SHODHPATRA: INTERNATIONAL JOURNAL OF SCIENCE AND HUMANITIES
E-ISSN: 3048-6041 | Volume-1, Issue-12 | December 2024

Isolation of Albumin Protein from Egg

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General Introduction

Albumin is any of numerous simple heat-coagulable water-soluble proteins that occur in blood plasma or serum, muscle, the whites of eggs, milk, and other animal substances, and in many plant tissues and fluids . It is a family of globular proteins also moderately soluble in concentrated salt. It is a highly water-soluble protein composed of more tha 500 amino acids with small globular protein. There are several types of albumin, i.e., human albumin (~67 kDa), bovine serum albumin (~69 kDa), and ovalbumin (egg white, ~47 kDa) . As a plasma protein in blood human, albumin has an average half-life of 19 days] and shows stability at a pH range of 4–9 [.Factors that affect albumin coagulation include heating, pH conditions close to the isoelectric point of albumin, and salt concentration . For the first time, albumin was discovered in human urine in 1827 by Richard Bright through experiments on heating urine and producing coagulants that look like egg white when heated. Albumin is produced in the liver . The liver of an adult produces about 12 g of albumin per day. The normal albumin level in adult humans is 3.4–4.7 g/dL, about 60% of total plasma protein]. As a plasma protein, albumin functions include maintaining osmotic pressure. Albumin helps keep fluid from leaking out of your blood vessels into other tissues by maintaining the plasma osmotic pressure up to 70–80% . Osmotic pressure occurs due to the water present in different concentrations in the body, which is influenced by the body's salt content and other nutrients. Albumin also functions as a guardian of the acid-base balance in the body because it has many electrically negative charges . Albumin can also act as an antioxidant that inhibits the production of free radicals . Another function of albumin is to transport drugs, such as digoxin, warfarin, and anti-inflammatory drugs, throughout the body and help metabolism.

In medical fields, albumin has been used to transport bilirubin, fatty acids, ions, hormones, and minerals throughout the body through blood circulation . Moreover, albumin as a life-saving drug has been applied since the second world war to the COVID-19 pandemic . A recent publication reported that albumin could dampen hypercoagulability in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) . COVID-19 is unusual pneumonia caused by SARS-CoV-2 that can be treated with ther. Albumin infusion to COVID-19 patients shows anticoagulation behavior. A low dose of albumin can act as an anticoagulant through its negatively charged groups, binding to positively charged groups . Compared with conventional preventive anticoagulation therapy, a low dose of heparin can increase D-dimer's plasma level . Albumin is also required in several medical procedures, such as a therapeutic agent in hypovolemia, hypoalbuminemia, and chronic liver In addition, other researchers reported that albumin has an important role in the context of cardiovascular disease It shows that albumin plays a very important role in the medical and pharmacy sectors to treat or prevent shock following serious injury, bleeding, surgery, or burns by increasing blood plasma volume On the other hand, albumin can also be a supporting material for corrosion inhibitors and biosensors.

The magnitude of the benefits of albumin for humans makes the existence of albumin interesting and important to discuss. The United States Food and Drug Administration (FDA) approves the use of human serum albumin (HSA) that is extracted from human plasma or produced recombinantly. Unfortunately, the use of HSA remains several problems, such as the high production cost, issues of viral infection, finding viable donors, and all factors that result in a high cost to the customer. Substituting HSA with another source of albumin is one solution to solve the problem . Scientists reported that albumin also could be found in animals and plants . In animals, the albumin content is about 35%–50% of the total serum protein. To get albumin benefits, separating albumin from its source is necessary. Various methods have been reported in albumin separation, including chromatography, solvent extraction, electrophoresis, and adsorption. Before separation is performed, qualitative and quantitative determination of albumin is important to know the presence of albumin from the source. Recently, research on the determination of albumin is progressing to get better in selectivity and sensitivity. The use of albumin for analytical purposes is inseparable from the reactivity of albumin to other compounds. Albumin has a binding site that can bind with other compounds and be measured spectroscopically and electrochemically

This paper reports the progress of the latest news on albumin, including its sources and its preparation and determination methods. The applications of albumin in medical and pharmacy purposes and analytical measurement are explained in this paper. In addition, the challenges and prospects in albumin research are also discussed.

