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Impact of pH on the interactions between whey and egg white proteins as assessed by the foamability of their mixtures

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ABSTRACT

The impact of protein–protein interactions on foaming properties of mixtures consisting of egg white proteins (EWP) and whey proteins (WP) with total protein content of 60 g/L was examined at pH 5, 7 and 9. The ratio between EWP and WP in the mixtures was varied between 67:33, 50:50 and 33:67 (in %; w/w). The ionic strength was adjusted to that of milk (I = 176 mM). The foamability of the protein products was characterized by the foam capacity, stability and firmness. In addition, the hydrophobicity in the protein solutions was assessed as a measure for the physical behaviour and ability of proteins to adsorb at the air–water interface.

The individual egg white proteins and whey proteins each showed the best foaming properties at pH 5 and pH 9, respectively. At pH 9 a synergism was observed in the capacity and stability of the foams from EWP/WP-mixtures. This effect appears to be caused by the electrostatic interactions between egg white and whey proteins which occur in the bulk solution after the pH adjustment prior to the foaming. In contrast, at pH 5 no positive influence of foaming the components in a mixture as well as no indication of intermolecular interactions was found. At pH value near the pI of ovalbumin the protein interactions occur when the proteins have adsorbed at the air–water interface. The protein systems foamed at pH 7 showed intermediate foamability compared to the values obtained at alkaline and acidic pH.

1. Introduction

Foams are known as thermodynamically instable colloidal systems comprised of a consistently dispersed gas phase in a liquid matrix (Dickinson, 1992). The selection of the appropriate amphiphilic substances for the foam stabilization is frequently pre-determined by the desired foam properties. Many protein-containing products are aerated foods, such as baked products, extruded and expanded cereal products, egg products, confectionaries and dairy products.

During foam formation proteins diffuse from the aqueous phase and adsorb at the air–water interface due to the compatibility of their hydrophobic groups with the hydrophobic character of the interfaces. During adsorption, protein molecules can unfold to a certain degree and reorient at the interface with polar groups exposed towards water phase and the non-polar groups towards the air phase (Zayas, 1997). This in turn, leads to the decrease of the interfacial tension and to the formation of more or less stable interfacial protein films. The adsorption rate of proteins, as the most important factor for foam formation, depends on the protein concentration, the molecular weight and the structure of the proteins used (Martin, Grolle, Bos, Stuart, & van Vliet, 2002).

Foaming properties of proteins are influenced by a large number of parameters including thermal or chemical pre-processing conditions, method of foaming, whipping time and the physical and chemical properties of the proteins as well as the environmental factors like ionic strength or pH.

According to Lakkis and Villota (1990) the effect of pH on proteins is usually explained by the net charge of the molecules and the protein conformation. The final protein conformation results on only from the internal interactions between the amino acids side chains, but also from their interactions with water molecules. Water may be associated by the proteins via hydrogen bonding with their hydrophilic groups, dipolar interactions with the ionic groups or may be structured around hydrophobic groups. It is and low generally known that an increase of the pH in the protein solution away from the isoelectric point (pI) results in negative protein charges, however, at different levels, depending on pI of the protein. At alkaline pH, most protein molecules possess negative net charges inducing repulsion between them, resulting in a decrease of protein–protein interactions and an increase of protein–water interactions, which tend to reduce the association of proteins with

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each other. In contrast, the proteins with no net charge at their isoelectric point show minimal interactions with water molecules hydration and solubility. Due to the low electrostatic and steric repulsion between the proteins, the attractive forces predominate, what results in the protein association.

Whey proteins have their pI in the pH range between 4.2 and 5.3 (Kilara & Harwalker, 1996). Variations of pH cause similar changes in the electrostatic charges of the whey protein molecules. In comparison, egg white is a more complex system of proteins with different physicochemical properties, e.g. their isoelectric point. Ovalbumin, for instance, has a pI at pH 4.6 while lysozyme at pH 10.7 (Kilara & Harwalker, 1996). The high pI of lysozyme and its positive charge under physiological conditions is responsible for its potential for electrostatic interactions with other, oppositely charged proteins in the mixture.

The potential of lysozyme to interact with oppositely charged proteins was already observed in mixtures with whey protein components by Howell, Yeboah, and Lewis (1995). The authors reported the formation of aggregates between the positively charged lysozyme and negatively charged whey proteins due to the electrostatic interactions leading to an increase of the solution's turbidity. According to Le Floch-Fouere et al. (2009) ovalbumin and lysozyme exhibit different interfacial behaviour when foamed separately, but there is a synergy in interfacial adsorption between ovalbumin and lysozyme when they are foamed in mixture.

Generally, the extent of intermolecular interactions of the proteins at the interface largely affects the formation and stabilization of protein based foams. The negative net charge of proteins adsorbed at the outer film lamella helps to stabilize foams via net repulsion between adjacent films at the air–water interface and inhibits the coalescence of gas bubbles (Phillips, Whitehead, & Kinsella, 1994). However, electrostatic repulsion within the film may also inhibit the foamability of the proteins (Townsend & Nakai, 1983).

