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AMYLASE EXTRACTION FROM THE HEPATOPANCREAS OF *Mytilus galloprovincialis.* LMK.

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ABSTRACT:

The amylase extraction from the hepatopancreas of Mytilus galloprovincialis, is presented in the paper. By testing different extraction media it was found that CaCl2 solutions solubilises best the amylase. The enzymatic preparation presents maximum activity at 35°C when 0,4 % CaCl2 solution is used for extraction. The extraction temperature do not influence significantly the activity of the obtained preparations.

During the last period, the enzymatic study of mussels has been developed in connection with their physiological aspects. In 1970, GOROMOSOVA and SAPIRO (2) studied the amylase activity of the muscle and hepatopancreas from mussel in close relation with their glycogen content, sexual cycle and the temperature variations of the sea water. On the same mussel there are studies of other enzymes like transaminases (6), acid phosphatase (5), acid proteases (1) and so on.

The purpose of the present paper is to establish the

optimal extraction conditions of amylaze from the hepatopancreas of Mytilus galloprovincialis in connection with the study of the action parameters of this important enzyme involved in the glucide metabolism.

MATERIAL AND METHODS

The enzymatic activity was determined by the method of METAIS and BIETH (4) adapted to the optimum activity conditions of amylase from the sea mussel hepatopancreas. The reaction mixture contains: 8 mg starch in acetate buffer 5 x 10^{-3}M, pH 6 and 0.6 - 1.1 mg solubilized protein. The reaction was realized at 35°C for 30 minutes and stopped with 5 ml of 6% acetic acid. After the addition of 5 ml of N/1,500 iodine the optical density was determined at 500 nm.

The experimental results were established by the following relation:

$$\frac{O.D._{control} - O.D._{sample}}{O.D._{control}} \cdot 8.f \quad \text{amylase units/ml}$$

where:

- O.D. represents the optical density of sample and control (which was not incubated for 30 minutes at 35°C);
- 8 mg of starch in the reaction mixture;
- f represents the dilution factor of total proteic extract.

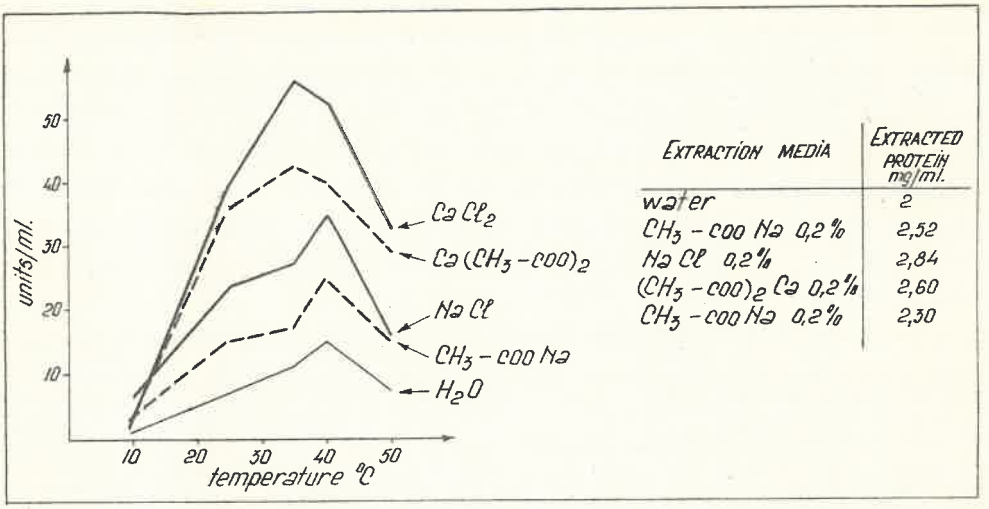
An amylase activity unit is defined as that enzyme quantity which hydrolyzed 1 mg of starch in 30 minutes at 35°C.

The proteic extract was obtained by the macerating the hepatopancreas of the sea mussel in a Potter homogenizer (1 g tissue with 10 ml extraction medium). The tissular homogenate was centrifuged for 15 minutes at 10.000 r.p.m., the supernatant representing the total proteic extract.

The proteic concentration was dosed by the method of LOWRY et al. (3).