Introduction

Chicken eggs are considered a perfect food due to their ability to sustain life. They have a largescale consumption worldwide since egg proteins have a complete amino acid profile.They may be used as a thickener or emulsifier in baking. Structurally, the three main components of eggs are eggshell, egg white and yolk that make up around 10%, 28% and 60% (by weight) of the chicken egg respectively (Mine and Zhang 2013). Ovalbumin (OVA, 54%), ovotransferrin (OVT, 11%), ovomucin (OVM, 3.5%), and lysozyme (LSZ, 3.4%) are the major proteins of egg white, by weight; these proteins have many uses in foods, dietary supplements, vaccines, pharmaceuticals, antibacterial agents, and so on, after they are isolated from egg white

Ovalbumin, (OVA) was one of the first proteins to be extracted in its pure form from egg white by salting out, using large quantities of ammonium sulfate and acetic acid. It is a phosphoglycoprotein comprising of 385 amino acid residues with a molecular weight of 45 kDa .albumin has been widely used for emulsification as well as for its nutritional properties. Many different methods are employed for the extraction of albumin like precipitation by salting out/solvent, at isoelectric pH, and liquid chromatography.

Abstract

Eggs are an excellent source of nutrients. Egg whites are used in food, cosmetic, and biotechnology industries for a plethora of physiological functions that they demonstrate due

to their proteins. Although these proteins look promising, they can be utilised only after they have been isolated/extracted from the egg white. The objectives of this study were to develop a procedure for the extraction of the major egg white proteins, lysozyme (LSZ), ovomucin (OVM), ovotransferrin (OVT), and ovalbumin (OVA), and to simplify the developed method in order to execute a step wise scale-up from a lab scale (100 g egg white) to a pilot scale (20 kg egg white). The specific focus of this study was to improve the existing protocols in order to reduce the experimental setup requirement and thus reduce the cost of extraction. This was achieved by optimizing the extraction conditions for LSZ (cation exchange resin based separation), applying the traditional siphon filtration for the separation of resin particle, OVM, and OVT precipitates, and elimination of any heat treatments for OVA purification.

In case of LSZ, the extraction was achieved in a shorter time (180 min), with lesser resin dosage (2 g/100 g egg white) and without any pH buffers. For OVM, a modification of the 2-step method was performed and for the scale up study, siphon filtration was used. OVT was separated by the ammonium sulfate and citric acid salting out method and OVA was obtained as a supernatant at the end of the process and needed no further purification. The siphon filter used was designed indigenously in the lab using food grade transparent vinyl tubing, stainless steel mesh (100 mesh size for resin separation and 400 mesh size for precipitate separation), and waterproof silicone sealing tape to hold the filter in place with the tube opening and avoid any leakages. The proteins extracted in this study had comparable purities and yields than the previously reported method.

For example, from 100 g egg white, an average yield of 58% for LSZ, 83% for OVM, 75% for OVT, and 99% for OVA were achieved, and the average purities were 92%, 85%, 69%, and 94%, respectively. The process developed in this study is easy to adapt and has a potential for scale-up to an industrial scale as was proven from our stepwise scale up batches of 1kg and 20kg which achieved similar average yields and purities ($p>0.05$)

Objective :

Many of the published articles/literatures claim the easy scalability of the process but no evidence is available to support these claims. Some articles have scaled up to 3 kg egg white which can be considered small scale, generally 1-5 kg, but not pilot/bulk scale (generally 10 kg and above) . Hence, we hypothesized that by optimizing the current methods and by employing siphon filtration as a separation technique, we can achieve a pilot level scale up within a laboratory setup, thereby proving the ease, efficiency, and cost effectiveness of our separation scheme and scale-up. The overall objectives of this study were:

1. To establish a scheme for sequential extraction of egg white proteins namely, Lysozyme, Ovomucin, Ovotransferrin, and Ovalbumin

2. To scale up the extraction 7 The specific objectives of this thesis research were: 1. To develop a process for the extraction of egg white proteins, that is economical, sustainable, and produces comparable results to the existing methods. This would involve the optimization of certain steps in the current methods using 100 g egg white.

