Effect of wet-curing on physical properties and proteins of cured ham

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Abstract: Eating the right quality and quantity of protein is a very important part of a balanced and healthy diet. Sterling proteins are those in which all essential amino acids are present. In meat products, like hams, come off many different reactions during ageing and storage. Examples include the production of free amino acids or the production of biogenic amines from them by decarboxylation. In this study I investigated the presence of these amino acids and biogenic amines, as well as the quality properties of cured hams during curing and ageing experiments.

In this study the wet-cured ham were analysed for changes in NaCl concentration (at 3 parts: surface, core, bottom layer), color, water activity, denaturation temperature and enthalpy (Differential Scanning Calorimetry), free amino acids (FAAs) and biogenic amines (BAs). The meat samples were immersed into 100 g L^{-1} NaCl brine. The curing took 20 days, followed by smoking and ageing for 35 days (12°C, 75% RH).

Keywords: wet-curing, proteins, amino acids, biogenic amines

Introduction:

Meat is primarily a protein food, containing high amounts of protein with high biological value. Some essential amino acids are naturally present in sufficient quantities only in meat (Toldrá, 2002). Meat is also a major source of vitamins and minerals and contains most of the B vitamins. Of these, vitamin B12 is essential. With a balanced diet, meat and meat products can provide up to 70% of vitamin B12 (Dublecz, 2011). Both traditional and modern preservation methods are currently used to ensure that the product retains its organoleptic properties and valuable components for as long as possible.

Curing has been used for centuries to preserve food, especially meat. During the curing process, salt, sugar, nitrite or nitrate and other substances are added to the product to preserve it and to develop its flavour and color (Keenan, 2016). There are two main forms of traditional curing: dry- and wet-curing. The traditional curing is usually immersed in simple brine, which reduces the water content of the meat due to osmotic dehydration (Lawrie & Ledward, 2006). Perhaps the biggest disadvantage of traditional methods today is that they are

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time-consuming, some products can take years to cure. In the case of curing, both physical and chemical processes take place in the product, which strongly determine the final product. These changes play an important role in the development of flavour, texture, color and also proteins.

Mayer et al (2010) found that biogenic amines are small molecular weight organic bases. They can have aliphatic (putrescine, cadaverine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) structures that result in metabolic processes in animal, plant and microbial cells. They are found in high amounts in foods where microbial activity is present, such as in wine, fermented meats, fish, vegetables and cheese. Typically, these nitrogen-containing compounds are produced by microbial decarboxylation and transamination of amino acids to aldehydes or ketones. During maturation or possible degradation, if the reaction conditions are favourable in terms of enzyme activity, the growth of microorganisms leads to the decarboxylation of free amino acids, resulting in the production of biogenic amines (Fiechter et. al., 2013). Therefore, it was also important to investigate the presence of biogenic amines in the present study.

Materials and Methods:

Experiment

Pork loin (Longissimus dorsi) obtained from a local slaughterhouse was used for all investigations. Pork loin was chosen as it can be considered as a relatively homogenous muscle. The meat samples were cured in brine containing 100 g L^{-1} NaCl for 20 days. After the wet-curing the samples were smoked on beech wood at about 27°C. The smoking was followed ageing for 35 days (12°C, 75% RH). During the experiment the NaCl content, color, water activity, denaturation temperature and enthalpy (Differential Scanning Calorimetry) were measured on day 0., 1., 4., 8., 13., 20., 27., 34., 41., 48. és 55. The free amino acids (FAAs) and biogenic amines (BAs) were measured only int he ageing period.

NaCl content determination

The determination of the amount of NaCl penetrated into the meat was carried by Mohr method (there were no added other additives which contain chlorine) as described by Volpato et al. (2007). The following formula was used to calculate the amount of salt:

$$NaCl\ content\ (\%) = \frac{V * N * F * 0{,}0585 * 100}{m}$$

V: consumption of 0,1 N $AgNO_3$ in titration (mL)

N: normality of $AgNO_3$

F: factor of 0,1 N $AgNO_3$

m: mass of sample (g).

For NaCl content was measured at 6 point of the sample. The sample was cut at 2 areas (side and middle of sample). These areas were separated to 3 parts. This sampling was able to determine the NaCl content on the surface, in the core and in the bottom part (above fat) (Fig 1.). The fat layer was approx. 15 mm, the meat layer was approx. 80 mm.

