PURIFICATION AND EXTRACTION OF EGG WHITE LYSOZYME

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INTRODUCTION

The lysozyme is an enzyme (EC 3.2.1.17) that catalyses the hydrolysis of glycoside bonds β->1,4 of the polysaccharides from bacterial walls. It´s a globular protein, found in many living organisms, basic, with high isoelectric point and 14kDa of molecular mass. The goal is to propose a method to purify the enzyme of egg white lysozyme **[1]**.

MATERIALS AND METHODS

1. Acid treatment (E1): egg white was mixed with 0.1M acetic acid, then it was filtrated and centrifuged (4500rpm) for 5 minutes.

2. Heat treatment (E2): supernatant obtained in the previous step was incubated in water at 60˚C, then it was centrifuged and conserved in ice.

3. Ionic exchange chromatography in Amberlite CG50 (E3): fractions of 10mL were obtained eluting with 0.1M phosphate buffer until the absorbance measurement was zero. Then 3mL fractions were eluted with 0.6M phosphate buffer.

4. Enzymatic assay: the absorbance of 0.3mL of E3 and 3mL of *Micrococcus lysodeikticus* walls was performed at 450nm every 30 seconds during 3 minutes **[2]**.

5. Dialysis: 2mL of E3 were dialyzed with 0.01M phosphate buffer and dried in polietilenglicol 35000.

6. Protein concentration determination: Bradford method **[3]** was developed using 0.3mL of different concentrations of BSA and 2.7mL of Bradford reagent to build the calibration curve. The absorbance was measured at 595nm. The same process was performed for E1, E2 and E3.

7. Electrophoresis: 20µL of the samples were mixed with sample buffer 2X and heated. Then 15µL of each one and 10µL of protein markers were applied in SDS polyacrylamide gel. The gel was stained with blue of Coomassie R-250 **[4]**.

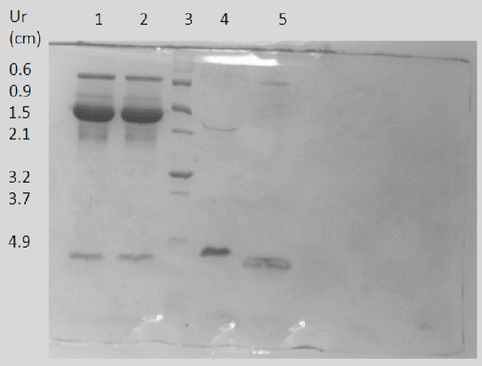

Fig 4. Electrophoresis gel. Electrophoretic mobility (cm) for the proteins markers in well 3. Well 1 corresponds to E1, well 2 is E2, well 4 is dialyzed E3 and well 5 is E3.

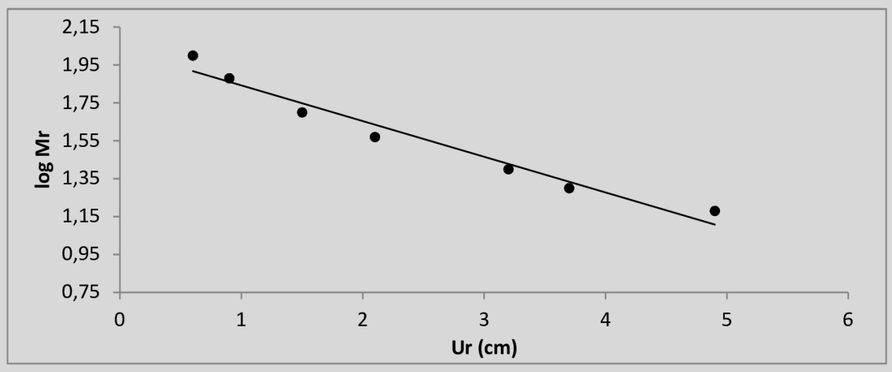

Fig 5. Calibration curve of the electrophoresis. Interpolating the mobility of well 4, the molecular mass of lysozyme obtained is 12.02kDa.