2.1 To scale up the operation in a step wise manner to ensure that the designed process gives similar yields and purity to the small-scale results, considering factors like the new separation technique 'siphon filtration' and the associated setup requirements for it. T

he aim was to keep the cost of setup as low as possible thereby reducing the cost of extraction. The extraction process and setup were to be designed in such a way that they are easy to adapt on industrial large-scale operations for which extractions with 1 kg and 20 kg EW were to be conducted in an ordinary lab setup as evidence for easy scale up.

LITERATURE REVIEW

Egg: Eggs are a perfect food, containing almost every nutrient essential to sustaining life. They contain everythingthat a bird needs to and complete all the stagesof embryonic development.Chicken eggs are a very common food commodity that we see in kitchens and can be used in various ways. Eggs are one of the few foods that are consumed throughout the world because their proteins have a complete amino acid profile. They may also be used as a thickener or emulsifier in baking Eggs consist of 3 main components: eggshell, egg white and yolk. Egg's shell is built of 8000-10,000 pores which ensure that oxygen can penetrate inside, and carbon dioxide and other gases can escape out. The shell represents about 10% of weight of egg and consists of calcium carbonate and calcium phosphate mainly.

When the eggshell is cracked open, we see 2 portions – the yellow one is called the yolk whereas the white/transparent portion is egg white . The fat content of yolk consists primarily of triglycerides, cholesterol, and phospholipid lecithin. The amount of fat and cholesterol as well as the composition of fat is influenced by the diet of the hen. The spermatogenic membrane (VM) is a multi-layered structure that protects and shapes the egg yolk and separates it from the egg white (Mann 2008). Along with chalazae, the VM keeps the egg yolk in the central part of the egg, thus preventing its fusion with the shell membrane. In addition, it acts as a diffusion barrier by transporting water and nutrients between the egg yolk and the egg white. It protects the embryo during the first 96 hours of incubation against the strongly alkaline nature of the egg (12%), lipids (12%), and carbohydrates and minerals.

Egg white and its Protein :

1. Egg white predominantly comprises of water (89%) and protein (10%), with the rest consisting of carbohydrates (0.4%), ash (0.5%), and trace amounts of lipids (0.03%).
2. The protein composition of egg white by weight is ovalbumin (OVA, 54%), ovotransferrin (OVT, 11%), ovomucin (OVM, 3.5%), and lysozyme (LSZ, 3.4%) which are the major proteins and therefore, the most studied.
3. The rest of the fractions are minor proteins, ovomucoid (11%), G2 globulin (4.0%), G3 globulin (4.0%),ovoinhibitor (1.5%),ovoflavoprotein (0.8%),ovoglycoprotein (1.0%), ovomacroglobulin (ovostatin) (0.5%), cystatin (0.05%), and avidin (0.05%).
4. The four major proteins of egg white have numerous applications in the fields of food, nutraceuticals, vaccines, pharmaceuticals, and antimicrobials Some of these egg white proteins in their isolated forms also have the potential to prevent the spread of COVID-19.

Properties and Applications of Major Egg White Protein :

Albumin :

1. Albumin one of the first proteins extracted from egg white, is a phosphoglycoprotein with a molecular weight of 45 kDa and comprising 386 amino acid residues.
2. It has four cysteine residues and a single cystine disulphide bridge. When egg white proteins are separated by electrophoresis, three ovalbumin bands appear these correspond to the dephosphorylated, mono- and di-phosphorylated forms, and the sites of phosphorylation have been identified as serine residues 68 and 344.
3. In addition, albumin has two further sites of modification: the N-terminus is acetylated, and the carbohydrate moiety is linked through asparagine 292 .
4. An interesting feature of the structure of albumin that 16 became evident from its sequence is its homology with a group of proteinase inhibitors known as serpins.
5. It was found to have 30% sequence homology with the archetype member of the family α1-antitrypsin (Hunt and Dayhoff 1980).
6. Most members of the serpin family have what is described as a stressed (S) and a relaxed (R) conformation.

7. Proteolytic cleavage converts them from the S to the R conformation. It is also found that the S and R forms exhibit different heat stabilities.

