LIPIDS IN MUSCLE AND ADIPOSE TISSUES, CHANGES DURING PROCESSING AND SENSORY PROPERTIES OF MEAT PRODUCTS

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SUMMARY

Dry-cured meat products represent a large part of the meat products on the European market. The technologies develop for these products lead to the production of a large scale of meat products with typical sensory traits. Numerous studies have been devoted to optimise the quality traits of these products which are considered as traditional products by the consumer and provide a high added value to the producer. Among the components of the raw material, lipids play a key role in the final quality of these products. Many sensory traits of dry-cured meat products depend on lipid traits of muscle and adipose tissues of fresh meat and on their degradation through a complex set of lipolytic and oxidative reactions during processing. Lipid traits of both muscle and adipose tissues of fresh meat are strongly related to pig rearing conditions, mainly genotype and feeding strategy. During processing, lipids undergo intense lipid hydrolysis controlled by both lipases and phospholipases, which remain active all along the process. Lipids are also subjected to oxidation, which generates numerous volatile compounds. These volatiles contribute to some typical aroma notes of dry-cured meat products such as rancid, aged ham and dry-cured odours. This paper reviews the recent knowledge on the influence of lipid traits of fresh meat, lipid hydrolysis and oxidation on the development of sensory traits of dry-cured meat products.

1 - Introduction

Drying after salting or fermentation and before a long ripening period is a process used since centuries to preserve meat from spoilage. In the present, these traditional technologies are used to produce high quality dry meat products with a large variety of eating quality (Flores, 1997). The meat industry produces through these processes a wide class of products which are appreciated by the consumers because of their sensory traits and their image of traditional products. These products hold a large place on the market, specially in the Mediterranean countries (Chizollini, Novelli & Zanardi, 1998). The most famous products arising for these technologies are dry-cured hams (Spanish Serrano and Iberian hams, Italian Parma ham, French Corsican and Bayonne hams) and dry fermented sausages (Milano salami, Spanish chorizo, French saucisson sec). These dry meat products provide a large added value to the producer and, at a lesser extend to farmers, because they are regarded as high quality products and consequently are often very expensive (Lopez-Bote, 1998; Bosi, Cacciavillani, Casini, Lo Fiego, Marchetti & Mattuzzi, 2000). That is why during the last decades, numerous studies were devoted to optimise the quality traits of these products. The quality of dry-meat products is related to both the quality of raw matter (adipose tissue and muscle) and the control of complex biochemical reactions which take place during processing (Buscailhon & Monin, 1994b; Toldra & Flores 1998). The quality of the raw matter is strongly related to the rearing conditions of pigs (Lopez-Bote, 1998; Buscailhon & Monin, 1994c). The control of the biochemical reactions which contribute to the development of the typical sensory traits of dry products depends largely on the process (Toldra et al., 1998).

Among the components of the raw matter, lipids have been widely studied for at least 2 reasons (Gandemer, 1999):

- lipids in muscle and adipose tissues largely vary both quantitatively and qualitatively according to the rearing system of pigs and are subjected to intense degradation during processing, namely lipolysis and oxidation ;
- lipids play a key role in many quality traits of meat products including nutritional value and sensory properties, mainly flavour because they are both solvent and precursors of aroma compounds.

This paper is an overview of the recent knowledge on the relationships between lipid traits of adipose tissues and muscles, their changes during processing and the quality of dry-cured meat products.

² - Lipid traits of muscle and adipose tissues

In European countries, most of the dry-cured and dry fermented meat products are manufactured from muscles and adipose tissues from pigs reared in intensive systems. Pigs are from industrial genotypes (Large White, Pietrain, Landrace and their crossbred) reared indoors and fed a commercial concentrated diet. These diets mainly consist in cereals and soya meal and contain a low amount of fat (3-5%). Pigs have a fast growth rate and are slaughtered at 100-120 Kg around 5-6 months of age. Selected against fatness since half a century, carcasses of these animals are very lean (Girard, Bout & Salort, 1988b). Sometimes, pigs are slaughtered heavier (160-180 kg) and older (9-12 months) (i.e. Parma area) (Bosi et al., 2000). Muscle and adipose tissues of these pigs show very similar compositional traits. In some limited areas of Europe, pigs are produced in traditional rearing systems : the most famous ones are Corsica and Iberian ones (Benito, Vasquez, Menaya, Ferrera, Garcia Casco, Silio, Rodriganez, & Rodriguez, 2000; Coutron-Gambotti, Casabianca, de Sainte Marie & Gandemer, 1999). These systems rely on local breeds (Iberian, Corsican) reared outdoors and on a feeding strategy based on the availability of natural resources (acorns, grass, roots, oaks). The pigs are slaughtered between 18 and 24 months of age because they have a low growth rate and are subjected to alternating periods of scarcity (summer) and abundance (autumn) (Lopez-Bote, 1998; Coutron-Gambotti, Casabianca, de Sainte-Marie & Gandemer 1999c). Pigs are fattened under chestnut grooves or oak plantations eating large quantities of chestnuts or acorns for 4-6 months during the winter. During this period, adipose and muscle tissues acquire their typical chemical traits which are characterised by a large development of subcutaneous adipose tissue and a sharp increase in intramuscular lipid content leading to raw matter very different from these of industrial pigs (Lopez-Bote, 1998; Coutron-Gambotti et al., 1998).

2.1. Adipose tissue

Adipose tissues of industrial genotype pigs are mainly subcutaneous adipose tissues : backfat accounts for at least 80% of the total adipose tissues of the carcasses (Girard et al., 1988a). That is why this adipose tissue traits were widely studied. On average, backfat contains 75-80% lipids, 5-15% water and a small proportion of proteins as collagen. Lipids are mainly triacylglycerols (TAGs)(at leat 99%) with a small amount of cholesterol and degradation products of triacylglycerols (diacyl and monoacyl-glycerols, free fatty acids) (Girard et al., 1988a). Backfat from Iberian and Corsican pigs are very developed (23-30 mm thickness for Iberian pigs and 40-50 mm for Corsican ones)(Benito, J. et al. 2000; Coutron-Gambotti, et al., 1998; Secondi, Gandemer, Luciani, Santucci & Casabianca, 1992) and contain more lipids (> 90 %) (Secondi et al., 1992; Coutron-Gambotti & Gandemer, 1999a).

