EGG WHITE LYSOZYME PURIFICATION

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ABSTRACT

The purification of the egg white lysozyme was carried out following two separate paths: molecular exclusion chromatography and ion exchange chromatography. The objective was to find the best purification method. With the data obtained in the different tests carried out, it was determined that of the two purifications carried out, the one in which ion exchange chromatography was used was more efficient. However, it was concluded that doing both chromatographies in a specific order, the best purification was achieved.

MATERIALS AND METHODS

Initially the first extract (E1) was obtained by treating the egg white from *Gallus gallus* eggs with acetic acid 0.1 M and centrifuging, eliminating the supernatant. After this the second extract (E2) was obtained by heat treating E1 at 60ºC 5 minutes, and then centrifuging, taking the solution.
By using an Amberlite GC50 column to carry out an ion exchange chromatography of E2, fractions of lysozyme were obtained (E3) (*Fig. 1*). Also a Shephadex G75 column was used to carry out a molecular exclusion chromatography of E2, obtaining E3* (*Fig. 2*).

The enzymatic activity of all the extracts obtained was measured at 450 nm, following the disappearance by hydrolysis of *Micrococcus lysodeikticus* cell walls, which were used as the substrate for the enzyme. The data obtained were analyzed by several representations, and using these representations to determinate the activity using the equation showed (Equation 1):

$$Activity\left(\frac{UAL}{ml}\right) = SLOPE * \left[\frac{1UAL}{-(0{,}001/min)}\right] * \left(\frac{1}{0{,}3ml}\right) * Dilution\ factor$$

To determinate the grade of purification of E3 and to compare it to the other extracts, that were supposed to be less purified, two SDS electrophoresis (*Fig. 3 and 4*)were carried out (one for E3 and the other for E3*) **[1]**.
The gel was charged following the next pattern: 1. Nothing, 2. E1, 3. E2,4. diluted E3/E3*, 5. E3/E3* and 6. the molecular mass marker.

RESULTS

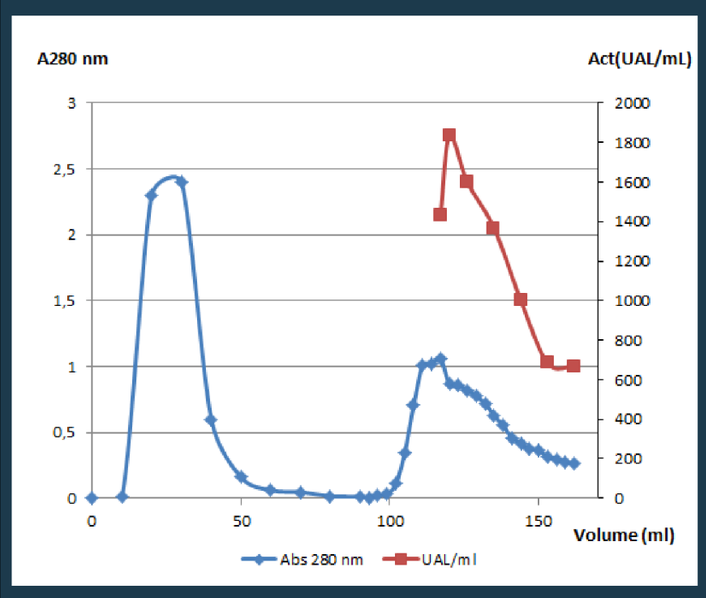

Fig. 1: Amberlite chromatography elution profile.

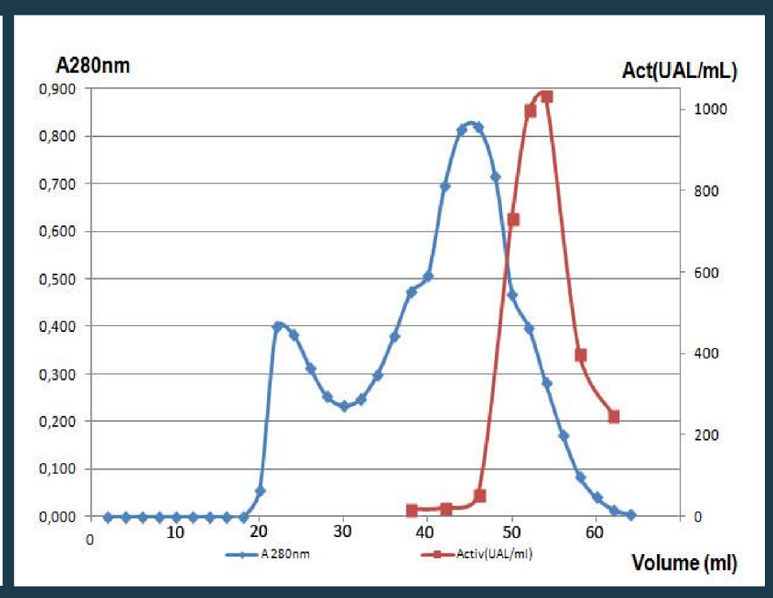

Fig. 2: Sephadex chromatography elution profile.