Ovatransferrin :

1. ovotransferrin, as the name suggests, is a protein that belongs to the transferrin family (Superti et al. 2007). OVT, initially known as conalbumin, comprises of 686 amino acid residues and has a molecular weight of 76 kDa

2. It is a single glycopeptide chain that is folded into two globular lobes linked by an alpha helix structure .

3.This protein is 40-70% homologous to mammalian (serum) transferrin and lactoferrin when comparing 17 their domains (lobes).

Ovamucin :

1. OVM is a high molecular weight sulfated glycoprotein that confers the gel-like properties to egg white, thus maintaining the viscosity and structural integrity of egg albumen.
2. 18 OVM has a varying molecular weight that depends on its form .
3. A single unit (monomer) of OVM has a molecular weight of 163 kDa (Donovan et al. 1970) whereas native, soluble, and insoluble OVM have the molecular weights 8000 kDa, 8300 kDa, and 23000 kDa, respectively.
4. Ultracentrifugation can separate these fractions into precipitate (consisting of the insoluble fraction majorly) and supernatant (consisting of the soluble fraction majorly) (Kato et al. 1970a). Furthermore, OVM has two subunits: α, a mixture of α1 and α2, and β. The α subunit is carbohydrate poor and has around 15% dry matter whereas the β subunit is carbohydrate rich and has around 50% dry matter.

Lysozymes :

Lysozymes are a group of enzymes extensively spread around in various aspects of life like organs, tissues, secretions of vertebrates as well as bacteria, phages, and plants . Their molecular weights may differ depending on the source, but they all exhibit the same property of lysing the bacterial cell walls instantly through the action as muramidase, hence the name ‘lyso’ (lysis) and ‘zyme’ (enzyme) . Egg white has LSZ with a molecular weight of 14.2 kDa, one of the lowest molecular weight egg white proteins, and an isoelectric point ranging 10.5-11. Hen lysozyme is a monomeric, secretory protein containing four disulfide bonds: Cys 6-127, 30-115,64-80, and 76-94, which cause high 20 thermal stability of the enzyme together with six helix regions; its tertiary structure is quite stable in aqueous solutions.

Extraction Method of Major Egg White Protein :

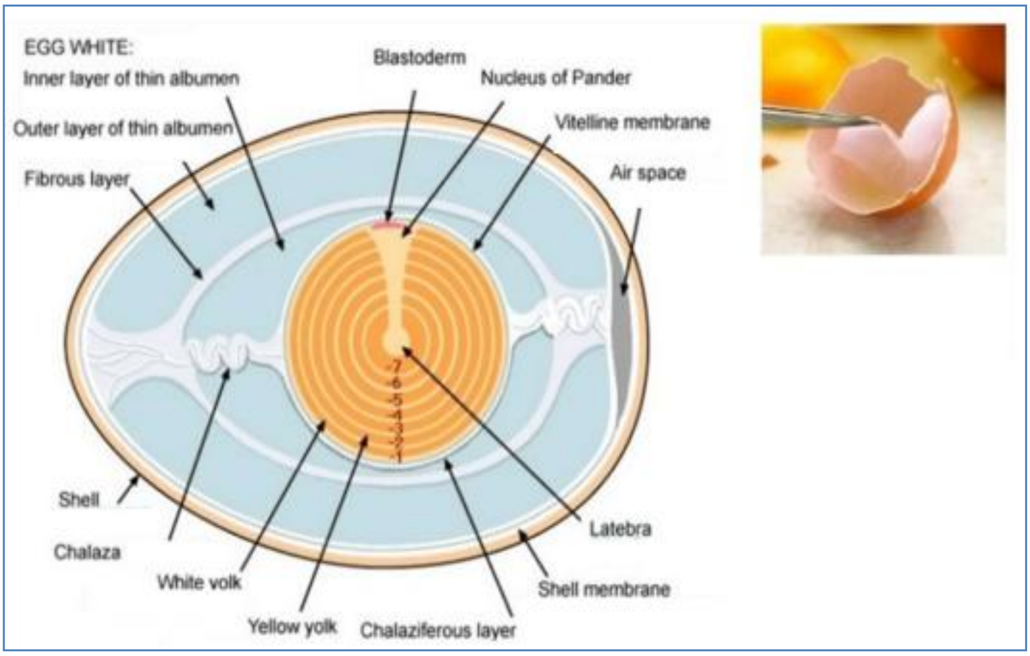
Albumin :

