Food Research International 41 (2008) 522–531

Contents lists available at ScienceDirect

Food Research International

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

Enzymatic hydrolysis of ovomucin and effect on foaming properties

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ARTICLE INFO

Article history:
Received 15 October 2007
Accepted 3 March 2008

Keywords:
Ovomucin
Egg albumen
Hydrolysis
Solubility
Surface hydrophobicity
Foaming

ABSTRACT

An ovomucin fraction was isolated from egg albumen by isoelectric precipitation, however, of low solubility. To improve solubility, the ovomucin was hydrolysed with four proteases (pronase E, alcalase, flavourzyme, neutrase) for 0–24 h. The hydrolysed ovomucin was analysed for degree of hydrolysis (DH), solubility, surface hydrophobicity (S_0), surface tension, and foaming properties: capacity and stability. The solubility of hydrolysed ovomucin increased logarithmically with DH. The pronase E exposed highest activity and neutrase the lowest, which correlated with the effect on ovomucin solubility. Hydrolysis with flavourzyme and neutrase increased the S_0, primarily during 0–6 h, but pronase E and alcalase hydrolysis reduced the S_0 compared with the unhydrolysed ovomucin. Foaming capacity reached an optimum at DH of 15–40%, and correlated highly with the initial surface tension drop. Enzymatic hydrolysis did no significantly affect the foam stability.

1. Introduction

Ovomucin is a high molecular weight glycoprotein, which makes out ~1.5–3.0% of the total protein in raw egg albumen. The ovomucin consists of three subunits, i.e. α_1-, α_2-, and β-ovomucin, differing in where they are represented in the albumen, molecular mass, and solubility (Burley & Vadehra, 1989). The α_1-ovomucin subunit has an apparent molecular mass (AMM) of 135–150 kDa (Hiidenhovi, Aro, Huopalahti, Suominen, & Kankare, 1995; Itoh, Miyazaki, Sugawara, & Adachi, 1987), the α_2-subunit has a molecular mass of 231 kDa (Watanabe et al., 2004) and the β-subunit has a AMM of 400–720 kDa (Hayakawa, Kondo, Nakamura, & Sato, 1983; Itoh et al., 1987; Robinson & Monsey, 1975). The ovomucins are distributed with 80% in the firm albumen and 20% in the thin albumen, and an average egg albumen contains 73% α-ovomucins and 27% β-ovomucin (Burley & Vadehra, 1989). Egg albumen is often used in foods due to its functional properties such as gelling and foaming. The foaming behaviour of egg albumen exhibits both a high foam volume and a high stability of the foam against liquid drainage due to the rapid unfolding and surface tension lowering of the egg albumen proteins (Hammershoj, Prins, & Qvist, 1999; Hammershoj & Qvist, 2001). Especially ovomucin may stabilize foams due to its long protein strands linked with oligosaccharide chains (Kato, Hirata, & Kobayashi, 1978) that for β-ovomucin can be as high as 65% (Watanabe, Tsuge, & Shimoyamada, 1998), which may support water retention in the foam. Previous studies have shown that firm egg albumen is superior regarding foam stability compared with the thin egg albumen (Nakamura & Sato, 1964; Nau, Gestin, Protais, Awade, & Thapon, 1995). This is regardless of the macroscopic structure providing the high viscosity of the firm part, as reduction hereof by homogenisation has little effect on the foam stability analysed as liquid retention in the foam (Hammershoj & Larsen, 1999). On the other hand, reduction of disulfide bonds reduces the foam stability significantly, which shows that ovomucin in its native state has high impact on the foam stability. Ovomucin have previously been isolated by isoelectric precipitation and centrifugation (Kato, Nakamura, & Sato, 1970), which however results in very low protein solubility. Replacing the centrifugation step with a sieving step has proven to avoid packing of the protein and thereby increase its solubility (Hiidenhovi, Huopalahti, & Ryhänen, 2003). Especially, the firm part of the egg albumen contains insoluble ovomucin, which can be separated by ultracentrifugation into a soluble protein in the supernatant and an insoluble ovomucin fraction in the precipitate (Watanabe et al., 1998). The low solubility of ovomucin can be increased by adding denaturants and reducing agents such as 2-mercaptoethanol (2-ME) (Itoh et al., 1987), or 2-ME plus urea or guanidinium hydrochloride (Robinson & Monsey, 1971). On the other hand, reduction with 2-ME has negative influence on the foaming properties (Hammershoj & Larsen, 1999) and is not suited for application in human foods. Others have solubilised the insoluble ovomucin using sonication (Hayakawa & Sato, 1977, 1978). An alternative method to improve the solubility of precipitated ovomucin may be hydrolysis with different proteolytic enzymes (Hiidenhovi, Hietanen, Makinen, Huopalahti, & Ryhänen, 2000, 2005; Moreau, Nau, Piot, Guerin, & Brule, 1997). By enzymatic hydrolysis of proteins the amide bonds are cleaved and peptides or free amino acids are released by the application of proteases with

