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Enabling egg white protein fractionation processes by pre-treatment with high-pressure homogenization

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ABSTRACT

Egg white (EW) includes several proteins with high potential for fractionation processes (lysozyme, ovotransferrin). However, the high viscosity of EW that is caused by the fibrillar protein ovomucin is a limiting factor concerning processability. The common procedure to reduce this viscosity is the precipitation of ovomucin, which, however, results in undesirable dilution effects and loss of proteins. So, the objective of this work was to develop a method to decrease the viscosity of EW without the described disadvantages. Therefore, a high-pressure homogenization process was used. It was demonstrated that it is possible to destroy the EWs' fibrillar network by homogenization treatment, and thereby, to decrease the viscosity significantly. Additionally, filtration was enabled, which allows the use of EW for e.g. chromatographic fractionation. Simultaneously, it was shown that lysozyme that was entrapped in the fibrillar network was released, and therefore, it is available for fractionation processes in higher amounts.

1. Introduction

Egg white (EW) has excellent interfacial properties, and therefore, it is extensively used as functional ingredient in food industry. For some processes, e.g. for fractionation, some of the physical EW properties are limiting factors. One of them is the inhomogeneous structure, which is caused by the different viscous layers in EW. Directly below the egg membrane and around the egg yolk are low-viscous layers, while there is a high-viscous layer in between, which provides nearly 60% of the total egg white (Li-Chan and Nakai, 1989; Ternes et al., 1994). This typical highly-viscous, gel-like structure is caused by the glycoprotein ovomucin (OVN) (Ternes et al., 1994; Strixner and Kulozik, 2011). OVN represents only 1.5–3.5% of the total egg white protein (EWP), but has a remarkable influence on its overall properties. In contrast to other EWP that having a globular structure, OVN is a linear filamentous biopolymer (Offengenden and Wu, 2013), with a disulfide bridge stabilized structure (Fig. 1). More specifically, EW contains two forms of OVN, a soluble and an insoluble one with high molecular weights of 5.6–8.3 $\times 10^3$ and 23 $\times 10^3$ kDa, respectively (Hiidenhovi, 2007; Tominatsu and Donovan, 1972; Lanni et al., 1949; Miller et al., 1981; Hayakawa and Sato, 1976). The soluble form is present in both high-viscous and low-viscous egg white, while insoluble OVN is only found in the high-viscous part (Hiidenhovi, 2007; Sato and Hayakawa, 1977). Both forms consist of the same two subunits, the carbohydrate-poor α-OVN (11–15% carbohydrate) as well as the carbohydrate-rich β-OVN (50–57% carbohydrate) (Itoh et al., 1987). Only their ratios differ with 67% α-OVN and 33% β-OVN for the insoluble form vs. 87% α-OVN and 13% β-OVN for the soluble one (Hiidenhovi, 2007; Hayakawa and Sato, 1977). On average, the glycoprotein OVN consists of 33% carbohydrates (Mine, 1995).

The two proteins that are of interest regarding the previously named fractionation are the EWP ovotransferrin and lysozyme. Mason et al. (1996) demonstrated that ovotransferrin is capable to bind two Fe^{3+}-ions per molecule with high affinity. This results in two different applications. First, it can be used as a nutritional ingredient in iron-fortified products such as iron supplements (Superti et al., 2007). Secondly, ovotransferrin shows antimicrobial effects, based on its ability to sequester the iron that is essential for bacterial growth (Bullen et al., 1978). Additionally, it binds to bacterial membranes, crosses the outer membrane and causes damage to the biological function of the cytoplasmic membrane, whereby it is able to kill gram-negative bacteria (Superti et al., 2007; Ibrahim et al., 2000). The combination of these two effects makes ovotransferrin an effective antimicrobial agent. The content of ovotransferrin in EW is 13% of the total EWP content (Strixner and Kulozik, 2011; Ternes, 2008).

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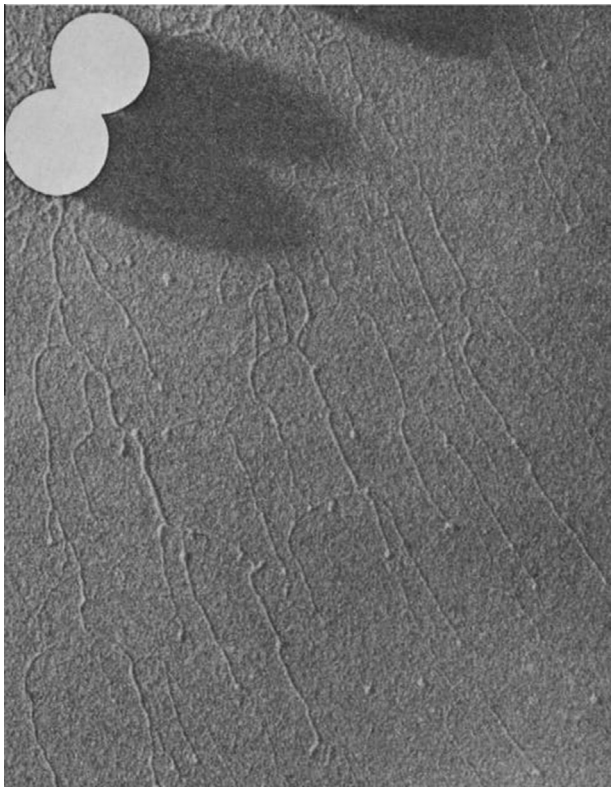

Fig. 1. Electron micrograph of ovomucin shadowed with uranium. The latex spheres are 0.264 μm diameter (Donovan et al., 1970).

