

# Separation of Ovotransferrin from Chicken Egg White without Using Organic Solvents

## A.S. Leaflet R2990

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### Summary and Implications

Ovotransferrin from chicken egg white was separated without using organic solvents. Egg white was diluted with 1 vol. of distilled water (DW), and then homogenized. After removing ovomucin by centrifugation after adjusting the pH to 4.5-5.0, the resulting supernatant was added with ammonium sulfate and citric acid, and then centrifuged after holding overnight at 4 °C. The precipitant, which contains ovotransferrin, was dissolved in DW and the ovotransferrin was re-precipitated using ammonium sulfate and citric acid. The precipitant collected after centrifugation was dissolved with DW, and then desalted and concentrated. The purity of ovotransferrin was determined, the protein identified, and the yield calculated after freeze drying. Over 85% purity and over 83% yield were obtained from the combinations of 5.0% (w/v) ammonium sulfate and 2.5% (w/v) citric acid followed by 2.0% (w/v) ammonium sulfate and 1.5% (w/v) citric acid. The method was simple and cost effective. The isolated ovotransferrin can be used as is or after modifications for various applications such as antimicrobial, anticancer treatments, and iron supplementing agents for human.

### Introduction

Ovotransferrin is a monomeric glycoprotein consisting of 686 amino acids and 15 disulfide bonds. The molecular weight of ovotransferrin has 76 kDa and an isoelectric point of 6.1. Ovotransferrin is known to have a strong antimicrobial activity, and thus, can be used to improve the safety of foods, and the peptides derived from ovotransferrin also have an ability to control microorganisms. Therefore, both ovotransferrin and its peptides can be used as antimicrobial agents in foods.

Ovotransferrin is found in two main forms - apo- (iron free) and the holo- (iron bound) forms. The chemical and physical properties of these two forms of ovotransferrin differ significantly. Apo-form (iron free) is colorless, while holo-form (iron bound) has a salmon pink color. The holo-form is more resistant to chemical and physical conditions than apo-form. Iron ion ( $\text{Fe}^{3+}$ ) can be easily attached to

ovotransferrin at pH > 7.0, but is released at pH < 4.5.

Ovotransferrin has similar functions to lactoferrin found in milk, and both have iron scavenging and iron delivery functions.

Over the past years, different techniques have been developed to separate ovotransferrin from chicken egg white, but most of the methods developed were laboratory scale. Recently, we have developed a large scale production of ovotransferrin using 43% ethanol extraction followed by 59% ethanol precipitation. Since ovotransferrin is highly susceptible for extreme pH and temperature conditions, apo-form of ovotransferrin in egg albumin was converted to holo-form before treatment with ethanol. After separating the holo-form of ovotransferrin, iron was released from ovotransferrin using pH adjustment and the released iron was removed using AG1-X<sub>2</sub> resin. However, the removal of the released iron from ovotransferrin requires an extra step and raises the costs.

The objective of this work was to develop a simple and easy separation method of ovotransferrin (apo-form) from egg white without using organic solvent.

### Materials and Methods

- Egg white was diluted with 1 vol. of DW, homogenized, and then centrifuged to remove ovomucin after adjusting the pH to 4.5-5.0
- The resulting supernatant was treated with 4 different levels of ammonium sulfate and 3 levels of citric acid combinations to precipitate ovotransferrin.
- After kept overnight at 4 °C and centrifugation, the precipitant was dissolved with 2 volumes of DW, and then desalted using ultrafiltration unit.
- Ovotransferrin was re-precipitated from the solution by adding 4 levels of ammonium sulfate and acid combinations.
- The purity of the separated ovotransferrin was evaluated using SDS-PAGE.
- Western blot was used to identify the protein.

