LYSOZYME PURIFICATION FROM HEN EGG WHITE

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INTRODUCTION

Lysozyme (N-acetylmuramide glycanhydrolase; EC 3.2.1.17) is one of the enzymes which form part of the egg white. It catalyses hydrolysis reactions, cleaving β-(1, 4) glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid from bacteria's cell walls, such as Fleming established [1].
The lysozyme is characterized by being a globular protein formed by only one polypeptide chain. It is a basic protein (pI=10.5 – 11.0), with low molecular mass (14 KDa) and it is stable at acidic pH.

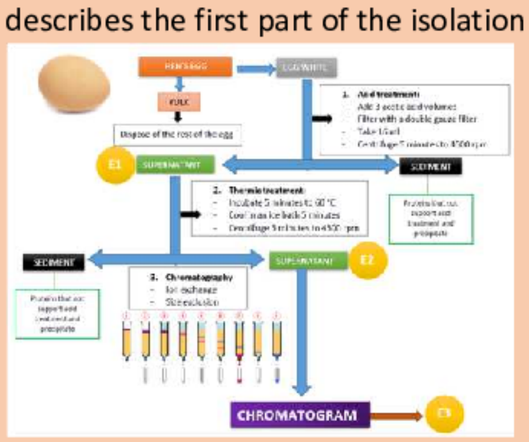
Thanks to these characteristics, the protein could be subject of different techniques as acid and thermal treatment, size exclusion chromatography and ion exchange chromatography, enzyme and colorimetric assay and SDS-PAGE electrophoresis. In this way, the analysis of the obtained results allowed to decide if the method being employed was the most appropriate to purify the lysozyme; and if that is not the case, suggesting an improvement to get high yield.

OBJECTIVE

To propose a good and effective method to isolate and purify the lysozyme from the rest of protein which form egg white.

MATERIALS AND METHODS

The following diagram describes the first part of the isolation of lysozime:



After that, E1, E2 and E3 samples were analyzed by:

- **Dialysis** to shift the initial E3 solvent for phosphate buffer and **concentrate** this sample in polyethilenglycol.
- **Enzymatic assay** to locate and determine its enzymatic activity, thorugh the use of a solution of bacterial cell walls from *Micrococcus lysodeikticus.* [2]
  Colorimetric assay to determinate protein concentration of each samples by Bradford method [3]
- **SDS-PAGE electrophoresis** according to Laemmli method [4] to define lysozyme molecular weight and to test the degree of purity achieved.

RESULTS

1. **ISOLATION OF LYSOZIME. ACID AND THERMAL TREATMENTS.**
   Eggwihite was subjected to acid treatment with acetic acid and 15 mL (called E1) of supernatant were obtained. After a thermal treatment at 60ºC, 14 mL (called E2) of supernatant were obtained. No visible precipitate was obtained by any of the methods.

2. **CHROMATOGRAPHICS RESULTS:**
On the left, the image shows three peaks that correspond to fractions 6, 9 and 25. Fraction 25 is an intuited peak, it is selected as average of every protein concentrations that form E3. In these fractions there will be more protein concentration and therefore greater activity. Fractions 6, 9 and 25 are studied by enzymatic essays to determine the location of the lysozyme.

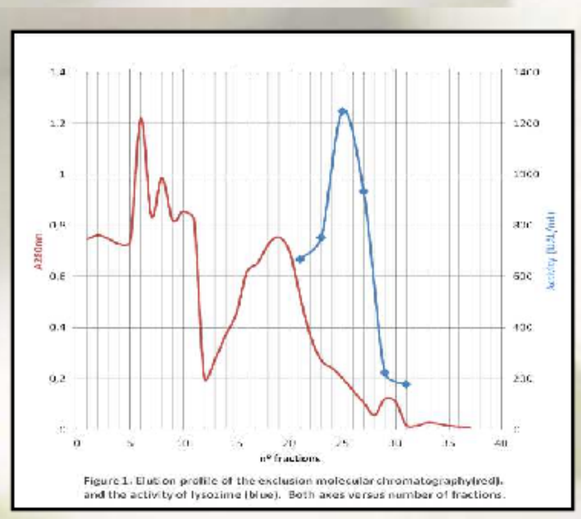


Figure 1: Elution profile of the exclusion molecular chromatography(red), and the activity of lysozime (blue). Both axes versus number of fractions.

