ENZYMATIC ACTICITIES OF DRY-CURED MEAT PRODUCTS. 1) COPPAS C 9

Maggi,E., Fasella,P., Dazzi,G., Rossi,G.L.

The events taking place in the muscular tissue from the moment of slaughtering of the animal until its transformation in finished product, have been studied from several points of view, particulary from a macroscopic, microbiologic, and chemical point of view.

Most of the previous chemical analyses have particularly considered the low molecular wight components.

What is known about the muscle biochemistry, howerer, tends to suggest that the chemical modifications relative to the low molecular weight components are largely due to the enzymatic activities present in the meat during the salting and curing processes.

Therefore, we decided to study the time dependence of several enzymatic activities during and curing of a typical product /coppa/ and to compare these data with these obtained with the corresponding group of fresh muscles.

We studies the activities of a group of enzymes including two Hydrolases, two Dehydrogenases and two Transferases. Among the Hydoolases we chose the Acid and Alkaline Phospnatases, which are of intereset in view of the important biochemical role of the organic phosphoesters hydrolized by these enzymes.

Lactic and Malic Dehydrogenases were studies with the purpose of testing the survival of metabolic routes connected with the processes of oxireduction, leading to the formation of ossiacids and chetoacids, products which are presumed to be important for the organoleptic characteristics of the mature product. Among the

Transferases we chose Aspartic and Alanine Transaminases which are important because of their role in the interconversion of proteic and glucidic metabolites.

EXPERIMENTAL CONDITIOND

As a first approach we determined the specific enzymatic activities on ten samples of coppas bought in the stores of the city of Parma, of different maturation ages and weighing approximately 150 grams. The exact times of maturations of these samples were not known.

Later on, a single coppa /coppa A / has been divided in ten transversal sections and each one has been used for enzymological studies. This experiment was perfomed to control possible variations due to lack of uniformity of the tissue, since several muscles contribute to the finished product.

For the sake of comparison we also studied the enzymatic activities in each one of ten transversal sections of fresh muscles taken two hours after the slaughtering of the animal.

At last, to account for possible variations in enzyme activities during the maturation process, linked to surface dehydration and high salts and drugs concentration,we selected another coppa (coppa B) for differential studies of peripheral and central portions of several transversal sections (sections n.1, 3, 5, 7, 9).

The coppa samples were freed from the external envelope made of bovine colon and finely minced in a meat grinder.

The material (15 %) was then homogenized in ATO MIX (MSF with distilled water, in several steps, and for a total time of five minutes. Care was taken to avoid heating of the material.

The homogenate was then centrifuged (20 cm radius) for thitry minutes at 2°C at 5000 r.p.m. and the supernatant was filtered on paper to remove the small fat particles in suspension.

Part of the filtrate was then used to evaluate the various enzymatic activities,while the remaining part was used for protein nitrogen determination according to the method of Kjeldhal.

Phosphatases

We used the method of Bergmejer (1963) for the Acid Phosphatase activity assay.

To 1 ml of buffer solution (0,05 M sodium citrate, pH 4,8), already containing the substrate (Disodium P- nitrophenylphosphate 5.5×10^{-3} were added 0,02 ml of fresh muscle or coppa extract. The mixture was incubated at 37°C.

The reaction was stopped by addition of 4 ml NaOH 0.1 N after 0, 30, 60, 90, 120, 150 minutes. The optical density of the solution was measured in a Beckman DB - G spectrophotometer at 405 nm, after dilution 1 : 10 in NaOH 0.1 N.

The method employed in the determination of Alkaline Phosphatase activity differed from the previous one for the use of a buffer solution Tris HCl 10^{-1} M, pH 9.

The samples did not reguire dilution before use in the spectrophotometer.The specific enzyme activity was measured in Moles of transformed substrate per mg of protein per minute.

Dehydrogenases

For the study of Lactic Dehydrogenase activity we followed the method of Wroblewski et La Due (1955).

In a 1 cm cuvette the following reagents were mixed: Tris HCl 10^{-1}, pH 7.5 buffer solution, sodium pyruvate 10^{-3} M, NADH 10^{-4} M and variable amounts of extract (0.02 ml for determinations of enzyme activity in the fresh muscle and 0.01 ml for extracts from coppa samples that had been previously diluted 1:20 times). The rate of NADH disappearance at 340 nm was then recorded with the Beckman Spectrophotometer. Enzyme activities were then expressed in the same units as before.

