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Article *in* European Food Research and Technology · November 2015
DOI: 10.1007/s00217-015-2490-2

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REVIEW PAPER

Manufacture of dry-cured ham: a review. Part 1. Biochemical changes during the technological process

Inna Petrova[1] · Inga Marie Aasen[2] · Turid Rustad[3] · Trygve Magne Eikevik[1]

Received: 10 April 2015 / Revised: 18 June 2015 / Accepted: 20 June 2015

Abstract Dry-cured ham is a traditional meat product highly appreciated by consumers. Production of dry-cured ham is a time-consuming process which varies between different ham types. There are many factors affecting the final characteristics of dry-cured ham. The quality of the raw material and the process conditions mainly influence the rate and the extent of biochemical reactions which are in turn responsible for the formation of specific flavor and texture. This review paper highlights the characteristics of the raw material, the enzymatic and chemical processes taking place during dry-cured ham manufacture and the compounds formed by these reactions. The rates of the enzymatic changes from fresh meat to the stage of final product are also described.

Keywords Dry-cured ham · Enzymatic activity · Proteolysis

Introduction

Dry-cured ham is a traditional food product which is well known all over the world; however, different countries and areas have their own styles. The differences between various types of dry-cured ham are due to pig breed, feed of pigs, their weight and age, as well as differences in the production process. High-quality dry-cured hams, with a production length longer than 1 year, have distinct organoleptic characteristics: a rich, unique, and recognizable flavor and color in the range from rosy to maroon or brown red marbled with white fat. However, the sensorial, physical–chemical, aromatic, morphological, and textural characteristics of dry-cured ham vary significantly depending on the alterations in the technological process from producer to producer [1–5].

The traditional technology for the production of dry-cured ham mainly consists of salting, postsalting (resting), and drying–ripening stages. In Northern Europe (Germany, Scandinavia), smoking is frequently applied. Salting and drying–ripening are the most important steps in the manufacture where the flavor of the final product is mainly formed.

The duration of the postsalting and the drying–ripening stages varies depending on the type of dry-cured ham. The drying–ripening step lasts from 2–3 months to 2–3 years for the highest quality dry-cured hams. Increased time of ripening gives a higher degree of enzymatic degradation, contributing to taste and flavor of the final product and as a consequence of higher quality of dry-cured ham [6]. Shorter processing time allows faster production of dry-cured ham, but the quality characteristics will suffer. The technology for each particular kind of dry-cured ham is adjusted according to the desired priority: quality or high production capacity.

During ripening, endogenous enzymes degrade proteins and lipids to amino and fatty acids correspondingly, which are mainly responsible for the flavor of dry-cured ham [7]. Free amino and fatty acids are further degraded and converted by enzymatic and chemical reactions, including oxidation, to volatile compounds. Free amino acids contribute

✉ Inna Petrova
inna.petrova@ntnu.no

[1] Department of Energy and Process Engineering, Norwegian University of Science and Technology (NTNU), Kolbjørn Hejes v 1B, 7491 Trondheim, Norway

[2] SINTEF Materials and Chemistry, Richard Birkelands vei 3, 7491 Trondheim, Norway

[3] Department of Biotechnology, Norwegian University of Science and Technology (NTNU), Sem Sælandsvei 6/8, 7491 Trondheim, Norway

Published online: 01 July 2015

directly to taste [8], while further protein degradation products participate in generation of many odorants [9, 10]. A total of twenty-eight odorants were identified in Iberian ham by Carrapiso et al. [11] including aldehydes, sulfur- and nitrogen-containing compounds, ketones, esters, and alcohol.

During the drying–ripening stage, the vapor pressure gradient which occurs between the meat surface and the environmental drying air causes water evaporation from the surface and simultaneous diffusional water transport from the inner meat tissues toward the interfacial layer [12]. The reduction in water content will increase the salt concentration in the muscle tissues. This affects the rates of the enzymatic reactions influencing the final organoleptic characteristics of dry-cured ham [13–19]. The duration of the period when salt concentration in the tissues is low enough to allow the activity of enzymatic reactions is crucial for the sensorial properties, especially for the development of flavor [1, 4–6, 20]. The rates of enzymatic reactions are also determined by temperature [20–22].

Muscle enzymes have been well characterized, but despite this, knowledge about their activities as a function of the process conditions is still lacking. It is a difficult task to define any dependence between the rates of enzymatic reactions and the process parameters. Drying kinetics has also been studied for dry-cured ham, and models have been developed by Clemente et al. and Gou et al. [23, 24]; however, as far as we know there has been no attempt to combine the two aspects of dry-cured ham manufacture: drying kinetics based on mass transfer and biochemical changes occurring throughout the process. The aim of this review is to investigate the existing information about both aspects of the process to identify the dependences and relations between biochemical changes and drying mechanisms. The first part of the review focuses on biochemical mechanisms within dry-cured ham, especially on enzymatic activity during ripening. The second part focuses on drying mechanisms and modeling.

