Egg lysozyme purification

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Lysozyme (EC 3.2.1.17) is an enzyme that catalyse the rupture of the β(1→4) glycosidic bonds in polysaccharides of bacterial cellular walls. This enzyme can be found in human secretions like saliva, tears or mucus.

The main objective of this study is to establish the most efficient method to purify and isolate the lysozyme from hen egg white. In the experiment, it has been purified using an ion exchange and size exclusion chromatography and enzymatic assay. The results of each chromatography have been analysed by PAGE-SDS electrophoresis. After that, some purification tables were made. The two chromatographies were compared to determinate which one was the most efficient in the lysozyme purification process.

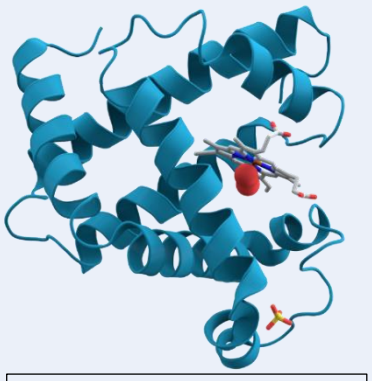
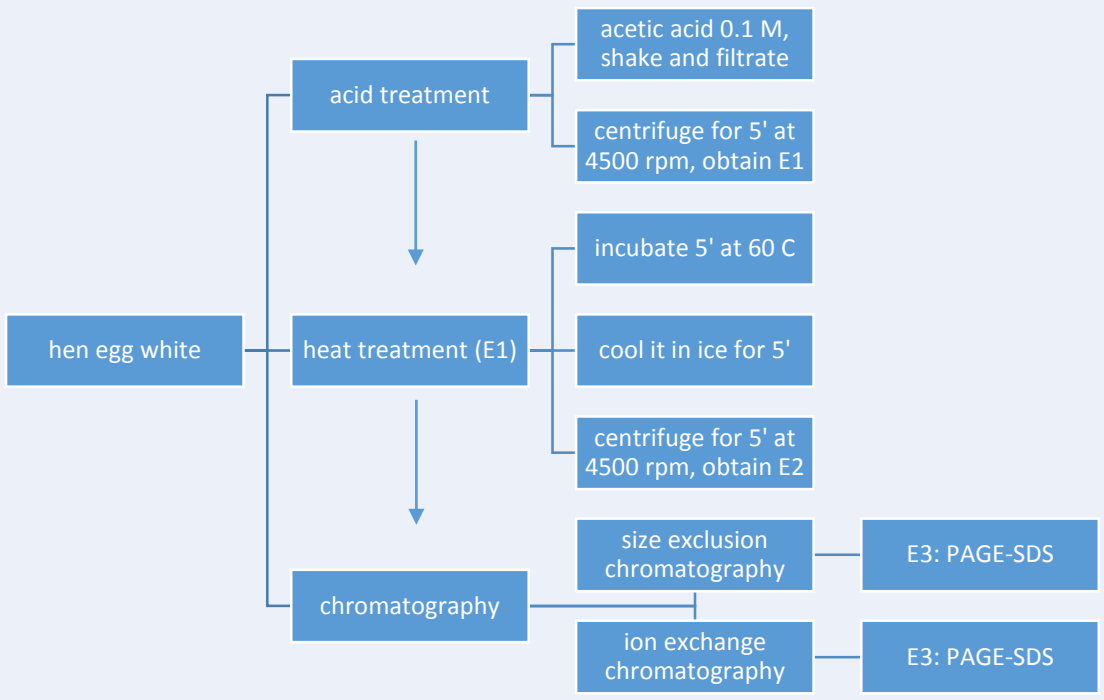


Figure 1: Lysozyme structure

1. Purification process



The scheme above must be follow. After the performance of both chromatographies the fractions with the most activity will be studied, the activity is given by the highest absorbances results, those fractions are **E3**.

To purify E3 a **dialysis** will be performed. The dialysis bag will be introduced into a big recipient with water. The pore of the bag is not big enough to let the lysozyme pass through it. The final volume of E3 after the dialysis will be the most purify fraction of the lysozyme.

In order to determinate the quality of the purification a **PAGE-SDS electrophoresis** will be performed. With the results of the electrophoresis the molecular mass can be calculated.

The concentration of E1, E2 and E3 can be determinate with a **BSA standard line**. To elaborate this, different concentrations of BSA will be use and mixed with Coomasie blue and measure at 595 nm.

2. Results

After the measurement of the fractions of the size exclusion and ion exchange chromatography, the results have been represented below.