On average, fatty acid composition of backfat from industrial pigs are : 36% saturated fatty acids (SFA), 44% of monounsaturated fatty acids (MUFA) and 12% polyunsaturated fatty acids (PUFA)(Davenel, Riaublanc, Marchal & Gandemer, 1999) (Table 1). This composition varies according to numerous rearing factors including diet, breed, sex, age, physiological stage (Girard et al., 1988a). Except those related to

dietary lipids, differences in fatty acid composition are explained by differences in fatness of the carcasses related genotype, sex or rearing conditions. More developed is the backfat in the carcass, less is the content in PUFA. Thus, more developed is the backfat, higher is the part of fatty acids stored in the adipose tissue arising from, de novo, fatty acid synthesis (which produces SFA and MUFA) and lower is the proportion of PUFA provided by dietary lipids. Many experiments have demonstrated that fatty acid composition of backfat depends on dietary lipids. However in practice, the lipid content of diets is low and no fat is added to the raw matter. So the effect of dietary fat on backfat is not marked except in some particular case such as Iberian pigs where the backfat shows a high proportion of oleic acid. This is explained by the high proportion of this fatty acid in acorns eaten by pigs during the fattening period (Ruiz, Cava, Antequera, Martin, Ventanas, & Lopez-Bote, 1998).

More recently, the attention was focused on triacylglycerol (TAG) composition of backfat because TAGs represent the chemical forms in which the fatty acids are in adipose tissues. So most of the physical and chemical properties of backfat lipids are related to these of TAGs. The main TAGs in backfat of industrial pigs are POO (38%), PSO (24%), POL (13%) and OOO, PSL, PPO, OOL (3% each) where P, S, O, L mean palmitic, stearic, oleic and linoleic acids respectively (Davenel et al., 1999). TAG composition of backfat is strongly correlated with fatty acid composition. Despite very little is known about the factors involved in TAG composition differences between pigs, we can assumed that they are very similar to those of fatty acid composition. However, differences in TAG composition between backfat are more marked than those in fatty acid composition (Davenel et al., 1999). So TAG composition is more efficient for distinguishing adipose tissues according to the rearing conditions (Riaublanc, Gandemer, Gambotti, Davenel & Monin, 1999; Gandemer, Viau, Navarro, Sabio and Monin, 2000). The main interest in the knowledge of TAG composition is that it determines the melting point and the solid fat content of adipose tissues, parameters strongly correlated with the consistency of adipose tissue. Thus each TAG possesses a melting point depending on its fatty acid composition and on the position of each fatty acid on the glycerol moiety. Recently, it was established that solid fat content at 20°C is strongly correlated with disaturated TAG, namely PSO (R² > 0.90) because these TAGs are solid at ambient temperature (Davenel et al., 1999) On average, solid fat content of adipose tissue is around 20% with a large variation (from 7% to 30%). Backfat with a solid fat content at 20°C lower than 15-17% lacks of consistency (Davenel et al., 1999). Because of the low amount of PSO and the high amount of OOO in the backfat of Iberian pigs which contain a low proportion of stearic acid and a high proportion of oleic acid, the adipose tissues of these animals present the low solid fat content at ambient temperature (10-12%) (Gandemer et al., 2000).

2.2. Muscle

Intramuscular lipids refer to lipids contained in both intramuscular adipose tissue and muscle fibres. The intramuscular adipose tissue is comprised of cells located along the fibres and in the interfascicular area (Cassens & Cooper 1971). The fat cells are isolated or in clusters. They contain almost exclusively TAGs. The lipids of the fibres consist of cytosolic droplets of TAGs and membrane lipids, phospholipids (PL) and cholesterol. The amount of TAGs in the fibres only accounts for a small part of the total intramuscular TAGs.

Thus, intramuscular lipid content is low in muscles of industrial genotypes (2.5 - 3.5%)(Girard et al., 1988b). Pigs reared in traditional systems exhibit a high intramuscular lipid content. Thus, muscles of Iberian pigs and, to a lesser extent, of Corsican pigs have higher intramuscular lipid content than these of industrial pigs (7-12%)(Coutron-Gambotti et al., 1998 ; Secondi et al., 1992; Antequera, Cordoba, Ruiz, Martin & Ventana, 1993; Andrés, Cava, Mayoral, Tejeda, Morcuende & Ruiz., 2001; Tejeda, Gandemer, Antequera, Viau & Garcia, 2002). These differences are mainly related to TAG content, PL content is very similar in the muscles of all types of pigs. TAG content of muscles depends on numerous factors. However, recently, it has been postulated that intramuscular TAG content is under the control of a major recessive gene, named HIMF gene (Janss, Van Arendink & Brascamp, 1994). Only the double carriers of the HIMF allele exhibit a high intramuscular TAG content. It seems that the frequency of double carriers is higher in traditional unselected breed such as Meishan, Iberian and Corsican breeds than in industrial breeds selected against fatness during many years. For pigs reared in extensive systems, the main cause of the high lipid content of muscles is the fattening period which takes place when pigs are old (16-18 months) and have a low capacity to deposit muscle in the carcass and consequently deposit a large amount of the feed energy as fat in both adipose tissue and muscles. PL content of muscles is not greatly affected by rearing conditions(Gandemer, 1997). The main factor determining PL content of muscles in pigs is the metabolic type of the fibres (Leseigneur- Meynier & Gandemer, 1991). Glycolytic muscles contain less PL than oxidative ones. Fatty acid composition of TAG in muscles are very close to that of adipose tissues. In contrast, PL is characterised by high PUFA proportions (45-55%) where at least 1/3 are long chain PUFA with 4, 5 or 6 double bounds. The presence of these fatty acids explains why PL are the main substrate of lipid oxidation in muscles (Gandemer, 1999). Differences in fatty acid composition of PL in muscles are very small whatever the genotypes and the rearing conditions of pigs which suggests that the potential sensitivity of intramuscular lipids to oxidation is very similar whatever the origin of raw meat (Gandemer, 1997).