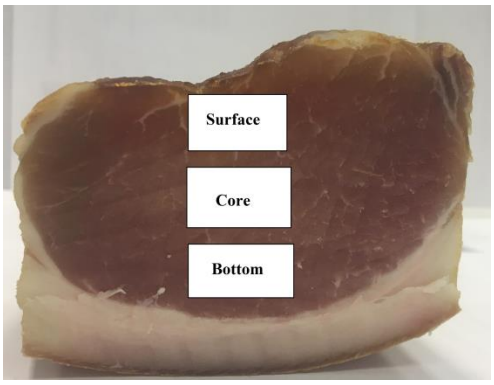

Figure 1. Sampling of the meat

Color measurement

Objective color was measured with Minolta ChromaMeter CR-400 (Konica Minolta Inc., Japan) at 3 different points on the surface and cross-section of meat samples. The results of the color measurement were evaluated in the CIE Lab system, in which the three color factors were lightness (L *), redness (a *) and yellowness (b *).

Water activity measurement

The water activity of the samples was measured using a LabMaster AW equipment. The measurement was performed on a sample taken from 3 different points (2 sides and centre of the loin). The equipment performs the measurement automatically.

Differential Scanning Chalorimetry

The differential scanning calorimetric (DSC) measurements were performed in a Micro DSC II CS32 Calorimeter Vessels Setaram (Setaram Inc. Caluire-et-Cuire, France). Samples of 0.220 ± 0.005 g were obtained from the centre of each meat sample. The samples were placed in metal pans and hermetically closed. The heating rate of the scans was 1°C min^{-1} from 25 to 90°C. Distilled water was used as reference.

Chromatographic determination of amino acids and biogenic amines

The qualitative and quantitative determination of amino acids and biogenic amines was carried out with an AAA 400 equipment. This Automatic Amino Acid Analyzer is a compact automatic liquid chromatograph based on ion exchange column chromatography. Using buffers provided by the producer, separation is performed by step gradient elution. The post-column derivatisation was performed by a ninhydride reaction followed by detection at 570 nm and for proline at 440 nm. The evaluation was performed with CHROMULAN V. 0.82 programm. About 5 gram sample is required to perform the measurement.

Results:

NaCl content

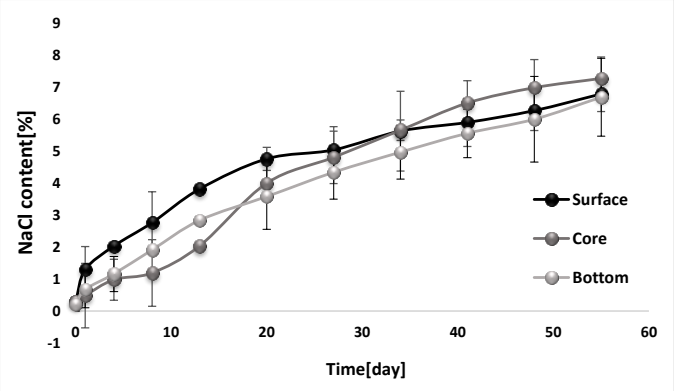

Figure 2: Changes in NaCl content measured on the surface, core and bottom of cured ham during the experiment

The NaCl content was measured at three different points in the sample to observe salt diffusion. The curves are almost linear. It is clear that the changes in NaCl content measured in the different layers show a similar trend. The increase was significant ($p<0,05$) in all layers. As expected, the salt content of the surface layer increased more rapidly during the curing phase (day 20), followed by a more significant increase in these values measured in the core during the ageing period. This can be explained by the fact that during curing, the salt diffusion starts more slowly and therefore the salt content of the surfaces in direct contact with the brine is higher. So from the surface to the bottom of the sample, the salt content was progressively lower, but there was no significant difference ($p>0,05$) between the average NaCl content of the layers. During the ageing, however, the salt content of the different layers becomes almost equal by the end of the experiment. At day 55, the salt content of the

cured ham was above the required concentration of 5% (average salt content of about 6.8%), but as the water activity had not reached the safe level of less than 0.9 before, the ageing could not be stopped.

