PURIFICATION AND EXTRACTION OF EGG WHITE LYSOZYME

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INTRODUCTION

Lysozyme (EC 3.2.1.17) belongs to a group of enzymes that catalyses the hydrolysis of glycoside bonds β $(1 \rightarrow 4)$ of the polysaccharides from bacterial cellular walls. Although this enzyme can be found in many different organisms like virus, plants or bacteria or even in different human secretions [1], in the experimentation egg white lysozyme was used.

Lysozyme is a globular protein (14 kDa) that shows stability at high temperature and acid pH conditions, and enzymatic activity against *Micrococcus lysodeikticus*. Furthermore, the enzyme has got a basic isoelectric point (10.5)

The goal of the experimentation was to find and propose an effective method for the purification of egg white lysozyme.

MATERIALS AND METHODS

The biological material that was used in this experiment was a hen egg, although chemical reagents were also needed like acetic acid, Amberlita CG-50, *Micrococcus lysodeikticus* walls or BSA. Once we had the egg, the white was removed from the yolk and a series of procedures were followed:

1. Acid treatment → the egg white was mixed with acetic acid (0.1M) and then it was filtrated and centrifuged, obtaining supernatant E1.
2. Heat treatment → to denaturalize part of the proteins that were in the egg white, a 60º C heat treatment took place on E1, obtaining supernatant E2.
3. Ionic exchange chromatography → until absorbance 0 values were obtained, 10 ml volume fractions were collected eluting with 0,1 M phosphate buffer; then 3 ml volume fractions were collected in a phosphate buffer with a higher ionic force (0,6M).
4. Enzymatic assay → to quantify lysozyme activity using the Morsky Method [2], an enzymatic assay was performed using *Micrococcus lysodeikticus* cell walls as natural substrate of lysozyme. E3 was obtained collecting fractions which showed higher activity.
5. Dialysis → a 3 ml volume of E3 was taken and it was dialyzed in a 5 L beaker that contained phosphate buffer (0,01M).
6. Protein concentration quantification → making use of the Bradford Method [3], the protein concentration of E1,E2 and E3 was quantified. A calibration curve was built using a constant volume of Bradford reagent (2.7 ml) and another constant volume of BSA but changing its concentration. Then the A_{595} was measured.
7. Electrophoresis → finally with the aim of quantifying the egg white lysozyme molecular mass, an electrophoresis in denaturing conditions was held following the Laemmli Method [4], so a 15% acrylamide gel was prepared.