Production technologies and styles of dry-cured ham

This section gives an overview about different styles of dry-cured ham and technologies used for their manufacture. A general technological process of dry-cured ham manufacture is shown in Fig. 1.

Different styles of dry-cured ham

The most famous species of dry-cured ham are Spanish Iberian, Celta, and Serrano hams; Italian Parma, San Daniele, and Toscano dry-cured hams; French Bayonne hams and Chinese types such as Jinhua and Xuanwei hams. They

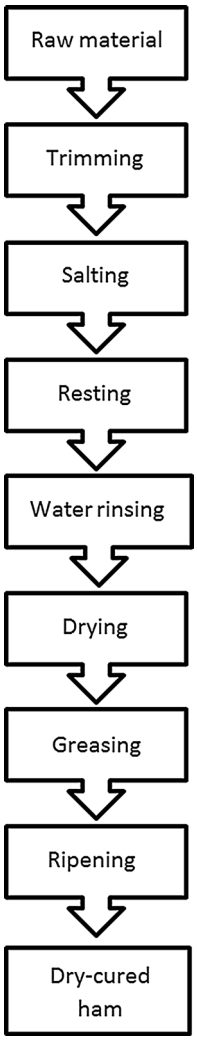

Fig. 1 A common technological process of dry-cured ham manufacture

are characterized as high-quality products that are ready to be consumed without any further treatment or cooking. The main characteristics of some of the most typical dry-cured hams (Parma [25], Toscano [26], Iberian [6, 27, 28], Santa Kristina [29], Bayonne [6, 30–32], and Jinhua [33, 34]) are described in Table 1. The comparison of the chosen ham types is nominal because the information has been obtained by different researchers using different methodology and is shown for general understanding of varieties between ham types.

Technological process

Salting and salt equilibrium

Salting of hams can be provided by the two main techniques—undetermined salt supply and exact salt supply.

Table 1 Characteristics of different styles of dry-cured hams

Ham	Breed	Product parameters					
		Parameters	Weight (kg)	Color when sliced	Aroma	a_w	pH
Italy ≥12 months of manufacturing							
Parma	Large White, Landrace and Duroc by the Italian Herd Book; other breeds carried out with aims consistent with those pursued by the Italian Herd Book	NaCl content: 4.2–6.2 %; Moisture content: 59.0–63.5 %; Proteolysis index: 24.0–31.0 %	8–10	Uniformly ranging from pink to red, marbled with white fat	Mild and delicate flavor, slightly salty with a fragrant and distinctive aroma	0.94	5.7
Toscano	Large White, Landrace and their hybrids; other breeds which are not in incompatibility of the genetics of these two according to the Herd Book	NaCl content: maximum 8.3 %; Moisture content: maximum 61 %; Proteolysis index: maximum 30 %	8–9	From bright red to light red with the presence of subcutaneous white fat with light pink veins	Mild and delicate flavor with a fragrant and distinctive aroma	0.87	5.5
Spain 12–48 months of manufacturing							
Iberian ham	Pure Iberian (females and males) and Duroc (males) enrolled to the Herd Book; crossbreeds which are corresponding to the genetics of Iberian and males corresponded to Duroc identified individually	NaCl content: 6.5 %; Moisture content: 49 %	8	Bright red with a high degree of marbling	Exquisite typical flavor	0.87	5.1
Norway 24 months of manufacturing							
Santa Kristina	Crossbreeds of 50 % Norwegian Duroc, 25 % Norwegian Landrace, and 25 % Yorkshire	–	8	Dark red marbled with fat	Salty and intensive	–	–
France ≥9 months of manufacturing							
Bayonne	Pie Noir	NaCl content (salt used is from Adour basin): 7.7 %; Moisture content: 56 %; Proteolysis index: 29.2 %	8–9	From bright red to light red with the presence of subcutaneous white fat and veins of intramuscular fat	Mild and slightly sweet	0.89	5.8
China about 11 months of manufacturing							
Jinhua	Liangtouwu	NaCl content: 8–15 %; Proteolysis index: 14–20 %	2.5–4	Rose-like muscle, golden yellow skin, and pure white fat	Distinctive and intense	–	–

Salting is accompanied by an osmotic dehydration process. While salt is diffusing into muscles, the moisture is going out at the same time.

Undetermined salt supply is the most common method. The hams are placed in stainless steel or plastic bins and totally covered by salt. The bins have holes in the bottom to let the moisture drain off. Since excess salt is added, the salting time determines the final salt content of the ham. Generally, the salting time for hams of Mediterranean style is from 17 to 48 h per kilogram of weight at 0–4 °C at high relative humidity up to 95 % [35, 36], while Scandinavian style hams are salted upon approximately the same conditions up to 5–6 days per kilogram [29].

