ENZYME PURIFICATION: HEN EGG LYSOZYME

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INTRODUCTION

Hen egg white lysozyme (EC 3.2.1.17) is an important enzyme model. It's active site is able to recognize and hydrolize the glicosidic polymer, murein, forming the bacterial wall, defending the organism from infections. Lysozyme is a basic small protein and, thanks to this characteristics, we are able to separate it from the rest of components forming the egg white.

The objective of this study is to propose an isolation and purification method for the lysozyme. The proposed purification should be as high yield as possible.

MATERIALS AND METHODS

The enzyme, obtained from a local market hen egg, follows this scheme of purification:

1) **Acid treatment** and 2**) heat treatment** selecting the supernatant. This will give us two different extracts, E1 and E2 consecutively.

3) **An ion exchange chromatography** is then carried out. An enzymatic assay is made to find out the aliquots that are kept forming the extract E3.

Extracts were then analyzed:

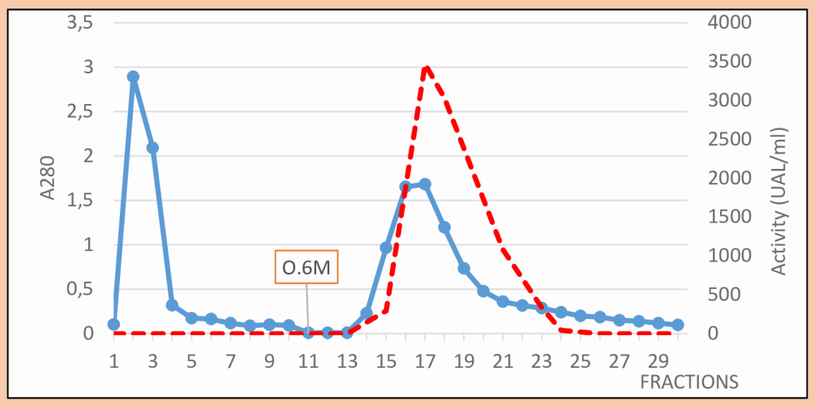
4) An **activity essay** with Micrococcus lysodeikticus for the three extracts and a protein [**concentration determination** (Bradford method [5]) allow us to decide the enzyme yield for each process.

5) A **SDS-PAGE electrophoresis** (Laemmli method [7]) give us more information about the degree of purify and the molecular weight of the principle proteins.

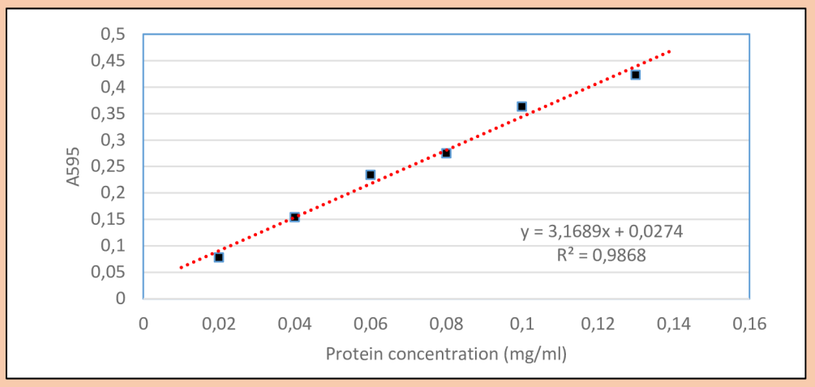
RESULTS

- ACID AND HEAT TREATMENT**:** The eggwhite with acetic acid 0.1 M was centrifugated (E1). The supernatant was carried to 60ºC and after 5 minutes it was centrifugated too (E2). After this isolation, it´s visible a thin turbid film at the bottom of the tubes 1 and 2. This is an indicator of little precipitation.

- CHROMATOGRAPHY: As it is an ion exchange chromatography, proteins elute in order of it´s acid-basic component. The blue line indicates the eluted protein and the red line give us information about the activity for the lysis of Micrococcus lysodeikticus walls. Fractions 15 to 23 were selected to conform the extract E3.



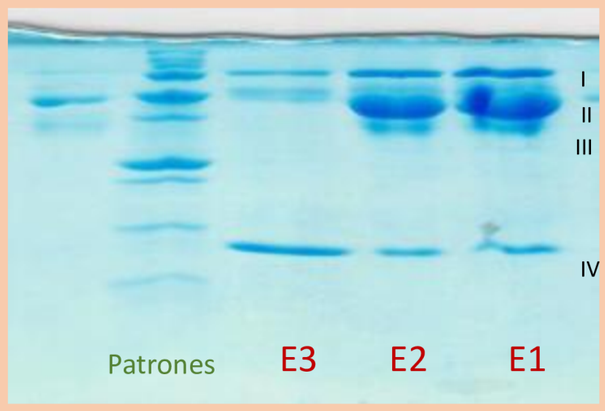
- DETERMINATION OF PROTEIN´S CONCENTRATION: BSA concentration standard curve allow us to determine the concentration of the different extracts.



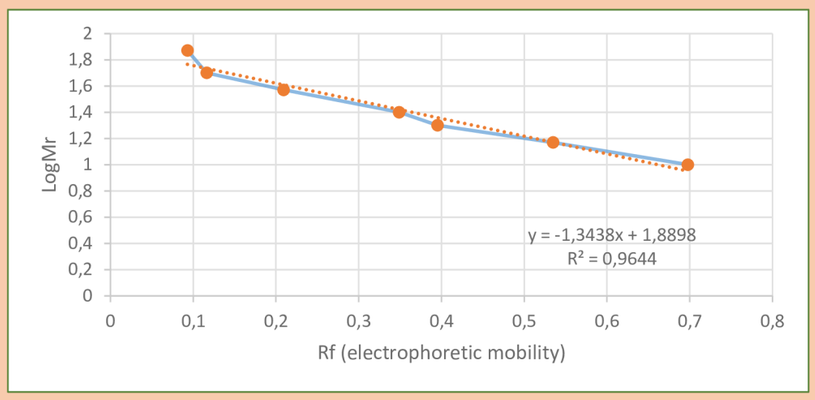
PURIFICATION TABLE:

Extracto	Volumen total (ml)	Proteína total (mg)	Actividad (UAL/ml)	Actividad total (UAL)	Actividad específica (UAL/mg)	Rendimiento (%)	Purificación (Nº de veces)
E1	16	295,2	9166,7	146667,2	496,84	100	1
E2	15	229,5	7500	112500	490,2	76,7	0,986
E3	11	1,177	1016,6	11182,6	9500,93	17,14	25,92

- SDS-PAGE: The electrophoretic mobility shows a small protein (IV) that has been purified, being it´s strip wider in E3 than in E1. Is remarkable, how the bigger proteins such us II (Ovoalbumin) almost disappear in E3



Lysozyme
Mr= 13 KDa



CONCLUSION

The purification method used in this study give us some clues in order to develop a new high yield method. The changes to implement are:

First of all, as the heat treatment doesn´t increase the purification, we can discard this step.

Using two different chromatographies seems to be the best way to isolate the lysozyme. An ion exchange chromatography after an exclusion molar chromatography would give us better yields.

Finally, the possibility of dialyzing the final sample should be considered in order to take out the salts used in the ion exchange chromatography.

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