Malic Dehydrogenase activity was assayed by the same method, after replacing pyruvate with oxalacetate, and properly changing the dilution of the extract to obtain an optimal course of reaction, i.e. a linear dependence of optical density change from time for at least 2 minutes.

The angular coefficient of the straight line representing the progress of the reaction was always directly proportional to the enzyme concentration.

Transaminases

The Aspartic Amino Transferase activity was studied by the following method. To Tris HCl 10^{-1} M (pH 7.5) buffer were added l-Aspartate 3×10^{-2} M, MDH 2 g/ml , NADH 10^{-4} M, variable amounts of extract (0.02 ml for determinations of enzyme activity in the fresh muscle and 0.05 ml for extracts from coppa samples) and α-Ketoglutarate 2×10^{-4} M.

The rate of desapearance of NADH was measured by the optical density change at 340 nm.

In the Alanine Amino Transferase assay, MDH was replaced by LDH at a concentration of 10 g/ml and l-Aspartate by l-Alanine 10^{-3}M

The specific activity is measured in the same units as before.

Materials

The reagents used in the enzymatic activity assays are the following commercial products: Disodic p - Nitrophenylphosphate, oxalacetate , ketoglutarate from Fluka AG , Buchs.

NADH , MDH , LDH , from Boehringer a. Soehne G mbH , Mannheim.

Tris , Sodium pyrivate from Merck AG , Darmstadt.

1 - Aspartate from Carlo Erba , Milan

1 - Alanine from Bayer , Leverkusen.

RESULTS

The enzymatic activities, that we are reporting in Table 1, are referred only to the amount of proteins present in the material that was water extractable under our experimental conditions.

For illustration purpore,we are also showing the percent composition determined in samples number 5 of coppas A and B. In the case of coppas bought in the various city stores and the corresponding group of fresh muscles each samples was a transversal section including both the peripheral and central portions of the mature product (Table 2).

In Table 3 we show that the Acid Phosphatase activity in the coppas from city stores (average value 45.8×10^{-10}) is not significantly different from the value in the fresh muscle (52.3×10^{-10}) or in coppa A (56.8×10^{-10}). Data relative to coppa B also show that there are no significant variations between the external and inte-

nal portions of each section (44.8×10^{-10} and 45.9×10^{-10}) .

The values of Alkaline Phosphatase activity in coppas are only slightly different from those in the fresh muscle: the average values are 5.2×10^{-10} in coppa A and in the ten commercial samples against 4.1×10^{-10} in the fresh muscle.

The data for coppa B show that the central portion of each section presents a lower specific activity than the peripheral portion (4.5×10^{-10} against 5.9×10^{-10}), (Table 4).

Lactic Dehydrogenase (Table 5) shows the same general beha vior of Alkaline Phosphatase, but the activity increase is much more marked. The specific activity is 1.5×10^{-6} in the fresh muscle, 5.4×10^{-6} in coppa A and 11.6×10^{-6} in the samples from the city stores. Conversely the figures relative to central and peripheral portions of the various sections are essentially the same.

The Malic Dehydrogenase activity is also higher in coppa A and in the ten samples (average value: 6.9×10^{-6}) than in the fresh muscle (4.0×10^{-6}) (Table 6) .

The average values for coppa B are slightly higher in the external than in the internal portions of each section (7.9×10^{-6} against 6.5×10^{-6}).

The two Transaminase activities which have been studies show a pattern opposite to the one presented by Dehydrogenases . The specific activity of Aspartic Amino Transferase Decays from an average value of 471×10^{-9} in the fresh muscle to very low values (32.1×10^{-9}) in coppa A and in the commercial samples (19.2×10^{-9}). Coppa B is also showing some significant difference

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between the external (76.9 x 10^{-9}) and internal portions (93.10^{-9}) (Table 7).

Alanine Amino Transferase decays less markedly than Aspartic Amino - Transferase (from 21.9 x 10^{-9} in the fresh muscle to 4.3 x 10^{-9} in coppa A and 2.4 x 10^{-9} in commercial samples). Data for coppa B show again small significant differences between central and peripheral and portions, with higher specific activity in the central portion (8.6 x 10^{-9} and 6.5 x 10^{-9} respectively) (table 8).