Color

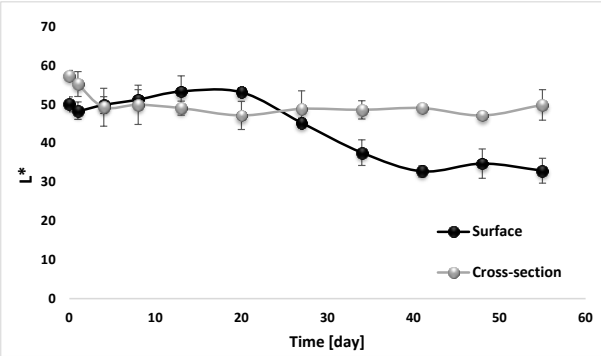

Figure 3: Changes in the L* (lightness) value of cured ham on the sample surface and on the cross-section

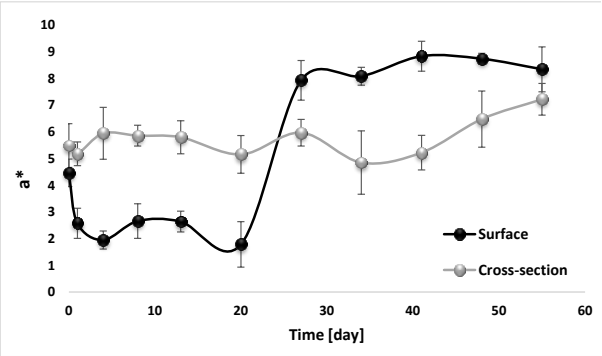

Figure 4: Changes in the a* (redness) value of cured ham on the sample surface and on the cross-section

The changes in the lightness (L*) are shown in Figure 3. It can be seen that the cross-section remained darker to the end of the curing period, as the salt content of the brine made the surface of the meat paler. From day 20 onwards, the surface area values showed a decreasing trend. This is due to the smoking process, which is responsible for the sudden darkening of the samples through color development. Also, of course, the colors have deepened during ageing, as shown by the further decrease in L* values. This is explained by the fact that during the ageing period the samples are constantly drying, their moisture content decreases and their color is growing dark. The different in the lightness between the surface and the cross-section was significant ($p<0,001$).

The changes in the redness (a*) are shown in Figure 4. The values measured on the surface are also higher in this case. After day 20, a significant change is seen, which can also be explained by the smoking. The coloring effect of smoking is striking, as it results the ham the reddish color typical of a smoked products. However, similarly to the L * values, there was no significant change in a* values for the cross-section. In the ageing phase, the values measured show an increase, which can be explained by the continuous penetration of salt and its effect on color.

The change in b* is not shown because it is not relevant for meat.

Water activity

Water activity means the fraction of the total water content in food that is available to microbes and is therefore closely related to the microbiological stability and shelf life of the product. In simple terms, it is the amount of "free" water in food. The lower the aw value, the more protected the product is against microbiological spoilage.

The diagram shows that during the curing process the brine diluted the muscle cells by day 4, and from day 8 onwards, the dehydrating property of NaCl can be seen. The aw value decreased to a greater extent in the ageing phase. This can be explained by the drying of the samples and the ageing process. However, the safe level below 0.90 was reached only on day 55. During curing, the aim is to achieve this value (<0.9) as soon as possible. This can perhaps be accelerated by changing the ageing parameters.

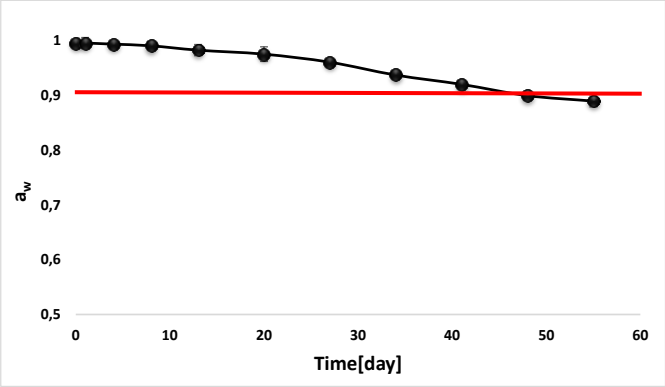

Figure 5: Changes in water activity of cured ham during the experiment

Differential Scanning Calorimetry (DSC)

The DSC thermograms of cured hams were analysed to establish the effect of curing on protein denaturation (Fig 6). The diagram shows the heat flow curves of the samples measured on days 0, 20 and 55. DSC can be used to study the structural changes in the proteins of the sample, i.e. denaturation, coagulation and aggregation can be well monitored. In the case of raw meat three thermal transitions were detected with endothermic peak (T_{max}) values at 58°C, 66 °C and 80°C. These correspond to myosin, sarcoplasmic proteins and collagen and actin. Fernández-Martín (2007) concluded that the evolution of the denaturation peaks obtained from DSC measurements may be related to the denaturation of individual muscle proteins and to changes in the structure of the meat. This suggests that as the salt content increased, the proteins were probably denatured or aggregated.

