Separation and Purification Technology 161 (2016) 44–52

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

A novel approach for lysozyme and ovotransferrin fractionation from egg white by radial flow membrane adsorption chromatography: Impact of product and process variables

Janina Brand [a,*], Evelyn Dachmann [a], Manuel Pichler [a], Silke Lotz [a], Ulrich Kulozik [a,b]

[a] Chair for Food Process Engineering and Dairy Technology, Technische Universität München, Weihenstephaner Berg 1, 85354 Freising, Germany
[b] ZIEL Institute for Food and Health, Technische Universität München, Weihenstephaner Berg 1, 85354 Freising, Germany

ARTICLE INFO

Article history:
Received 14 September 2015
Received in revised form 22 January 2016
Accepted 23 January 2016
Available online 25 January 2016

Keywords:
Non-precipitated egg white
Lysozyme
Ovotransferrin
Chromatography
Radial flow membrane adsorber

ABSTRACT

Fractionation of egg white proteins like lysozyme and ovotransferrin is an option to separately exploit the functionalities of the individual fractions. Methods for egg white protein fractionation published so far imply limitations either with regard to the used substrate (precipitated, mucin-free egg white) or the chromatographic system (packed bed columns).

Thus, the aim of the present study was isolate lysozyme as well as ovotransferrin with a process that overcomes these limitations. Therefore, egg white was pre-treated with high-pressure homogenization to reduce its viscosity without losing proteins. Additionally, to avoid the diffusion limitations existing in common ion exchange processes, adsorptive membranes were used as stationary phase. In the first step of the fractionation, lysozyme was separated by a cation exchange process. Thereby, the effect of pH, conductivity and elution profile were determined. Using a fractionation pH of 9.8 and a sample conductivity of 7.8 mS cm^{-1} resulted in a lysozyme purity of 96% and a yield of 99%. Thus, a complete binding of the target protein is possible. In the second step, the flow through resulting from the lysozyme fractionation was taken as substrate for the subsequent ovotransferrin fractionation via cation exchange. With the application of a fractionation pH of 4.9 an ovotransferrin purity of 84% and a yield of 97% were achieved. Thus, the two-stepped cation exchange process resulted in a purification factor of 21 for lysozyme and 5 for ovotransferrin. Further, the achieved yields and purities were shown to be flow rate independent using radial flow membrane adsorbers. Hence, the maximal possible flow rate can be used, whereby process time and costs are reduced. This is an essential aspect regarding scale-up to industrial level.

Summing up, it was possible to fractionate lysozyme as well as ovotransferrin from the high-pressure homogenized egg white using adsorptive membranes with high purity and yield. Hence, radial flow membrane adsorbers offer a suitable possibility for egg white protein fractionation.

1. Introduction

Egg white is a very complex protein source with ~90% of dry matter consisting of various proteins, but low contents of fat, minerals and carbohydrates. Many of the egg white proteins have bio-functional properties. Thus, there is an increasing interest to obtain them in an isolated, pure form by means of an effective and affordable process. There are two egg white proteins that are known to be particularly valuable, lysozyme and ovotransferrin.

Lysozyme is an ubiquitous enzyme, which has the ability to inactivate bacteria by hydrolyzing the β-linkage between N-acetyl muramic acid and N-acetyl glucosamine contained in the bacterial cell walls [1]. Hence, it is an antimicrobial agent that can be diversely applied, especially against gram-positive bacteria. In the food industry, the bacteriostatic and bactericidal properties of lysozyme are commonly used to prevent late blowing of cheese induced by *Clostridium tyrobutyricum* [2–4]. Further food applications are in the area of preservation with a natural agent such as the extension of poultry meat shelf life under refrigerated storage conditions by means of surface treatment with lysozyme [5]. Antimicrobial packaging systems can be created to extend the shelf life of minimally processed foods by incorporating lysozyme in edible films or packaging materials [6,7]. In the beverage industry, under certain conditions, lysozyme can control lactic acid bacteria during fermentation process for beer and wine production [8–10]. Moreover, in the pharmaceutical industry or in the medical area it is used prophylactically against dental caries in tooth paste and

* Corresponding author.
E-mail address: janina.brand@tum.de (J. Brand).

http://dx.doi.org/10.1016/j.seppur.2016.01.032
1383-5866/

mouth wash [11,12]. Furthermore, it can be incorporated in aerosols treating bronchopulmonary diseases or as a component in therapeutic creams for tissue and skin protection, e.g. against cytopathic effects induced by *herpes simplex virus* [13,14]. It was demonstrated as an effective agent against inflammations, e.g. in the case of inflammatory bowel disease [15]. Hence, it can be concluded that the application of lysozyme has a diverse and industrially relevant potential in a broad range of different applications.

The second component of interest is the glycoprotein ovotransferrin. It is an iron-binding protein, whereby it is applicable in various iron-fortified products [16]. Until now, mainly lactoferrin from milk is considered for this purpose. However, lactoferrin in milk is lowly concentrated and, thus, the high demand, especially in Asian countries, cannot be satisfied so far. An advantage of ovotransferrin compared to lactoferrin is that its amount in egg white is about 140-times higher than the one of lactoferrin in milk. Besides the application for supplementation the iron-binding properties can be utilized with regard to antimicrobial activities. This is based on the ability to sequester Fe^{3+}, which is essential for bacterial growth. Moreover, other studies indicate that the bacteriostatic activity is not only due to the removal of iron, but that it is also based on the capability of a cationic domain (OTAP-92) to bind to and to cross the outer membrane of bacteria. This process leads to a damage of the biological function, and thereby, the bacteria are destroyed, especially gram-negative bacteria [17]. Additionally, ovotransferrin shows antifungal and antiviral effects [18–20]. An overview concerning the mechanisms and functions of ovotransferrin is given in the review by Giansanti et al. [18].