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0963-9969/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2008.03.004

*endo*protease or *exo*protease activity, respectively. Until now, the effect of hydrolysis on foaming properties of ovomucin has not been reported. However, enzymatic modification of whole egg albumen with pancreatin and thermitase (Behnke, Kiss, Nadudvari, & Ruttloff, 1986) or pepsin (Bamforth & Cope, 1987) apparently increases the foaming capacity of egg albumen. Furthermore, enzymatic hydrolysis of other proteins such as caseins (Panyam & Kilara, 1996), β-lactoglobulin (Davis, Doucet, & Foegeding, 2005; Ipsen et al., 2001), and a rapeseed isolate (Larre et al., 2006) also has resulted in altered foaming properties.

The aim of the present study was to isolate ovomucin by isoelectric precipitation together with sieving followed by hydrolysis with four enzymes; alcalase, flavourzyme, neutrase and pronase E, respectively, and study the effects on solubility, degree of hydrolysis, surface hydrophobicity, air-water interfacial pressure and foaming properties of the obtained ovomucin fraction.

2. Materials and methods

2.1. Egg albumen samples

Different egg albumen sources were obtained to evaluate the effect of freshness and origin on the yield of ovomucin by the precipitation. The egg albumen samples were (A) total egg albumen >30 d of age obtained from a commercial egg processing company (Sanovo Foods A/S, Odense, Denmark) and stored at −20 °C, (B) same as A but stored at 4 °C, (C) total egg albumen from 60 eggs stored 5 d at 12 °C after lay from Lohmann LSL hens (the hen housing at the University), (D) firm part of egg albumen from 66 eggs collected immediately after lay (egg age < 24 h) from same Lohmann LSL hens as in C and separated by sieving through a 1 × 1 mm mesh and collected as the retentate, and (E) thin albumen part of same eggs as in D i.e. collected as the egg albumen running through the mesh. This initial part of the experiment was only performed once.

2.2. Proteolytic enzymes

The following four proteases were selected in order to cover a wide range of activities and proteolytic cleavage mechanisms:

