GLYCOLYTIC ACTIVITIES IN A SALTED AND MATURED PORK PRODUCT

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Introduction

In previous studies (1,2) we have investigated the time course of several enzymatic activities during the maturation of an Italian pork product (Coppa). Several muscles from the neck region of the pig contribute to this product. The fresh muscles are treated with NaCl, nitrates, nitrites and drugs, and the finished "coppa" becomes commercially available after two to four months of maturation. In this communication we report results concerning the fate of glycolytic activities in muscle after salting and maturation in another typical italian pork product, the " Prosciutto di Parma ".

This product consists of the leg of the pig, from which only the distal portion below the intertarsal articulation has been removed. Only NaCl is used in the salting process, while nitrates and nitrites are normally not employed. The microbial flora does not markedly effect the maturation of this product, which remains almost sterile throughout the process. Microbial enzyme activity does not, therefore, interfere with the residual tissural enzyme activity in the finished product (3).

Experimental Conditions

After slaughtering, the pig leg is kept at 0°C and 95% Relative Humidity for 24 hrs. The leg is then kept under salt at 2°C and 85% Relative Humidity for one month. The salt is then washed away and the maturation of the product takes about one year at room temperature (20°C ± 10°C). During this time, the Prosciutto acquires the organoleptic qualities, savour, flavour and consistency, which make of it one of the best Italian pork products. All the"prosciutti" were bought in Meat Plants belonging to the "Consorzio del Prosciutto di Parma".

The enzymatic activities were measured in the fresh muscle after the cooling time, in the muscle after the salting process, and in the finished product.

All the samples used for the determinations of pH, enzyme activity, percent composition and bacterial counts were taken from the "biceps femoris" ~~exposed by~~ in a large, 5-6 cm thick, transversal section, ~~through~~ near the medial portion of the femur; this muscle was selected because it is voluminous, homogeneous surrounded by subcutaneous fat and skin, and therefore not greatly affected by external agents.

Materials and Methods

The reagents used in the assays of enzymatic activities were analytical grade commercial products, used without further purification: Adenosine 5'- monophosphate, Glucose-6-phosphate Dehydrogenase; Glucose-1,6-diphosphate, Phosphoglucomutase; Nicotinamide-Adenine Dinucleotide Phosphate, Fructose-1,6-diphosphate, Glycerol-1-phosphate Dehydrogenase/Triosephosphate Isomerase,Glycerate-3-phosphate, 3-Phosphoglycerate Kinase,Adenosine-5'-diphosphate,Lactate Dehydrogenase, Nicotinamide-Adenine Dinucleotide,reduced form, Triethanolamine hydrochloride were from Boehringer GmbH, Mannheim;Ethylenediaminetetraacetic acid tetrasodium salt, Imidazole, Tris (hydroxmethyl)aminomethane,

Sodium pyruvate were from Merk AG,Darmstadt; Iodoacetic acid, s-Collidine from Schuchardt, Munchen; Mercapto-ethanol from Sigma, S.Louis, Missouri; Glycogen (Rabbit liver) from K and K, Plainview N.Y.; Mg SO_4, H_3PO_4, KCl, NaOH, $AgNO_3$, NH_4SCN, phosphosulfuric acid from Carlo Erba, Milan.

The samples were water extracts of muscle homogeneized for a total time of ten minutes, in several steps and in the cold.The muscle to water ratio was the following: 10g of muscle suspended in 40 mls of bidistilled H_2O. The homogenate was then centrifuged for 1 hr in a refrigerated (2°C) Sorvall RC-2B (4,25 inc. radius) at 18.000 rpm. The supernatant was then used for the determination of the following specific activities: Phosphorylase (4), Aldolase (5), Glyceraldehide 3-P Dehydrogenase (6), Pyruvic Kinase (7) and Lactic Dehydrogenase (8). A Beckman DB-G u.v. spectrophotometer was employed for all spectrophotometric determinations. The pH's of the fresh, salted and aged muscular tissues were measured using a Radiometer pH-Meter PHM26, equipped with an infission electrode Beckman.

The percent composition of the muscle has been determined measuring the weight lost by evaporation of water in oven at 110°C and the total ash left after burning the organic matter at 550°C. NaCl has been measured according to the method of Charpentier-Wohlard. The fat has been extracted in Soxhlet and the protein content has been determined according to the Kjeldahl method.

The bacterial count (total aerobes and anaerobes /gm) was carried out as previously described (9).

Experimental Results

Table 1 shows the figures obtained for the specific activities of the glycolytic enzymes investigated,with the average standard deviation, and also the pH, the percent composition and the bacterial counts relative to fresh muscle, salted muscle and finished product.

pH

It is knoun that the pH of the muscle falls rapidly after the death of the animal and reaches its ultimate value within 24 hrs at 0°C. The slight variations between fresh muscle, salted muscle, and matured product are not very significant.