By the exact salt supply, a certain amount of salt is added to the surface and hand-rubbed. This method takes longer time than the undetermined technique, since all the salt should be absorbed. The time of salt diffusion, in this case, depends on the size of the ham, but generally it is between 14 and 21 days [6].

After salting, the hams are maintained at low temperatures to allow the salt to be distributed uniformly in the meat tissues. The whole process of salting and postsalting usually takes from one to several months for Mediterranean styles. However, process duration depends on a ham size, the ratio of lean surface to mass, pH, an amount of intramuscular fat, the presence of subcutaneous fat, a temperature of curing room, technology, etc.

Nitrite/nitrate treatment

Nitrite and nitrate are widely used in dry-cured ham manufacture as curing salts. The quality and safety of dry-cured ham are considerably affected by nitrite treatment. Nitrite provides several functions simultaneously: delaying oxidative rancidity, maintaining typical cured color, and inhibiting the growth of spoilage and pathogenic microorganisms (e.g., *Clostridium botulinum*) [37–39]. Nitrite and nitrate salts in meat can transform into nitrosamines [40] which have carcinogenic properties [41]. However, according to Demeyer et al. [42], the level of nitrosamines in dry-cured meat is generally less than the safety limit value, which is too low to inflict harm to human health.

Drying–ripening process

The drying of dry-cured hams is carried out in drying chambers with convective air-drying at an appropriate temperature and relative humidity (Table 1). High relative air humidities prevent extensive dehydration of the surface of the product and narrowing the pores. Narrowing the pores leads to surface hardening and considerable reduction in the drying rate. Convective drying is generally limited by air velocity, but in dry-cured ham manufacture, the velocity is kept very low (0.1–0.5 m s^{-1}) [6]. Although a forced air flow increases the driving force for mass transfer and speeds up the drying process, high air velocities can influence badly on the quality of dry-cured ham. The surface layer of ham dries out (and collapse) in such case. Thus, internal and external diffusions should be the same to achieve an efficient and uniform drying process. The air velocity ought to be kept low, but the air circulation must be uniform to ensure uniform air temperature and relative humidity through the curing chamber. Otherwise, the meat could be spoiled by microorganisms.

Factors affecting the mass transfer during drying and the drying kinetics are discussed in detail in Part 2 of the review.

Smoking

Smoking is not a general technological part for dry-cured ham manufacture; it is not applied to the most of the dry-cured hams. However, in Northern Europe, smoking is used to give the product a small trace of smoked flavor. Under these conditions, smoking is not expected to work as a preservation agent or to influence the protein or lipid degradation profiles, but can reduce the number of surface bacteria greatly due to the bactericidal and bacteriostatic properties of smoke [43]. If smoking is the main technological part of the manufacture, the meat is called smoke-cured ham and is not described in the present article devoted to dry-cured ham.

Characterization of raw material

Muscle characteristics and composition

Hams which are approved to be used for dry-cured ham manufacture are classified mainly according to pH level, weight, and fat content [6]. Soon after slaughtering, the level of pH decreases as the result of ATP (adenosine triphosphate) hydrolysis. The rate of pH decline is a marker of postmortem glycolysis; the rate of postmortem glycolysis is associated with meat quality problems [44]. The rate of pH decline can be unexpectedly rapid when the temperature of carcass is high. It leads to the phenomenon called "pale, soft, exudative (PSE)" meat which has a very low water-holding capacity that has serious economic consequences for the producers. The basis of the problem is mainly in antemortem stresses. Preslaughter stresses lead to another problem—"dark, firm, and dry (DFD)" meat, which has a low rate of pH decline in comparison with PSE. DFD meat has a higher water-holding capacity when compared with normal meat or PSE meat, but the advantage is eliminated by the high susceptibility to microorganism growth