### Results and Discussion

- Ammonium sulfate + citric acid performed better than ammonium sulfate + acetic acid in separating ovotransferrin.
- 5.0% (w/v) ammonium sulfate and 2.5% (w/v) citric acid combination at stage I and 2.0% (w/v) ammonium sulfate and 1.5% (w/v) citric acid combination at stage II were the best conditions for purifying ovotransferrin from chicken egg white.

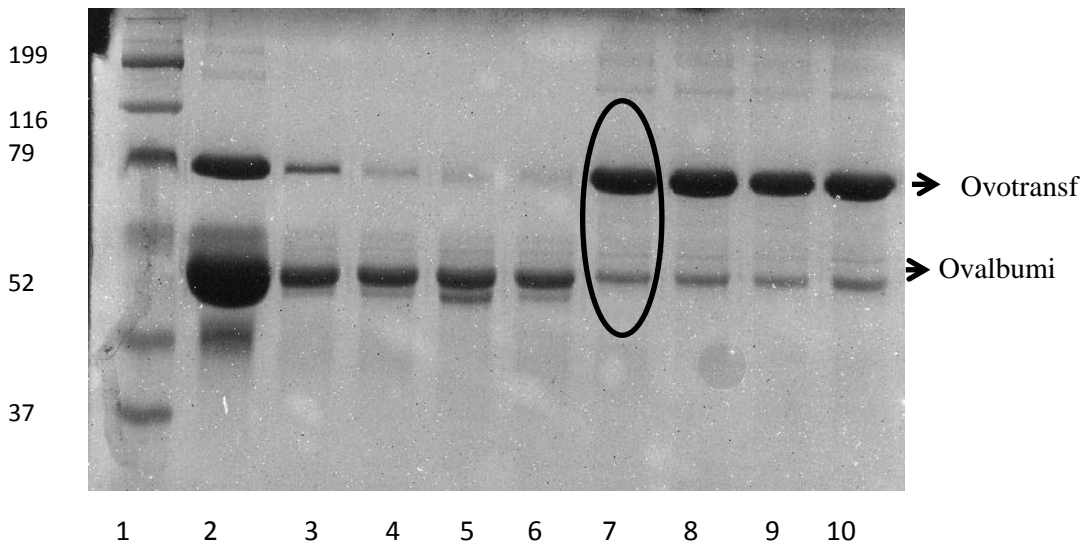
- The yield of ovotransferrin separated using the current method was 83% and the purity was >85%.

ovotransferrin from chicken egg white was a two-stage precipitation of ovotransferrin using 5.0% (w/v) ammonium sulfate and 2.5% (w/v) citric acid combination in step I and a 2.0% (w/v) ammonium sulfate and 1.5% (w/v) citric acid combination in step II. The yield of the ovotransferrin was 83% and the purity was greater than 85%.

**Conclusion**

Ovotransferrin can be separated from egg white without using solvents. The best combinations for purifying

**Figure 1:** Effect of citric acid on the separation of ovalbumin from ovotransferrin. Lane 1= Marker, Lane 2= Diluted egg white, Lane 3 to 6= Supernatant obtained from 1.0, 1.5, 2.0 and 2.5% citric acid with fixed level (2.0%) of ammonium sulfate, Lane 7 to 10 = Dissolved precipitant from 1.0, 1.5, 2.0 and 2.5% citric acid with 2% ammonium sulfate combinations.



**Table 1. Yield of ovotransferrin using the reported value in egg white.**

Sample	Weight (g)	Yield (%)
Egg white ovotransferrin <sup>1</sup>	3.17	100
Final ovotransferrin <sup>2</sup>	2.64 ± 0.17	83.4 0 ± 5.33

<sup>1</sup>The theoretical amount of ovotransferrin in 2x diluted egg white solution (egg white protein is 11% of total egg white and ovotransferrin is 12% of total egg white proteins (Stadelman and Cotterill, 2001)). The original amount of egg white was 240 g per replication.

<sup>2</sup>Apo-ovotransferrin produced with ammonium sulfate and citric acid combination; n=3