LIPID COMPOSITION OF TWO RABBIT MUSCLES OF OPPOSITE METABOLIC TYPE.

ALASNIER C., VIAU M. and GANDEMER G.

Institut National de la Recherche Agronomique, LEIMA, Unité "Lipides-Flaveur", Nantes, France.

S-IVB.44

SUMMARY

The comparison of the lipid composition of an oxidative muscle, *Semimembranosus propriosus* and of a glycolytic one, *Longissimus dorsi* showed that the oxidative muscle contained more total lipids, more triglycerides and more phospholipids than the glycolytic one. The phospholipids of *Semimembranosus propriosus* exhibited a higher proportion of cardiolipin and phosphatidyl-ethanolamine and conversely a lower proportion of phosphatidyl-choline and phosphatidyl-inositol. The fatty acid composition of triglycerides was similar in both muscles. The phospholipids had the same proportion of polyunsaturated fatty acids in the two muscles, but they contained more saturated fatty acids and less monounsaturated ones in the *Semimembranosus propriosus* than in the *Longissimus dorsi*.

Introduction

Carcasses of farm animals are formed at least of 200 muscles. Most of the muscles are composed of a mixture of two or three types of fibres. Ashmore and Doerr (1971) distinguished three types of fibres according to their metabolic and contractile properties (aW, aR and bR). It is generally accepted to name oxidative muscle the one containing a large amount of aR + bR fibres and glycolytic muscle the one mainly formed of aW fibres. Many papers suggested that metabolic type is one of the main factors involved in the variability of meat quality. Thus, it was stated that oxidative muscles gave more tasty and juicy meat than glycolytic ones (Valin *et al.*, 1982). However lipids and myoglobin oxidized faster in oxidative muscles than in glycolytic ones (Wilson *et al.*, 1976 ; Renerre et Labas, 1987). That is the reason why it is very important to characterize the composition of the muscles in relation to their metabolic type. The knowledge of these parameters is the preliminary step to understand the mechanisms involved in the determinism of meat quality. For this purpose we have chosen to work on rabbit muscles. Despite rabbit meat is not frequently eaten in European countries, it is a very convenient model for this kind of study because rabbit is cheap to produce and easy to handle. Moreover, an accurate determination of metabolic type of several muscles have been performed using enzymatic activity measurement or histochemical method (Briand and Briand, 1986 ; Leberer and Pette, 1984). These studies showed that some muscles of the rabbit carcass are practically of pure metabolic type. In the present study, we have determined the lipid composition of two rabbit muscles of opposite metabolic type : an oxidative one, the *Semimembranosus propriosus* and a glycolytic one, the *Longissimus dorsi*.

Materials and Methods.

Animals : Eighteen rabbits (female, hybrid N2 Californian 67) fed a standard commercial diet were slaughtered at 11 week-old. They were bled, skinned and eviscerated. The *Longissimus dorsi* at the level of the 1-4th lumbar vertebra and the entire *Semimembranosus propriosus* were dissected from the carcasses. These muscles were carefully trimmed and minced in a blender. Lipids were immediately extracted.

Lipid extraction : Lipids were extracted from fresh muscle with chloroform/methanol (2:1) according to the method of Foliate *et al.* (1957). The extracts were dryed under vacuum on a rotary evaporator. The total lipid content was weighed and expressed in g / 100 g of meat.

Lipid extract fractionation : The total lipid extracts were separated into neutral and polar lipids on silica cartridges (Sep-Pack, Waters) following the procedure described by Juaneda and Rocquelin (1985). The phospholipid content was calculated (P x 25) after phosphorus had been determined in the total lipid extract by the method of Bartlett (1959). The neutral lipid content was estimated by the difference between total lipid and phospholipid contents. Since the neutral lipid fraction contained mostly triglycerides, we shall refer to triglycerides in the text. Results were expressed in g / 100 g of muscle.

Phospholipid composition : The analyses were performed by high performance liquid chromatography using a Gilson binary solvent delivery system equipped with a rheodyne valve injector. Phospholipids were quantified with an evaporative light scattering detector (DDL 10, Cunow, France) working in the following optimum conditions : nebulizer gas pressure : 2.2 bar, auxiliary gas pressure : 0.5 bar, temperature of the evaporator chamber : 40°C. The detector gave a linear response for phospholipids over the range 5 to 200 mg (Leseigneur *et al.*, 1989). It was paired with a suitable computer and software (Apex, Stang- France). We used a stainless steel column (25 cm long x 4.5 mm i.d) packed with silica (5 mm Licrospher Si 60, Merck). The main phospholipids were eluted in 25 minutes using a gradient mode according to the procedure of Leseigneur *et al.* (1989). The eluants A and B were respectively pure $CHCl_3$ and a mixture containing CH_3OH, H_20, NH_4 and $CHCl_3$ in the following proportions : 92:5:2:1. The amount of eluant B increased from 0% to 20 % in 10 min and then from 20% to 100 % over a further 10 min. It was held at 100 % for 10 min. The flow rate was 1.5 ml/min. Individual phospholipid classes amounts were expressed in mg /100g of muscle and in percent of total phospholipid content.

