COMPARISON OF METHODS FOR LYSOZYME HEN EGG WHITE PURIFICATION

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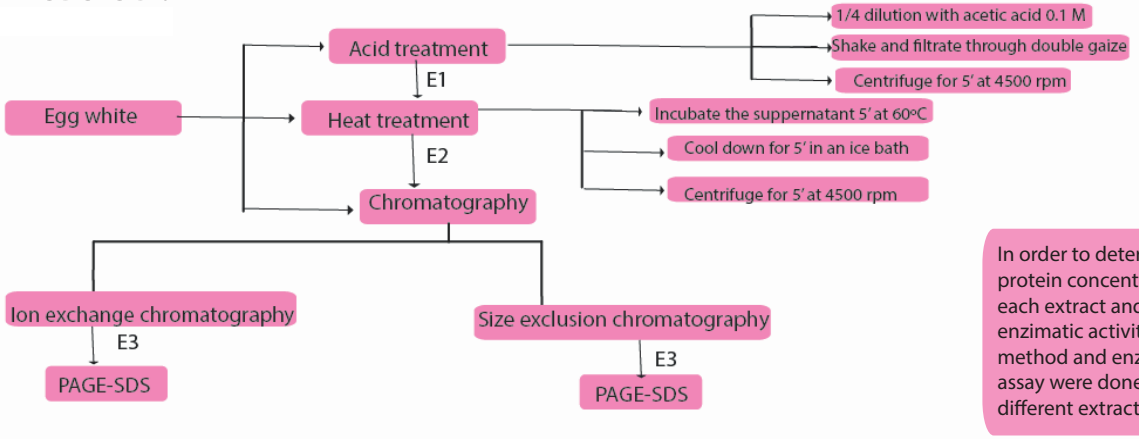
INTRODUCTION:

Lysozyme (EC 3.2.1.17) is an enzyme that appears in a lot of living organisms, and has bactericidal function as its activity allows the enzyme to break β(1-4) glycosidic bonds from bacterial walls. The main objective of this study is to establish the most efficient method to purify and isolate the lysozyme from hen egg white. Heat and acid treatments have been used, followed by two different types of chromatography: ion exchange chromatography and size exclusion chromatography. The results of each chromatography have been analyzed by polyacrylamide gel electrophoresis with SDS, and included in purification tables. At the end of the study it is suggested a more accurate procedure to be used in further studies.

METHODOLOGY:



In order to determine the protein concentration of each extract and its enzimatic activity, Bradford method and enzimatic assay were done to the different extracts obtained.

RESULTS:

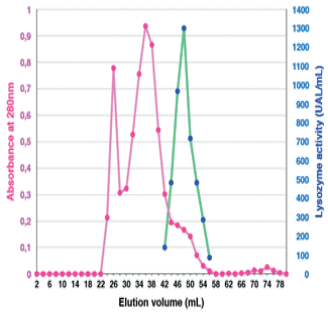


Figure 1: elution profile of size exclusionchromatography. The elution profile shows two peaks that correspond to the elution volume of proteins with a higher molecular mass than the lysozyme. This peaks were used to form L1 fraction. Using an activity assay, lysozyme activity is found between 42 and 56 mL and the peak of activity is found at 48 mL. Lysozyme's elution volume has been found between 44 and 50 mL.

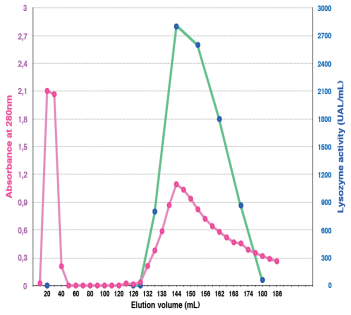


Figure 2: elution profile of ion exchange chromatography. The elution profile shows two peaks: the first one (elution volume = 20 mL) shows no lysozyme activity; the second one shows lysozyme activity and its elution volume is found at 144 mL.

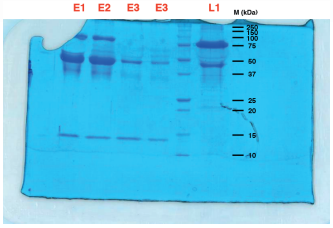


Figure 3: PAGE-SDS of E1, E2, E3 and L1 after size exclusion chromatography.

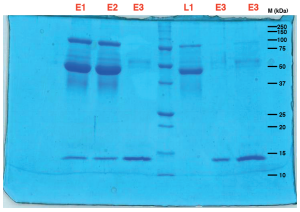


Figure 4: PAGE-SDS of E1, E2, E3 and L1 after ion exchange chromatography.

Table 1: purification table of size exclusion chromatography

Stage	Volume (mL)	Total protein (mg)	Protein concentration (mg/mL)	Total activity (UAL)	Specific activity (UAL/mg)	Yield (%)	Purification (fold)
E1	15	140	9,3	150000	1075	100	1
E2	14	147	10,5	130662	889	87	0,83
E3	13	8,01	0,044	81900	10227	54,6	9,5

Table 2: purification table of ion exchange chromatography

Stage	Volume (mL)	Total protein (mg)	Protein concentration (mg/mL)	Total activity (UAL)	Specific activity (UAL/mg)	Yield (%)	Purification (fold)
E1	16	196	12,23	170667	872	100	1
E2	15	173	11,54	150000	867	88	0,99
E3	33	5,9	0,18	75900	12778	44	15

The results show that ion exchange chromatography is a better method for lysozyme purification because the contaminant band in E3 is weaker than in size exclusion chromatography gel. In both cases, L1 fraction doesn't contain lysozyme. Therefore, lysozyme hasn't been lost in that stage of purification. Using PAGE-SDS gels, lysozyme's molecular mass has been established: 12.2 kDa (size exclusion chromatography) and 14.5 kDa (ion exchange chromatography). In addition, E1 and E2 contain ovotransferrin (78 kDa) and ovoalbumin (47 kDa), apart from lysozyme. In both purification tables, E1 and E2 don't show significant differences.However, specific activity of E3 is higher in table 2, as it is lysozyme purification, which means that ion exchange chromatography is a better method for lysozyme purification.

CONCLUSION:

This study proves that ion exchange chromatography is a more accurate procedure for lysozyme isolation and purification. However, this method isn't enough for a complete purification because after all purification stages lysozyme was contaminated with other egg white proteins. Therefore a final purification stage is proposed: an affinity chromatography in which the gel is made up of peptidoglycan, that is lysozyme's substrate. Besides, the heat treatment could be omitted because no significant purification is seen between E1 and E2, as both samples contain heat resistant proteins.