1. Albumin is any of numerous simple heat-coagulable water-soluble proteins that occur in blood plasma or serum, muscle, the whites of eggs, milk, and other animal substances, and in many plant tissues and fluids.
2. It is a family of globular proteins also moderately soluble in concentrated salt. It is a highly water-soluble protein composed of more than 500 amino acids with small globular protein.
3.There are several types of albumin, i.e., human albumin (~67 kDa), bovine serum albumin (~69 kDa), and ovalbumin (egg white, ~47 kDa).
4. As a plasma protein in blood human, albumin has an average half-life of 19 days and shows stability at a pH range of 4–9.
5. Factors that affect albumin coagulation include heating, pH conditions close to the isoelectric point of albumin, and salt concentration
6. The liver of an adult produces about 12 g of albumin per day. The normal albumin level in adult humans is 3.4–4.7 g/dL, about 60% of total plasma protein .
7. As a plasma protein, albumin functions include maintaining osmotic pressure. Albumin helps keep fluid from leaking out of your blood vessels into other tissues by maintaining the plasma osmotic pressure up to 70–80%.
8. Osmotic pressure occurs due to the water present in different concentrations in the body, which is influenced by the body's salt content and other nutrients.
9. Moreover, albumin as a life-saving drug has been applied since the second world war to the COVID-19 pandemic.
10.A recent publication reported that albumin could dampen hypercoagulability in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
11. COVID-19 is unusual pneumonia caused by SARS-CoV-2 that can be treated with therapy .
12. can Albumin infusion to COVID-19 patients shows anticoagulation behavior. A low dose of albumin act as an anticoagulant through its negatively charged groups, binding to positively charged groups .
13. Compared with conventional preventive anticoagulation therapy, a low dose of heparin can increase D-dimer's plasma level .
14. Albumin is also required in several medical procedures, such as a therapeutic agent in hypovolemia, hypoalbuminemia, and chronic liver.
15. In addition, other researchers reported that albumin has important role in the context of cardiovascular disease.

Procedure ;

Add about 100ml distilld water or deionised water . the egg white and stir until a white precipitate form. These are the egg globulin . now you can either centrifuge the mixture and pour all and save the supernatant which is your albumin ,or you can simply filter the mixture and keep the filtrate.

Isolation of Albumin:



Cross Section of Egg

Result & Discussion :

This experiment is composed oftwo parts. First part is extraction of proteins and the second is determining protein concentration.The experiment started withextraction of proteins needed for theexperiment.

Two common proteins wereextracted, namely egg albumin and milkcasein. In extraction of egg albumin andcasein, saltation using ammoniumsulfate and acid respectively wasconducted. The egg albumin yielded inour extraction weighed less than 0.1grams while casein weighed 2.1 grams.In extraction of egg albumin,saturated ammonium sulfate, estimated25% (w/v), and 50% saturatedammonium sulfate, estimated 13% (w/v)was used. The egg white solution withacetic acid was added first with saturatedammonium sulfate then centrifuged thenadded with 50% ammonium sulfateagain and centrifuged.

The principle ofadding ammonium sulfate andcentrifuging it is the formation of precipitate of proteins that precipitates atcertain percent and below and separatingit to other proteins that did not precipitate at such concentration viacentrifugation.Salting-out process starts withthe determination of the percentage ofammonium sulfate of which the desired protein precipitates the most.

The proteinsolution with impurities is first addedwith the concentration of ammoniumsulfate lower than the optimalconcentration needed for the precipitation of the desired protein. Afterthat, the solution will be centrifuged toseparate the precipitate and thesupernatant. Since the desired protein will not yet be precipitated, the first precipitate will disposed and the supernatant will be added with a higherconcentration of ammonium sulfate, ofwhich it will achieve the concentrationthat is optimal for precipitating thedesired protein, and will be centrifugedagain for the separation of the filtrateand precipitate.

This time, the filtratewill be disposed while the precipitatewill be stored because the desired protein was already in the precipitate.

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