The heat flow curves that at the end of curing, the denaturation peak of actin had completely disappeared, while at the end of ageing, only the peak of sarcoplasmic proteins remained.

Increasing the salt content therefore clearly affects the structure of proteins. This can be seen from the change in denaturation enthalpy values (Table 1.). As the salt content increased, this value decreased significantly, suggesting that the amount of denaturable proteins in the meat is decreasing.

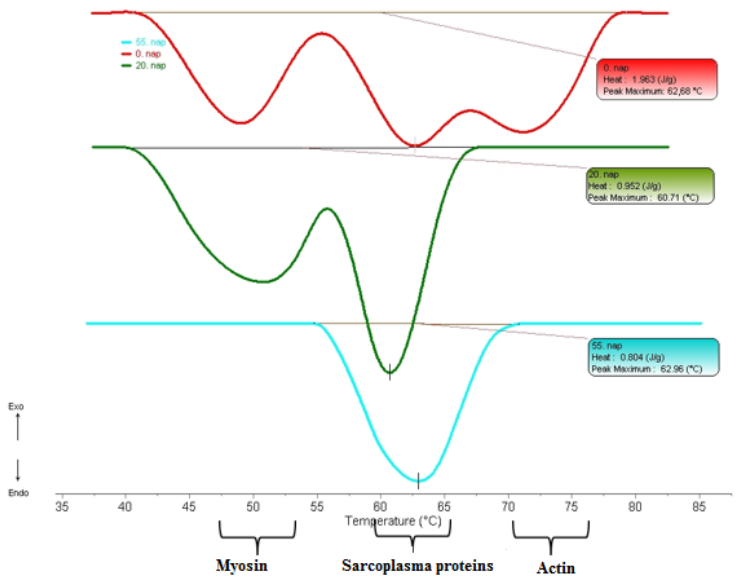

Figure 6: The heat flow curves of cured ham measured on days 0, 20 and 55

Table 1: Denaturation enthalpy values obtained from DSC measurements on days 0, 20, and 55 and the NaCl content of the samples on those days days

Entalphy [J/g]	NaCl content [%]
1,963	0,29
0,952	4,76
0,804	5,90

Changes in the amount of free amino acids

During maturation, 20 free amino acids were determined in the samples and are shown in Table 2. Amino acids directly contribute to the flavour and quality of meat products, and the compounds they produce (ammonia, amines, carbonyls, acids) are also very important in the development of the flavour of the product. The decarboxylation of free amino acids results in the production of amines. During decarboxylation, the following transformations occur: histidine to histamine, tyrosine to tyramine, ornithine to putrescine, lysine to cadaverine,

tryptophan to tryptamine, phenylalanine to phenylethylamine. The highest amounts of histidine and asparagine were found in the samples. There were also higher levels of alanine, arginine, glutamic acid, glutamine, glycine and leucine. Glutamic acid, glycine and cysteine are precursors of glutathione, the most important low molecular weight antioxidant in the human body (Simon-Sarkadi, 2019). Tyrosine and tryptophan are aromatic amino acids, which were only present in small amounts in the samples. Meat usually contains a lot of threonine, but it was not present in high levels in the samples. The lowest amounts were lysine, cysteine and cystathionine. Lysine is generally present in high amounts in foods of animal origin.

Table 2: Changes in free amino acids during ageing

Name	measured values of free amino acids during ageing (mg/g)						
	week 0	week 1	week 2	week 3	week 4	week 5	week 6
Aspartic acid	16,408	18,388	19,913	27,680	38,627	48,326	54,715
Treonine	18,154	25,653	34,722	60,865	61,899	63,459	65,621
Serine	23,169	28,969	47,770	74,947	100,352	109,275	127,417
Asparagine	77,678	70,915	119,677	412,115	335,351	433,328	501,517
Glutamic acid	45,121	52,427	100,866	138,296	152,674	181,664	183,073
Glutamine	50,569	54,202	17,698	24,382	30,823	80,894	141,245
Proline	13,978	6,543	41,570	23,511	73,308	83,548	97,575
Glycine	35,056	31,703	46,858	80,424	84,819	104,923	129,928
Alanine	55,384	57,590	96,496	149,715	171,507	201,456	251,886
Valine	25,321	31,039	39,492	70,937	105,503	115,326	133,792
Cysteine	0,594	0,547	8,172	20,024	17,433	23,453	32,151
Metionine	20,928	22,276	37,414	56,626	75,761	87,721	104,709
Cystathionine	0,689	0,437	1,579	4,386	2,086	5,136	6,859
Isoleucine	13,565	17,031	31,278	53,115	63,000	69,945	74,934
Leucine	37,760	46,145	74,825	124,252	171,116	196,986	226,145
Tyrosine	22,096	24,124	46,312	72,464	105,308	125,678	133,094
Phenylalanine	18,713	20,358	35,169	55,000	72,233	87,736	101,534
Lizin	7,873	9,080	55,148	70,059	102,451	114,952	128,068
Histidine	293,984	131,326	169,751	267,030	228,309	298,365	371,112
Arginine	66,163	31,165	47,481	90,088	122,058	152,946	177,871
Total	843,204	679,919	1072,191	1875,914	2114,618	2585,117	3043,243

