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Affinity Separation of Lysozyme from Quail Egg *(Coturnix ypsilophora)* and its Antimicrobial Characterization

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Abstract: Quail eggs are small in size, but it has more vitamins and minerals. Nutritional value of quail egg is 3 to 4 times greater than chicken eggs. Quail eggs contain 13% protein compared to 11% in chicken eggs. Egg white proteins have many pharmacological properties like anti ulcer, strengthen the immune system, increase brain activity and stabilize the nervous system. Lysozyme is an important egg white protein, which has 129 amino acids and molecular mass of 14.3kDa.Isoelectric point of Lysozyme is 11.35. Lysozyme is worn as cell, disrupting manager for removal of bacterial intracellular goods. The Lysozyme drugs are utilized for the cure of ulcers plus infections. The present work concentrates on the separation and purification of the proteins from Quail egg. Reverse micelle extraction yielded a considerable amount of the proteins from the quail egg white and was noted to be around 60-70 %. The purification factor can be further increased by performing gel filtration Chromatography. Molecular weight was found to be ~14.3kDa by SDS-PAGE.
Key Words: Lysozyme, Quail egg white, Reverse micelle extraction.

Introduction

Egg white proteins are such as ovalbumin and Lysozyme. Lysozyme is an enzyme. It is also called as a N-acetylmuramide glycanhydrolase. Lysozyme was first observed by Laschtschenko in 1909.The name Lysozyme was carried by Alexander Fleming (1881-1955). It have a molecular weight of 14.7kDa[1].Isoelectric point of Lysozyme is 11.35 and optimum pH range from 6to9 the maximum activity is in 6.2.Lysozyme was stored at -20◦c and it is stable for 4 year. A source of lysozyme is shown in table.1. Lysozyme is used for the cell disrupting agent for extraction of bacterial intracellular products. Lysozyme classified into three types based on prokaryotes and eukaryotes, chicken type, goose type, invertebrate type. It is used to lyse bacteria by hydrolyzing the β linkage between the muramic acid and acetyl Glucosamine of mucopolysaccharides in the bacterial cell wall. It is likewise used to inactivate the virus. Lysozyme is used to prevent the late gas blowing in chase for reduction of the sterilizing temperature required in food canning. Lysozyme is one of the most frequently used in the antimicrobial packaging[2].

Quail eggs are little in size, but it has more vitamins and minerals. Nutritional value of quail egg is 3 to 4 times greater than chicken eggs. Quail egg contains 13% protein compared to 11% in chicken eggs. These eggs are helped with a natural treatment like ulcers, strengthen the immune system, increase brain activity and stabilize the nervous system. Treatment of anemia with increasing level of hemoglobin in the body[3].

Table 1.Sources of Lysozyme

Natural sources	Viruses, bacteria, plants, insects, birds etc.
Major source	Egg white, Eggs are like Native hen egg, Quail egg, Emu egg, love bird egg etc. Percentage of lysozyme in egg white is 3.5%.
Other sources	Human milk, tears, saliva, mucus, urine and plants.

Experimental

Materials

Quail eggs are collected from local area Nammakkal.cost of the egg is 8 rupees. Packed in a box containing sand.

Micrococcus Luteus purchased from MTCC.

Potassium chloride, sodium dodecyl sulphate, isooctane, NaH_2po_4,Na_2Hpo_4,Nacl,potassium dihydrogen orthophosphate, sodium carbonate,coper sulphate,potassium sodium tartarate,folins phenol reagent, nutrient broth, agar agar, nutrient agar,30kDa membrane,100kDa membrane desalting column G250, Butyl sepharose column, Acryl amide,Bisacryl amide,tris hcl,sds,deionized water, ammonium persulphate,TEMED,tris base,glycine,coomassie G250,methanol,acetic acid, distilled water are purchased from Himedia.

Hydrolysis of Lysozyme

Quail egg white was split from egg yolk with by manually at room temperature. Then diluted with 3.3 fold of 0.05M Nacl solution. After that pH 4 was adjusted by adding of potassium dihydrogen phosphate. After that incubate at room temperature for 2-8hrs.Then the above mixture was centrifuged at 8000rpm for 15 minutes. Then collected the supernatant for further use[4].