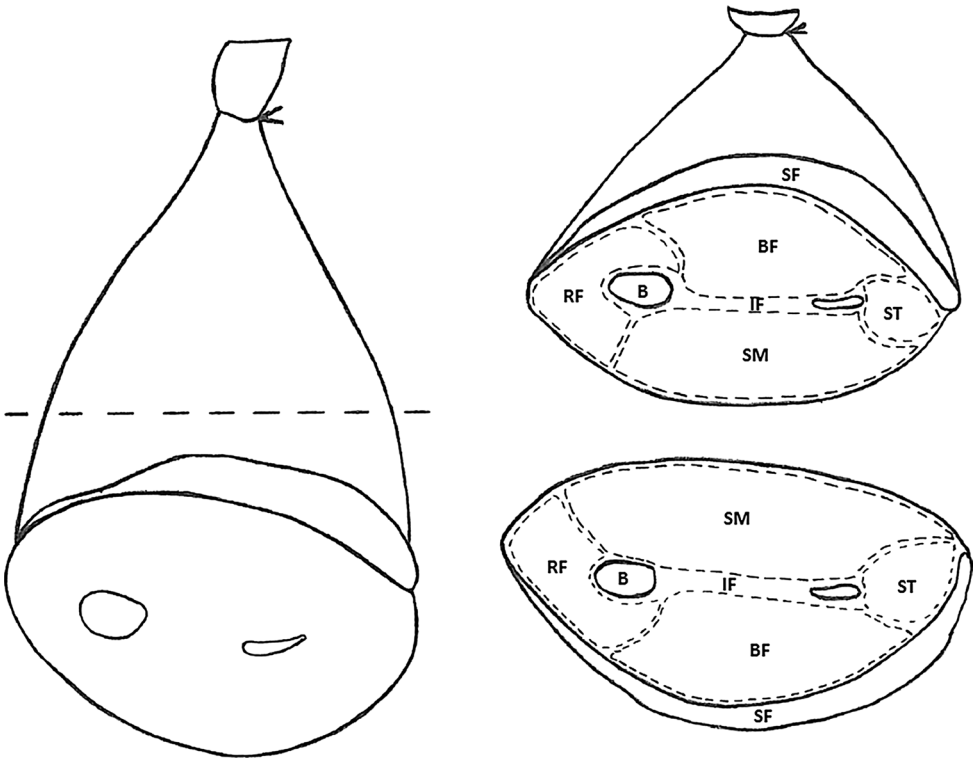

Fig. 2 Main muscular areas: SM—*semimembranosus* muscle; ST—*semitendinosus* muscle; BF—*biceps femoris* muscle, RF—*rectus femoris* muscle, B—bone; IF—internal fatty area; SF—subcutaneous fatty area

[44]. The pH at 24 h postmortem of 5.6–6.1 should be chosen to be appropriate for the successful production process of hams [6]. Close to the lower border of the appropriate pH region, the establishment of rigor mortis occurs due to ATP depletion. That influences the meat tenderness, which should also be taken into account. However, pH measurements are still under consideration at many producing facilities. They prefer to sort the hams only according to fat content and weight. Appropriate weight and fat contents are decided individually according to the type of dry-cured ham produced.

The most important muscles in a ham are shown in Fig. 2. In this paper, the main emphasis is on *Biceps femoris* and *Semimembranosus* muscles with regard to chemical composition and biochemical reactions taking place during dry-cured ham processing. *Biceps femoris* is an internal muscle covered with a thick layer of subcutaneous fat on one side; this slows down salt uptake, and salt content slowly increases throughout the process. The slow increase in salt contributes to higher proteolytic activity in this muscle, which influences the final textural properties [45]. On the other hand, *Semimembranosus* muscle is situated close to the surface without fat covering. It results in a fast salt uptake during the salting stage [46]. Since the extent of biochemical changes during the manufacturing process is different for these two muscles, they can be used as the "marker muscles" for the comparison. Ruiz-Ramirez et al. [16] showed that proteolysis index (degree of proteolysis) which is calculated as the percentage of nonprotein nitrogen divided by total nitrogen was 25 and 18 %, respectively, for *Biceps femoris* and *Semimembranosus* muscle (10 months of manufacturing). Similar results were obtained by Buscailhon et al., Flores et al. and Harkouss et al. [47–49].

Table 2 Composition of *Biceps femoris* and *Semimembranosus* pork muscles

Chemical component	Content (g/100 g)	
	Semimembranosus	*Biceps femoris*
Moisture	71–77	73–78
Protein	17–23	18–22
Fat	1.5–8.9	1.8–7.1
Ash	0.7–1.5	0.9–1.8

The main components of muscle meat are water, proteins, lipids, minerals, and trace quantities of carbohydrates, Table 2 [50]. Proportions of the components vary significantly depending on many factors including pig breed, age, feed, etc.

Proteins

Proteins are the major component of muscle, which constitute about 80 % of the muscle dry weight or 15–22 % of the muscle wet weight [51, 52]. Muscle proteins are usually classified based on solubility or biological function.

Table 3 Characterization of the main proteins of muscle tissues

Protein	Solubilization	Approximate content in the total protein fraction (%)
Actin	>0.3 M NaCl	12–15
Myosin		35–40
Tropomyosin		2.5
Troponin		<1.0
Sarcoplasmic proteins	<0.3 M NaCl	25–35
Collagen	Insoluble	10–15

The solubility category is based on solubilization of muscle proteins at different salt concentrations that gives three groups of proteins: myofibrillar, sarcoplasmic, and stromal proteins [44], Table 3 [6, 51, 52]. Enzymatic degradation of muscle proteins is important for the development of flavor and texture during dry-cured ham production.

