ACTIVITY OF AMINOPEPTIDASE B IN CURED MEAT PRODUCTS

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OBJECTIVE

To determine the role of aminopeptidase B in the generation of free amino acids in cured meat products

METHODS

Aminopeptidase B was isolated and purified according to the procedure described by Flores et al (1993). The activity was assayed in a 50 mM phosphate buffer, pH 6.5, containing 200 mM NaCl and 0.1 mM L-arginyl-7-amido-4-methyl coumarin (Arg-AMC) as substrate. The enzyme was incubated at 37ºC for 15 min and the fluorescence measured at 355 and 460 nm as excitation and emission wavelengths, respectively. One unit of activity is defined as the amount of enzyme hydrolysing 1 µmol of substrate/hr at 37ºC. Four replicates were measured for each experimental point. Half-life was determined by incubation at pH 6.5 at different temperatures. Free amino acids were determined by the PICO-TAG method as described by Aristoy and Toldrá (1991).

RESULTS AND DISCUSSION

The last step in the proteolysis observed in postmortem muscle is the generation of free amino acids. In fact, there are several studies showing an increase in free amino acids during the postmortem ageing/storage of meat and contributing to the improvement of meat taste (Nishimura et al., 1988). This increase has been attributed to the action of muscle aminopeptidases, active at neutral pH, since they hydrolyse peptide bonds near the amino terminus of many proteins and polypeptides. One of these enzymes is aminopeptidase B (EC 3.4.11.6.), which is located in the cytosol and represents about 11% of the total aminopeptidase activity in the cytosolic fraction of skeletal muscle. This enzyme has a molecular mass of 76 KDa and an optimal pH around 6.5 in the presence of 0.2 M of NaCl.

Aminopeptidase B purified from porcine skeletal muscle has been characterized as a chloride-activated enzyme hydrolyzing basic termini (Flores et al., 1993). This enzyme catalyzes the release of arginine, lysine and, although at a lower rate, phenylalanine, valine, proline and alanine (see table 1). Its activity in raw meat is around 1.26 U/g but is not so stable as other aminopeptidases even though its half-life is also significative at the temperatures shown in table 2.

A noticeable increase in those free amino acids generated by aminopeptidase B has been observed in processed pork cured products such as dry-cured ham and cooked ham as shown in table 3. The generation of free amino acids in dry-cured ham is incredibly high, confirming an intense degree of proteolysis. There are very large increases of lysine (731.5 mg/100 g), alanine (374.8 mg/100 g), valine (312.1 mg/100 g), proline (285.5 mg/100 g),, arginine (226.9 mg/100 g), phenylalanine (207.1 mg/100 g) and tyrosine (169.7 mg/100 g) as shown in table 3. The high content of lysine (734.6 mg/100 g) indicates a high degree of digestibility of ham proteins since this amino acid reflects availability for absorption without need for further digestion. There is still some small activity (0.07 U/g) of aminopeptidase B at the end of the process. In the case of cooked ham there are large increases corresponding to alanine (12.7 mg/100 g), arginine (10.4 mg/100 g) and lysine (9.5 mg/100 g). The activity of the aminopeptidase B at the end of the process is almost negligible (0.002 U/g), which is logical taking into account that its half-life is 2.2 hours at 50ºC (see table 2),

CONCLUSION

Processed pork meats present higher levels of free amino acids than raw postmortem muscle. Cooked ham and very especially dry-cured ham constitute a concentrated source of free essential amino acids. In both processes, aminopeptidase B might play an important role for the generation of amino acids such as arginine, lysine, proline, alanine and phenylalanine.

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Table 1: Activity of the purified soluble aminopeptidase B on aminoacyl-aminomethylcoumarin derivatives (expressed as a % relative to arginine). The enzyme was purified from porcine skeletal muscle.

Amino acids-AMC	Activity
Phenylalanine-	5.9
Lysine-	47.0
Methionine-	0.0
Alanine-	2.5
Leucine-	0.2
Arginine-	100.0
Tyrosine-	0.0
Serine-	0.0
Proline-	5.1
Glycine-	0.0
γ-Glutamic acid-	6.0
Pyroglutamic acid-	0.0
Valine-	7.4
Gly-Arg-	0.0
Arg-Arg-	0.0
Lys-Ala-	0.0
N-CBZ-Phe-Arg-	0.0
Z-Arg-Arg-	0.0

Table 2: Half-life, expressed in hours, of the purified soluble aminopeptidase B incubated at pH 7.0 and different temperatures.

Temperature(°C)	Half-Life (h)
5	126.800
15	75.500
25	68.600
35	25.700
50	2.200
65	0.008

Table 3: Changes in the free amino acids contents in the muscle *Biceps femoris* during the processing of cooked and dry-cured ham. Results are expressed as means of 6 samples (mg free amino acid/100g muscle) and net increments (Δ) in relation to raw meat.

Amino acids	Raw ham	Cooked ham	Δ	Dry-cured ham	Δ
Proline	3.18	7.28	4.10	288.63	285.46
Alanine	14.50	27.25	12.75	389.33	374.83
Arginine	3.88	14.30	10.43	230.80	226.93
Valine	3.40	7.60	4.20	315.47	312.07
Phenylalanine	2.03	6.98	4.95	209.17	207.14
Lysine	3.12	12.60	9.48	734.57	731.45