(1) Neutrase (EC 3.4.24.28) (Sigma–Aldrich Denmark A/S, Brøndby, Denmark) with an activity of 0.8 U/g (Anson units). Neutrase – also called bacillolysin – is a metalloendopeptidase produced by *Bacillus amyloliquifaciens*, with optimum activity at pH 7.0 and 50 °C, i.e. the conditions applied for the present experiments (http://www.chem.qmul.ac.uk/iubmb/enzyme/).
(2) Flavourzyme (EC 3.4.11.1) (Sigma–Aldrich Denmark A/S, Brøndby, Denmark) with an activity of 500 U/g (LAP units). Flavourzyme – also known as leucyl aminopeptidase – contains both *endo*- and *exo*peptidase activity and is produced by *Aspergillus oryzae*, with an optimum activity at pH 5–7 and 45–50 °C. The hydrolysis results in release of an N-terminal amino acid of the sequential order Xaa + Yaa-, in which Xaa represents the first hydrolysed amino acid and is preferably Leu, but may be other amino acids including Pro although not Arg or Lys, and Yaa represents the secondly hydrolysed amino acid and may be Pro (http://www.chem.qmul.ac.uk/iubmb/enzyme/). The conditions applied in the present study were pH 7.0 and 50 °C.
(3) Alcalase (EC 3.4.21.62) (VWR International ApS, Albertslund, Denmark) with an activity of 2.4 U/g (Anson units). Alcalase – also called subtilisin – is a serine-endopeptidase produced by *Bacillus licheniformis* with optimum activity at pH 8.0–8.5 and 55–60 °C. Alcalase has a broad specificity for peptide bonds, and a preference for a large uncharged residue in the P1 position of the P1–P1′ peptide bond (http://www.chem.qmul.ac.uk/iubmb/enzyme/), which interacts with the catalytic system of the serine protease according to the nomenclature of Schechter and Berger (1967), where the amino residues on the N-terminal side of the scissile bond are numbered P3, P2, P1 and those on the C-terminal side are numbered P1′, P2′, P3′, etc. The applied experimental conditions were pH 8.0 and 50 °C.
(4) Pronase E (EC 3.4.24.31) (VWR International ApS, Albertslund, Denmark) with an activity of ∼4000 U/g (P units). Pronase E – also known as mycolysin – is a metalloendopeptidase, which is a non-specific protease from *Streptomyces griseus* containing at least 10 proteolytic components (five serine-type proteases and five different Zn^{2+} peptidases). Pronase E has optimum activity at pH 7.0–8.0 and 37 °C with preferential cleavage of bonds with hydrophobic residues in P1′ (http://www.chem.qmul.ac.uk/iubmb/enzyme/). The experimental conditions were pH 8.0 and 37 °C.

2.3. Isoelectric precipitation and sieving (IEPS) of ovomucin

The procedure originally described by Kato et al. (1970) and later modified (Croguennec, Nau, Pezennec, Piot, & Brule, 2001; Hiidenhovi et al., 2003) was essentially applied for isolation of ovomucin. In brief, the egg albumen samples were diluted with three volumes of deionised H_2O and stirred for 1 h at room temperature (r.t.). Hereafter, the pH was adjusted by 1 M HCl to pH 6.0 and the solution stirred again for 1 h. The precipitated ovomucin was retained by sieving through a 125 μm mesh, and washed in three steps with deionised H_2O, 2% KCl and deionised H_2O, respectively. The washing was continued until the permeate was essentially protein free which was monitored by UV-absorbance at 280 nm to be less than 0.01 AU. The retained ovomucin was lyophilised and weighed.

2.4. Protein solubility of unhydrolysed ovomucin

An experiment was performed to analyse the solubility of unhydrolysed ovomucin in different solute media. The egg albumen sample C (5 d at 12 °C) was subjected to IEPS. Two different fractions were collected; the ovomucin before the first washing step (unwashed) and the ovomucin after the final washing step (washed). The lyophilised ovomucin was suspended to a calculated concentration of 10 g/L based on the dry matter content of the lyophilised powder in four different media: deionised H_2O, 0.1 M NaCl, 4 M urea, or 6 M guanidinium–HCl (GuHCl), respectively. The suspensions were left stirring at r.t. for 30 min followed by centrifugation at 1500*g* for 15 min. The protein content in the supernatant was analysed as AU by UV-absorbance at 280 nm using an average protein extinction coefficient of $E^{1\%}_{280} = 10.0$ (Aitken & Learmonth, 1996).

2.5. Protein composition by gel electrophoresis

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (Laemmli, 1970) was performed on lyophilised egg albumen and ovomucin samples diluted to 1 g/L in sample buffer (40% glycerol, 8% SDS, 0.5 M Tris and bromphenol blue, pH 6.8) containing 0.2 M dithioerytritol (DTE) as reducing agent. The samples were heated 5 min at 100 °C, and 30 μL corresponding to 30 μg protein were subsequently applied to each lane on a standard 7.5% polyacrylamide gel (BIORAD, Hercules, CA, USA). The electrophoresis was run in the Mini Protean II cell of BIORAD and the protein bands were stained with 0.25% Coomassie Brilliant Blue R-250 for