Myofibrillar proteins are the main constituents of muscle's fibers, which are 50–60 % of the total protein content in muscle tissues. They are soluble at high ionic strength (salt-soluble proteins). The major myofibrillar proteins, myosin and actin form the actomyosin complex [44, 52]. A group of cytoskeletal proteins (actin, myosin, titin, nebulin, desmin, vinculin, tubulin, dynein, spectrin, clathrin, keratin, vimentin, and many others) are mainly myofibrillar proteins; they contribute to the formation of meat texture [44]. At the microstructural level, it has been shown that myofibrillar proteins are the proteins which are mostly affected by proteolytic activity [53].

Sarcoplasmic proteins are in the range between 25 and 35 % of the total protein in muscle and include most of the muscle's enzymes [6]. Many of the enzymes are involved in energy metabolism, but proteases and lipases compose a significant part of this fraction.

Stromal proteins are the basic elements of muscle connective tissue (10–20 % of the total muscle protein content). The most abundant stromal protein in meat tissues is collagen, while elastin is found in smaller amounts [44]. They are insoluble at usual extraction conditions such as near-neutral pH, low temperature, and low or high salt concentration. Stromal proteins are also important for meat texture. The content and properties of stromal proteins may vary significantly due to different factors such as pork species, age, and muscle type [44].

Lipids

The content of lipids in pork muscle varies significantly depending on the degree of fattening and the presence of adipose tissue, but generally constitutes from 1 to 13 % of the total muscle weight [6]. Intramuscular lipids are divided into two main groups: lipids which are stored in fat cells and membrane lipids. The first group contains mainly nonpolar lipids such as triglycerides. Phospholipids belong to the second group.

Protein degradation

Proteolytic enzymes

Since proteins constitute as much as 80 % of the meat dry weight, the proteolytic reactions during manufacturing are important for the properties and the quality of dry-cured ham [49]. Muscle tissues contain a high number of various enzymes which contribute to the ripening process.

Proteolytic enzymes are classified according to their effect and location. According to the action, the most important proteolytic enzymes are proteases, which are associated with breakdown of proteins to large peptides and peptidases, which hydrolyze the large peptides to smaller ones and to free amino acids. The peptidases are classified into endo- and exopeptidases, and the exopeptidases into aminopeptidases and carboxypeptidases [6]. Proteolytic enzymes are classified regarding their location: in lysosomes or in cytosol. Lysosomal and cytosol enzymes have been studied both in fresh meat and in various meat products [54–60].

The major proteases located in lysosomes are the cathepsins, which are endoproteases. Cathepsins B, H, and L are cysteine proteases, and cathepsin D is an aspartate protease. Myofibrillar proteins are mainly broken down by cathepsins B, D, H, and L, which retain their activity for several months during the production of dry-cured ham [6]. The optimum temperature for cathepsins B, D, H, and L is in the range between 30 and 40 °C. Cathepsins B, H, and L have a neutral pH optimum, while cathepsin D works at around pH 4.0.

Cystatins are protein inhibitors that control the activity of cathepsins in vivo. Cystatins are cytosolic proteins; they have an ability to bind tightly and reversibly to cathepsins B, H, and L. Their activity is a part of the control mechanism responsible for protein degradation since their action may protect meat cells from unwanted endogenous or external proteolysis [61, 62].

Tripeptidylpeptidases and dipeptidylpeptidases are exoproteases which mainly continue the degradation of proteins and degrade polypeptides to smaller peptides. The smaller peptides can be further broken down by aminopeptidases.

Tripeptidylpeptidases hydrolyze different polypeptides to tripeptides. Tripeptidylpeptidases I are located in lysosomes and work at acidic pH; tripeptidylpeptidases II have optimal pH range at neutral values [6]. The temperature optima for tripeptidylpeptidases I and II are 37 and 30 °C, respectively.

Dipeptidylpeptidases are classified as type I, II, III, and IV; type I and II are located in lysosomes, type III is found in cytosol, and type IV is found in the plasma membrane. Dipeptidylpeptidases degrade polypeptides to dipeptides at pH 5.5 for types I and II and at pH 8.0 for types III and IV. All the types have optimal temperature between 45 and 65 °C and are stable for several months [6].

Aminopeptidases, which are located in lysosomes, continue protein degradation and break down peptides to free amino acids. Only five aminopeptidases have been separately analyzed; they have a neutral or basic pH optima and optimum temperatures in the range between 37 and 45 °C [6]. Small peptides and free amino acids are the main products which affect the specific flavor of dry-cured ham [17].

Proteolytic enzymes, which are found in cytosol, are represented by cysteine endopeptidases (particularly by calpains located in Z-disk region of cytosol) and by a family of cysteine proteases called caspases. Calpains have slightly basic optimum pH, and they degrade proteins to polypeptides. Calpains are stable for some days after slaughter and have been found to be active only in the first stage of curing [63, 64]. Caspases have a pH optima between 6.8 and 7.4 [65], and they are only active during the early postmortem changes within days. Caspases are not considered as the contributors to proteolysis at the later stages of dry-cured ham manufacture [66, 67].