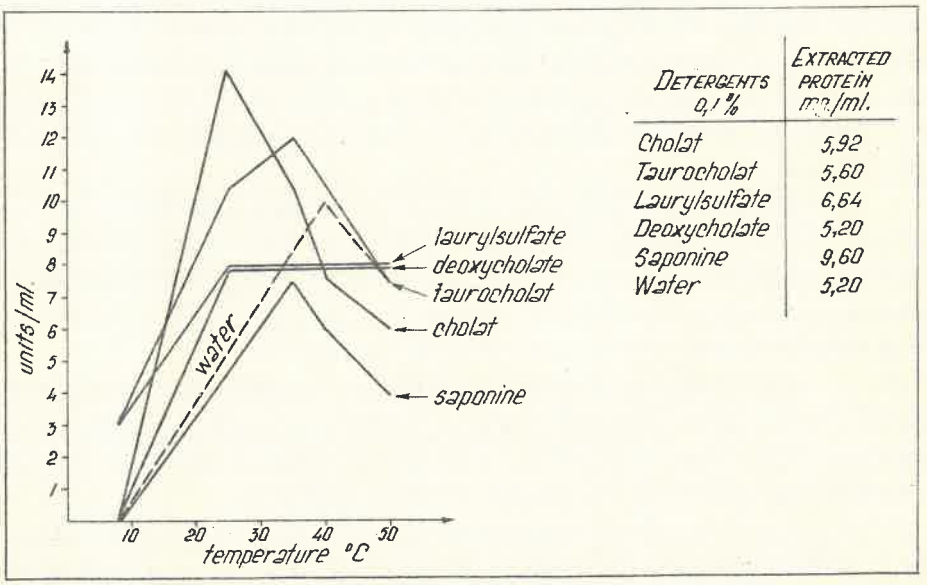
RESULTS AND DISCUSSIONS

In Figures 1 and 2 are presented the determination of optimum activity temperature for samples realised with different extraction media.



Extraction media	Extracted protein mg/ml.
water	2
$CH_3 - COO\ Na$ 0,2 %	2,52
$Na\ Cl$ 0,2 %	2,84
$(CH_3 - COO)_2\ Ca$ 0,2 %	2,60
$CH_3 - COO\ Na$ 0,2 %	2,30

Fig.1 - The determination of optimum temperature action of amylase from mussel hepatopancreas, obtained with 0.2 % saline solutions, against a control with distilled water



Detergents 0,1 %	Extracted protein mg/ml.
Cholat	5,92
Taurocholat	5,60
Laurylsulfate	6,64
Deoxycholate	5,20
Saponine	9,60
Water	5,20

Fig.2 - The establishment of optimum temperature action of amylase from Mytilus galloprovincialis hepatopancreas obtained with 0.1 % solutions of different detergents as extraction agents

As is result from Fig.1 the presence of Ca^{2+} in extraction medium (indifferently of the anion nature) induces the obtaining of maximum enzyme activity at 35°C. The proteic extracts achieved in distilled water or in saline solutions which contains sodium ion, shows a maximum amylase activity at 4o°C. The four o.2% saline solutions achieve a better proteic extraction and especially that of amylase; the best results were obtained with o.2% $CaCl_2$ as extraction medium.

In Fig.2 are presented the results of the same experiment but realised with five different o.1% detergent solutions. The profile of the curves are quite different, being impossible to establish any relationships between the chemical structure of detergents and the behaviour of enzymatic prepatations at the different temperatures; optimum temperature reaction varying between 2o°C (for the extraction with cholate) and 35°C (in the taurocholate case). Noteworthy is the fact that when laurylsulfate and deoxycholate solutions (an onic detergents) are used as extraction media, the amylase activity is constant from 2o°C to 4o°C. It is evident, from Figure 2 that maximum activity was obtained with cholate as extraction media. The utilisation of saponine leads to a highly concentrated proteic extract but its activity is low.

Because the protein extracts in $CaCl_2$ solution are very active, the relationship between extraction and $CaCl_2$ concentration was studied. It was found that at o.4% $CaCl_2$ the protein extracts contains the highest enzymatic activity (Fig.3). More concentrated $CaCl_2$ solutions acts as inhibitors.

Utilising o.4% $CaCl_2$ solutions, extractions are performed at -15°C, +4°C and 24°C. The results presented in Fig.4 demonstrate that the extraction temperature dosn't act significantly upon the activity of enzymatic preparations.

