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Surface hydrophobicity measurement as a tool in malting and brewing – a mini-review

Foam and foam stability are indispensable signs of beer quality. Foam stability is influenced by a great number of factors. Surface hydrophobicity of proteins counts among the most crucial factors in this respect. Up to now, little use has been made of surface hydrophobicity measurements in brewing. The significance of surface hydrophobicity of proteins in food is explained, followed by a description of the principles of surface hydrophobicity and of surface hydrophobicity measurement. An overview of interrelationship of surface hydrophobicity with cosolutes, such as sugars, is given. The importance of technological and chemical influences on surface hydrophobicity and possible outcomes of surface hydrophobicity measurements in brewing science are discussed.

Descriptors: surface hydrophobicity, protein, beer, ANS

1 The importance of hydrophobic effects

Foam plays a unique role in the consumer acceptance and sensorial properties of beers. For beers, a stable foam head is a must and cling, *i.e.* adhesion of foam to the glass wall, is additionally desired. Beer foam, as important food structure in brewing technology, is a gas in water type 'emulsion'. Other food products, whose structure is essentially determined by foam are meringues, soufflés, whipped products and milk- or cream-based ice cream. These products are gas-in-water or ternary gas/fat/water emulsions. Stabilized emulsions of the 'fat in water' type play also a role in food technology, e.g. milk, cake batters, coffee whiteners, mayonnaise or products from comminuted meats. In all of these emulsions and foams, proteins are found in the interface of the phases where they take part in the separation of the continuous and the discontinuous (or dispersed) phase of the emulsion. After adsorption to gas bubbles or droplets of hydrophobic substance, *e.g.* oil, proteins exert repulsive forces by electrostatic, steric and hydration effects. Additionally, unfolding of the proteins adsorbed to the interface results in the development of a highly viscoselastic membrane around the bubbles. Thereby coalescence of the droplets of the hydrophobic, discontinuous phase and the subsequent destruction of the emulsion are avoided.

Hydrophobic domains on the molecular surface mediate the anchoring of the protein to the hydrophobic phase, whereas their hydrophilic domains stay in contact with the hydrophilic (water) phase. Surface hydrophobicity is a functional property of proteins directly related to their capability to stabilize interfaces of hydrophobic-hydrophilic nature, which occur quite frequently in food emulsions or foams, as mentioned above. Surface hydrophobicity of proteins has also been found positively correlated with surface activity (Kato & Nakai, 1980; Cornec et al., 2001). Without the effects exerted by surface hydrophobicity of proteins, stable emulsions or foams in food would be impossible. Thus, knowledge about hydrophobic effects in food systems is crucial for the understanding of the stabilisation of emulsions or foams in food. However, surface hydrophobicity is not an innate and unchangeable property of the molecule itself, but can be modified by environmental, technological and enzymatic influences. Surface hydrophobicity measurement could be a means of understanding and quantification of factors, which influence modification of protein functionality during malting and brewing processes and thus influence beer foam stability. In the following, a closer look on the principles of surface hydrophobicity in emulsion stabilization by proteins is given.

2 Methods of surface hydrophobicity measurements

One method to determine the hydrophobicity of a given protein is calculation of total hydrophobicity as a function of amino acid sequence and side chain hydrophobicity. A lot of confusion exists, because at least sixteen different scales are used, e.g. Kyte & Doolittle, Bull & Breese, etc., which are either derived from solubility measurements or calculated empirically from molecular structure. The hydrophobicity values determined this way are not comparable from one scale to another, nor have they a good correlation to effective hydrophobicity, since they do not discriminate between amino acid residues on the molecular surface and those buried in the molecule (Nakai and Li-Chan, 1988).

For the experimental determination of protein hydrophobicity, phase partitioning coefficients and chromatographic procedures, such as reversed phase chromatography (RPC) and hydrophobic interaction chromatography (HIC) have been evaluated in the past. Chromatographic procedures worked well for small peptides with mostly accessible amino acid residues, but in the case of proteins, RPC and HIC could not be compared (Faunagh et al., 1984). Apart from its use in protein preparation, HIC may be a suitable technique to detect conformational changes of a protein induced by chemical denaturation in comparison to the unmodified protein (Bramanti et al., 2003). The determination of phase partitioning coefficients is

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not always suitable, since a lot of proteins do not enter the water-immiscible, organic phase (Nakai and Li-Chan, 1988).