Except for the electrostatic charge also the molecular conformation influences in a decisive way the ability of proteins to adsorb at the air–water interface and to stabilize the foam structure. Phillips et al. (1994) reported that proteins tend to foam best at pH levels where the molecules are flexible and less compact. Flexible proteins, such as β-casein, can rapidly reduce the surface tension and show good foamability, whereas highly ordered globular molecules, such as lysozyme, are relatively difficult to denature at the air–water interface and yield less favourable foam properties (Phillips, 1981). The rate of protein folding depends on the amount of stabilising intramolecular bonds. Already existing disulfide bonds demonstrably reduce the intrinsic flexibility of the native protein (Mine, 1995) and are usually referred to as the main structural barriers against the free change of protein conformation e.g. at the air–water interface (Phillips, 1981). On the other hand, these bonds, if newly formed at the interface, can stabilize foam structures by promoting strong coherent protein layers.

According to Pezennec et al. (2000) the pH value influences the sulfhydryl-disulfide exchange rate. The pK_a value of cystein sulfhydryl groups is close to pH 9. Hence, in the range of alkaline pH, the reactivity of sulfhydryl groups and the rate of intermolecular interactions are higher, while disulfide bonds are weakened. Among the whey proteins β-lactoglobulin contains two intramolecular disulfide linkages and one free sulfhydryl group. Ovalbumin, the major egg white protein contains four free sulfhydryl groups and one disulfide bond (Stadelman & Cotterill, 1995). Therefore, these proteins possess a potential to interact with each other by sulfhydryl/disulfide reaction when their functional groups are exposed. The exposure of functional groups may occur due to the unfolding at the air–water interface and increases. Intermolecular interactions strengthen the stability of interfacial protein films.

The pH increase may also affect the molecular properties of proteins by causing an excess of electrostatic charge. An irreversible change of the í-lactoglobulin conformation may be induced at pH values exceeding 8.0 (Kilara & Harwalker, 1996). In areas of high charge density, the intramolecular repulsion may be strong enough to induce unfolding of the protein. When protein unfolding is extensive enough to expose reactive groups such as hydrophobic or sulfhydryl groups, protein adsorption at the air–water interface and intermolecular sulfhydryl/disulfide exchange within the protein film is facilitated.

The effect of these factors on the foaming properties of isolated protein fractions such as ovalbumin, í-lactoglobulin, í-casein, conalbumin or lysozyme was shown in several studies (Lechevalier et al., 2003; Martin et al., 2002; Pezennec et al., 2000). The majority of the previous studies assessed the foaming properties of model systems comprised of single, isolated proteins. In contrast to that, in the food industry mostly complex protein systems, e.g. egg white or whey protein isolates containing a mixture of various proteins, are used to produce foamed products. However, there is only little knowledge about the impact of the interactions between egg white proteins and whey proteins in complex mixtures on their foam properties. Synergistic protein–protein interactions achieved by foaming of protein mixtures from different sources may improve the foamed product structure. This could be an alternative method versus an optimisation of product structure by increasing the protein concentration, dry matter level or by adding polysaccharides.

The objective of this work was therefore to characterize the impact of simultaneously offering whey proteins and egg white proteins to produce foams enhanced by interactions between the proteins in terms of foam capacity, stability and firmness at pH 5, 7 and 9. The pH values were chosen to represent characteristically different environmental conditions. At pH 5 the major egg white and whey proteins in the mixture are at the isoelectrical point and have a neutral net charge. pH 7 shows intermediate state of electrostatic charging, while at pH 9 the proteins possess high net charges and may interact electrostatically. The variation of pH from acidic to alkaline values allows also observing the effect of increasing reactivity of sulfhydryl groups of proteins on their foamability.

2. Materials and methods

2.1. Materials

Fresh eggs were obtained from the University's research farm and used within 48 h. The eggs come from a defined population of Leghorn hens that always receive the same composition of feed. The egg white was completely separated from the yolk by hand.

Whey protein isolate "Bipro" containing 94% (w/w) protein was supplied by Davisco Foods International, Inc. (Minnesota, USA).

2.2. Preparation of the protein solutions

All traces of egg shell, blood and the chalazae were removed from the egg whites. Afterwards it was gently stirred with a magnet stirrer for approximately 5 h to ensure a complete mixing of low and high viscosity egg white fractions.

Solutions of egg white protein (EWP) and whey proteins (WP) as well as mixtures of the two protein components were prepared. The ratios of EWP:WP in the mixtures in % (w/w) were 67:33, 50:50, 33:67.

To simulate the ionic environment of milk, synthetic milk ultra filtrate (SMUF) buffer with ionic strength of 176 mM and pH 6.8 as developed by Jenness and Koops (1962) was used. The solutions with a total protein concentration of 60 g/L and the ionic strength of milk were used for the foam formation by whipping.

In order to avoid the formation of large aggregates and lack of homogeneity in the model solutions, which can affect uniform