Affinity Separation: Reverse Micelle Extraction

The extractants such as surfactant, solvents act as fatty acid soap. And the micelles formation occurs and bound around the aqueous phase by way of reverse micelle structure.

Forward Extraction:

Surfactant such Sodium Dodecyl Sulfate (SDS) was used for the extraction. Aqueous solution was developed by adding salt 0.1M potassium chloride (Kcl) into the 5ml of crude enzyme. For organic phase add surfactant (50mM SDS) in 5ml of solvent isooctane. Aqueous and organic phase were mixed and vortex for 10 mins. The mixture was centrifuged at 3000rpm for 10 mins[5].

Backward Extraction:

From forward extraction took 2ml of organic phase, then 1M Kcl was dissolved in 2ml of distilled water both was mix and vortex for 10min.centrifuge the mixture for 10min at 3000rpm. The aqueous phase was used for the determination of protein concentration and enzyme activity[6].

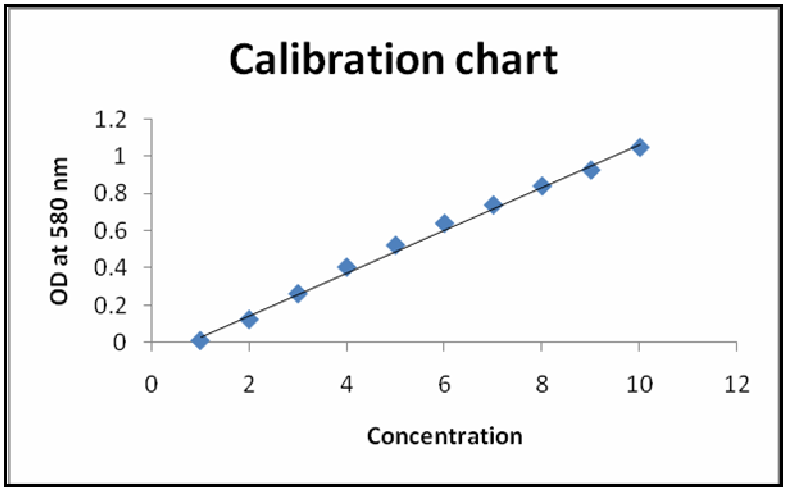
Protein Determination

Protein attention was determined using the Lowry's method with the help of UV spectrophotometer at 580nm. 1ml of sample was taken and to that 2ml of Lowry's buffer was added. Then, the mixture was allowed for incubation for 10 min, after that 0.2ml of diluted Follin's phenol reagent was added and then it was vortex immediately. Incubated at room temperature for 30 min. Absorbance was measured at 580nm[7].The standard calibration chart for BSA was shown in figure 1.

Enzyme Activity

Enzyme activity was measured using a culture of *Micrococcus Luteus* as a substrate. 50µl of sample solution was taken to this add 3ml of culture solution in pH 7 buffer adjusted to OD 1 at 660nm.decrease in absorption at 660nm was measured[8].

Fig 1.Standard calibration chart for BSA



Gel Filtration Chromatography

Protein purifications were conducted by way of semi preparative chromatography AKTA Prime plus system. The eluent was monitor with UV spectrophotometer at 280nm.The chromatography column was filled with Sephadex G 100 column. And the volume of flow rate is 0.5ml[9].

Molecular Weight Measurement

To analyze the purity and molecular mass of protein with the help of poly Acryl amide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS PAGE) was performed as described by Laemmli using 12% of separating gel with 4.5% of stacking gel. Coomassie brilliant blue was used for staining then the standard was used as a Lysozyme 14.3kDa. The gels be transferred keen on a clarification of 0.1%CBB R250 for 30 min at room temperature. Later they were destained with destaining solution pending the background clean and protein bands appeared[10].

Antimicrobial Activity

Prepare Nutrient agar plate. The 24hr culture of *Micrococcus lutues* was streaked with sterilized cotton swab. The three gels were cut with gel cutter. One is in control, another one for the organism, and the third well is sampled. 0.5µlof sample was poured to each gel, kept in a incubator at 32◦c for 24hr[11].