Fluorescence spectroscopy has gained by far the greatest importance due to its ease and accuracy. Probing with hydrophobic fluorescent chemicals is thus a possibility to determine the effective hydrophobicity. A defined quantity of fluorescent dye is added to a serial dilution of the protein sample. The probe in aqueous solution has a low quantum yield. If bound to an accessible hydrophobic region, fluorescence increases and the increase can be used to determine effective surface hydrophobicity. The initial slope S_0 of the fluorescence intensity vs. protein concentration plot is generally used as the index of surface hydrophobicity (Kato and Nakai, 1980). In this type of assay, the constant quantity of probe is titrated by rising protein concentration.

Three dyes are currently in use: 1-anilinonaphtalene-8-sulfonate (ANS), 9,11,13,15-cis, trans, trans, cis-octadecatetraenoic acid (cis-parinaric acid, CPA) and 6-propionyl-2-(dimethylamino)-naphtalene (PRODAN). ANS and CPA are anionic, whereas PRODAN is uncharged. ANS is the most commonly used dye and has the longest history in hydrophobicity measurement. PRODAN in contrast, has been brought in use more recently by Haskard and Li-Chan (1998). ANS is an aromatic structure, whereas CPA is a fatty acid with an aliphatic backbone. The differences in the structures may influence the type of binding site in terms of aromatic or aliphatic amino acids (Nakai and Li-Chan, 1988). Apart from differences in the targeted binding sites, a dependence of surface hydrophobicity measurements from pH value exists. The pH value not only influences the charge of ionic dyes and of the proteins, but also leads to conformational changes in the protein structure (Alizadeh-Pasdar and Li-Chan, 2000). In this respect, measurement should be performed at product pH, with the necessary dilutions made up in buffer of appropriate ionic strength.

3 Surface hydrophobicity measurements in brewing science

Bamforth et al. (2001) reported first experiments to relate ANS fluorescence to foaming properties of beer. The authors observed high background fluorescence and found a complex behaviour of the fluorescence vs. protein concentration curve. Upon high dilution (fifty- or hundredfold) of some beers, a correlation between foam stability and ANS fluorescence was found. In the cited study, the fluorescence was measured at beer pH without further buffering, which may explain the observed fluctuations of fluorescence. We found that from pH 3 to pH 5, a region comprising beer pH values, strong differences in the ANS fluorescence of malt proteins can be observed upon pH modification (data not shown). An additional influence can be exerted by ionic strength of the environment. Dilution in distilled water should be substituted by dilution in buffer, in order to maintain pH and ionic strength. However, a positive correlation of foam stability and ANS fluorescence was found for barley albumins and hordeins in a model system (Kapp and Bamforth, 2002).

4 Physical factors influencing surface hydrophobicity

Surface hydrophobicity is not an innate property of the protein molecule. It can be altered dramatically by technological factors, such as denaturation resulting from heat treatment or modification by Maillard reactions (Voutsinas et al., 1983). In general thermal denaturation tends to increase surface hydrophobicity, as unfolding and subsequent release of previously buried hydrophobic domains occur (Raymundo et al., 1998; Ju et al., 2001; Kim et al., 2005). Degree of thermally induced unfolding depends also on the concentration of proteins, where lower increases of surface hydrophobicity are found at higher concentrations (Sorgentini et al., 1995). Surface hydrophobicity can decrease upon thermally induced aggregation of proteins as hydrophobic domains, set free in initial thermal unfolding, are buried upon aggregation (Mohamed et al., 2005). In some cases, high surface hydrophobicity obtained by denaturation was found to have an adverse effect on emulsion stability (Palazolo et al., 2003).

In general, protein adsoprtion to air-water interface is favoured at pH values in the proximity of the isoelectric point (pI) or at high salt concentrations due to the reduction of the surface charge of the protein molecules (Foegeding et al., 2006). Transient exposure to extreme pH values can cause unfolding due to the extreme environmental conditions, paired with increase of surface hydrophobicity (Petrucelli and Anón, 1996). High hydrostatic pressures, which are currently discussed as a means for non-thermal pasteurization, can likewise influence surface hydrophobicity. Hydrostatic pressure of 800 MPa applied for 20 min caused an increase of β-lactoglobulin and a decrease of bovine serum albumin surface hydrophobicity (Galazka et al., 1996). Leguminose proteins exhibited generally a strong increase of surface hydrophobicity upon high pressure treatment, which was, however, not beneficial to emulsion stability as aggregation of the proteins was favoured under these conditions (Galazka et al., 2000; Puppo et al., 2004; Molina et al., 2001).