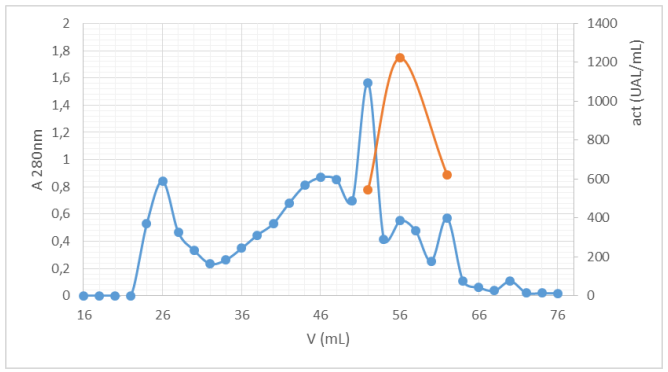


Figure 1. Elution profile of size exclusión chromatography. Using the activity assay (orange), lysozyme activity is between 50 and 63 mL and the higher activity peak is around 56 mL.

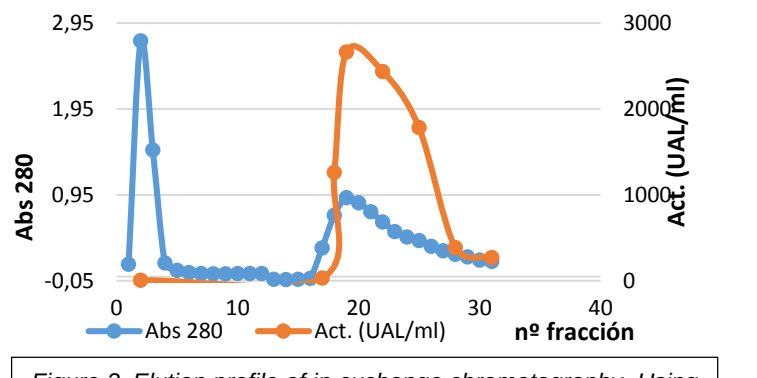


Figure 2. Elution profile of in exchange chromatography. Using the activity assay (orange), lysozyme activity is between 19 and 30 mL and the higher activity peak is around 20 mL.

Results of the PAGE-SDS electrophoresis

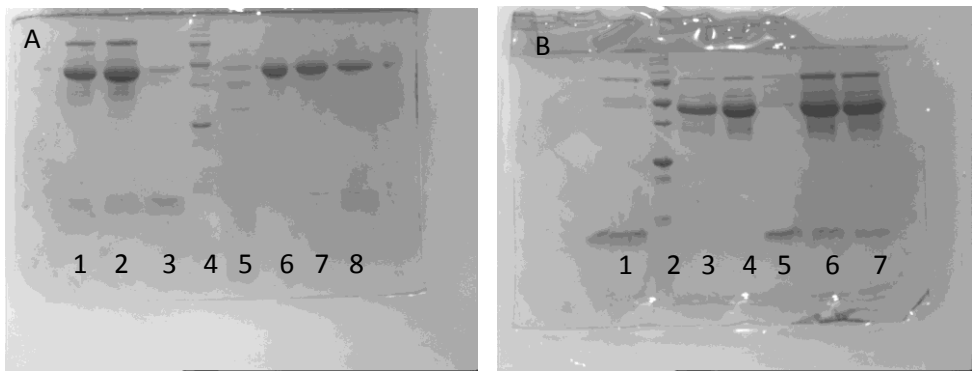


Figure 3. Resulting gels from size exclusion chromatography (A) and ion exchange (B). E1 and E2 are similar (ion exchange well 6: E2, well 7: E1, size exclusion chromatography well 1: E1, well 2: E2), they have two stains: the darkest is ovotransferrin, the medium one is ovoalbumin and the lightest is lysozyme. The lysozyme appears more clearly with E3 (ion exchange well 5 and size exclusion chromatography well 3), the gel of the size exclusion has less stains, but the ion exchange chormaotagraphy's gel has a more intense stain of the lysozyme, showing that probably has more enzyme that the other one. So, A has the lysozyme more purificated, but less concentration than B. In A the patron corresponds to the well 4 and in B to the well 2. The rest of the wells were filled with fractions of the chromatographys, but it won't be taken in consideration.

Sample	Total activity (UAL)	Protein (mg)	Specific activity (UAL/mg)	Yield (%)	Purification
E1	219167	173	1271	100	1
E2	141483	150	946	74,5	0,74
E3	42694	1,6	268513	19,4	211

Table 1. Size exclusion chromatography data

Sample	Total activity (UAL)	Protein (mg)	Specific activity (UAL/mg)	Yield (%)	Purification
E1	150133	147	1020	100	1
E2	92500	138	670	90,7	0,66
E3	75973	7	10792	50,6	10,6

Table 2. Ion exchange chromatography data

3. Conclusion With all these experiments we can determite that ion exchange chromatography is an accurate procedure for lysozyme isolation. However, this method is not enough for a complete purification, because, as it was seen in PAGE-SDS there were more stains that they are from other egg protein that were contaminated the sample. One solution to obtained a complete purified sample is putting together both of chromatographies: ion exchange chromatography give us more enzyme quantify and then, size exclusion one leave us to separate better different components.