LYSOZYME PURIFICATION FROM HEN EGG WHITE

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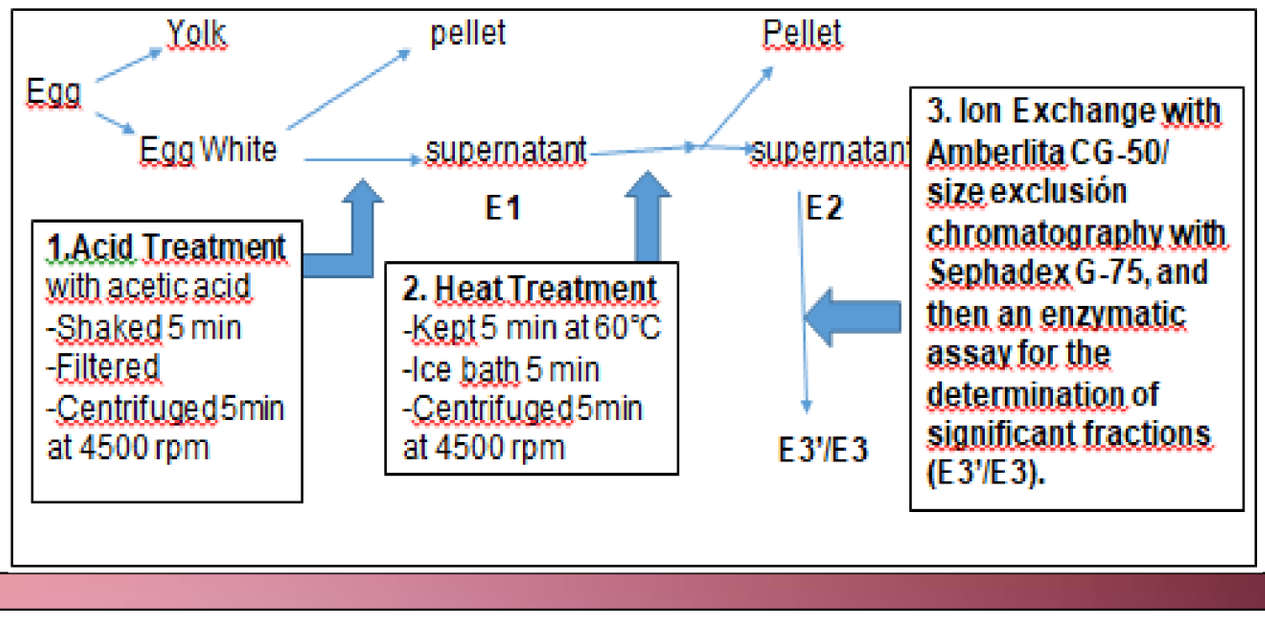
INTRODUCTION

Lysozyme (N-acetylmuramide glycanhydrolase; EC 3.2.1.17) corresponds to the 3% of egg white proteins [1]. It catalyses the hydrolysis of glycoside bonds β-1,4 of the polysaccharides from bacterial cell walls, such as Fleming established in 1922 [2]. It's a globular protein formed by one polypeptide chain, and it's a basic protein (pI=10.5-11.0), with low molecular mass (14kDa). It stands out for its stability at acid pH and its thermal resistance. Has great interest in the pharmacological industry and food [3], besides that it is easy and cheap to obtain. It also belongs to the innate defense system of many organisms.

The main objective of the work was to achieve an effective method to purify this enzyme, starting from the knowledge of its differential characteristics with the other proteins of the egg white.

METHODS

PURIFICATION OF THE LYSOZYME



After the purification, a **dialysis** was made to shift the initial E3/E3' solvent for phosphate buffer and concentrate this sample in polyethilenglycol.

EVALUATION OF THE PROCESS WITH E1, E2 AND E3/E3' SAMPLES

- Enzymatic assay to locate and determine its enzymatic activity, using a solution of bacterial cel walls from *Micrococcus lysodeikticus* [4], measuring the absorbance at 450nm every 30 seconds during 3 min. The activity was quantifed with this equation (eq.1):

$$Actividad\left(\frac{UAL}{mL}\right) = \left(\frac{\Delta A450}{\min}\right) \times \frac{1UAL}{\frac{0,001}{\min}} \times \frac{1}{0,3mL} \times factordilución,$$

- **Colorimetric assay** by Bradford method [5] to determinate protein concentration of each samples. with the previous preparation of a standard curve of BSA from a concentration of 0.2mg / mL. It was added to 0.3 mL of each stage sample 2.7mL of Bradford, and the absorbance at 595nm was measured.

