Analysis of vitelline membrane proteins of fresh and stored eggs via HPLC

Annette Schäfer, Wiebke Drewes and Fredi Schwägele

Federal Centre for Meat Research, Institute for Chemistry and Physics,
E.-C.-Baumannstr. 20, 95326 Kulmbach, Germany

Summary

The outer layer of the vitelline membrane of hen's eggs was investigated with respect to changes in dependence upon storage temperature and duration of storage. At a temperature of 20°C the amount of VMO1 and VMO2 in the salt soluble fraction decreased during storage, whereas eggs stored under refrigerated conditions did not show significant changes. Membrane weight and protein amount of the salt soluble fraction seemed not to be influenced during storage while the lysozyme percentage in the salt soluble fraction increased. This leads to the conclusion that the deterioration of the vitelline membrane during storage may be related to disintegration of the proteins VMO1 and VMO2 from the membrane.

Introduction

The outer layer of the vitelline membrane formed after ovulation in the upper part of the oviduct consists of sublayers, which are composed of fibrils. It consists of ovomucin, lysozyme and at least two other proteins - the vitelline membrane outer proteins VMO1 and VMO2 [1, 2]. The vitelline membrane deteriorates during storage. Investigations on the protein composition revealed a loss of VMO1 as well as the formation of a lysozyme dimer in the outer layer [3]. To investigate changes in the components of the outer layer of the vitelline membrane during storage our studies focused on lysozyme, VMO1 and VMO2.

Materials and methods

Hen's eggs (grade A, weight 60 - 65 g) were stored up to 30 days at temperatures of 5, 10 and 20°C at a relative humidity of 60 %. The vitelline membrane was separated and the proteins lysozyme, VMO1 and VMO2 were extracted by concentrated NaCl-solution. The proteins of the salt soluble fraction were fractionated by HPLC on a RP-C_4-column and subsequently quantified.

Results and discussion

The salt soluble fraction was separated into 7 distinct peaks via HPLC (Figure 1). The molecular weights of the substance peaks eluted at retention times of 8.8 min., 15.7 min. and 18.1 min. were determined by means of SDS-polyacrylamide-gelelectrophoresis (SDS-PAGE). According to the areas of the peaks lysozyme, VMO1 and VMO2 a relation of 80:15:5 was calculated.

In the case of vitelline membranes from fresh eggs the lysozyme content was about 55 % of the protein content in total (Figure 2). During storage of 30 days it increased up to 70%. The determined content of lysozyme showed a high variation during storage. The differences were only significant during storage at 20°C.

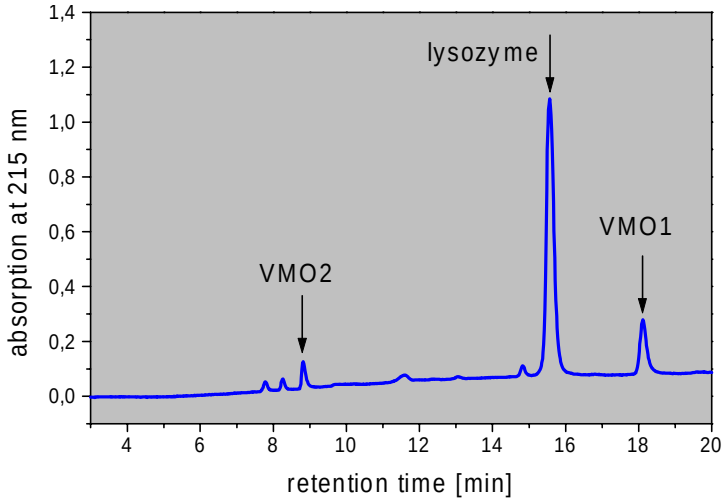

Figure 1: Separation of the salt soluble fraction of the vitelline membrane by HPLC on a RP-C_4-column.