3 - Lipid changes during meat processing.

During dry-cured ham or fermented sausage processing, lipids are progressively altered through both lipolysis and oxidation. In the European southern countries, the processes include the same steps : salting, fermentation drying and ripening. However, large differences are observed in the time-temperature- relative humidity cycles, mainly during drying and ripening according to the process used for each product in each country (Toldra et al., 1998). Obviously these large variations in processing conditions affect the kinetics of reactions of lipolysis, and oxidation to a large extend.

3.1. Lipolysis in adipose tissues and muscles

Lipolysis is one of the main process of the degradation of lipids in fresh meat during processing (Toldra et al., 1998). Lipolysis is governed by a set of specific enzymes, namely lipases and phospholipases and leads to the formation of free fatty acids (FFA). Both endogenous enzymes of fat cells and muscle fibres and enzymes of bacteria are involved in lipolysis. However, it was established that the contribution of bacteria in lipolysis in dry fermented sausages is weak because the medium conditions are far from the optimal conditions of bacterial lipases (Molly, Demeyer, Johanson, Raemaekers, Ghistelinck & Geenen, 1997).

In fat cells of both adipose tissues and muscles and in muscle fibres, TAGs are hydrolysed by two important lipase systems : lipoprotein lipase (LPL) and hormono-sensitive lipase (HSL)(Belfrage, Frederikson, Strälfors & Tornqvist, 1984). The former acting at the capillary endothelium, is responsible for the degradation of lipoprotein TAGs and permits fatty acid uptake by the cell. The latter, located in the cytosol, hydrolyses TAGs and diacylglycerols permitting fatty acid mobilisation. A monoacylglycerol lipase ends the process by hydrolysing the monoacylglycerols. The activity of the monoacylglycerol lipase is higher than that of HSL (Belfrage, Frederikson, Strälfors & Tornqvist, 1984). This explains why monoacylglycerols do not accumulate in tissues. A third lipase system, an acid lipase located in the lysosomes, was described in adipose tissue and muscles but its activity is low (Belfrage et al., 1984).

Two lipases and three ones have been detected in backfat and in muscles of pigs, respectively (Motilva, Toldra & Flores, 1993) (Motilva et al., 1993a). In both backfat and muscles, lipases have been described as neutral and basic lipases corresponding probably to HLS and LPL. In addition, muscles present an acid lipase activity which is probably related to lysosomal lipase (Motilva et al., 1993a). Despite the activity of these lipases decreases during dry-cured processing, these enzymes remain active during the whole process for dry sausages and a large part of process for dry-cured hams (Hernandez, Navarro & Toldra, 1999; Motilva, Toldra, Nieto & Flores, 1993). In adipose tissues, neutral lipase remains active over 12 months in dry-cured hams suggesting that neutral lipase, namely HSL is the main enzyme involved in lipolysis in adipose tissue TAGs during dry-cured ham processing (Motilva, Toldra, Aristoy & Flores, 1993; Toldra et al., 1998). In dry-cured ham muscle, neutral and basic lipases are very active during the first 3-4 months of the process, then their activities decrease slowly. In contrast, acid lipase have low activity during the entire process (Motilva et al., 1993c). All these lipases exhibit an activity equal to 10-20% of their maximal activity up to 15 months of processing (Toldra, Flores, & Sanz, 1997). Acid and basic esterase activities have been described in ham adipose tissues and muscles. They have a higher activity than lipases and are more stable during processing (Motilva et al., 1993c). Very little is known about the factors affecting the activity of these enzymes. The activity of these enzymes varies according to the anatomical location of the muscles (Hernandez, Navarro, & Toldra, 1998; Flores, Alasnier, Aristoy, Navarro, Gandemer, & Toldra, 1996) and oxidative muscles have a higher activity of both acid and neutral lipases than glycolytic muscles (Flores et al., 1996). The activity of these enzymes are similar in light and heavy pigs (110-110 kg versus 160-170 kg) (Toldra, Flores, A

PLs are hydrolysed by specific enzymes named phospholipases. Phospholipases are divided into 3 main groups according to the ester bounds they hydrolyse (Waite, 1987). Phospholipases A_1 and A_2 hydrolyse fatty acids in 1 and 2 of the glycerol backbone of Pls, respectively. The lipolysis of PLs is ended by lysophospholipases which hydrolyse the remaining fatty acid after phospholipases A action. Phospholipases of mammal tissues have been widely studied because they play a key role in the metabolism of phospholipids (Waite, 1987). However, data on muscle phospholipases and lysophospholipases are limited. Most studies have been devoted to the mammalian heart phospholipases because of the role of these enzymes in ischemia. In heart, except those from lysosomal origin, the phospholipases and lysophospholipases have a neutral or basic pH optimum, are often Ca²⁺ dependent and membrane associated (Nalbone & Hostetler, 1985). Studies devoted to the enzymes in skeletal muscles are rare.