-**SDS-PAGE electrophoresis** according to Laemmli method [6], with 15% of acrylamide, in which 20µL of the different samples to be analyzed were mixed with 20µL of 2x charge buffer and warmed up. 15µL of the samples E1, E2, E3, E3' and the maximums of the chromatographic profile, and 10µL of standard proteins were loaded in the gel, and 25mA / gel was applied for one hour. The gel was subsequently stained with Coomassie blue.R-250.

RESULTS

1. *Acid and heat treatment*. No sediment was found.

2. *Chromatographic results*

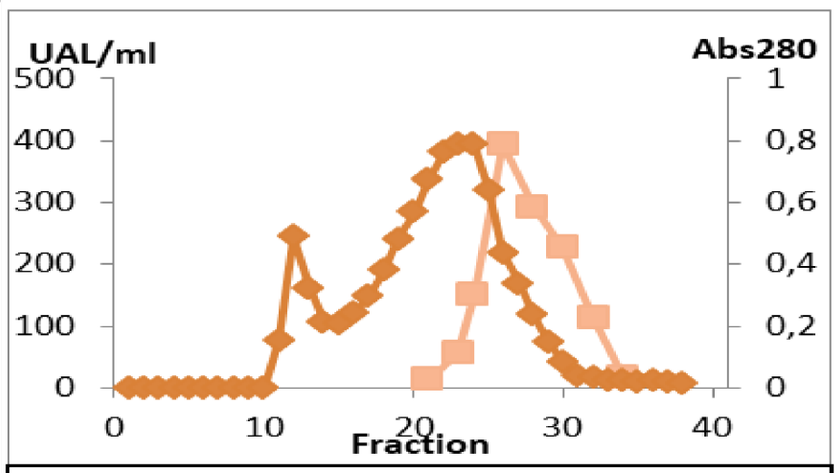


Figure 1. Chromatogram of fractions eluted by size exclusion chromatography (right axis, orange). Activity profile of lisozyme (left axis, pink).

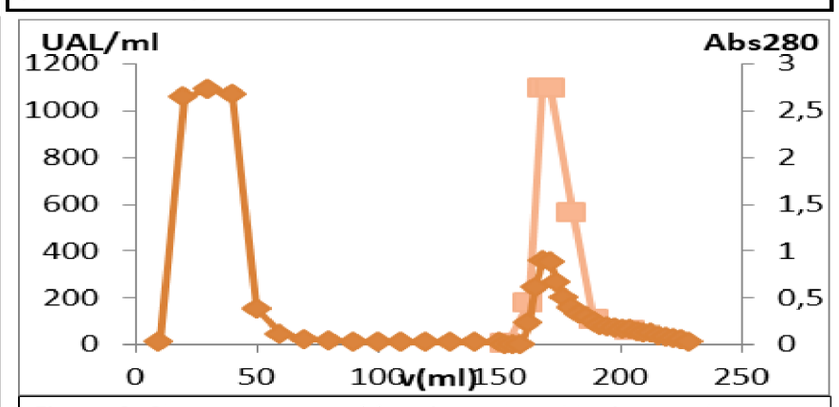


Figure 2. Chromatogram of fractions eluted by ion exchange chromatography (orange). Activity profile of lysozyme (pink).

3. Enzimatic assay

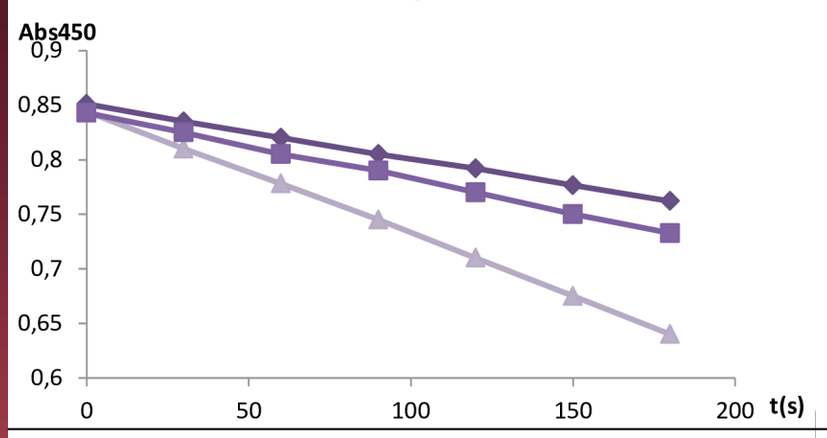


Figure 3. Enzymatic activity of lysozyme. E3 (Δ), E1 1/100 (◊) y E2 1/100 (□). E1= 10.333,33 UAL/ml, E2= 11.333,33 UAL/ml, E3= 233,33 UAL/ml