Exogenous proteases from lactic acid bacteria and yeasts also contribute to proteolytic activity during the ripening period, but not so significant, when compared with cathepsins, dipeptidylpeptidases, tripeptidylpeptidases, and aminopeptidases [68, 69].

Proteolysis during dry-cured ham manufacture

The amount of proteolytic enzymes of pork muscle depends on the pig breed and genetics [4, 70–75]. Between the stages from fresh meat to the final dry-cured ham, there is a loss of enzyme activity due to denaturation or degradation of the enzymes. The enzyme activity also decreases due to the decreasing water activity during the drying–ripening, as soon as water evaporates and the salt concentration increases [16, 22, 49, 76, 77].

Activity of proteases

The first step of the protein hydrolysis is caused by cathepsins B, L, H, and D, calpains, peptidases, and cytosolic enzymes, which degrade the muscle proteins to polypeptides [49, 78, 79].

Since the optimum temperature for endogenous enzymes is higher than 25 °C, the relatively low temperatures of the salting and the postsalting stages do not allow the maximum possible enzymatic activity. The temperature is usually adjusted during the drying–ripening stage to optimize the temperature with the aim to increase enzyme activity. Morales et al. [77] showed that proteolysis index for *Biceps femoris* muscle, which was ripened at 30 °C, was higher when compared with muscles which were ripened at 5 °C (20.9 vs. 15 % correspondingly). The pH of the raw material also influenced the extent of proteolysis. Proteolysis level of the ripened *Biceps femoris* muscle was 18.9 % (pH less than 5.66) and 17.2 % (pH higher than 6.00). Skrlep et al. [80] also found for "Kraski prsut" dry-cured ham that a lower pH of the raw meat (from 5.51 to 5.63) had a positive effect on proteolysis compared to higher pH (from 5.80 to 6.18). This occurred due to the increased cathepsin activity at lower pH, which affects the extent of proteolysis [16, 49, 81, 82]. Morales et al. [77] also showed that proteolysis index decreased from 19.3 to 16.7 % when the salt concentration increased from 1 to 4 %.

Zhao et al. [76] claimed that cathepsins and calpains are, possibly, the main endopeptidases which take part in proteolysis during dry-cured ham manufacture. However, calpain-like activity was not found by Sarraga et al. [64] after the postsalting step; thus, only cathepsin-like activity will be evaluated as the main protease which takes part in proteolytic changes during the drying–ripening stage.

Cathepsins B, H, L, and D lose their activities gradually with time [46, 76, 83]. The studies of the enzyme activity were performed in muscle extracts, which reflect the stability of the enzymes in the salted ham. Generally, the enzymes remain active longer in *Biceps femoris* than in *Semimembranosus* muscle due to easier salt uptake by external *Semimembranosus* muscle and faster suppression of enzymatic reactions as a result [46]. However, the remaining activity of the enzymes decreases together with salt penetration into the tissues of *Biceps femoris* muscle.

Table 4 shows that activities of cathepsins B, L, H, and D are still observed at the end of drying–ripening stage. This means that they can be active during the whole manufacturing process [46, 76, 83].

In the beginning of the curing process (between the stage of raw material and the postsalting stage), cathepsin L is the most active protease [63, 64]. Cathepsin B has an intermediate role for protein degradation into amino acids [46]. According to Parreno et al. [46], cathepsin L lost its activity more rapidly when compared with cathepsin B. The contribution of cathepsin H to the proteolysis is very low [46].

Summarizing, the protease activity is the highest at the beginning of dry-cured ham manufacture due to higher amounts of active enzyme and due to higher water activity.

Table 4 Residual enzymatic activity (% of original activity) of dry-cured ham compared to fresh pork (studied in extracts)

Dry-cured ham	Muscle used	Length of manufacture (months)	Cathepsin B	Cathepsin H	Cathepsin L	Cathepsin D
Jinhua	Biceps femoris	9	9	–	14	–
Spanish style	Biceps femoris	8	50	1.1	–	–
Spanish style	Semimembranosus	8	30	1.5	–	–
Serrano*	Semimembranosus	8	14	22	–	23

* The values give the percentage of residual activity in dry-cured hams compared to fresh pork after correction for differences in moisture content

Activity of aminopeptidases

During dry-cured ham production, the action of proteases is followed by peptidases, generating small peptides and finally free amino acids. Zhao et al. [22] found that aminopeptidases remain active during the whole dry-cured ham process, but low temperatures reduce their activity.

Alanyl aminopeptidase is responsible for 83 % of the total porcine muscle aminopeptidase activity [22]. Alanyl aminopeptidase activity decreased gradually during the manufacturing of Jinhua ham, and at the end (262 days) of processing, about 3 % of the original activity remained [22].

