ISOLATION AND PURIFICATION OF HEN EGG-WHITE LYSOZYME

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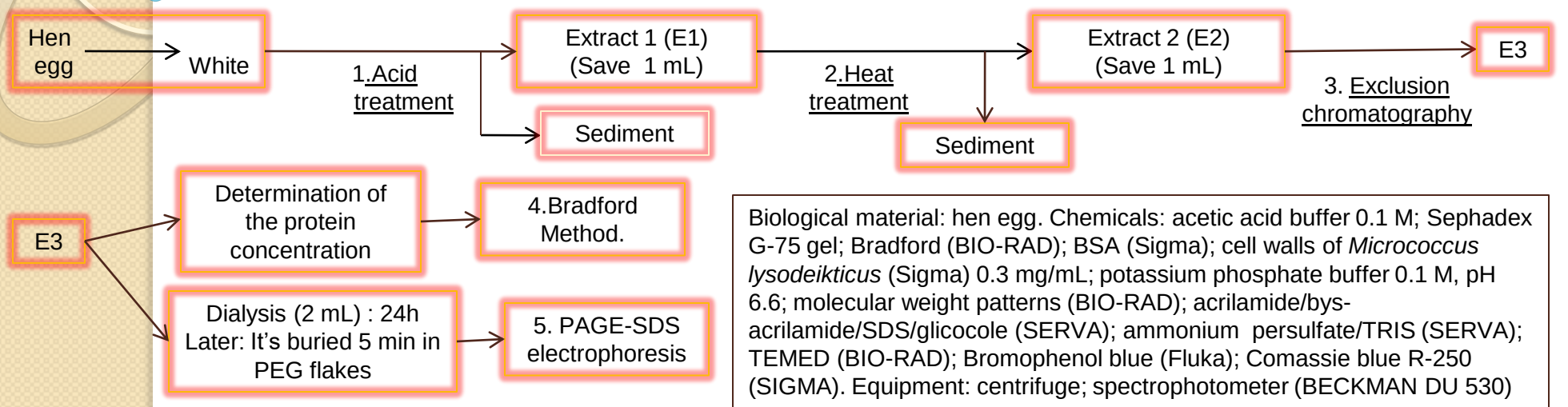
INTRODUCTION

The term lysozyme includes a whole group of enzymes which catalyses the hydrolysis of glycosidic bonds β(1-4) of the bacterial walls, precisely between polymers N-acetylmuramic and N-acetyl-D-glucosamine in a peptidoglican. The isolation and purification of lysozyme have been achieved using an acid treatment, a heat treatment and a molecular exclusion chromatography with G-75 Sephadex. Alternatively, a ionic exchange chromatography was done in order to compare the results. Then, to determine the activity and concentration of the lysozyme a dialysis was done. Next, the composition of proteins was analysed by a PAGE-SDS electrophoresis. Finally, a purification table was elaborated. The aim of this investigation is proposing a method to isolate and purificate lysozyme from the white of a hen egg according to its properties.

MATERIALS AND METHODS



Biological material: hen egg. Chemicals: acetic acid buffer 0.1 M; Sephadex G-75 gel; Bradford (BIO-RAD); BSA (Sigma); cell walls of *Micrococcus lysodeikticus* (Sigma) 0.3 mg/mL; potassium phosphate buffer 0.1 M, pH 6.6; molecular weight patterns (BIO-RAD); acrilamide/bys-acrilamide/SDS/glicocole (SERVA); ammonium persulfate/TRIS (SERVA); TEMED (BIO-RAD); Bromophenol blue (Fluka); Comassie blue R-250 (SIGMA). Equipment: centrifuge; spectrophotometer (BECKMAN DU 530)

RESULTS

1. Acid treatment: slightly yellow extract, negligible sediment.

2. **Heat treatment**: see-through extract, sediment was cloudy.

3. Exclusion chromatography.

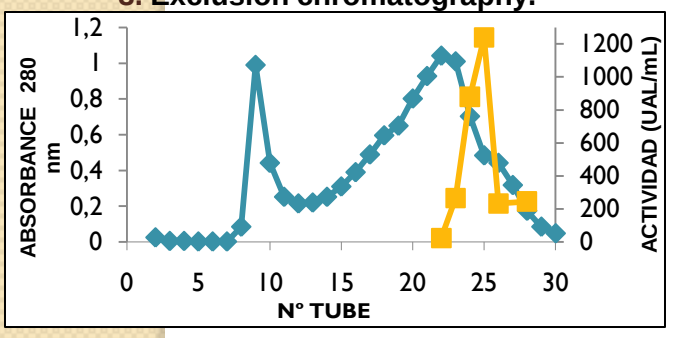


Figure 1. Chromatografic behaviour of E2 on Sephadex G-75.