Changes in boigenic amines

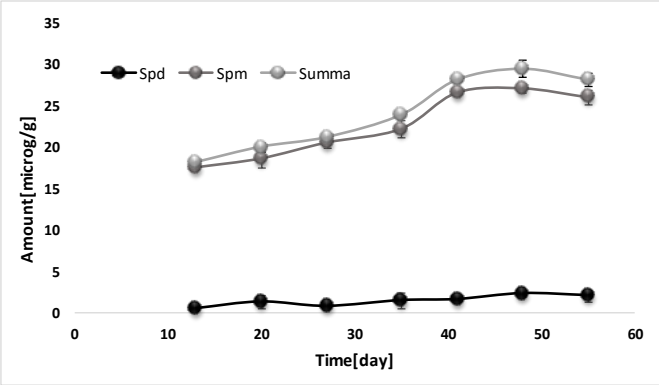

Figure 7: Changes in biogenic amines during ageing

The changes in biogenic amine content measured in cured hams during the ageing period are shown in Figure 6. The identification of biogenic amines is important as they can cause harmful physiological effects when consumed in higher quantities. Polyamines, such as putrescine, cadaverine and the spermin and spermidine found in the sample, increase the toxicity of histamine and tyramine. Although histamine was not detected in the sample, histidine was present in high amounts among the free amino acids. This histidine, if storage had continued, would probably have turned into histamine. Histamine can cause low blood pressure, indigestion and headaches in humans at high levels.

Spermine is produced from spermidine, which is produced from putrescine. In general, food made from vegetables contains more of them. The diagram clearly shows that the amount of spermidine is minimal. Spermine was present in higher amounts, but it was also negligible. The average amount of total biogenic amines in the hams was 0.024 mg/g. This is very low, probably due to the short ageing time for the biogenic amines to appear.

Conclusion

Our results provided the information about the salt content, the color, the water activity, the protein denaturation and the free amino acids and biogenic amines in case of wet-cured ham during the curing and ageing process. During the curing phase, the NaCl content of the surface increased faster than in the core and in bottom of the samples. This can be explained by the fact that salt diffusion is initially slow and since the brine is in direct contact with the surface, it is present in higher concentrations. By the end of the experiment, however, there was salt equalization between the different layers.

The results of the color measurements reflect the fact that the cross-section remained darker compare to the surface. The value of the lightness (L*) was therefore lower, which can be explained by the fact that the direct contact of the brine caused some fading. This operation

also affected the redness (a*), but again mainly for surface values. Smoking makes the meat redder and deepens its colour.

The water activity decreased continually, especially during the ageing phase. However, the product reached the safe level only on day 55. Therefore, it may be necessary to change the ageing conditions in order to shorten the ageing period.

Based on the DSC measurements, it can be concluded that an increase in NaCl content clearly affects the structure of the proteins, which is also reflected in the change in denaturation enthalpy values.

The analysis of free amino acids showed that the amount of histidine in the starting samples was extremely high. In addition, the asparagine and arginine contents were found to be slightly higher. During the six-week study, the amount of each of the free amino acids determined in the samples increased. Of the many biogenic amines, only two have been identified, spermin and spermidine. Overall, the biogenic amine content of the samples was very low during ageing. This can probably be explained by the short time of 6 weeks for the production of biogenic amines.

Acknowledgement

The Project is supported by the MATE Doctoral Scool of Food Sciences and European Union and co-financed by the European Social Fund (grant agreement no. EFOP-3.6.3-VEKOP-16-2017-00005).

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