Fig 2.Reverse micelle extraction

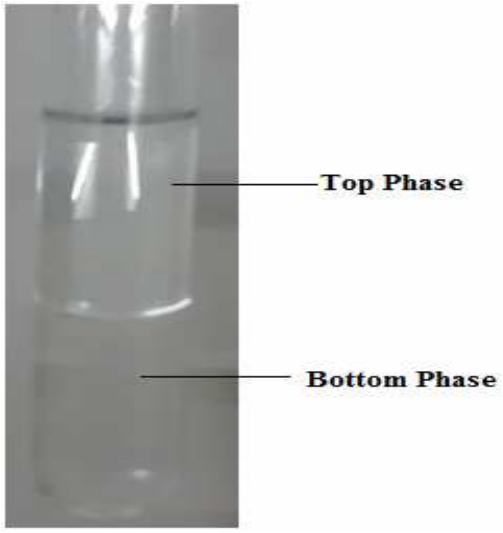


Fig 3.Colour changes in Lowry's method

Results and Discussions

The reverse micelle extraction of total protein estimation was carried out using two different types of surfactants such as CTAB and SDS as shown in the table 2 and 3. From the table the greater partition coefficient value was obtained for CTAB using RME in Forward extraction when compared to SDS and also the backward extraction. So forward extraction was done using CTAB reverse micelle extraction.

The choosy partition and cleansing of objective proteins in a combination of both alike and unlike proteins, Reverse Micelles are thermodynamically constant, nanogauge-sized gathering of surfactants to encapsulate minute pools of H_2O in a mass organic phase. These allow proteins and additional hydrophilic molecules chosen solubilised in the aqueous microenvironment as organic reactants and products stay in the bulkiness of crude phase. As seen in table 2, the maximum partition coefficient of 12.5 was obtained for low concentration of CTAB whereas k value gets decreased when CTAB concentration was high. RMs is vibrant quantities; collide among each further in solution with occasionally exchange contents. Around single collision in one thousand consequences in a swap of RM contents. Collisions happen lying on a timescale of nanoseconds even as exchanges of substance occur each little microsecond.

Extraction of proteins with CTAB reversed micelles has been typically carried out from the protein aqueous solution containing KCl. Ionic surfactant (CTAB) are the majority usually used, because of their capability to solubilise a broad variety of proteins. Though, the sturdy electrostatic connections among proteins also ionic surfactants contact origin the denaturation of proteins and next small capitulate of aim proteins[12].

Table 2.Reverse Micellar extraction of total protein estimation using CTAB

	System	Top phase	Bottom phase	Partition coefficient
Forward extraction	CTAB(50mM), KCL(0.1M)	0.75	0.06	12.5
Backward extraction	CTAB(50Mm), KCL (1M)	0.23	0.38	0.6

Table 3.Riverse micelle extraction of total protein estimation using SDS

	System	Top phase	Bottom phase	Partition coefficient
Forward extraction	SDS(50mM), KCL(0.1M)	0.12	0.14	0.85
Backward extraction	SDS(50Mm), KCL (1M)	0.2	0.3	0.66

Gel Filtration Chromatography

Protein was purified using gel filtration chromatography column was packed with Sephadex G100 before injecting sample column was equilibrated with phosphate buffer. The injection volume of sample is 0.5ml it should be diluted with binding buffer. Then the sample protein was bind with the column will give a peak. The **figure 4 shows** protein present in a sample. After that the column was equilibrated with elution buffer because the remaining proteins are eluted. Then the fraction was collected and monitored in UV Spectrophotometer at 280nm[13].

Molecular Weight Measurement

The sample was run on a 12% SDS-PAGE. Standard Lysozyme was used as a reference. Molecular weight of Lysozyme 14.3kDa was found. The SDS-PAGE figure 5 Shows so as to the Lysozyme obtain by gel filtration chromatography had high clarity (as indicated through single band) and the bulk protein fractions restricted nearly no lysozyme[14].