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Very little is known on the *post-mortem* activity of phospholipases in muscles. Phospholipases and lysophospholipases are active *post-mortem* in fresh muscles. The main activities are related to basic phospholipases A and lysophospholipases (maximum activity at pH 8-9) which are probably membrane-bound enzymes (Alasnier & Gandemer, 2000). Muscles, more specially glycolytic ones, exhibit also a low phospholipase A activity with acid pH optimum suggesting the presence of lysosomal phospholipases A. The activity of lysophospolipases is far higher than that of phospholipases in muscles (Alasnier et al., 2000). This result is consistent with the low proportion of lysophospholipids in the muscles during dry-cured ham processing because lysophospholipids formed by phospholipases are immediately hydrolysed by lysophospholipases. These enzymes are more active in oxidative muscles than in glycolytic ones (Alasnier et al., 2000). No data are available on the evolution the activities of these enzymes during dry-cured meat processing. However, we can postulate that these enzymes remain active because the proportion of long chain PUFA in the free fatty fraction increases for at least 6 months in dry-cured hams, giving evidence of PL hydrolysis (Buscailhon, Gandemer & Monin, 1994).

Free fatty acid (FFA) amount increases during processing both in dry-cured sausages (Molly, Demeyer, Civera & Verplasetse, 1996; Hernandez et al., 1999) and in dry cured hams (Coutron-Gambotti & Gandemer, 1999b ; Motilva et al., 1993c). Low in fresh meat products, FFA amounts sharply rise during dry-cured processing. Thus in dry cured hams FFA amount rises from 1-2% to 10-12% of the total. lipids in 10 months in adipose tissue. In muscle, the rate of lipolysis is fast during the first six months, but then slows towards the end of the process of dry cured hams (12 - 24 months). At the end of the process, FFAs account for 8 to 20% of total lipids in muscle according to the technology used and the raw material (Buscailhon, Gandemer & Monin, 1994a ; Motilva, Toldra, Nadal & Flores, 1994 ; Gandemer et al., 2000). then it increases slowly. In dry sausages, FFA content rises from 1-2% in raw matter to 4-5% in one month (Molly et al., 1996). In adipose tissue, the kinetic of FFA release is consistent with the decrease in neutral lipase activity during processing. The larger proportion of linoleic acid in FFA compared to that in TAGs (14% *versus* 8%) suggests that lipolysis preferentially affects the TAGs containing linoleic acid such as palmitoyl-oleyl-linoleyl-glycerol (Coutron-Gambotti et al., 1999a). These results could be explained by the liquid state of this TAG which differs from most of the TAGs of pig adipose tissue which are solid at the temperature of dry-cured ham processing (Davenel et al., 1999).

In muscle, both TAGs and PLs contribute to FFA generation and the relative contribution of these lipids depends on the TAG content of the raw material. In most cases, PLs are the main substrates for lipolysis in dry-cured hams (Buscailhon et al., 1994a ; Motilva et al., 1994 ; Flores, Nieto, Bermell & Miralles, 1987). This conclusion is supported by the fact that the FFA composition is closer to the fatty acid composition of PLs than to that of TAGs whatever the type of ham (Gandemer et al., 2000). The hypothesis of a PL origin of FFAs is consistent with the decrease in PL content in muscle during dry-cured ham processing (Buscailhon et al., 1994a ; Flores, Nieto, Bermell & Miralles, 1985 ; Coutron-Gambotti, Gandemer, Rousset, Maestrini, & Casabianca, 1999 ; Flores, Nieto, Bermell & Alberola, 1987) (Figure 1). However, TAGs also provide a significant amount of FFAs (30-50%) in muscles with very high TAG content such as muscles from Iberian and Corsican pigs (Martín, Córdoba, Ventanas & Antequera, 1999; Alasnier, David-Briand & Gandemer, 1999). Thus the contribution of TAGs to lipolysis is low in Bayonne, Parma and Seranno hams while it is more important in Iberian and Corsican hams (Gandemer et al., 2000).

Very little is known about the effects of *ante mortem* factors and technological parameters on lipolysis. Oxydative muscles contains more FFA than glycolytic muscles (Alasnier et al., 1999) which is consistent with the higher activity of phospholipases, lysophospholipases and both neutral and acid lipases in oxidative muscles (Flores et al., 1996 ; Alasnier et al., 2000). Lipolysis is not significantly affected by the VPe of pig diets during fattening (Cava, Ruiz, Ventanas, & Antequera, 1999). Heavy and light pigs have similar pattern of lipolytic enzymes (Toldra et al., 1996). Regarding technological parameters, it is clear that the time/temperature cycles of the different stages of processes greatly affects FFA content and lipolytic enzyme activity. The longer is the stage and higher is the temperature, the higher is the FFA content of the hams. Other parameters have obtained less attention. The use of frozen raw matter instead of refrigerated one and reducing salt content

of ham by reducing the time of salting stage have no subsequent effect on lipolysis during dry-cured ham processing (Motilva et al., 1994; Coutron-Gambotti et al., 1999b). Muscles with a low initial pH (<6.1) have a higher FFA content all along the process indicating that a low initial pH in meat promotes lipolysis (Buscailhon, Berdagué, Gandemer, Touraille & Monin, 1994d).

Many authors have postulated that lipolysis promotes lipid oxidation during dry-cured ham processing (Buscailhon, et al., 1994a; Cava et al., 1999; Antequera, Lopez-Bote, Cordoba, Garcia, Ascensio, Ventanas, Garcia-Regueiro & Diaz, 1992). This assertion is based on the concomitant increase in the amounts of both lipolysis and oxidation compounds during the first stages of processing. However, nobody have clearly established a close relationship between the two processes of lipid degradation. Moreover some recent data support the hypotheses that the two processes would not be interrelated or lipolysis protects long chain PUFA against oxidation. Thus, several authors underline that the amounts of various volatile compounds arising from lipid oxidation decrease during the last months of processing whilst FFA amounts always increase (Buscailhon, Berdagué & Monin, 1993; Ruiz, Ventanas, Cava, Andres, & Garcia, 1999) and some parameters promoting lipolysis have no effect on volatiles arising from lipid oxidation or on aroma notes related to oxidation products (Buscailhon, Berdagué, Bousset, Cornet, Gandemer & Touraille, 1994e). In addition the amount of FFAs reaches 8-20% of total lipids in muscles at the end of the dry-cured process and FFAs contain almost all the long chain PUFAs initially esterified in PL of fresh meat (Buscailhon et al., 1994a; Coutron-Gambotti et al., 1999b). These results strongly suggest that the hydrolysis of PL during processing protects the long chain PUFAs from oxidation. However, the exact mechanism remains unknown.

