after which time they were pressed. The mean consumption of salt totalled 6.5-7% of the leg weight. Salting was conducted in cooling chambers at a temperature of 0-5°C and a relative humidity of 80-90%, for a period of 21 days, including the pressing for 7 last days.

Following the mechanical removal of excess salt from the surface of the hams, half the hams (10 left hams of each hog) were desalted (D) by being soaked in pure, cold water for 24 hours. After 12 hours, the water was replaced. On the second day, the hams were removed from the water to drain and the surfaces dried. Prior to drying, all hams (both desalted-D and non-desalted-N) were sprinkled with a mixture of herbs in the following composition: 92.5% ground black pepper, 5.55% ground dried garlic and 1.95% ground bay leaf.

After desalting, all hams (N and D) were subjected to the same processing procedures: Drying in drying chambers with controlled microclimatic conditions (air circulation-10-20 cm/sec; temperature-12–16°C; humidity was gradually reduced from 90 to 70%) lasting 158 days; Ripening of hams takes place in cellars with a stable microclimate and the possibility for complete darkness, and an air temperature which does not exceed 18°C in summer (between 12 and 18°C year round) and relative air humidity between 65 and 75% until they become 14 months old.
Sampling for analysis

Sampling for chemical analysis and white precipitates counting was conducted on the 20 hams taken from 10 hogs, divided into 2 groups (N and D) of 10 hams each.

The surfaces of the dry-cured hams were thoroughly cleaned, particularly the caudal side which was removed from the remainder of the dry-cured ham by section. The section was made by a longitudinal cross-section of the muscle on the caudal side of the leg, from the *tuber ischiaticum* to the *tuber calcanei*, and the following muscles were cross-sectioned: *m. semimembranosus, m. semitendinosus, m. gracilis, m. biceps femoris*, and the flexors *m. flexor digitalis pedis superficialis* and *m. flexor digitalis pedis profundus*. On both sides of the longitudinal cross-section, white precipitates were counted. Another parallel longitudinal cut was made on this removed section of dry-cured ham to obtain a sample weight of approximately 200 g, which was homogenized by grounding. Prior to grinding, all visible fat was removed. These samples were used for the chemical analysis.

Chemical analysis of samples

Moisture content and dry matter were determined by drying the homogenized sample at a temperature of 105°C to a constant mass, and the loss of mass was expressed as a percentage of moisture in the sample. The total protein content was determined using the Kjeldahl method, using a Kjel-Foss 16200 nitrogen analyser. Intramuscular fat content was determined using the Soldt method (Hungarian Standard No. 6830-66). The NaCl content was determined by titrimetrical method (James, 1999). Cholesterol and fatty acid content was determined using gas chromatography HPLC (Chrompack CP 9000, column 300 x 0.25 mm, quartz capillaries, active aquatic phase Cp-Sil-88) according to Csapó et al. (1986, 2000). Ash content was determined by combusting organic matter and burning the remains at a temperature of 550°C to a constant mass, while the remainder of sample mass was expressed as percentage of ash. Mineral content (micro and macro elements) was determined using an atomic absorptive spectrophotometer. Amino acid content was determined according to Csapó et al. (1986), in an automatic amino acid analyser (LKB Model 4101 automatic AA analyser) and HPLC of the previously hydrolyzed proteins using the hyrolizing Pyrex tube (Pierce Chemical Company, Rockford, IL, USA). Proving the presence of amino acid tyrosine in the white precipitates in muscle tissue of dry-cured ham was carrying out using the xanthoprotein reaction (treatment with HNO₃ and NaOH).

Statistical analysis

Statistical data analysis was conducted by using the SAS software (SAS, 1999). The chemical composition of dry-cured ham (basic chemical composition, composition of macro and micro elements, amino acids and fatty acids) was assessed using the model of ANOVA with the weight of raw hams as the covariable and random effect of animal, as follows:

\[ Y_{ij} = \mu + T_i + bx_{ij} + a_{ijk} + e_{ijk} \]

where:
- \(T_i\) – effect of treatments (i=1, 2)
- \(b\) – regression coefficient
- \(X_{ij}\) – weight of raw hams centered to zero mean
- \(a_{ijk}\) – random effect of animal
- \(e_{ijk}\) – error

The intensity and frequency of appearance of white precipitates was established using the method of comparison of two samples (Table 2) (Savić et al., 1969). This method consists of scoring the muscle cross-section in terms of the abundance of present
precipitates as follows: 1 point = more than 8 precipitates; 2 points = 4 to 8 precipitates; 3 points = 2 to 4 precipitates; 4 points = 1 to 2 precipitates and 5 points = no precipitates.