RESULTS

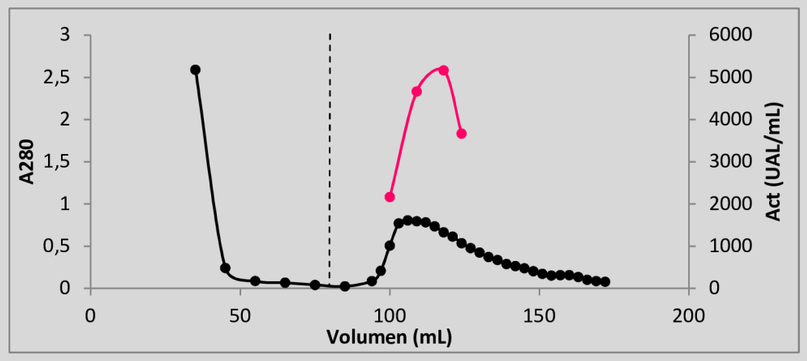

Fig 1. Ionic exchange chromatography. Chromatogram of fractions eluted in Amberlite column (black profile, right axis). The line indicates buffer change. Activity profile of the lysozyme (pink dots, left axis).

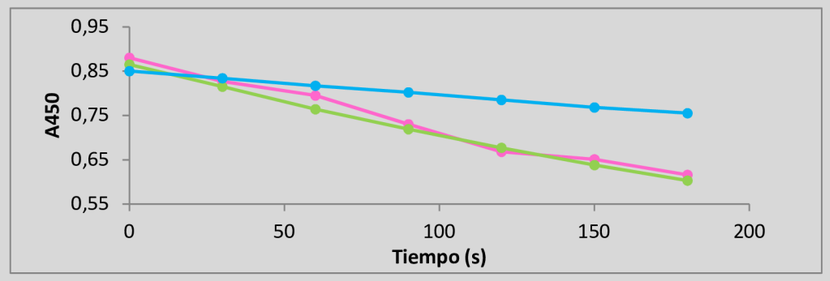

Fig 2. Enzymatic assays of fractions E1 (pink), E2 (green) and E3 (blue) diluted 1/30.

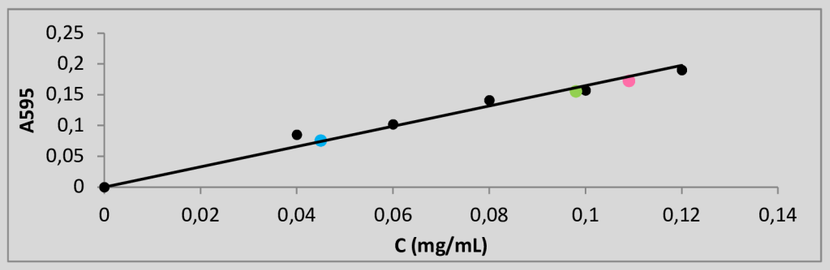

Fig 3. Calibration curve of known BSA concentrations. Blue dot corresponds to E3 (1/5) which concentration is estimated in 0.045mg/mL. Green dot corresponds to E2 (1/100) with a concentration of 0.109mg/mL. And pink dot corresponds to E1 (1/100), with a concentration of 0.098mg/mL.

CONCLUSIONS

	Activity (UAL)	Protein (mg)	Specific Act. (UAL/mg)	Yield (%)	Purif (folds)
E1	127500	147	867	100	1
E2	116100	141	826	91,1	0,95
E3	105000	6,8	15556	82,4	18

- As observed in the table, steps E1 and E2 conclude with the same protein mass but there is a loss in enzyme activity and yield.

- The process achieves an 18 fold purification in E3. As it can be seen in the electrophoresis results (Fig 4), dialyzed sample is more purificated.

- The lysozyme molecular mass estimated was 12.02kDa, very close to the real one.

- Comparing to the results obtained in Sephadex G75 purification (not shown), this procedure ends with more contaminating proteins and the estimated lysozyme molecular mass is smaller than the real one. However, with either method, pure lysozyme is not obtained.

- To improve the purification, heat treatment could be eliminated and Sephadex chromatography could follow Amberlite´s.

REFERENCES

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[4] B.D Hames, D.Rickwood (1986) "In Gel Electrophoresis of Proteins: A practical Approach" IRL, Oxford, 1-86.