The results also show that there are significant variations in the various transversal sections of both the fresh muscle and coppas A and B. This finding may be retionalized noting that the muscular tissue is not uniform, as a single samples may contain "pars muscularis " and "pars tendinea".

It follows that, in the various sections of a sample , tissues with quite different structure and biochemical characteristics may be present together in various proportions.

The interpretation of the variations observed in the commercial samples is more difficult, since these variations may arise also from different conditions of maturation.

CONCLUSIONS

In the present study we have only considered enzymatic activities which are water extractable. Studies are now in prodress to evaluate the a mount of enzymatic activities present in the water insoluble fractions. Even with this limitation the data suggest several considerations.

Inspection of Tables 1 and 2 shows that the presence of

higher concentration of proteins in the water extract from coppa than in the water extract from fresh muscle only depends from differences in the initial content of water in coppas and fresh muscle.

It seems therefore that the salting process has not significa ntly altered the total amount of soluble proteins.

The overall behavior of the specific activities of the three classes of enzymes which have been studied seems to be as follows: the Hydrolases are not significantly altered in the coppa with respect to the fresh muscle: the Dehydrogenases increase and the Transaminases decay significantly.

Our experimental results may be of intereset from at least there points of view. First of all they suggest the necessity of investigating the modifications occurring in low molecular weight compounds. Chemical transformations of these compounds may be catalyzed by enzymes retaining high specific activity in the maturing product. As examples, we may mention the lactic, malic, pyruvic and oxalacetic acids.

In second place the data pose the guestion of the transformations occurring in the coppas during the salting and curing processes, which are responsible for the loss of Transaminase activity and increase of Dehydrogenase activity. The first event may be due to protein denaturation or coenzyme loss; the second one may be related to the solubilization of enzymatic material which is not easily water extractable in the fresh muscle. Studies are now in progress to a ascertain whether coenzyme loss may be fu lly or in part responsible for the decay of Transaminase activity. Coenzyme loss could be due to the Phosphatases which remain active

in the salted and cured product.These hydrolitic enzymes could remove the phosphate group from the coenzyme pyridoxal - phosphate.

The increase in specific activity of water extractable Malic Dehydrogenase may be attributed to an alteration of the mitochondrial structures and the solubilization of the lsozyme of Malic Dehydrogenase which is bound to these subcellular particles in the fresh muscle.

The increase in Lactic Dehydrogenase activity may be due to various phenomena, among which a redistribution of H and M hybrides, the presence of free subunits in solution and or to the fusion of inactive polymers (Clausen 1970), activation by small molecules and, finally, the presence of bacterial Lactic Dehydrogenase. Electrophoretic analyses are in progress to test these possibilities.

Finally the results obtained may suggest an enzymological method to evaluate the status of maturation of coppas and analogous finished products like"prosciutto" (italian ham) .

Studies are now in progress in our Laboratory to show the variations in typical enzymatic activities during the curing and the maturation processes of varions dry - cured meat products.

REFERENCES

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TABLE 1 - PROTEINS (md/ml of extract)

Section n°	Fresh muscle	Coppa A	Coppa B		Commercial Coppa samples
			external	internal	
1	5.9	8.8	9.3	9.6	7.3
2	6.2	9.3	-	-	7.7
3	6.1	9.6	11.5	10.4	9.9
4	5.8	9.7	-	-	8.7
5	5.6	10.6	10.9	10.6	10.2
6	5.6	10.7	-	-	10.2
7	5.3	11.9	12.3	9.9	10.9
8	5.3	11.6	-	-	12.5
9	5.2	11.6	11.9	10.5	9.5
10	4.2	11.0	-	-	9.0

TABLE 2 - COMPOSITION OF EDIBLE PORTION OF FRESH MUSCLE AND MATURED PRODUCT.

Samples	Water %	Fat %	Protein by dif. %	Ash %
Fresh muscle	55.9	25.3	17.9	0.9
Coppa A	39.5	32.3	23.4	4.8
Coppa B (external)	33.8	42.2	20.0	4.0
Coppa B (internal)	50.9	25.0	18.8	5.3
Commercial coppa n.1	39.8	25.6	27.4	7.2
Commercial coppa n.2	27.0	43.5	23.3	6.2
Commercial coppa n.3	37.4	33.1	22.9	6.6
Commercial coppa n.4	35.4	33.1	25.0	6.5
Commercial coppa n.5	36.9	35.1	21.5	6.5
Commercial coppa n.6	30.5	35.3	27.8	6.4
Commercial coppa n.7	30.4	41.7	21.7	6.2
Commercial coppa n.8	36.0	31.2	26.0	6.8
Commercial coppa n.9	34.9	34.8	24.2	6.1
Commercial coppa n.10	27.3	47.2	20.1	5.4

TABLE 3 .