The duration of the ripening process significantly affects the levels of free amino acids, as illustrated by the comparison of Iberian and Parma hams, with 24- and 12-month production processes, respectively, Table 5 [28, 84]. The ripening phase was around 12 months for Parma ham [3] and 23 months for Iberian ham. The increase in free amino acids by the end of dry-cured ham manufacture reported by the authors is similar to the results reported in other studies [8, 22, 85–87].

Table 5 Free amino acid content in Iberian and Parma hams (mg/100 g of dry matter)

Amino acid	Iberian*	Parma**
ASP	710	264
GLU	1142	735
SER	385	262
ASN	73	29
GLY	296	231
GLN	16	21
BALA	11	Not specified
ALA + TAU	753	540
HIS	198	240
THR	352	240
CAR	729	Not specified
ARG	478	324
PRO	375	Not specified
ANS	74	Not specified
TYR	190	190
VAL	507	338
MET	210	104
ILE	460	207
LEU	686	441
PHE	335	248
LYS	934	727
ORN	Not specified	91
TRP	Not specified	66
Total quantity	8914	5298

* The samples contained semimembranosus, semitendinosus, and biceps femoris muscles

** Muscle not specified

Lipid degradation

Lipolytic enzymes

Lipolytic enzymes in dry-cured ham are found in muscles and in adipose tissues. During manufacture of dry-cured ham, the triacylglycerols and phospholipids are hydrolyzed by lipases and phospholipases correspondingly. The resulting products of the degradation are free fatty acids [35] which are more easily oxidized than triacylglycerols [44].

Triacylglycerols in muscle are mainly degraded by a lysosomal acid lipase (pH optimum 5.0) in the pH range from 5.5 to 6.2 [15]. Phospholipase A, which is located in lysosomes, works in the same pH range and accompanies the lysosomal acid lipase activity. These two enzymes are the major participants of lipolysis in muscle and contribute to the long-chain free fatty acid formation at the postmortem stage. Neutral lipase is active at pH 7.0 and has been found to have a higher temperature optimum (45 °C) when compared to acid lipase and to phospholipase A (37 °C).

The main lipolytic enzymes of adipose tissue degrade triacylglycerols, monoacylglycerols, and lipoproteins. Cholesterol and glycerol esters of triacylglycerols are mainly hydrolyzed by hormone-sensitive lipase (pH optimum 7.0–7.5) [88]. Lysosomal acid esterase and cytosol neutral esterase of adipose tissue also break down triacylglycerols [15]; they are active at elevated temperatures (optimum

temperatures are 60 and 45 °C, respectively) [35]. Due to low availability of substrate, the activity of acid and neutral esterases is restricted. However, they are fairly stable and are able to hydrolyze short-chain fatty acids from tri-, di-, and monoacylglycerols [6]. pH optimum for acid and neutral esterases of adipose tissue is 5.0 and 7.5, respectively [35]. Monoacylglycerols of adipose tissue are mainly broken down by a monoacylglycerol lipase, which has a pH and temperature optimum of 7.0 and 37 °C, respectively. Lipoprotein lipase degrades lipoproteins of adipose tissue; this enzyme mainly works at basic pH (optimum pH 8.5), and temperature optimum is 37 °C [35].

Lipolysis during dry-cured ham manufacture

Lipolytic activity and free fatty acid generation in dry-cured ham production have been studied widely [28, 89–92].

Intramuscular lipids contribute to the formation of the final dry-cured ham flavor. Hydrolysis is the first step of the transformation of lipids to flavor compounds, which gives an increase in the amount of free fatty acids [93]. Phospholipids are considered as the most important fractions for the flavor formation in dry-cured ham due to free amino acids mainly originating from phospholipids [47, 94]. However, Gandemer [95] stated that up to 50 % of free fatty acids can be formed from triacylglycerols if their content is high enough.

A study of Iberian dry-cured ham hold by Flores et al. [28] showed that the activity of the main lipolytic enzymes was stable during the salting and the postsalting stages, Table 6. Another study of Parma ham performed by Vestergaard et al. [96] indicated the stability of lipolytic enzymes from the stage of fresh meat to the end of the ripening, Table 7.

Lipid oxidation is promoted by light, elevated temperature or the presence of salt [44]. The first products formed are hydroperoxides, which can be degraded to secondary oxidation products such as aldehydes, ketones, hydrocarbons, esters, alcohols, and lactones. Secondary products of oxidation are an important part of the flavor formation and contribute to the specific taste of dry-cured ham [4, 95].

Table 6 Activity (U/g of muscle) of lipolytic enzymes in *Biceps femoris* muscle of Iberian dry-cured hams at the end of the salting and postsalting stages

Lipolytic activity	Salting	Postsalting
Acid lipase	0.09	0.11
Phospholipase	0.056	0.041
Neutral lipase	0.49	0.40
Acid esterase	1.23	1.49

Degradation products formed during dry-cured ham manufacture

The main products of proteolysis and lipolysis, which influence the final flavor of Iberian dry-cured ham during the manufacture, are listed in Table 8 [8, 11].

