IPASE, ESTERASE AND AMINOPEPTIDASE ACTIVITIES IN RAW PORK ADIPOSE TISSUE

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BSTRACT

Adipose tissue contains different lipolytic and exopeptidase enzymes which are responsible of lipid and peptide metabolism. These enzymes might play an important role in flavour development during the processing of meat products containing adipose tissue as an important component.

The objective of this work is to study the lipase, esterase and aminopeptidase activities in this tissue in order to have an approximate idea of the hydrolytic changes which may be expected in the processing of meat products.

The assayed enzymes were neutral (pH 7.0) and basic (pH 8.5) lipases, acid (pH 5.0) and neutral (pH 7.5) esterases and leucyl, arginyl, alanyl, tyrosyl and pyroglutamyl hydrolysing activities. Specific fluorescent substrates were used for each enzyme.

The results indicate an important lipolytic activity : 2.1 and 0.8 U/g fat for neutral and basic lipases, respectively, and 12.4 and 1.4 U/g fat for acid and neutral esterases, respectively. In the case of the aminopeptidase activity, only the leucyl, arginyl and tyrosyl hydrolysing activities with 0.38, 0.31 and 0.11 U/g fat, respectively, are relevant. The alanyl and pyroglutamyl hydrolysing activities are very low, 0.02 and 0.005 U/g fat, respectively. In view of these important levels of enzyme activities, a relevant amount of free fatty acids and amino acids might be generated, depending on the conditions, during the processing of meat products and with special incidence on those dry-cured.

INTRODUCTION

Adipose tissue has a very important role in the regulation of the overall energy balance in mammals, covering most of the energy need during starvation. Three important enzyme systems, with important flavour consequences, have been detected in the adipose tissue : a) the lipase system consisting in the lipoprotein lipase and hormone-sensitive lipase, which hydrolyze triacylglycerols to free fatty acids and glycerol, and the monoacylglycerol lipase which hydrolyzes monoacylglycerols to free fatty acids and glycerol (Belfrage et al., 1984), b) the esterase system which also hydrolyze triacylglycerols to free short chain fatty acids and glycerol (Khao et al., 1979) and c) aminopeptidase activity which generates free amino acids from peptides (Toldrá et al.,1992a).

Adipose tissue is an usual component in the majority of meat products (Flores and Toldrá, 1992) which can experience , due to the endogenous enzyme system and depending on the processing conditions (Toldrá, 1992), very important hydrolytic changes such as those observed in dry sausages (Acton, 1977, Domínguez and Zumalacárregui, 1991) and dry-cured hams (Flores at al., 1988, Pezzani et al., 1988).

The objective of this work is to study the lipase, esterase and aminopeptidase activities in the pork adipose tissue in order to have an approximate idea of the endogenous hydrolytic changes which may be expected during the processing of meat products.

MATERIALS AND METHODS

Samples : Samples of subcutaneous adipose tissue, located just at the *Biceps femor* muscle, were removed from 6-month-old pork carcasses between 18-24 hours post-slaughter.

Preparation of enzyme extracts : The enzymes were extracted (dilution 1:10) with 0.2 Tris/HCl, pH 8.2, containing 2 mg/ml sodium deoxycholate, 0.08 mg/ml Nonidet p-40, 0. mg/ml heparin, 10 mg/ml bovine serum albumin and 25 mM saccharose as previously describ (Motilva et al, 1992, Toldrá et al., 1992a).

Assay of the enzymes activities : The enzymes were assayed at 37°C and pH 5.0 (acid lipas and esterase), pH 6.5 (Leucyl, arginyl and tyrosyl hydrolyzing activities), pH 7.0 (neutra lipase and alanyl hydrolyzing activity), pH 7.5 (neutral esterase and pyroglutamy hydrolyzing activity) and pH 8.5 (basic lipase). The respective 4-methylumbelliferyl and 7 (4-methyl)coumarylamide derivatives (Sigma) were used as substrates. Four samples a controls were measured for each experimental point.

Chemical analysis : Fat content was determined by the Soxhlet method (ISO, 1973).

RESULTS AND DISCUSSION

The activities of the assayed lipases and esterases are shown in table 1. As can b observed, all of them are active in the adipose tissue but especially the neutral lipas and the acid esterase. These enzymes may be very important in the dry-curing process sinc they have been detected in the first 7 months of processing (Toldrá et al., 1991) Furthermore, their optimal pH for maximum activity (Motilva et al., 1992) is very closed t the usual pH in most of the meat products. These enzymes might be the responsible of th reported increase in free fatty acids concentration during the processing of dry-cured ham (Flores et al., 1985, Pezzani et al., 1988) and dry sausages (Acton, 1977, Domínguez an Zumalacárregui, 1991).

Aminopeptidase activity is also detected (see table 2) in adipose tissue but not so high as in muscle (Toldrá et al., 1992a) where a strong generation of free amino acids has been observed during the dry-curing process (Aristoy and Toldrá, 1991a, 1991b). The most important seems to be the leucyl and arginyl hydrolyzing activities. These enzymes have an optimal pH around 6.5 although below that pH their activities sharply decrease down to almost negligible values at pH 5.5. Furthermore, both enzymes have shown a very good stability with high activities even after 7 months of dry-curing (Toldrá et al., 1991). The tyrosyl hydrolyzing activity has an intermediate activity and also a very good stability (Toldrá et al.,1991). On the other hand, alanyl and pyroglutamyl hydrolyzing activities are very low, almost negligible, in adipose tissue (table 2).

Table 1.- Lipase and esterase activities in fresh pork adipose tissue.

Enzyme	Activity *
Neutral lipase	2.1 ± 0.3
Basic lipase	0.8 ± 0.1
Acid esterase	12.4 ± 0.9
Neutral esterase	1.4 ± 0.3

Activities are expressed as U/g of fat and are the mean of 6 samples ± SD.

Table 2.- Amino acid hydrolyzing activity in fresh pork adipose tissue.

Enzyme	Activity *
Leucyl	0.379 ± 0.111
Arginyl	0.251 ± 0.059
Tyrosyl	0.110 ± 0.030
Alanyl	0.020 ± 0.005
Pyroglutamyl	0.005 ± 0.001

Activities are expressed as U/g of fat and are the mean of 6 samples ± SD.

CONCLUSIONS

The results indicate that the activities of neutral lipase and acid esterase are qui important in the adipose tissue and might be responsible of most of the hydrolytic chang happening in different dry-curing processes

There is also an important leucyl and arginyl hydrolyzing activitiy in the adipose tiss which could be also responsible for the generation of free amino acids.

All these enzymes might very important in flavour development during the processing of mea products.

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