3.2. Lipid oxidation in adipose tissue and muscle

Lipid oxidation is one of the main causes of deterioration in the quality of meat during storage and processing (Asghar, Gray, Buckley, Pearson, & Booren, 1988 ; Gray, Gomaa & Buckley, 1996 ; Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). However, it do not only contributes to off-flavour but it is also essential to the typical aroma of many meat products (Shahidi, Rubin & D'Souza, 1986). The overall mechanism of fatty acid oxidation is well established. The main process of lipid oxidation in muscle food is a chemical process named autoxidation. It is a free radical process involving initiation, propagation and termination steps (Frankel, 1982 & 1985). The initiation step takes place by the removal of a hydrogen from a methylene carbon in a fatty acid to form an alkyl radical (L*). This process affects polyunsaturated fatty acids (PUFA) preferentially because it is easier to remove a hydrogen from a methylene carbon as the number of double bounds in the fatty acid increases (Frankel, 1985 & 1984). This explains why PLs which contain a large a amount of these fatty acids, are the main substrates of lipid oxidation in muscles (Wilson, Pearson & Shortland, 1976 ; Gandemer, 1997). Lipid oxidation initiates by a large number of molecules in muscles including chemicals such as reactive oxygen species (OH*) and iron-oxygen complex (Asghar et al., 1988). The propagation step starts with a reaction between the L° radical and oxygen to form a peroxyl radical (LOO°) which then extracts a hydrogen from another fatty acid and forms hydroperoxides (LOOH), the primary products of autoxidation. The termination step starts by hydroperoxide decomposition which leads to the formation of numerous volatile and non volatile compounds through a very complex set of reaction pathways (Frankel, 1984). These reactions have been studied extensively and attention has been focused on volatile products because of their impact on aroma. The nature and the relative proportions of compounds in the volatile fraction extracted from foods depend on numerous factors. Among them, fatty acid structure is the most important because it affects the number and the proportion of hydroperoxide isomers (Frankel, 1982). Another important factor is the conditions in which peroxides are formed and decomposed, including the mechanisms of oxidation (autoxidation, thermo-oxidation, photo-oxidation, etc...) and the medium conditions (temperature, pH, presence of iron, etc) (Frankel, 1985 ; Grosch, 1987). A large variety of volatiles including alkanes, aldehydes, alcohols, esters and carboxylic acids arises from this process (Frankel, 1982). Among the numerous volatiles formed, the most important are those with a low odour threshold because of their impact on aroma of foods. These are aldehydes and several unsaturated ketones and furan derivatives (Grosch, 1987). They include C3-C10 aldehydes, C5 and C8 unsaturated ketones and pentyl or pentenyl furans. These compounds have a large variety of aroma notes and their odours have been described as oily, tallowy, deep-fried, green, metallic, cucumber, mushroom and fruity. In muscle food time course of lipid oxidation is currently evaluated using peroxide value and TBA-reactive substance measurements and more recently by volatile compound quantification. Low in fresh adipose tissue and muscle, the level of hydroperoxides rises rapidly to reach a maximum several months after the beginning of the process and then it decreases slowly to the end of the process. It is difficult to know exactly when peroxidation reaches its maximum during processing because of the instability of these molecules. However, peroxide value reaches its highest value generally 2-4 months after the beginning of the process. In constrat, TBA test value and volatile content of muscle and adipose tissues show a continuous increase during several months during dry-cured ham processing (Figure 2). At the end of the process, the general oxidation level in muscle and adipose tissues tends to decrease (Buscailhon et al., 1993 ; Ruiz et al., 1999 ; Hinrischen & Pedersen, 1995). Oxidation leads to a significant decrease in long chain polyunsaturated fatty acids in both FFA and PLs during dry-cured ham processing (Buscailhon et al., 1994a, Coutron-Gambotti et al., 1999b). Many factors are involved in the control of lipid oxidation in muscles (Morrissey et al., 1998). High temperatures and long time of drying and ripening favour lipid oxidation (Toldra and al., 1998). High salt content increases oxidation product content in dry cured hams (Coutron-Gambotti et al., 1999b). In contrast, high vitamin E content in muscles prevents dry-cured hams from oxidation (Cava et al., 1999). A low initial pH in muscles favours lipid oxidation in dry-cured hams (Buscailhon et al., 1994d). The genotype of pigs have a limited effect on lipid oxidation (Berdagué, Bonnaud, Rousset & Touraille, 1993a). Volatiles arising from lipid oxidation have been extensively studied in dry cured hams of various countries (French : Buscailhon et al., 1993 ; Berdagué et al. 1993a ; Italian : Bolzoni, Barbieri & Virgili 1996 ; Barbieri, Bolzoni, Parolari, Virgili, Buttini, Careri, & Mangia, 1992 ; Careri, Mangia, Barbieri, Bolzoni, Virgili & Parolari, 1993 ; Spanish Serrano and Iberian : Flores, Grimm et al., 1997 ; Dirinck, Van Opstaele & Vandendriessche 1997 ; Garcia, Berdagué, Antequera, Lopez-Bote, Cordoba & Ventanas, 1991 ; Garcia et al., 1991) and also in dry sausages (French saucisson : Berdagué, Monteil, Montel & Talon, 1993b ; Milano salame : Meynier, Novelli, Chizzolini, Zanardi & Gandemer, 1999). More than 100 volatile compounds were identified. They are formed from lipids, carbohydrates and amino-acids. Whatever the type of dry-cured hams, most of the volatiles are formed by lipid oxidation. In dry sausages, volatiles arising from lipid oxidation are not always the main volatiles because of the presence of spices which are often potent antioxidants (Meynier et al., 1999). The main volatiles generated through oxidation process are aldehydes. The contribution of these compounds to flavour depends on the chemical structure of molecules and on their concentrations (Shahidi et al., 1986). Aldehydes formed through fatty acid oxidation are linear saturated (C5 to C10 alkanals), unsaturated (C5 to C11 alkenals) with some polyunsaturated ones (2,4 nonadienal and decadienal). These adhedydes have a large impact on the overall aroma of dry cured meat products because of their typical aroma and their low odour threshold (Shahidi et al., 1986 ; Dirinck et al., 1997). These aldehydes exhibit unpleasant odours such as fatty, oily, rancid, deep fried (nonanal, t-2-heptenal, 2pentyl-furan, 2,4 decadienal) whereas other volatiles have more pleasant odour notes such as greenish odour (hexanal). Ketones arising from lipid oxidation are mainly methylketones (C5-C10). They exhibit a large variety of aroma notes such as fruity (2-heptanone, 2-decanone, 2undecanone) oily and fatty (2-dodecanone) or blue cheese (2-heptanone) (Shahidi et al., 1986). One of the possible origin of these methyl