Enzyme activities

Enzyme activities are measured in the following units: moles of substrate converted into product in one minute by a milligram of protein in the water extract.

Phosphorylase - Aldolase - Glyceraldehyde 3-P Dehydrogenase

The phosphorylase activity decreases from a mean value of

3×10^{-10} units in the fresh muscle to a mean value of 1.6×10^{-10} units in the salted muscle.

No activity is detectable in the finished product.

The Aldolase Activity, 1.6×10^{-6} units in the fresh muscle, decreases to 1.2×10^{-6} units in the salted muscle and falls to about 5×10^{-8} units in the finished product.

The Glyceraldehyde 3-P Dehydrogenase activity shows the same trend as the previous enzymes, a progressive fall during the time of salting and maturation of the product.

Pyruvic Kinase and Lactic Dehydrogenase

These enzyme activities remain constant during the salting time and maturation of the product, respectively about 3×10^{-6} units and 1.4×10^{-5} units.

Percent Composition

The water content decreases from a value of 73.3% to a value of 70.7 after salting and 62.5 after maturation. The percent water content does not therefore change drastically with time in the "biceps femoris".

The percent protein content increases from 23.7 to 24.2 to 29.8, depending on the dehydrating action of NaCl first, and the water loss accompanying the maturation at room temperature later on.

The slight decrease observed in the fat content does not seem to be significant.

Total ash and NaCl increase concomitantly in time, as expected from progressive diffusion of NaCl towards the muscular portion used in our experiments. Notice that the total ash values reported in Table 1 are approximately the sum of the [illegible] total ash from fresh muscle and the NaCl.

Bacterial Counts

In agreement with results from other laboratories (3) [illegible] no bacteria were detected either in the fresh muscle or in the salted or matured product.

All enzyme activities measured in our experiments are therefore of tissural nature.

	Fresh muscle	Muscle after salting process	Matured product
Phosphorylase	$3.0 \pm 0.2 \times 10^{-10}$	$1.6 \pm 0.2 \times 10^{-10}$	$< 10^{-13}$
Aldolase	$1.6 \pm 0.1 \times 10^{-6}$	$1.2 \pm 0.1 \times 10^{-6}$	$4.9 \pm 0.3 \times 10^{-8}$
Glyceraldehyde-3-P-Dehydrogenase	$1.5 \pm 0.1 \times 10^{-5}$	$4.4 \pm 1.1 \times 10^{-6}$	$1.4 \pm 0.1 \times 10^{-7}$
Pyruvate Kinase	$2.7 \pm 0.4 \times 10^{-6}$	$3.0 \pm 0.3 \times 10^{-6}$	$2.6 \pm 0.2 \times 10^{-6}$
Lactate dehydrogenase	$1.5 \pm 0.1 \times 10^{-5}$	$1.4 \pm 0.2 \times 10^{-5}$	$1.3 \pm 0.1 \times 10^{-5}$
pH	5.7 ± 0.04	5.8 ± 0.02	5.9 ± 0.03
Water %	73.3 ± 0.4	70.7 ± 0.3	62.5 ± 0.6
Protein %	23.7 ± 0.2	24.2 ± 0.2	29.8 ± 0.3
Fat %	1.7 ± 0.4	1.6 ± 0.3	1.5 ± 0.2
Ash %	1.4 ± 0.1	3.5 ± 0.1	6.8 ± 0.2
NaCl %	———	2.3 ± 0.1	4.7 ± 0.1
Total aerobic bacteria /gm	<10	<10	<10
Total anaerobic bacteria/gm	<10	<10	<10

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Discussion

It should be noted that the first enzymes in the glycolytic pathway loose most of their activity within the first month. The last enzymes in the pathway, instead, retain the level of activity measurable after the first 24 hours for several months after the slaughtering of the animal.

This could be rationalized as an example of enzyme stabilization by ligands. The lactic acid accumulated during the post mortem glycolysis could act as a stabilizer of Lactic Dehydrogenase and in turn effect the Pyruvic Kinase.

Moreover Lactic Dehydrogenase and Pyruvic Kinase are known to be stable at the ultimate pH reached in the post mortem glycolysis, while Phosphorylase, Aldolase and Glyceraldehyde 3-P Dehydrogenase are known to be inactivated at low pH values.

The observations that we have reported indicate that some enzyme activities are fully retained in the muscle cell for a long time after the death of the animal, at least in cases in which there is no contamination by external agents.

It is remarkable that an irreversible denaturation does not take place even though the product remains at relatively high temperature (approximately 15°C) for the several months requested for maturation. At this temperature the purified enzymes would undergo heat inactivation; moreover they would be susceptible to the action of proteolytic enzymes. The cellular environment in the muscle cells seems therefore to be able to protect enzymes from these degrading processes for a very long time after death.

How much this depends on the process of rapid cooling and salting of it before maturation remains to be ascertained.

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