Free amino acids are directly corresponding to the taste of dry-cured ham [8]. The listed free amino acids were identified during the whole process of ham production, but the kinetics of their development varied with time. According to Jurado et al. [8], lysine (Lys), alanine (Ala), and glutamic acid (Glu) were the most abundant free amino acids of ham at the end of processing.

A great variety of odor qualities, such as fruity, cheesy, mushroom-like, nutty or cured ham-like, were identified by Carrapiso et al. [11]. Methanethiol, 2-methylpropanal, 3-methylbutanal, hexanal, 2-heptanone, and 1-octen-3-ol were the most readily identified odorants.

Control of enzymatic activity

As described above, the enzyme activity in the hams is directly affected by the water activity, pH, and temperature. Since the pH is determined by the quality of the raw material, the enzymatic activity during the manufacturing can be directly controlled only by the temperature, the salt

Table 7 Activity (nmol of released 4-methylumbelliferone h^{-1} g $protein^{-1}$) of lipolytic enzymes in *Biceps femoris* muscle of Parma dry-cured hams at 0, 3, 6, 10 months of the producing

Lipolytic activity	Aging time							
	0 Months		3 Months		6 Months		10 Months	
	BF*	SM**	BF*	SM**	BF*	SM**	BF*	SM**
Acid lipase	1.29	1.52	1.29	1.74	1.84	2.86	1.72	2.28
Neutral lipase	0.79	0.33	0.79	0.48	1.11	1.40	0.73	0.60

* *BF biceps femoris* muscle

** *SM semimembranosus* muscle

Table 8 Main degradation products formed during the manufacture of Iberian dry-cured ham

Organoleptic property	Compounds
Taste	ASP
	GLU
	SER-ASN
	GLY-CLN
	HIS
	ARG
	THR
	ALA
	PRO
	TYR
	VAL
	MET
	ILE
	LEU
	PHE
	TRIP
	LYS
Aroma	Hydrogen sulfide
	Methanethiol
	Unknown*
	2-Methylpropanal
	2,3-Butanedione
	Unknown**
	3-Methylbutanal
	2-Methylbutanal
	1-Penten-3-one
	2-Pentanone
	Pentanal
	Ethyl 2-methylpropanoate
	Unknown***
	Hexanalf/(Z)-3-hexenal
	Ethyl 2-methylbutyrate g/(E)-2-hexenal
	2-Methyl-3-furanthiol g/2-heptanone
	Heptanal f/3-mercapto-2-pentanone
	Methional f/2-furfurylthiol
	2-Acetyl-1-pyrroline
	(E)-2-heptenal
	Dimethyl trisulfide
	1-Octen-3-one g/1-octen-3-ol
	2-Propionyl-1-pyrroline i/octanal
	(E)-2-octenal

* The compound was not identified. It has cured, rancid, apple-like aroma

** The compound was not identified. It has fruity, toasted aroma

*** The compound was not identified. It has cured, nutty, almond-like aroma

content, and the drying rate. The drying rate is controlled by the relative humidity and the temperature of drying air.

Normally the temperature is kept low until the water activity will fell down to the levels that prevent microbial growth, which also implies a relatively low enzyme activity. During the drying, the temperature is increased, either in one step or gradually, which allows to increase the enzyme activities. In order to maintain enzyme activity and ripening for a longer period, the hams can be covered with wax/fat to reduce the evaporation. This helps to maintain desirable water activity in the end of the process.

Conclusions

Proteolysis and lipolysis are the main processes which contribute to the final quality of dry-cured ham. Proteolytic and lipolytic changes are generally ascribed to endogenous enzymatic activity. Small peptides and free amino acids, which are formed by the degradation of proteins, along with the secondary products of lipid oxidation are the compounds, which are mainly responsible for the flavor formation during the ripening of ham. The rates and the extent of ripening are determined by the water activity and the temperature. Thus, the duration and the extent of enzymatic ripening can be controlled by varying the initial salt content and controlling the drying rates. As this review has revealed, quantitative data for the "in situ" enzyme activity and product generation as a function of the drying conditions are relatively scarce. However, new, emerging mass-spectrometric methods enable quantitative analyses of peptides and flavor compounds, which are generated during the ripening. Coupled to studies of factors, which affect the drying kinetics (the topic of the second part of this review), this will provide better tools to control the ham manufacturing process and the product quality.

Acknowledgments The work was supported by the Research Council of Norway (Project 225262/E40—DryMeat). Many thanks to the Food Technology Group of NTNU and the Sintef Energy Research Group for cooperation, help, and support.

Conflict of interest None.

Compliance with Ethics requirements This article does not contain any studies with human or living animal subjects.

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