Fig 4. Purified Lysozyme of Gel filtration chromatography

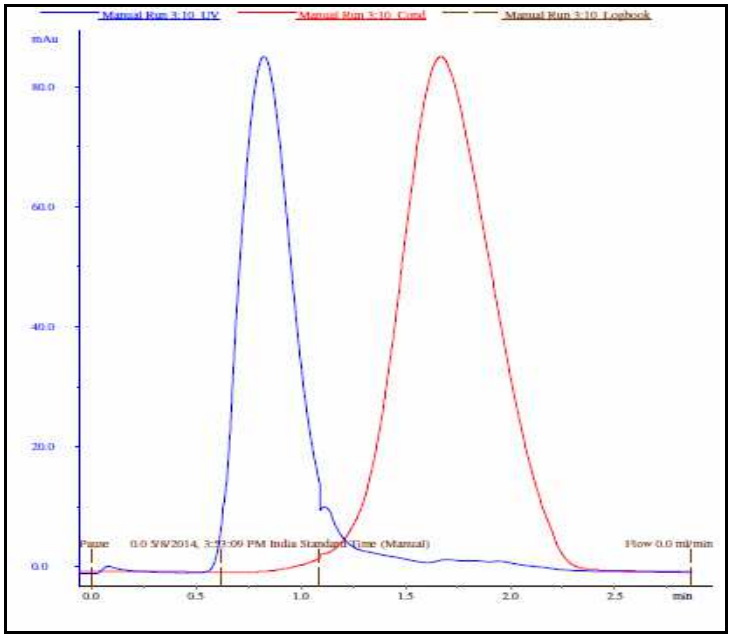
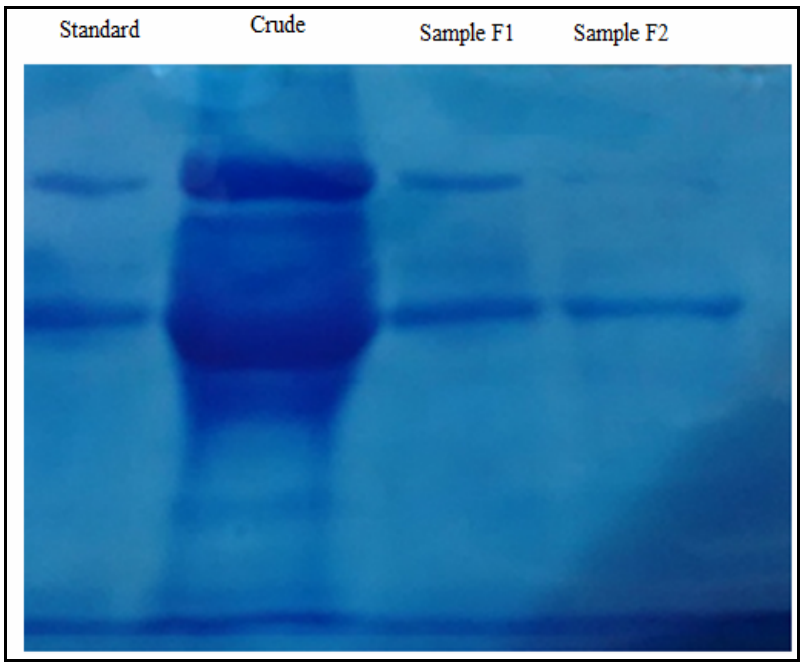


Fig 5. SDS-PAGE



Antimicrobial Activity

Standard Lysozyme

Zone of inhibition=18mm

Purified Quail egg white Lysozyme

Zone of inhibition=12mm

The activity of Lysozyme was determined for Quail egg white purified Lysozyme fraction collected from gel filtration chromatography using Bacillus Substilus as a substrate. Figure 6 shoes the Zone of inhibition of sample and standard. Based on the diameter of the lysis zones of purified Lysozyme sample was compared to the diameter of standard Lysozyme.

Fig 6. Antimicrobial activity of purified Lysozyme

Conclusion

Capable and economical lysozyme purification from Quail egg white can be achieved by using CTAB–salt Kcl, if the right salt is chosen. The salts contain a sturdy role to lysozyme partition actions. The partitioning of lysozyme able to be aimed at to upper-phase into a forward extraction of reverse micelle system via the adding of salt through solvent, such because isooctane. While the CTAB concentration is 125mM-salt Kcl 0.60M Concentration is was operated at pH 7 and room temperature (32◦C) with addition of 5ml of isooctane, approximately 70% of lysozyme preserve be extracted from the Quail egg white. Then the protein was purified using gel filtration chromatography. The purified protein was loaded in SDS PAGE for molecular weight measurement. Finally the pharmaceutical application of Antimicrobial activity was done.

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