With a view to determine the optimum extraction time the homogenates were allowed to stand at 4°C for 0 - 1 - 2 and 24 hours before to be centrifugate. As it is seen from Table 1 maximum proteic concentration is obtained after 24 h extraction, but the highest amylase activity is recovered in the experiment with zero hours of extraction.

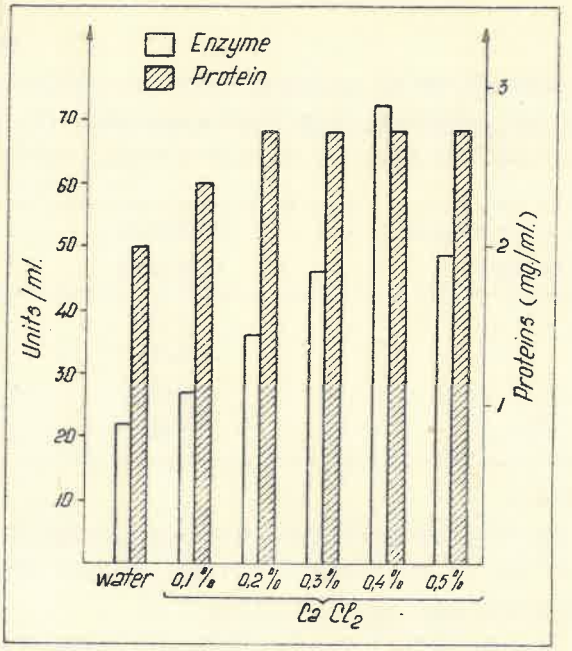


Fig.3 - The influence of $CaCl_2$ concentration upon the extraction efficiency of amylase

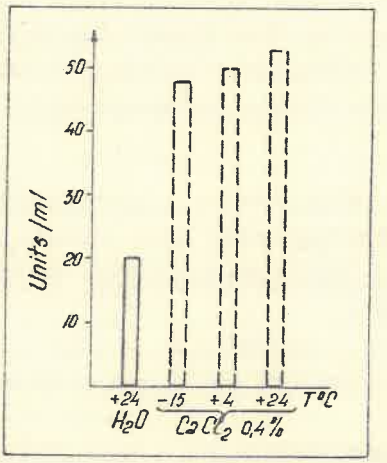


Fig.4 - The determination of optimum extraction temperature in the case of samples obtained with distilled water and $CaCl_2$ solution.

Table 1

The determination of optimum time extraction of amylase from Mytilus galloprovincialis hepatopancreas (1 g tissue was homogenized with 1o ml o.4 % $CaCl_2$ solution)

Time extraction (hours)	Protein (mg/ml)	Units/ml
0	3.34	58.2o
1	3.34	52.8o
2	3.34	5o.4o
24	4.8o	5o.1o

CONCLUSSIONS

By testing different extraction media it was found that $CaCl_2$ solutions solubilises best the amylase from the hepatopancreas of Mytilus galloprovincialis.

The enzymatic preparation presents maximum activity at 35°C when o.4% $CaCl_2$ solution is used for extraction.

The extraction temperature don't influence significantly the activity of the obtained preparations.

The amylase from mussel hepatopancreas is extremely soluble in saline solutions, its solublisation being achieved practically during the tissue homogenisation.

REFERENCES:

1. DUMITRU,I.F., NICULESCU,S., IORDACHESCU,D., GHITESCU,E. - 1975 - Evidencing some acid proteases in the sea mussel Mytilus galloprovincialis. Revue Roum.Biochim., 12: 159-165.

2. GOROMOSOVA,S.S., SAPIRO,A.Z. - 197o - Amylase activity in the hepatopancreas and muscles of mussels. Biol.moria, 18: 89-98.

3. LOWRY,O.H., ROSEBROUGH,L.A., FARR,R.J., RANDALL,R.J. - 1951 - Protein measurement with Folin reactiv.J.biol.Chem., 193: 265-275.

4. METAIS,P. BIETH,J. - 1968 - Determination de l'alpha amylase par une microtechnique.Ann.Biol.Clin., 26: 133-142.

5. MINAFRA,S. - 1968 - La fosfomonoesterasi acida in alcune specie di molluschi marini. Biol.lat., 21: 223-234.
6. SKARDIV MAGRIE E. - 1957 - Sull'attivita transaminasica nel Mytilus galloprovincialis. Distribuzione dell'attivita enzimatica nei vari organi. Boll.Soc.Ital.biol.sperim., 33: 1089-1090.