3. **ENZYMATIC ASSAYS.**
On the right; E3 was formed by uniting 21-27 fractions. Enzymatic essays verify that only E3 presents activity, so E3 contains lysozyme whose maximum activity is 1246.67 UAL/mL.

4. **DIALYSIS.**
A total volume of 2mL of E3 was dialyzed to exchange the buffer (in molecular exclusion chromatography) or to eliminate high salt content (ion exchange chromatography). Finally, a successful dialysis provided a volume of 730 µL of DIALYZED E3.

5. **DETERMINATION OF PROTEIN ´S CONCENTRATION**

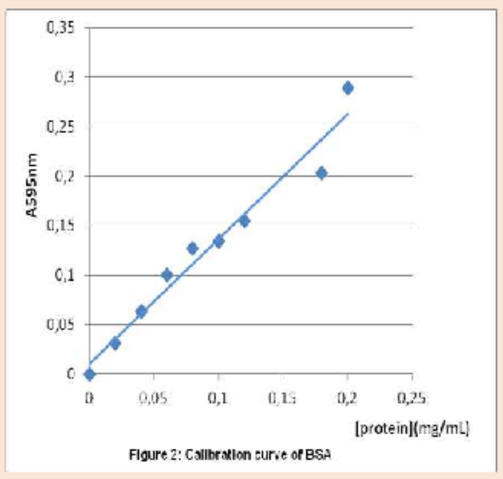


Figure 2: Calibration curve of BSA

Table1: E1, E2 and E3 concentrations. E1 and E2 should have the same concentration because through both methods no precipitate was obtained.

	C(mg/mL)
E1	11,8
E2	9
DIALYZED E3	0,184
E3	0,096

6. **ELECTROPHORESIS.**

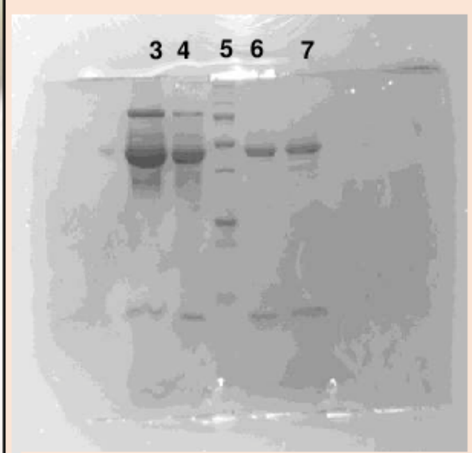


Figure 3: Electrophoresis gel. There is not a single band in any of the wells so it was not possible to purify the lysozyme in any of them.

Table 2: Wells' information.

Well	Sample	µg charged
3	E2	135
4	E1	177
5	Protein markers	-
6	Dialyzed E3	2,76
7	E3	1,74

Table 3: Results obtained in the standard curve of electrophoresis to calculate the molecular weight of: Lysozyme (8.71 KDa) and other contaminating proteins (P.C.) like Ovoalbumine (57.54 KDa).

	Mr (Kda)	LogMr	Rf
PROTEIN MARKERS	200	2,3	2,63
	150	2,18	4,6
	100	2	7,89
	75	1,88	11,84
	50	1,7	19,08
	37	1,57	26,97
	25	1,4	42,1
	20	1,3	48,68
	15	1,18	64,47
	10	1	-
P.C.1	95,5	1,98	9,1
P.C.2	72,44	1,86	15,79
P.C.3	57,54	1,76	21,71
P.C.4	41,7	1,62	30,26
LYSOZYME	8,71	0,94	69,74

7. **PURIFICATION TABLE.**

Table 4: Purification table of both types of chromatographies.

E3	Act. (UAL)	Prot (mg)	Sp. Act. (UAL/mg)	Yield (%)	Purif. number
Sephadex	97520	174	81020	20	61
Amberlita	94367	142	41961	52	35

CONCLUSIONS

Eliminate thermal treatment and replace the acid treatment for being ineffective. None of the chromatographies purified the Lysozyme, so combining both chromatographies would lead to best results. In addition, some modifications can be performed to get more effective chromatographies like increasing the length of the molecular exclusion column, changing the eluents of ion exchange chromatography by more powerful ones or even proposing another suitable electrophoresis gel for the Lysozyme.

REFERENCES

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4. Laemmli U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **277**, 680-685.