**Results and discussion**

*Chemical and physical characteristics of Istrian dry-cured ham*

The results of this study show that the technological process of desalting did not significantly influence the basic chemical composition of the dry-cured ham. A comparison of average salt content in non-desalted (6.85%) and desalted (5.31%) dry-cured hams showed a strongly significant difference ($P<0.0001$), as expected. Dry matter content as shown in Table 1 is similar in both desalted and non-desalted dry-cured hams and the difference in protein, fat and ash content are also very small. Higher dry matter content in Istrian dry-cured ham in comparison to other types of dry-cured ham (Eakes et al., 1975; León-Crespo et al., 1986; Baldini et al., 1992; Toldrá et al., 1997; Toldrá, 2002) are noticeable which could be explained by the specific processing of the raw ham, without rind and subcutaneous fat, as the ham surface is thus exposed to ambient conditions during processing, which accelerates dehydration.

The results of the counting of white precipitates in the dry-cured ham longitudinal cross-section are shown in the Table 2. The regularity of the appearance of precipitates on cross-sections was not constant. Occasionally, precipitates were present on cross-sections of all muscles or appeared without any order or only on individual muscles. However, regularity was noted in the appearance and non-appearance of precipitates on the left and right dry-cured ham, such that where there were no precipitates on the right non-desalted ham, nor were there any on the left desalted ham of the same hog. The desalting slightly (average of 0.7 points) reduced the number of precipitates on cross-sections of the studied muscles.

Statistical analysis of the macro and microelements content (Table 3) showed a statistically significant difference ($P=0.0124$) in the content of the macroelement calcium in desalted (0.27 g/kg) and non-desalted hams (0.22 g/kg).

*Free amino acids in dry-cured ham muscle tissue*

The most represented free amino acids in the experimental dry-cured hams are shown in Table 4, expressed in g/100 g of the sample. The total free amino acids content of the non-desalted hams was in average of 5.02 g/100g of the sample, whereas these were 4.72 g/100g in the desalted hams. In the majority of cases, the non-desalted hams had a higher individual amino acid content.

| Table 1. Basic chemical composition and salt content of Istrian dry-cured ham (LSM±SE). |
|---------------------------------|------|------|------|------|
| Chemical composition (%)        | Non-desalted | Desalted | SE   | P<   |
| Dry matter                      | 65.61 | 65.61 | 0.33 | 0.9926 |
| Proteins                        | 39.44 | 39.73 | 0.45 | 0.6817 |
| Lipids                          | 19.42 | 19.31 | 0.25 | 0.7982 |
| Ash                             | 8.11  | 8.18  | 0.09 | 0.6644 |
| Salt                            | 6.85  | 5.31  | 0.06 | ***0.0001 |

***: $P<0.001$. 

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Table 2. Number and frequency of appearance of white spots.

| Sample number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---------------|---|---|---|---|---|---|---|---|---|----|
| Number of white spots | Non-desalted | 7 | 0 | 4 | 2 | 6 | 0 | 8 | 0 | 2 |
| Desalted | 2 | 0 | 2 | 0 | 4 | 0 | 3 | 0 | 0 | 5 |
| Points | Non-desalted | 2 | 5 | 3 | 4 | 2 | 5 | 2 | 5 | 4 |
| Desalted | 4 | 5 | 4 | 5 | 3 | 5 | 3 | 5 | 5 | 2 |

*Bold: same number of white spots.*

| Treatment | Frequency of white spots (%) |
|-----------|-----------------------------|
|           | 0  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
| Non-desalted | 30 | 20 | -  | 10 | -  | 10 | 20 | 10 |
| Desalted | 50 | 20 | 10 | 10 | 10 | 10 | -  | -  |

Table 3. Macro- and microelements content of the Istrian dry-cured ham (LSM±SE).