Based on this background, it is desirable to isolate these two egg white proteins. For the fractionation of these two proteins ion-exchange methods are typically used, based on the chemical characteristics of lysozyme and ovotransferrin. Lysozyme with an isoelectric point (IP) of 10.7 (Table 1) is the only protein that is positively charged across a wide pH range. It can therefore be fractionated by a cation exchange process. Ovotransferrin also shows a relatively high IP of 7.2, 6.6 or 6.1 (Table 1), depending on the state of iron saturation (zero, one, two Fe^{3+}, respectively) [21]. Normally, in egg white, ovotransferrin mainly occurs in its unsaturated form (IP 7.2).

Previous chromatographic methods are mostly based on ion exchange, but there are some limitations when using conventional packed bed columns, mainly because of its high viscosity. Egg white is therefore not suitable for column chromatography in its natural form [22]. There are different possible methods to reduce the egg white viscosity. In literature, the most common method is the isoelectric precipitation of the protein ovomucin, which is responsible for the highly-viscous, gel-like structure of egg white [23–27]. The resulting substrate is generally named "mucin-free" egg white. Although this method leads to the desired structural egg white characteristics, it also entails some disadvantages. One aspect is the loss of the texturing protein ovomucin. Further, the loss of the target proteins lysozyme and ovotransferrin due to co-precipitation is not negligible in terms of yield. Alternative pre-treatment methods avoiding these protein losses were already described by Brand et al. [28,29]. It was demonstrated that mechanical methods like high-pressure homogenization decrease the egg white viscosity enormously by destructing the ovomucin network, which is responsible for its gel-like structure. An additional advantage of this process is that lysozyme, which is partly bound in the ovomucin network is released, and therefore, available for fractionation. In contrast to what was frequently used in previous studies ("mucin-free" egg white), we used high-pressure homogenized egg white named "mucin-containing" egg white.

Another disadvantage of the hitherto published methods is that many of them work with non-food-grade buffers [30–33], which limits their application.

Further to that certain limitations are based on the used chromatographic media. Mostly, the authors applied conventional bead based columns. These are restricted in their scalability due to high back pressure at high flow rates. Additionally, they are also limited in their rate of adsorption, which is controlled by the diffusion of the protein into the pores of the beads [34].

To overcome the named restrictions of the classically applied column chromatography, adsorptive membranes can be used, where the ligands are not bound to beads, but to the surface of a porous microfiltration membrane. Several types of membrane adsorber constructions already exist [34–37]. Initially, membrane adsorbers with stacked layers were developed, resulting in an axial flow through the column. However, this was found to result in an inhomogeneous flow distribution especially at high flow rates, whereby the binding was limited [35,38]. To overcome this demerit, radial flow membrane adsorbers were generated afterwards. There, the membrane is coiled leading to a radial product flow from the outer rim of the module through the pores of the membrane to the center tube (Fig. 1). Hence, the transport of the protein molecules to the ligands in membrane adsorbers is based on a convective flow instead of diffusional transport mechanisms, which occur in classical chromatography. Based on the existing convective flow a complete depletion of the target protein from the sample is possible. Otherwise, in classical bead chromatography a concentration gradient is required to ensure the diffusive transport into the pores. Compared to packed bed columns the flow rate in membrane adsorbers can significantly be increased [39,40]. The recommended flow rate of classical packed bed columns is mostly 1 column volume (CV) min^{-1}, whereas it is typically between 5 and 10 CV min^{-1} for membrane adsorbers.

Table 1
Major egg white proteins and some characteristics [21].

Protein fraction	Average amount (mg mL^{-1})	Molecular weight (kD)	IP (–)
Ovalbumin (OVA)	60.9	45.0	4.6–4.8
Ovotransferrin (OVT)	15.8	76.0–77.7	6.1–7.2
Ovomucoid (OVD)	11.6	28.0	4.1
Lysozyme (LYS)	3.7	14.3	10.7

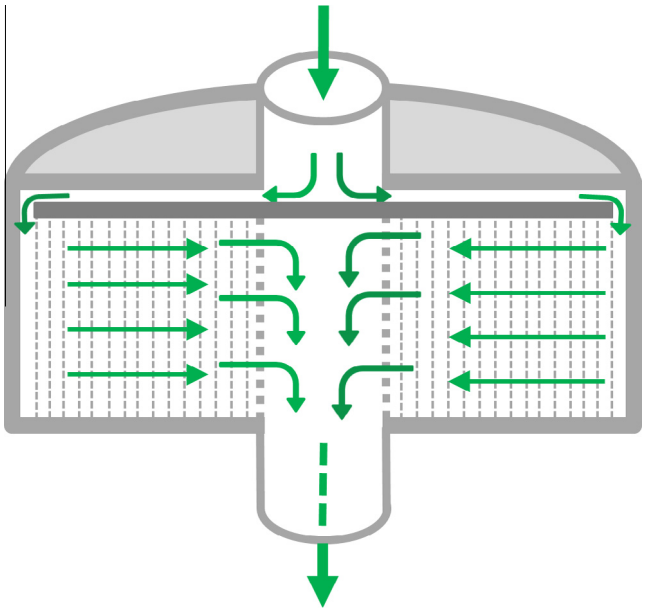

Fig. 1. Scheme of the applied radial flow membrane adsorber and the corresponding flow direction.