Lysozyme is known as a hydrolytic enzyme that cuts β-1-4-glycosidic linkage between N-acetyl muramic acid (NAM) and N-acetyl glucosamine (NAG) contained in bacterial cell walls. It thus shows antibacterial activity especially against gram-positive bacteria (Li-Chan et al., 1986). Hence, it is used to prevent late blowing in cheese or as a preservative in food industry as well as for several further applications in packaging and pharmaceutical industries (Lesnierowski and Kijowski, 2007; Proctor et al., 1988). Lysozyme provides 3.5% of the total EWP content, whereby part of it is bound to OVN fibrils by electrostatic interactions. These electrostatic interactions are mainly based on the positive charges of the lysyl-ε-amino group in lysozyme. This group interacts with the negative charges of the terminal sialic acid residues in OVN (Kato et al., 1975, 1976), which leads to lysozyme–ovomucin-complexes (Omana et al., 2010a; Tilgner, 2010). Additionally, the mentioned OVN network leads to water embeddings in which especially small proteins like lysozyme (14.3 kDa) can be entrapped. Therefore, the availability of lysozyme for fractionation processes is limited.

For EWP fractionation, chromatographic methods are typically used, in lab scale as well as in industrial scale (Guérin-Dubiard et al., 2005; Awadé et al., 1994; Awadé and Efstathiou, 1999; Awade, 1996; Vachier et al., 1995; Tankrathok et al., 2009; Croguennec et al., 2000, 2001; Lesnierowski and Kijowski, 2007; Levison et al., 1999; Levison, 2003). However, these chromatography processes are mainly based on the ion exchange with beads. These methods are very time consuming due to the diffusional transport mechanisms and the high back pressures. Besides, the up-scale is limited and there are microbial risks concerning the hygienic design (Levison et al., 1992; Levison, 2003; Kreuß, 2010; Kreuß and Kulozik, 2009). Additionally, the resulting purities and recoveries especially at industrial scale are not yet satisfying. Hence, new high-throughput methods resulting in high purities and recoveries that can easily be scaled-up need to be developed. Caused by the naturally highly viscous structure of egg white with its fibrillar protein OVN, it would not be suitable for column chromatography in its natural form (Levison, 2003). Therefore, it is necessary to decrease the viscosity of EW as well as to enable a pre-filtration step to avoid pore blocking. In the industry, a combination of pumps and filters with large mashs is mainly used to decrease the viscosity. However, the achieved results are not yet satisfying in terms of the extent of OVN fibril destruction and repetitious accuracy. Additionally, until now, there is a lack of knowledge, if these processing steps are responsible for damage to egg white functionality (Lechevalier et al., 2007). An alternative procedure, commonly used in industrial scale as well as in lab scale is to separate the texturing protein OVN by an isoelectric precipitation process (Omana et al., 2010a; Croguennec et al., 2000; Guérin-Dubiard et al., 2005; Omana and Wu, 2009; Hiidenhovi et al., 1999). For this method, EW is diluted with three volumes of water or NaCl solution and the pH is adjusted to 6.0. Afterwards, the EW suspensions are stored at 4 °C over night. Then, a centrifugation step separates the supernatant from the precipitated OVN. However, this precipitation process is limited by several disadvantages. First, the dilution of EW that is necessary for the complete precipitation of OVN is undesirable, because thereby a subsequent concentration step is indispensable. Secondly, although OVN causes disadvantages for the processability of EW, it despite is a structuring, and thereby, valuable techno-functional protein. Consequently, significant losses are undesirable. The third disadvantage is the unavoidable co-precipitation, and therefore, losses of the valuable EWP ovalbumin, ovotransferrin and lysozyme. As mentioned above, ovotransferrin and lysozyme are the main target proteins regarding fractionation processes, why the loss of them is negative. Also, the extent of this loss is not negligible. Depending on the method, it is up to 10% for ovotransferrin, 12.5% for ovalbumin and 22.5% for lysozyme (unpublished data). As a consequence, these points show that the common procedure leads to a loss of value concerning EW, and therefore, an alternative is required.

Therefore, the objective of this study was to develop a method that ensures the processability of EW, especially concerning the fractionation processes, by creating a homogenous structure with a decreased viscosity. Additionally, the filtration that is not possible using untreated EW should be enabled, because this is an indispensable requirement for subsequent chromatographic separations. Further, the entrapped and bound lysozyme should be released from the fibrillar network to be available for fractionation. This should be achieved without any loss of protein or dilution effects.

In order to fulfill the given requirements, EW was treated by a high-pressure-homogenization process. The hypothesis behind was that the mechanical forces disintegrate the fibrils into smaller pieces, which increases the processability, without any loss of valuable EWP. The work of Li-Chan et al. (1986) already indicated that the viscosity can be decreased by use of a homogenizer. It is known that the processing steps like homogenization contribute to 30% to the variability in functional properties (Lechevalier et al., 2005). Hence, there is a need for a fundamental knowledge concerning the effects of a processing step. Thereby, the methods can be improved regarding e.g. required intensities and repetitious accuracy. So, the present work evaluates the effect of high-pressure homogenization of EW with pressures between 0 and 100 MPa on its viscosity, particle size distribution, filterability as well as on lysozyme content.

2. Materials and methods

2.1. Raw material

Freshly laid eggs from "Lohman Tradition" hens were collected from the University's research farm (Thalhausen) and used within