| Minerals | Non-desalted | Desalted | SE  | P< |
|----------|--------------|----------|-----|----|
| Macroelements: | | | | |
| Ca       | g/kg | 0.22 | 0.27 | 0.01 | *0.0124 |
| P        | "    | 3.26 | 3.25 | 0.07 | 0.9366 |
| Mg       | "    | 0.41 | 0.41 | 0.01 | 0.4348 |
| Na       | "    | 25.96| 26.59| 0.29 | 0.1997 |
| K        | "    | 6.16 | 6.16 | 0.06 | 0.9861 |
| Microelements: | | | | |
| Mn       | mg/kg | 1.95 | 1.75 | 0.08 | 0.1353 |
| Cu       | "    | 4.09 | 3.94 | 0.09 | 0.3139 |
| Zn       | "    | 46.39| 44.90| 0.56 | 0.1295 |
| Fe       | "    | 22.38| 22.17| 0.58 | 0.8313 |

*:P<0.05.

than the desalted hams; however the differences in the arithmetic means were not statistically significant, with the exception of methionine which was lower (P=0.0041) in D (0.14 g) than in N hams (0.17 g). It is known that salt strongly influences the activity of all muscular enzymes, regardless of whether it stimulates or inhibits its activity (Toldrá, 2002), and so it could be expected that salt concentrations in dry-cured ham also influenced the creation of free amino acids during processing. Especially at high...
concentrations, salt shows an extremely inhibitory effect on cathepsins and aminopeptidases, with the exception of aminopeptidase B. Accordingly, resalting in the salting stage is a simple way of repressing possible high cathepsin activity in the hams, which could lead to excessive softness (Parolari et al., 1994; Toldrá, 2002). Salt also inhibits the action of neutral lipase, neutral and acid esterase whereas it stimulates the action of acid lipase. Arginil aminopeptidase (aminopeptidase B) is chloride-active enzyme and its activity increases to a concentration of 0.35 M NaCl. Similarly, m-calpain is also active at a concentration of 0.50 M NaCl, though a higher salt concentration inhibits its activity (Toldrá, 2002).

Free fatty acids and cholesterol in dry-cured ham muscle tissue

Free fatty acids expressed as methyl ester fatty acids (%) of muscle tissue in the experimental dry-cured hams are shown in Table 5. A high presence of cholesterol was established (N: 85.66 mg/100g and D: 85.37 mg/100g) in dry-cured ham muscle tissue, as well as oleinic (N: 41.81% and D: 41.00%), palmitic (N: 22.13% and D: 22.52%), stearic (N: 12.53% and D: 12.70%) and linoleic (N: 12.34% and D: 11.94%) fatty acids. The effect of salt on lipolysis is still not completely clear. Several studies have shown a positive effect of salt on lipolysis (Motilva and Toldrá, 1993), while others have not shown this correlation (Coutron-Gambotti and

| Amino acids     | Non-desalted | Desalted | SE  | P<     |
|-----------------|--------------|----------|-----|--------|
| Aspartic acids  | 0.31         | 0.31     | 0.22| 0.9450 |
| Treonine        | 0.23         | 0.20     | 0.02| 0.3151 |
| Serine          | 0.24         | 0.22     | 0.02| 0.3747 |
| Glutamic Acid   | 0.72         | 0.66     | 0.02| 0.1201 |
| Proline         | 0.29         | 0.25     | 0.02| 0.1792 |
| Glycine         | 0.26         | 0.23     | 0.02| 0.4001 |
| Alanine         | 0.42         | 0.37     | 0.04| 0.4013 |
| Cistine         | 0.04         | 0.03     | 0.01| 0.5885 |
| Valine          | 0.26         | 0.32     | 0.03| 0.2182 |
| Methionine      | 0.17         | 0.14     | 0.01| **0.0041 |
| Isoleucine      | 0.23         | 0.22     | 0.02| 0.4932 |
| Leucine         | 0.40         | 0.37     | 0.02| 0.4985 |
| Tyrosine        | 0.16         | 0.17     | 0.01| 0.3355 |
| Phenylalanine   | 0.23         | 0.26     | 0.01| 0.2652 |
| Lysine          | 0.63         | 0.49     | 0.06| 0.1711 |
| Histidine       | 0.18         | 0.18     | 0.01| 0.5359 |
| Arginine        | 0.26         | 0.31     | 0.03| 0.4345 |
| Total amount    | 5.02         | 4.72     | 0.19| 0.3497 |