4. Bradford Method.

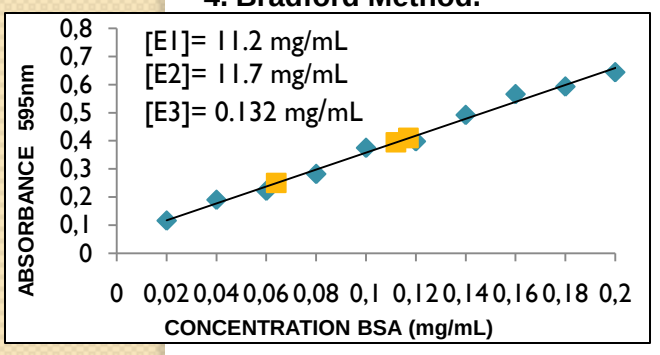


Figure 2. BSA calibration curve to determinate protein concentration in each of enzymatic extracts.

5. PAGE-SDS electrophoresis.

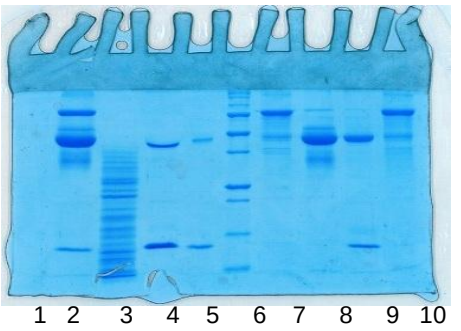


Figure 3. SDS-PAGE gel electrophoresis (15% acrilamide). **Lane 2**: E1 at 1/20 dilution (15 µL). **Lane 3**: E2 at 1/20 dilution (15 µL). **Lane 4**: E3 (20 µL). **Lane 5**: E3 (5 µL). **Lane 6**: MW standard proteins (10 µL).

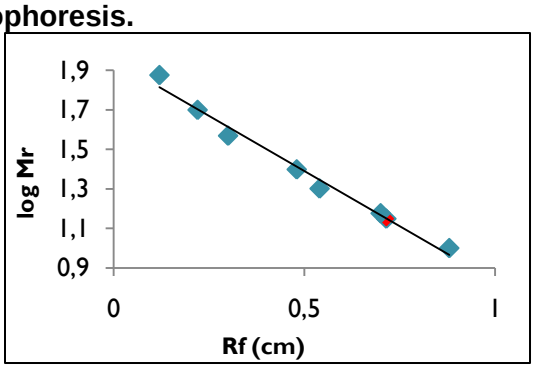


Figure 4. Standard curve of log molecular weight vs relative mobility (Rf) of "MW standard proteins". Rf of lysozyme: 0.715 (red point); molecular weight determinated is 14.1 KDa.

Table 1. Purification table of lysozyme (using exclusion chromatography)

STAGE	TOTAL VOL. (mL)	TOTAL PROTEIN (mg)	TOTAL ACT. (UAL)	SPECIFIC ACT. (UAL/mg)	YIELD (%)	PURIFICATION (fold)
E1	15	171	116000	678.36	100	1
E2	13.6	149.6	105170	703.00	90.66	1
E3'	36	5.04	26400	5238.09	22.76	7.72

Table 2. Purification table of lysozyme (using ionic exchange chromatography)

STAGE	TOTAL VOL. (mL)	TOTAL PROTEIN (mg)	TOTAL ACT. (UAL)	SPECIFIC ACT. (UAL/mg)	YIELD (%)	PURIFICATION (fold)
E1	15	156	150000	961.54	100	1
E2	13	124.8	112666	902.78	75.11	0.94
E3	28	4.872	96133	19731.78	64.09	20.52

CONCLUSIONS

Table 1: low yield for E3 (>25%) considering the purification (7.72). **Table 2**: high yield for E3 (>50%) considering the purification is greater (20.52); E2 has been contaminated in heat treatment (purification is 0.94). In both process, the lysozyme has been partially purificated; therefore, the specific activity increase in each stage. Comparing the results, in ionic exchange chromatography, the results are reasonably better than the results of exclusion chromatography. **Improvements for isolation and purification**: elimination of heat treatment, considering the sample can be contaminated; connect both chromatographies, to get a better isolation and purification.

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