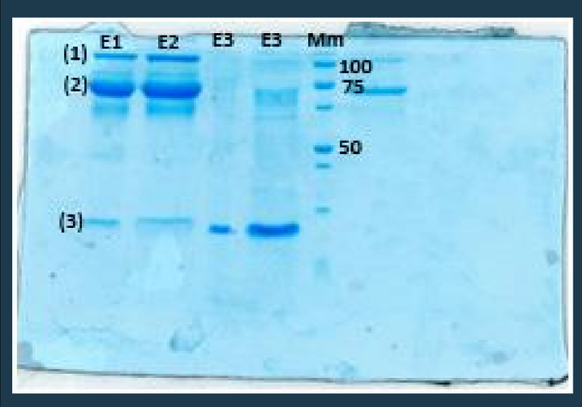

Fig. 3: Amberlite electrophoresis gel.

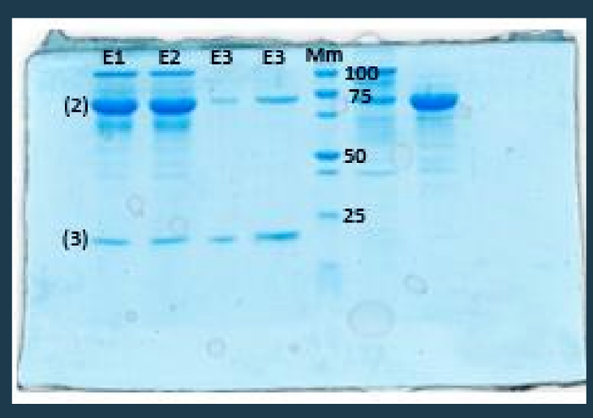

Fig. 4: Sephadex electrophoresis gel.

Table 1: Purification table:

STAGE	AMOUNT OF PROTEIN (mg)	TOTAL ACTIVITY (UAL)	SPECIFIC ACTIVITY (UAL/mg)	YIELD (%)	PURIFICATION (number of times)
E1	249	170062	753	100	1
E2	226	143772	717	88,5	0,9
E3 (SEPHADEX)	26,3	58907	4285	41,2	4,9
E3 (AMBERLITE)	16,8	76870	3869	36,4	7,4

DISCUSSION

The data obtained in the purification chart shows that in E2 the enzyme wasn't apparently purified, whereas the yield decreased. This indicates that thermal treatment isn't a relevant step in the purification of lysozyme.
Comparing both chromatographies, the ionic exchange chromatography was shown to purify 7,4 times the enzyme and size exclusion chromatography only 4,9 times. According to these results, Amberlite chromatography is more effective than Sephadex. However, the yield was higher in the Sephadex chromatography.

The results from the polyacrylamide electrophoresis gel indicated that there was a different contaminating agent in the different methods. In the method that involved the ion exchange chromatography were identified two proteins: lysozyme and a protein of >100 kDa molecular mass which presumed to be ovotransferrin **[2]**. The method that contained the size exclusion chromatography showed two different proteins too: one with a molecular mass between 75 and 50 kDa, identified as ovalbumin and lysozyme.

CONCLUSION

It was determined that ion exchange chromatography achieved a better purification. As mean, the lysozyme was obtained 2.4 times more purificated in Amberlite.

In spite of this the purification process could be improved. In the electrophoresis in the E3 wells it was observed that apart from lysozyme there was ovotransferrin, and in the case of the E3* wells was observed lysozyme and ovalbumin. Probably one part of ovotransferrin was associated to the amberlite, and a part of ovalbumin was eluted with the lysozyme.

Considering this the following improvement was proposed:

More impurities would be eliminated if firstly an ion exchange chromatography is carried out. In the results of the electrophoresis gel bands of lysozyme and ovotransferrin would appear. After this a molecular exclusion chromatography would be carried out, knowing that in the sample there are only lysozyme and ovotransferrin, the bigger molecular mass of this last in comparison of the molecular mass of the lysozyme will cause them to elute in different fractions.

BIBLIOGRAPHY

[1] Laemmli U.K. (1970). "*Cleavage of structural proteins during the assembly of the head of bacteriophage T4.*" Nature. 227, 680-685

[2] Alleoni A.C.C. (2006). "*Albumen protein and functional properties of gelation and foaming*" Sci. Agric. 63, 291-298