**P<0.01.**
Gandemer, 1999). Statistical analysis of the presence of free fatty acids in muscle tissue of dry-cured ham in this study showed that desalting had a statistically significant impact on the content of only two fatty acids in the intramuscular fat of dry-cured ham. A statistically significant difference (P=0.0203) was found for the content of heptadecanoic (C17:0), which had a lower content in non-desalted (0.18%) than in the desalted dry-cured ham (0.24%) and for the content of docosapentaenoic - DPA (C22:5ω-3) acids (P=0.0373), which had a lower content in non-desalted (0.39%) than in the desalted dry-cured ham (0.49%). Certain studies (Motilva and Toldrá, 1993; Vestergaard et al., 2000) showed that the concentration of free fatty acids in dry-cured ham increase with processing time and that it is highest in superficial muscles (m. semimembranosus).

| Fatty acids methyl esters                  | Non-desalted | Desalted | SE  | P<  |
|-------------------------------------------|--------------|----------|-----|-----|
| Capric, C10:0                             | 0.09         | 0.07     | 0.01| 0.1926|
| Lauric, C12:0                             | 0.08         | 0.09     | 0.01| 0.3112|
| Myristic, C14:0                           | 1.10         | 1.15     | 0.03| 0.2491|
| Pentadecanoic, C15:0                      | 0.08         | 0.08     | 0.00| 0.4920|
| Palmitic, C16:0                           | 22.13        | 22.52    | 0.34| 0.4811|
| Palmitoleic, C16:1ω-7                     | 2.50         | 2.65     | 0.15| 0.5444|
| Heptadecanoic, C17:0                      | 0.18         | 0.24     | 0.01| *0.0203|
| Stearic, C18:0                            | 12.53        | 12.70    | 0.29| 0.7145|
| Oleic, C18:1ω-9                           | 41.81        | 41.00    | 0.99| 0.6181|
| Linoleic, C18:2ω-6                        | 12.34        | 11.94    | 0.85| 0.7682|
| Arachidic, C20:0                          | 0.27         | 0.31     | 0.01| 0.0724|
| Gadoleic, C20:1                           | 0.86         | 0.83     | 0.03| 0.5869|
| Linolenic, C18:3ω-3                       | 0.25         | 0.27     | 0.01| 0.2803|
| Eicosadienoic, C20:2ω-6                   | 0.50         | 0.45     | 0.03| 0.3218|
| Behenic, C22:0                            | 0.07         | 0.07     | 0.01| 0.8445|
| Erucic, C22:1ω-9                          | 0.34         | 0.31     | 0.02| 0.3769|
| Arachidonic, C20:4ω-6                     | 2.19         | 1.84     | 0.23| 0.3534|
| Eicosapentanoic - EPA, C20:5ω-3           | 0.30         | 0.24     | 0.02| 0.0966|
| Docosapentanoic - DPA, C22:5ω-3           | 0.49         | 0.39     | 0.02| *0.0373|
| Docosahexaenoic - DHA, C22:6ω-3           | 0.45         | 0.41     | 0.03| 0.4726|
| Nervonic, C24:1                           | 0.27         | 0.27     | 0.02| 0.8735|
| Indeterminate                             | 2.23         | 2.14     | 0.11| 0.6156|
| Cholesterol                               | 85.66        | 85.37    | 1.20| 0.8791|

*:P<0.05.
which contain more salt and less moisture than internal muscles (m. biceps femoris).

Conclusions

Desalting treatment highly affected (P<0.0001) the decreasing of salt quantity in the muscle tissues of the ham as expected. Although differences were established in the content of calcium, methionine, heptadecanoic and docosapentanoic acid, it is difficult to conclude that this is solely a consequence of the desalting treatment. A slight decrease in the appearance of white precipitates (average of 0.7 points) in desalted hams was established. The regularity of appearance of precipitates on both the desalted and non-desalted hams suggests that the appearance of the white crystals is also connected with raw ham characteristics.

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