Fatty acid composition : Fatty acid composition of triglycerides and phospholipids was determined by gas chromatography. Methyl esters were prepared as described by Morrison and Smith (1964). The gas chromatograph was a DANI 6500HR apparatus equipped with a split injector and a flame ionization detector. It was paired with a CR3A integrator (Shimadzu). We used a capillary column (30 m long, 0.32 mm internal diameter, 0.1 mm film thickness) containing a polar stationary phase (Superox II, Alltech). The split flow rate was set at 40 ml/min. The head pressure of the hydrogen carrier gas was 0.6 bar. The oven temperature was held at 150°C for 4 min, increased to 200°C at 10°C/min, and then maintained at 200°C until the end of the analysis. The individual fatty acid peaks were identified by comparing their retention times with those of standard fatty acid mixtures. The results were expressed in percent of the amount of methyl esters present.

Statistical analysis : The lipid composition of the two muscles were compared by a t test.

Results and Discussion.

Lipid content : The *Longissimus dorsi* had lower total lipid, triglyceride and phospholipid contents than the *Semimembranosus propriosus* (1.08 versus 2.86%, 0.47 versus 2.03% and 0.61 versus 0.83% respectively)(Table 1). Despite these results suggest that oxidative muscles contain more total lipids than glycolytic ones, studies performed on a larger number of muscles indicate that the total lipid content of muscles is not strictly related to their metabolic type (Leseigneur-Meynier and Gandemer, 1991). Conversely, it is largely admitted that the oxidative muscles contain more phospholipids than the glycolytic ones in farm animals. This result is explained by the fact that the oxidative muscles are formed of fibres smaller in diameter and containing more organelles such as mitochondria than the glycolytic ones. Consequently the oxidative muscles contain more membrane and correlatively more phospholipids per unit of weight as compared to the glycolytic ones. This conclusion should be extend to the rabbit muscles according to the results published on the diameter of the oxidative and glycolytic fibres and on the activity of mitochondrial enzymes in muscles.

Phospholipid composition : As described in the pork, beef and chicken muscles, the oxidative muscle of rabbit showed a higher proportion of cardiolipin and phosphatidyl-ethanolamine (PE) than the glycolytic one (7.3 versus 3.3% and 27.8 versus 23.6% respectively)(Table 1). Conversely it contained a lower proportion of phosphatidyl-choline (PC) and phosphatidyl-inisitol (PI) (58.8 versus 64.4% and 5.1 versus 7.1% respectively). Expressed as mg / 100 g of muscle, the *Semimembranosus propriosus* had a larger amount of cardiolipin, PE and PC (x 3, x 1.5, x 1.2, respectively). This result is related to the mitochondria content of the fibres. The membranes of these organelles are the only ones to contain cardiolipin and are richer in PE as compared to the other membranes (plasma membrane,

reticulum). Thus oxidative muscles which contain a large number of mitochondria showed a higher amount of cardiolipin and PE.

Fatty acid composition : The fatty acid composition of the total lipid extract of rabbit muscles was characterized by its high proportion of polyunsaturated fatty acids (PUFA) (29 to 34%). This value is far higher than these generally observed in chicken, pork and beef muscles. The main PUFA were 18:2 n-6 (19-24%) and 18:3 n-3 (3-4%) with appreciable amount of long chain PUFA (3.5-9%). The intramuscular lipids contained less PUFA in the *Semimembranosus propriosus* than in the *Longissimus dorsi* (28.8 *versus* 34.4%). This difference between muscles could be related to the triglyceride proportion in the total lipid extract. Indeed triglycerides contain less PUFA than phospholipids. So in muscles with a high lipid content such as *Semimembranosus propriosus*, the triglycerides accounted for a large part of the total lipid extract (68%) and consequently the intramuscular lipids of these muscles contained less PUFA than those of *Longissimus dorsi* in which triglycerides accounted for less than 50% of the total lipid extract.