ketones are an incomplete β-oxidation of free fatty acids by bacteria. Thus, some Staphylococci added as starters in dry-fermented sausages such as French saucisson produce methyl ketones in a significant amount (Talon, Leroy-Sétrin & Fadda, 2002). They are Staphylococci. carnosus and S. xylosus. Moreover these strains possess a catalase and superoxide dismutase activity which prevents fatty acids from autoxidation (Talon et al., 2002). So the choice of the starters affects the balance between oxidation products arising from autoxidation and β-oxidation of fatty acids. Some bifonctional ketones such as 3-hydroxy-2-bytanone have been detected (Careri et al., 1993). They are formed by oxidation of hydroxy fatty acids. They have buttery aroma notes. Linear, saturated or unsaturated alcohols (C4-C8) are also from lipid oxidation origin. They contribute to the overall aroma of dry-cured meat products, mainly unsaturated such as 1-octen-3-ol (mushroom) and 1-penten-3-ol (grass) (Shahidi et al., 1986). In contrast, hydrocarbons have probably no significant impact on aroma because of their high odour threshold (Shahidi. et al., 1986; Dirinck et al., 1997). Whatever the type of dry-cured products, volatile fraction show a similar set of oxidation products. It isn't surprising because the fatty acid compositions of TAGs and PLs which determine the type of volatiles formed during oxidation are similar in all the types of pigs used to produce these hams (see part 1)(Table 3). So the main differences in lipid oxidation products between dry-cured meat products are mainly related to the quantity of volatiles formed during the process (Buscailhon et al., 1993; Dirinck et al., 1997; Garcia et al., 1991) which depends on raw material traits such as intramuscular lipid content (Berdagué et al., 1993a) and on some processing traits such as the length and temperature of the process (Buscailhon et al., 1993; Ruiz, et al., 1999), the amount of salt (Coutron-Gambotti et al., 1999b), the antioxidation pattern of the raw meat or the mixture of bacteria strains in starters and degradation of amino acids and carbohydrates (Dirinck et al., 1997). So to evaluate the impact of lipid oxidation in aroma of dry-cured hams, it is essential to determine precisely the contribution of each volatile compound to the overall aroma of dry-cured hams.

4- Lipids and sensory traits of dry-meat products

4.1. Lipids and quality of dry sausages.

Adipose tissue traits determine several quality attributes of dry-cured sausages because this tissue accounts for at least 30% of the raw materiel of these products. The production of high quality dry cured sausages such as French saucisson or Milano salami requires adipose tissue with specific physical properties. The producers are looking for firm adipose tissue. The consistency of adipose tissue have been recognised as the most important quality trait to consider in the choice of adipose tissue for dry-cured meat product processing (Girard et al., 1988a; Whittington, Prescott, Wood & Enser, 1986) because lack of consistency induces many quality defaults in the final products. When adipose tissue lacks of consistency, dry meat products are difficult to cut and cohesiveness of cuts is poor because adipose tissue and muscle pieces of the sausages tend to separate. Cuts are oily because of the liquid fat which exudes from adipose tissue and covers the meat. In general, adipose tissue which low consistency has a yellow colour and a translucent appearance while firm adipose tissue appears white or slightly pink (Girard et al., 1988a; Davenel et al., 1999). Moreover, liquid fat exuded from fat cells covers the small meat cubes and prevents the product from drying. Because soft adipose tissue contains a high amount of linoleic acid with a low amount of SFA, it is very sensitive to lipid oxidation and becomes rancid faster than firm adipose tissue.

The overall aroma of dry sausages strongly depends on the type of starters used in the fermentation stage of the products. Among the bacteria used in the mixture of starters, *Staphylococci* play a key role in the formation of volatiles. Some orientate the fermentation process towards the production of carbohydrate degradation products (*S. warneri*), others towards linear oxidation products through classic autoxidation reactions (*S saprophyticus*) while others favour the generation of methylketones (*S. carnosus*) (Berdagué et al., 1993b; Talon et al., 2002). Thus, because of the specific aroma of each group of volatiles, the overall aroma of dry-sausages is very different according to the starter composition. Thus Berdagué et al (1993b) have established that French saucisson prepared with starters containing *S. carnosus* contains more methylketones and exhibit stronger aroma of dry-cured meat products. In contrast, those produced with *S warneri* producing higher amount of volatiles from carbohydrates such as 1,3 and 2,3 butanedione and diacetyl exhibit a more pronounced buttery aroma. In dry sausages, many others additives such as spices greatly affected the time course of formation of volatiles from lipid oxidation. Thus these additives are often potent antioxidant reducing the formation of volatiles arising from lipid oxidation and they have strong specific aroma, which can mask aroma notes of lipid oxidation volatiles (Meynier et al., 1999). Moreover, differences in aroma of various Milano salami collected on the local market are mainly related to the type of spices used by the manufacturer (Meynier et al., 1999).