ACID PHOSPHATASE - Specific Activity in $\frac{\text{Moles of substrate}}{\text{mg protein. min .}}$

Section n°	Fresh muscle	Coppa A	Coppa B		Commercial Coppa samples
			External	Internal	
1	29.9×10^{-10}	67.4×10^{-10}	40.3×10^{-10}	45.3×10^{-10}	43.8×10^{-10}
2	49.8×10^{-10}	68.1×10^{-10}	-	-	57.7×10^{-10}
3	50.5×10^{-10}	57.1×10^{-10}	46.5×10^{-10}	46.1×10^{-10}	55.6×10^{-10}
4	48.5×10^{-10}	54.9×10^{-10}	-	-	44.9×10^{-10}
5	52.2×10^{-10}	51.9×10^{-10}	40.6×10^{-10}	44.2×10^{-10}	50.2×10^{-10}
6	48.0×10^{-10}	52.5×10^{-10}	-	-	44.5×10^{-10}
7	61.9×10^{-10}	54.5×10^{-10}	48.9×10^{-10}	45.6×10^{-10}	52.8×10^{-10}
8	51.2×10^{-10}	58.6×10^{-10}	-	-	43.0×10^{-10}
9	66.8×10^{-10}	49.6×10^{-10}	47.7×10^{-10}	47.9×10^{-10}	43.3×10^{-10}
10	64.0×10^{-10}	53.0×10^{-10}	-	-	23.3×10^{-10}
$\bar{x}$	52.3×10^{-10}	56.8×10^{-10}	44.8×10^{-10}	45.9×10^{-10}	45.8×10^{-10}

TABLE 4.

ALKALINE PHOSPATASE - Specific Activity in $\frac{\text{Moles of substrate}}{\text{mg protein. min.}}$

Section n	Fresh muscle	Coppa A	Coppa B		Commercial Coppa samples
			External	Internal	
1	2.6×10^{-10}	4.9×10^{-10}	6.7×10^{-10}	4.0×10^{-10}	9.2×10^{-10}
2	4.5×10^{-10}	3.3×10^{-10}	-	-	7.9×10^{-10}
3	4.6×10^{-10}	2.5×10^{-10}	6.1×10^{-10}	5.6×10^{-10}	3.4×10^{-10}
4	3.9×10^{-10}	6.7×10^{-10}	-	-	4.3×10^{-10}
5	3.7×10^{-10}	7.2×10^{-10}	6.3×10^{-10}	4.2×10^{-10}	3.9×10^{-10}
6	4.8×10^{-10}	7.5×10^{-10}	-	-	6.3×10^{-10}
7	5.2×10^{-10}	5.7×10^{-10}	5.4×10^{-10}	4.5×10^{-10}	5.3×10^{-10}
8	2.9×10^{-10}	5.1×10^{-10}	-	-	1.9×10^{-10}
9	5.1×10^{-10}	4.5×10^{-10}	4.8×10^{-10}	4.2×10^{-10}	4.0×10^{-10}
10	3.6×10^{-10}	4.5×10^{-10}	-	-	5.8×10^{-10}
$\bar{x}$	4.1×10^{-10}	5.2×10^{-10}	5.9×10^{-10}	4.5×10^{-10}	5.2×10^{-10}

TABLE 5.