The fatty acid composition of the triglycerides exhibited a high proportion of PUFA (28-30%), mainly as 18:2 n-6 (21-23%) and 18:3 n- 3 (5-6%)(Table 2). This result should be linked to the fatty acid composition of the animal feed which is essentially made of dehydrated luzern. No difference was observed between the fatty acid compositions of the two muscles. This result is consistent with those obtained in pork and chicken muscles and indicates that the metabolic type of the muscles had only a weak influence on this parameter.

The proportions of saturated, monounsaturated and polyunsaturated fatty acids in the phospholipids were 34-36%, 19-20% and 43-45% respectively (Table 2). This results shows good agreement with those obtained in other farm animals (Gandemer, 1990). The main PUFA were 18:2 n-6 (27-28%) and 20:4 n-6 (8-9%). The phospholipids contained an appreciable amount of long chain PUFA of the n-3 serie (20:5, 22:5 and 22:6 : 2.7-3.5%). No significant difference was observed in PUFA proportions between the two muscles (45% in the *Longissimus dorsi versus* 43% in the *Semimembranosus propriosus*). However the phospholipids of the *Semimembranosus propriosus* contained less monounsaturated fatty acids, less 20:4 n-6 and less n-3 fatty acids as compared to those of the *Longissimus dorsi*. Conversely, they exhibited a higher proportion of saturated fatty acids (38.5 *versus* 34.5%). These results seem to indicate that the metabolic type of muscles affects the phospholipid fatty acid composition. However the results obtained from only two muscles don't allow to conclude definitively. Thus it is difficult to relate the differences in phospholipid fatty acid compositions between the two muscles to their metabolic type rather than to a particularity due to their anatomical location. So in a previous study on five pork muscles, we have demonstrated that the *Longissimus dorsi* exhibited an atypical phospholipid fatty acid composition but the fatty acid composition of phospholipids was similar in the four other muscles whatever their metabolic type. Hence to conclude on this point, this study should be extend to a larger number of muscles.

Conclusion

Within the lipid parameters studied, only phospholipid content and composition are related to the metabolic type of the muscles in rabbit. These results are in good agreement with those obtained with other farm animals such as pork, chicken, turkey or beef. This leads us to conclude that rabbit should be a convenient model for the study of the effect of the metabolic type of the muscle on meat quality traits.

References

Ashmore, C.R. and Doerr, L. (1971). Postnatal development of fiber types in normal and dystrophic chick muscle. Exp. Neurol., 30 : 431-446.

Bartlett, G. (1959). Phosphorus assay in column chromatography. J. Biol. Chem, 234 : 466-468.

Briand, M. and Briand, Y. (1986). Metabolic and contractile properties of rabbit muscles : A statistical approach. Comp. Biochem. Physol., 85B : 481-485.

Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226 : 497-509.

Gandemer, G. (1990). Les phospholipides du muscle : composition et altération au cours des traitements technologiques. Rev. Franç. Corps Gras, 5 : 75-81.

Juaneda, P. and Rocquelin, G. (1985). Rapid and convenient separation of phospholipids and no phosphorus lipids from rat heart using silica cartridges. Lipids 20 : 40-41.

Leberer, E. and Pette, D. (1984). Lactate deshydrogenase isoenzymes in type I, IIA and IIB fibres of rabbit skeletal muscles. Histochemistry, 80 : 295-298.

Leseigneur, A., Gandemer, G. and Marion, D. (1989) Fractionnement en classes des lipides alimentaires par HPLC à l'aide d'un détecteur à diffusion de lumière. In: *Actes du Congrès International Chevreul pour l'Etude des Corps Gras*, (ed.) ETIG, 1 : 311-318.

Leseigneur-Meynier, A. and Gandemer, G. (1991). Lipid composition of pork muscle in relation to the metabolic type of the fibres. Meat Sci., 29 : 229-241.

Morrison, W.R. and Smith, L.M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron-fluoride methanol. J. Lipid Res., 5 : 600-608.

Renerre, M. and Labas, R. (1987). Biochemical factors affecting metmyoglobin formation in beff muscles. Meat Sci, 19, 151- 165.

Valin, C., Touraille, C., Vigneron, P. and Ashmore, C.R. (1982). Prediction of lamb meat quality traits based on muscle biopsy fibre typing. Meat Sci., 6, 257-263.

Wilson, B.R., Pearson, A.M. and Shortland, F.B. (1976). Effect of total lipids and phospholipids on warmed-over-flavor in red and white muscle from several species as measured by thiobarbituric acid analysis. J. Agric. Food Chem., 24, 7-10.