4.2. Intramuscular lipids and dry-cured ham quality

Intramuscular lipid content affects several quality traits of dry-cured hams. The first one is the aspect of slices because intramuscular lipids become visible when their content in muscles exceeds 5% (Buscailhon et al., 1994c). The hams with high intramuscular lipid content such as muscles from Iberian and Corsican pigs have a higher marbling score than those of Parma and Bayonne Hams (Rousset & Martin, 1998). Intramuscular lipids strongly affect colour of ham slices. Thus, redness and brightness scores of ham cut decrease as intramuscular lipid content increases (Gou, Guerrero & Arnau, 1995). Intramuscular lipid content also affects texture of hams. Thus, High intramuscular lipid content has a positive impact on ham tenderness (Parolari, Rivaldi, Leonilli, Bellati & Bovis, 1988). Hams produced from genotypes with high intramuscular lipid content have more intense fat aroma (Hinrischen et al., 1995) because intramuscular TAGs are a good solvent for most aroma compounds (Shahidi et al., 1986). The higher is the intramuscular TAG content of muscle, the higher is the quantity of aroma compounds traps in the ham. Lipids play a key role in the overall aroma of dry-cured hams because of the volatiles generated through oxidation. The overall aroma of hams depends on the equilibrium between volatiles arising from lipid oxidation and those arising from amino acid and carbohydrate degradation reactions. There is some evidence of independence between the reactions producing volatile compounds from either lipid oxidation or amino acid degradation (Buscailhon et al., 1993; Ruiz et al., 1999). Many studies have tried to establish relationships between aroma traits of dry-cured hams described by panellists and volatile compounds extracted from the same hams. These studies lead to some consistent conclusions. Thus, dry cured hams produced through a long ripening process have the highest aroma intensity because they have the highest amounts of all kind of volatiles generated through both lipid and amino acid degradation (Ruiz, J. et al., 1999). During ripening, the aroma of dry-cured ham changes from fat, pork, fresh meat aroma notes to dry-cured and aged aroma notes (Flores et al., 1997; Ruiz et al., 1999; Dirinck et al., 1997; Buscailhon et al., 1994e). During the first steps of processing (salting, drying and first part of ripening), volatiles mainly arise from lipid oxidation while those formed during the second part of ripening stage are formed from both lipid and amino acid degradation (Hinrischen et al., 1995). Rancid aroma is correlated to oxidation products, mainly to aldehydes such as nonanal and 2-hexenal which exhibit a strong rancid odour (Ruiz et al., 1999; Berdagué et al., 1993a). Positive aroma notes such as "cured ham", "dry-cured ham" or "aged" aroma notes have been correlated to either branched aldehydes arising from amino acid degradation or methylketones arising from lipid oxidation (Flores et al., 1997; Hinrischen et al., 1995; Buscailhon et al., 1994e). All these results are consistent with the differences between the hams from different European countries. Thus, hams with a short processing (9-12 months) such as Italian country style, French Bayonne and Italian Parma hams exhibit an aroma characterised by a fresh meat and fat aroma. In contrast,

hams with a long ripening process (18-24 months) such as Corsican and Iberian hams have a more pronounced aroma with strong "aged" and "cured aroma" notes (Rousset et al., 1998). Aroma of Parma hams differs from that of other hams because of the high ester content of volatile fraction which have a pleasant fruity aroma (Barbieri et al., 1992; Careri et al., 1993).

5. Conclusion

Lipids are largely involved in the quality of dry-cured hams and dry-fermented sausages. Both the quantity and the composition of adipose tissues and intramuscular lipids have to be considered. Lipid traits of the fresh adipose tissue and muscle affected by rearing conditions, are lipid content of both tissues and consistency of adipose tissue. These traits influence mainly aspect of cuts such as marbling, oily aspect, colour and, also flavour of the final product because intramuscular fat traps volatile aroma formed in aqueous phase of the muscles. During processing, lipids undergo an intense lipolysis controlled by both lipase and phospholipases. Because of the long processing time, often at relatively high temperature, a large amount of the volatile compounds generated in dry-cured meat products are formed through fatty acid oxidation through both autooxidation process and bacteria incomplete β oxidation. These molecules are aldehydes, ketones, hydrocarbons, and alcohols. Aldehydes are responsible of rancid aroma while methylketones have a more positive impact on ham aroma (aged and dry-cured ham odours). The control of lipid oxidation and the balance between volatile compounds arising lipid oxidation, carbohydrate fermentation, amino acid degradation and additives such as spices, are the critical points to control for producing high quality dry-cured meat products. Further studies are required for a better understanding of the contribution of individual molecules and the processes involved in their generation to the typical aroma of dry-cured meat products such as aged flavour and dry-cured flavour.

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Table 1. Fatty acid and triacylglycerol compositions and solid fat content (SFC) of adipose tissues from pigs of various European areas (as % methyl esters or triacylglycerols)(adapted from Gandemer etal., 2000)