4. *Determination of protein concentration*

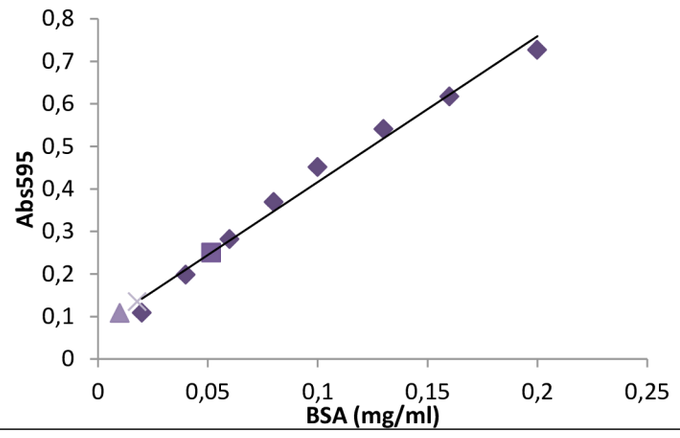


Figure 4. BSA concentration standard curve. E1 1/500 (□), E2 1/500 (Δ), E3 (x). Interpolations: E1=27mg/ml; E2=36mg/ml; E3=0,028mg/ml

5. *SDS-PAGE electrophoresis*

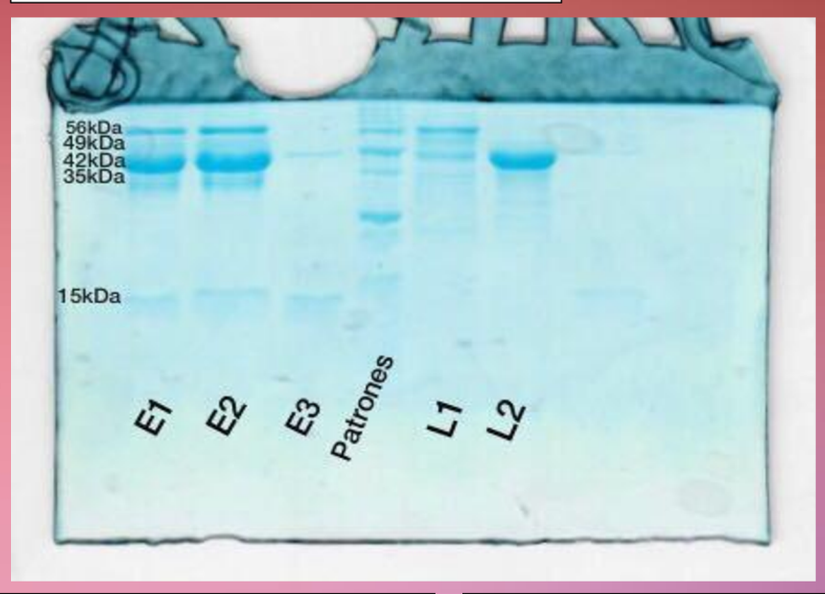


Figure 5. SDS-PAGE electrophoresis by preparing a 15% acrylamide gel. There is no difference between E1 and E2 so this purification step should be removed. Contamination by ovoalbumin (43kDa) is appreciated in E3. The molecular mass estimated for lysozyme is 15kDa. The difference between L1 and L2 is due to the different mechanisms of wash

CONLUSIONS

- No sediment was found after acid and heat treatment, so it was deduced that the proteins in hen egg are thermostable.

- The size exclusion chromatography provided a molecular mass for lysozyme that is more similar to the real one than the obtained by ion exchange chromatography. It also provided a greater efficiency (Table 1) so it was proposed that size exclusion is the best method for lysozyme purification.

- However, the result of the SDS-PAGE showed contamination by ovoalbumin.

- For a better result heat treatment could have been skipped because the purification factor was less tan 1. Also, it would be useful doing another size exclusion chromatography with a different gel in order to separate lysozyme from ovoalbumin.

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