RESULTS

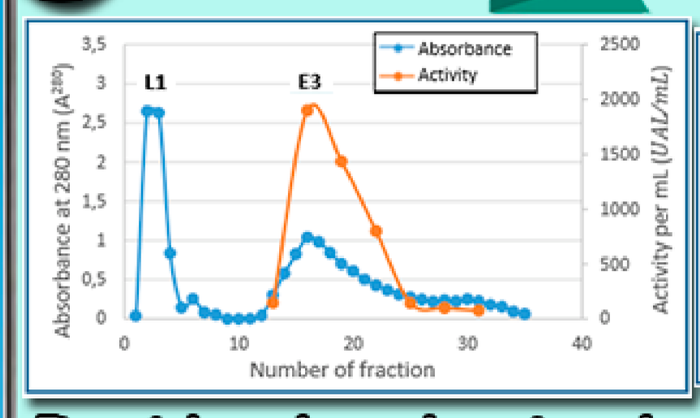

Figure 1. Ion exchange chromatography elution profile

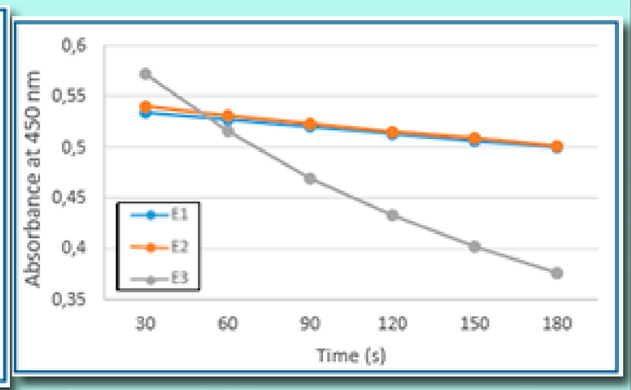

Figure 2. E3's activity assay after a ion exchange chromatography

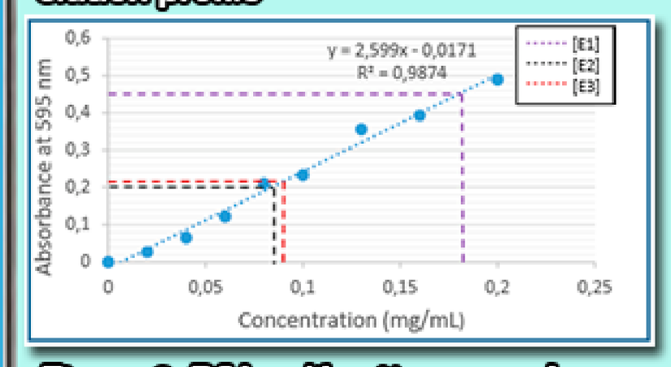

Figure 3. BSA calibration curve by Bradford method.

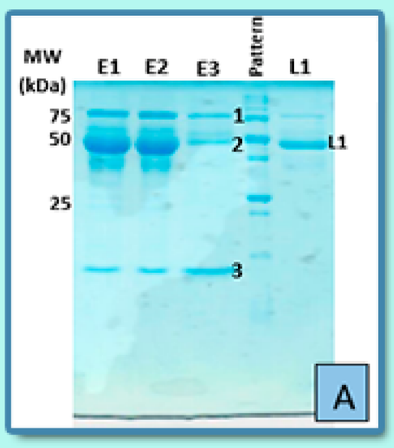

Figure 4. Electrophoresis agarose gel

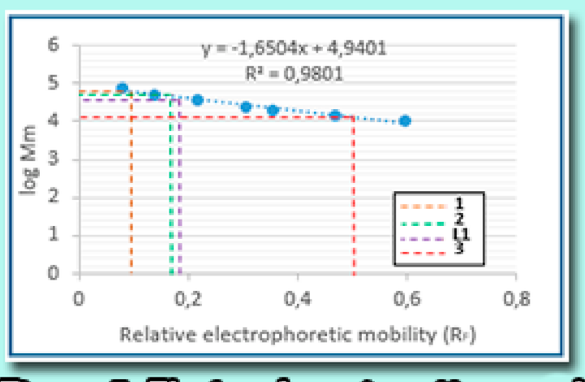

Figure 5. Electrophoresis patterns calibration curve

Table 1. Lysozyme purification table after a ion exchange chromatography

STAGE	Total Vol. (ml)	Total Prot. (mg)	Total Act. (UAL)	Specifc Act. (UAL/mg)	Efficiency %	Purification
E1	15.9	18,00	148399,47	518,51	100	1
E2	14	20,00	140000	500	94,7	0.96
E3	41	3,56	11343,06	768,5	7,6	1.48

CONCLUSIONS

As we can see in Table 1 and Figure 4, we conclude:

- The amount of protein (in weight) in E2 is very similar to the one in E1 so we can conclude that the heat treatment is not very effective for the enzyme purification.

- The enzyme obtains its maximum purification value in E3, so the extract in which a dialysis has been realized is way more purificated and the ion exchange chromatography is determinative to the purification process.

- In the electrophoresis gel (Figure 4), we found 3 characteristic bands which correspond to conalbumin (63 kDa), ovoalbumin (45 kDa) and lysozyme (14 kDa). This shows that the purification process need improvements due to the fact that we have two contaminant proteins.

- To improve the purification method of the lysozyme, the heat treatment could be eliminated and we could perform a size-exclusion chromatography followed by an ion exchange chromatography.

REFERENCES

1. Fleming A. & Allison V.D. (1922). Observations on a bacteriolytic substance (lysozyme) found in secretions and tissues. Br J Exp Pathol 13, 252-260

2. Morsky P. (1983). Turbidimetric determination of lysozyme with *Micrococcus lysodeikticus* cells : reexamination of reaction conditions. Anal. Biochem. 128, 77-85.

3. Bradford M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254

4. Laemmli U.K. (1970). Cñeavage of structural proteins during the assembly of the head bacteriophage T4. Nature 277, 680-685.