**Producing a Low Ovomucoid Egg White Preparation by Precipitation with Aqueous Ethanol**

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A novel method for producing a low ovomucoid egg white preparation is proposed. Egg white powder (0.5 g) was dissolved in a 10-fold weight of distilled water and adjusted to pH 5, and ethanol was added to the solution at a final concentration of 20% (v/v). The mixture was vigorously stirred and centrifuged. The precipitate was washed three times with 20% ethanol (6.25 ml each), with about 65% of egg white proteins occurring in the precipitate. The use of ELISA demonstrated that 70% of ovomucoid was recovered from the supernatant fraction. However, functionally important proteins such as ovalbumin, ovotransferrin, and lysozyme still remained in the precipitate. These results may be due primarily to the much higher solubility of ovomucoid in this aqueous ethanol. Food quality evaluation showed that high whippability and foam stability were retained in the low ovomucoid preparation as in its material egg white. This product would thus be applicable as a new processed food for ovomucoid-sensitive allergic patients.

**Key words:** allergy; egg white; ovomucoid; food processing

Hen’s egg is one of the staple food items that, however, frequently induce hypersensitivity. Many studies have focused on egg white proteins, which are reported to elicit allergic reactions more frequently than egg yolk. Egg white is a mixture of about 40 different proteins, among which ovalbumin (OA), ovotransferrin (OT), ovomucoid (OM), and lysozyme (LY) are the major allergens for patients with egg allergy. OA, OT, OM, and LY constitute 51, 12, 11, and 3.5 weight%, respectively, of the total protein in egg white. Some groups reported that OM plays a more important role in the pathogenesis of allergic reactions to egg white than other egg white proteins. OM is a highly soluble glycoprotein with a molecular weight of 28,000, and is soluble and antigenic even in boiled shell eggs. Urisu et al. reported that an ovomucoid-depleted egg white preparation from boiled egg white was not allergenic for 21 (55%) of 38 egg-sensitive allergic patients. In their method, OM was removed in consideration that it hardly precipitates even when boiled. However, the resulting preparation is poor in functional properties since all the other proteins were heat-denatured. We here propose a method for producing a low OM egg white with whipping properties by aqueous ethanol precipitation.

Egg white powder (0.5 g, Q. P. Co., Japan) was dissolved in distilled water (5 ml) and adjusted pH to 5 or 7 with a small amount of 1 N HCl. Ethanol was added to the egg white solution at the final concentration of up to 25%. The mixture was vigorously stirred at ambient temperature for 10 min and then centrifuged at 5,000 × g for 10 min. The precipitate was washed three times with the same volume and concentration of aqueous ethanol. The resulting precipitate was mixed with water (5 ml) containing 4 M urea, SDS (4%), and 2-mercaptoethanol (10%), heated in boiling water for 10 min, and the solution was used for a protein assay and SDS-PAGE analysis.

The protein content was determined using a protein assay kit (Bio-Rad, U.S.A.) and is shown in Table 1. Only 16% of the material protein was recovered from the precipitate when ethanol was added to

<p>| Table 1. Protein Recovery in Aqueous Ethanol Precipitates and ELISA Values of the Supernatant Fraction |
|-------------------------------------------------------|---------------------------------------------------|--------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Final ethanol concentration (%)</th>
<th>pH</th>
<th>Protein recovery (%)</th>
<th>ELISA value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>7</td>
<td>16</td>
<td>_b</td>
</tr>
<tr>
<td>25</td>
<td>7</td>
<td>60</td>
<td>0.68</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>65</td>
<td>0.70</td>
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<sup>a</sup> The material egg white was used as a control.

<sup>b</sup> Not tested since the precipitate was not obtained in good yield.

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the material (pH 7) at the final concentration of 20%. The protein content in the precipitate increased by using 25% ethanol or lowering the material pH to 5. At pH 5, OA (isoelectric point: 4.6), the most abundant egg protein, was more easily precipitated than at pH 7. On the other hand, OM (isoelectric point: 4.1) was soluble even at pH 5 as described later. For an industrial application, the use of a smaller amount of ethanol should be preferable. We thus set the pH at 5 and the ethanol concentration at 20%.

SDS-PAGE analysis revealed that the product contained OA, OT, and LY (Fig. 1). Since OM was not stained enough with Coomassie Brilliant Blue R-250 (Daiichi Pure Chemicals Co., Japan), ELISA was done to evaluate the precise OM content.

For ELISA, rabbits were used to prepare anti-OM antibody. OM was prepared from egg white by the method of Waheed et al., and further purified by anion- and cation-exchange chromatographies. Female rabbits (JW/CSK, about 2 kg) were subcutaneously injected with purified OM (0.1 mg) in Freund’s adjuvant. The injection was repeated at 10-day intervals until the antibody titer had risen enough. Ten days after the last injection, blood samples were taken. Sera were obtained by centrifugation, pooled, and stored at −80°C until use. The OM in the supernatant fraction (aqueous ethanol soluble fraction) was determined with the anti-OM serum and peroxidase-conjugated goat anti-rabbit IgG (Kirkegaard & Perry Lab., U.S.A.) by the usual ELISA method. As a result, 70% of the OM was recovered in the supernatant (Table 1).

To evaluate the functional properties of the product, a whipping test was performed. Egg white powder (5 g) was dissolved in distilled water (50 ml), adjusted to pH 5, and mixed with ethanol (12.5 ml). A precipitate was obtained from the mixture by centrifugation and washing in the procedure already described. The precipitate was dispersed in water (50 ml) and whipped for 2 min using a mixer (mode: beat egg whites, General Electric, U.S.A.). The whipped sample was immediately transferred into a cylinder and, 1 min later, the water drainage from the foam phase was measured for volume. The whipping ability was represented as (total foam volume−drainage volume after 1 min)/initial material volume. The whipped sample in the cylinder was allowed to stand for 1 hr at ambient temperature prior to measurement of the drainage volume. The foam stability was represented as (initial material volume−drainage volume after 1 hr)/initial material volume. The material egg white powder (5 g) in distilled water (50 ml) was whipped in the same manner and used as a control. The whipping ability and foam stability of the sample were almost the same as those of the material egg white (Table 2).

Fig. 1. SDS-PAGE Analysis of the Low Ovomucoid Egg White Preparation.
Lane A, low ovomucoid egg white; lane B, material egg white. Arrows indicate ovalbumin (OA), ovotransferrin (OT), ovomucoid (OM), and lysozyme (LY). SDS-PAGE was done using a 10 /20% gradient gel (Daiichi Pure Chemicals Co., Japan), and the proteins were stained with Coomassie Brilliant Blue R-250.

Table 2. Whippability* and Foam Stability* of the Low OM Egg White Preparation

<table>
<thead>
<tr>
<th>Property</th>
<th>Egg white</th>
<th>Low OM</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whippability</td>
<td>6.2</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Foam stability</td>
<td>0.82</td>
<td>0.75</td>
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* Standard errors were less than 5% of the values.

This is the first finding that a low OM egg white preparation can be produced without heat-denatura-

tion. Up until now, such preparation with high whippability and foam stability has not been available. Since the product was obtained in a reasonable yield by a very simple method, it would be widely applicable in the confectionery field for ovomucoid-sensitive allergic patients. It may also be effective for patients under hyposensitization immunotherapy. Clinical tests are being carried out to confirm the effectiveness of this low OM egg white preparation.

Acknowledgment

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