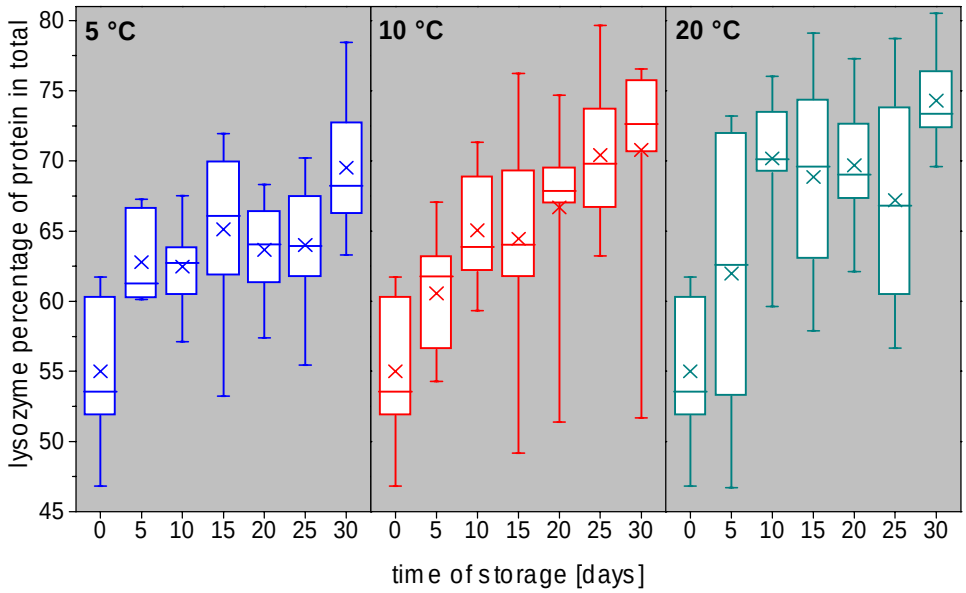

Figure 2: Changes of the lysozyme content during storage at different temperatures.

Under refrigerated conditions (5 and 10 °C) no significant decrease could be observed for the VMO1 content. Whereas at a storage temperature of 20°C a slight but significant decrease appeared (Figure 3). The percental peak area of VMO1 related to the total peak area of the three above mentioned proteins shows a decline from about 15% to 5% during 20 days of storage. The value for the VMO2 content decreases already at storage temperatures of 5°C and 10°C from about 5% to 3% within a period of 10 days and remains constant (Figure 3). Storage for 20 days at 20°C causes a continuous decline of the VMO2 content to about 1%.

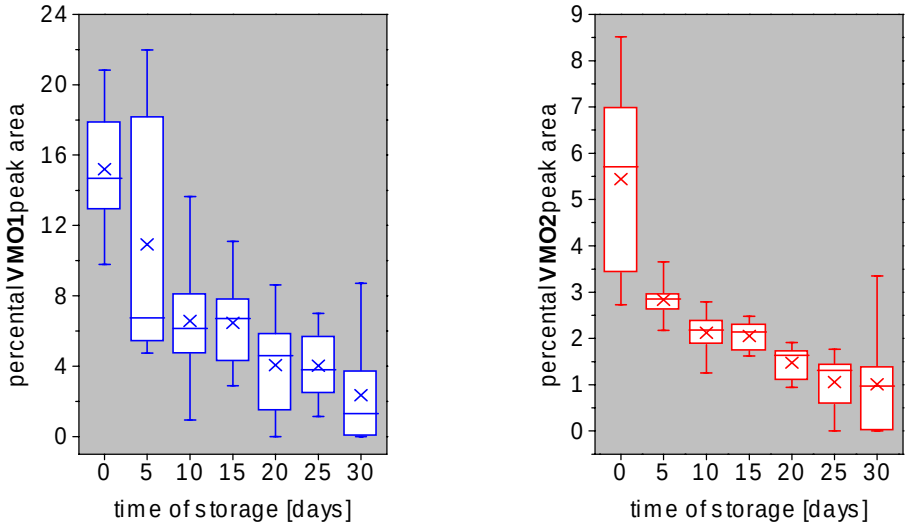

Figure 3: Changes of the peak areas of VMO1 and VMO2 during storage at 20°C.

It can be concluded that the dissociation of salt soluble proteins during storage is responsible for the disintegration of the membrane structure, which leads to a reduction of the membrane strength. The ratio of the salt soluble proteins changed throughout storage because of the different individual diffusion rates. As the changes during storage at room temperature were significantly higher than under refrigerated conditions, the membrane proteins might serve as an indicator for the applied storage conditions.

References

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