LACTIC DEHYDROGENASE - Specific Activity in $\frac{\text{Moles of substrate}}{\text{mg protein. min.}}$

Section n°	Fresh muscle	Coppa A	Coppa B		Commercial Coppa samples
			External	Internal	
1	0.7×10^{-6}	5.3×10^{-6}	10.0×10^{-6}	8.8×10^{-6}	18.3×10^{-6}
2	0.7×10^{-6}	4.3×10^{-6}	-	-	8.1×10^{-6}
3	2.0×10^{-6}	4.3×10^{-6}	10.8×10^{-6}	9.7×10^{-6}	12.6×10^{-6}
4	1.9×10^{-6}	4.5×10^{-6}	-	-	16.5×10^{-6}
5	3.0×10^{-6}	4.2×10^{-6}	11.4×10^{-6}	12.5×10^{-6}	9.9×10^{-6}
6	2.0×10^{-6}	4.8×10^{-6}	-	-	10.8×10^{-6}
7	1.8×10^{-6}	5.3×10^{-6}	13.5×10^{-6}	12.6×10^{-6}	12.2×10^{-6}
8	1.8×10^{-6}	6.7×10^{-6}	-	-	9.2×10^{-6}
9	0.6×10^{-6}	7.2×10^{-6}	13.7×10^{-6}	14.7×10^{-6}	10.6×10^{-6}
10	0.7×10^{-6}	7.2×10^{-6}	-	-	8.0×10^{-6}
$\bar{x}$	1.5×10^{-6}	5.4×10^{-6}	11.9×10^{-6}	11.7×10^{-6}	11.6×10^{-6}

TABLE 6.

MALIC DEHYDROGENASE - Specific Activity in $\frac{\text{Moles of substrate}}{\text{mg protein. min}}$

section n°	Fresh muscle	Coppa A	Coppa B		Commercial Coppa samples
			External	Internal	
1	2.7×10^{-6}	7.4×10^{-6}	8.2×10^{-6}	5.6×10^{-6}	9.8×10^{-6}
2	3.4×10^{-6}	5.9×10^{-6}	-	-	6.2×10^{-6}
3	3.7×10^{-6}	6.1×10^{-6}	7.0×10^{-6}	6.2×10^{-6}	6.6×10^{-6}
4	3.9×10^{-6}	6.0×10^{-6}	-	-	7.4×10^{-6}
5	4.9×10^{-6}	6.9×10^{-6}	6.2×10^{-6}	6.1×10^{-6}	6.6×10^{-6}
6	3.6×10^{-6}	5.9×10^{-6}	-	-	8.0×10^{-6}
7	4.2×10^{-6}	6.1×10^{-6}	9.1×10^{-6}	7.1×10^{-6}	6.9×10^{-6}
8	4.2×10^{-6}	6.7×10^{-6}	-	-	6.0×10^{-6}
9	4.6×10^{-6}	8.8×10^{-6}	9.1×10^{-6}	7.7×10^{-6}	7.0×10^{-6}
10	4.6×10^{-6}	9.0×10^{-6}	-	-	4.8×10^{-6}
$\bar{x}$	4.0×10^{-6}	6.9×10^{-6}	7.9×10^{-6}	6.5×10	6.9×10^{-6}

TABLE 7.

ASPARTIC AMINOTRANSFERASE-Specific Activity in $\frac{\text{Moles of subs}}{\text{mg Protein.mi}}$

Section n°	Fresh muscle	Coppa A	Coppa B		Commercial Coppa samples
			External	Internal	
1	329.6×10^{-9}	28.3×10^{-9}	30.5×10^{-9}	23.0×10^{-9}	26.5×10^{-9}
2	399.4×10^{-9}	20.3×10^{-9}	-	-	22.6×10^{-9}
3	434.7×10^{-9}	24.7×10^{-9}	75.9×10^{-9}	76.9×10^{-9}	29.6×10^{-9}
4	502.7×10^{-9}	33.5×10^{-9}	-	-	38.1×10^{-9}
5	497.2×10^{-9}	34.0×10^{-9}	92.3×10^{-9}	167.6×10^{-9}	14.3×10^{-9}
6	519.0×10^{-9}	41.0×10^{-9}	-	-	8.5×10^{-9}
7	465.2×10^{-9}	44.2×10^{-9}	128.3×10^{-9}	146.0×10^{-9}	18.0×10^{-9}
8	530.2×10^{-9}	34.6×10^{-9}	-	-	11.4×10^{-9}
9	484.1×10^{-9}	32.5×10^{-9}	57.3×10^{-9}	51.3×10^{-9}	17.9×10^{-9}
10	548.2×10^{-9}	28.4×10^{-9}	-	-	5.0×10^{-9}
x	471.0×10^{-9}	32.1×10^{-9}	76.9×10^{-9}	93.0×10^{-9}	19.2×10^{-9}