ta dagi kerinti yela berm mali farina kerinti yela berma mali farina kerinti bermana ke	Hams Parma n = 10	Italian Country style n = 9	Bayonne n = 10	Corsican n = 10	Serrano n = 10	Ibérian n = 10	Statistical effect
Fatty acids (%)	/	agents in B. Rent	ipo distriction de la construcción de la construcci		and a cost and	ala Sectional d	1. CONSTRAINT-1-2-1
16:0	22,7ª	22,6 ab	22,2 abc	21,6 ^{bc}	21,0 ^{cd}	20,4 ^{cd}	***
18.0	12.4^{a}	12.5 ^{<i>a</i>}	12,9 ^{<i>a</i>}	$12,1^{ab}$	$11,2^{bc}$	10,6 ^c	***
18 · 1	48.7 ^c	49.2 ^c	49.1 ^c	51,7 ^b	51,5 ^b	54,9 ^{<i>a</i>}	***
$18 \cdot 2 n_{-6}$	10.7^{a}	9.1 ac	9.9 ab	8.7 ac	$10,2^{ab}$	8,4 ^c	*
18 : 3 n-3	0,4 ^c	1,1 ^{<i>a</i>}	0,7 ^{<i>abc</i>}	1,0 ^{<i>ab</i>}	0,8 ^{<i>ab</i>}	0,6 ^{ac}	**
Friacylglycerols (%)	D. G. 199	B D Diversion	ran des la contra		and the law		
PSO	26.0 ^{<i>a</i>}	26,6 ^{<i>a</i>}	27,5 ^{<i>a</i>}	25,6 ^{<i>a</i>}	19,9 ^{<i>ab</i>}	18,9 ^b	***
200	43.0 bc	43.8 bc	41,6 ^c	$42,9^{bc}$	45,8 ^{ab}	47,8 ^{<i>a</i>}	**
000	4 0 ^c	4.1 ^c	4.4 ^c	6.5 ^b	$6,2^{b}$	10,9 ^{<i>a</i>}	***
000	1.8°	1.6 ^{bc}	1.9 ^c	2.2°	2,7 ab	3.0 ^{<i>a</i>}	***
SFC at 20°C (%)	16,7 <i>^a</i>	17,0 ^{<i>a</i>}	17,3 ^{<i>a</i>}	15,9 ^{<i>a</i>}	12,0 ^{<i>b</i>}	10,8 ^b	***

On the same row, means supperscript by different letters are significantly different at the level : 5 % (*), 1 % (**), or 0,1 % (***), ns : non significant.

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Table 2. Lipid composition of biceps femoris muscle from pigs of various European areas (adapted from Gandemer et al., 2000)

CRARKS PED STOLET SS	Ibérian n = 10	Corsican n = 6	Serrano n = 6	Bayonne n = 40
Lipid content (g/100g fresh meat)	and the state of the line	nie leade light 8 three	(Ganaliana)	1 A A A A A A A A A A A A A A A A A A A
Total lipids	93(29)	53(16)	35(05)	26(01)
Triacylglycerols	86(29)	46(16)	2,7(0,5)	2,0(0,1)
Phospholipids	0,0(2,))	-4,0(1,0)	2,7 (0,5)	2,0(0,1)
1 prob	0,72 (0,09)	0,71 (0,01)	0,75 (0,00)	0,00 (0,01)
Fatty acid composition (% methyl esters)				
Triacylelycerols				
Saturated	221(0.9)	276(66)	25.2 (1.5)	27.1.(1.2)
Monounsaturated	52.1(0,8)	27,0(0,0)	55,0 (1,5)	37,1 (1,3)
n-6	62,3 (0,8)	65,1 (5,6)	55,9 (2,8)	57,8 (2,9)
n-3	5,0 (0,4)	6,5 (1,8)	7,9 (2,1)	4,8 (2,6)
Polyungature	0,6 (0.04)	0,8 (0,3)	0,9 (0,4)	0,4 (0,2)
oryunsaturated	5,6 (0,5)	7,3 (2,1)	8,8 (2,3)	5,2 (3,1)
Phospholipids (% methyl esters)				
Saturated	30 5 (1 6)	29 4 (2 1)	314(0.5)	20 5 (3 6)
Monounsaturated	21.1(3.6)	15.6(1.3)	18.2(2.0)	29.5(5,0)
18:2n-6	21,1(3,0) 21,4(2,4)	13,0(1,3)	10,2(2,9) $20 \in (1,6)$	21,4 (4.0)
20 :4n-6	51,4(2,4)	55,6 (1,0)	30,0 (1,0)	32,2 (5,2)
n-6	11,8 (1,5)	14,6 (1,5)	12,1(1,7)	10,8 (2,0)
n-3	45,5 (2,5)	50,6 (1,5)	46,1 (1,6)	46,0 (6,8)
Polyupsatarí	2,9 (0,5)	4,5 (0,4)	4,3 (0,3)	3,1 (0,1)
Value	48,4 (3,1)	55,0 (1,8)	50,4 (3,2)	49,1 (6,8)

Values between brackets are standard deviation

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Table 3. Volatiles arising from lipid oxidation in dry-cured hams. (number in parentheses is the proportion of the components in % of total volatiles of ham) (adapted from Coutron, 1986)

		Amount	Typical odour
alter detto articular la		(μg d'eq. nonane/ 30 g of ham)	
Alkanes, alkenes	and a company of the space	5070 (39%)	
	Pentane	56	
	Hexane	45	
	heptane	445	
	octane	4296	
	nonane	114	
	deacane	16	
	undecane	7	
	1-octene	35	
	4-octene	44	
411	2-octene	12	
Aldehydes		4011 (31%)	
	Pentanal	80	bitter, almond
	hexanal	3021	green, bitter, fruity
	heptanal	533	oily, fruity, soapy-fruity
	octanal	228	fatty, soapy-fruity
	nonanal	107	tallowy, soapy-fruity, rancid
	decanal	11	orange peel
	t-2-heptenal	18	putty, fatty
Kota	cis-2-heptenal	13	
Actones		631 (5%)	
	butan-2-one	46	
	pentan-2-one	399	fruity
	hexan-2-one	46	
	heptan-2-one	124	blue cheese
	octan-2-one	11	fruity, green
Alash	3- octan-1-one	6	
riconols		663 (5%)	
	pentan-1-ol	320	fruity
	hexan-1-ol	54	green, floral
	1-penten-3-ol	242	buttery, green
Esters	1-octen-3-ol	47	mushroom
	Ethyl- butanoate	5	
	Ethyl- hexanoate	8	
Others	Ethyl- octanoate	2	
_	2-pentylfuran	24	buttery, rancid

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Figure 2 : Changes in volatiles arising from lipid oxidation during dry-cured ham proccessing (adapted from Buscaillon et al. [46]



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