5 Influence of hydrolysis on surface hydrophobicity

Furthermore, the degree of enzymatic hydrolysis has a distinct influence on surface hydrophobicity. In general, hydrolysis enhances surface hydrophobicity in the resulting product (Wu et al., 1998; Were et al., 1997). In this respect should be noted that peptides resulting from enzymatic hydrolysis of proteins exhibit a functionality different from that one of the high molecular weight fraction. The peptides may exhibit greater foaming capacity than the high molecular weight fraction, dependent also on the enzyme used (Mutilangi et al., 1996). Unusually, hydrolysis of soy proteins with uncommon plant proteases did not increase surface hydrophobicity (López et al., 1998). Konrad et al. (2005) demonstrated that depending on raw material and product purposes, optimal degrees of hydrolysis can be found, in which moderately increased surface hydrophobicity is beneficial to product stability. Beyond a certain degree of hydrolysis, extremely hydrophobic peptides favour rapid coalescence and thus impair product stability. Extremely hydrophic peptides lack the necessary polarity or charge essential for stable action in hydrophobic-hydrophilic interfaces (Caessens et al., 1999).

6 Maillard reaction as an influence on surface hydrophobicity

Maillard reactions, resulting in the covalent fixation of the sugar molecule, as well as length of the sugar molecule attached influence surface hydrophobicity (Antipova & Semenova, 1997; Semenova et al., 1999; Antipova et al., 1999). Achouri et al. (2005) found that Maillard reaction can lead to an initial decrease and, with progression of the reaction, increase of surface hydrophobicity within the 11S glycinin of soy. The foam stability of soy glycinin was found to be highest at strong glycosylation. Additionally, the foaming properties generated by Maillard reaction of proteins depend strongly on the type of sugar coupled to the protein (Chevalier et al., 2001). It should be noted that in most cases Maillard reaction

occurs under the influence of heat and thus Maillard influence on surface hydrophobicity cannot be evaluated fully independent from the effects of thermally induced unfolding.

7 Interrelationship of surface hydrophobicity with carbohydrate components of food

A complex influence on surface hydrophobicity is exerted by the interaction of proteins with sugars or dextrins, respectively. Interaction can result from hydrogen bonds between OH-groups of sugars with the hydrophilic regions of proteins. The effect increases with the length of the dextrin molecule and induces increasing hydrophily. A complex behaviour of interfacial pressure of 11S globulins of broad beans (*Vicia faba*) upon presence of sugars was observed by Antipova & Semenova (1997): a decrease in interfacial pressure was observed in the presence of 0.05 % sucrose, whereas presence of 2 % sucrose led to an increase of interfacial pressure. The prescence of 0.05 % maltodextrin (dextrose equivalent 10) caused a dramatic change of the time dependance of interfacial pressure of the proteins. The impact of high molecular hydrocolloids on the surface hydrophobicity of proteins is complex. Depending on the individual protein, enhancement as well as reduction of surface hydrophobicity have been observed with k-carrageenan and guaran, respectively (Alizadeh-Pasdar & Li-Chan, 2001; Uruakpa & Arntfield, 2006). As k-carrageenan is a charged molecule, an influence of pH, resulting in altered electrical charge, has to be expected. Opposite charge of the protein and the ploysaccharide can mediate better anchoring of the protein to the interface (Benichou et al., 2002).

8 Conclusions

Emulsion and foam stability are influenced by many factors, e.g. protein concentration and composition, degree of hydroloysis, pH, pI, Maillard reaction, concentrations of salts and low molecular weight cosolutes, such as sugars (Damodaran, 2005). Hence, the prognosis of foam stability should not be tried upon one single contributing factor, even if this factor is as crucial as surface hydrophobicity. With respect to maximum foam stability at least the degree of hydrolysis of proteins and the surface hydrophobicity will have to fit in an interrelated optimum. The state of maximal surface hydrophobicity must therefore not be in common with maximum foam stability, especially since a lot of different proteins and peptides contribute to the foam and its stability. Nevertheless, surface hydrophobicity measurements may be a beneficial tool to determine the modification of interfacial functionality of protein fractions during individual steps or upon altered conditions of the brewing process. Development of surface hydrophobicity could be observed for different protein fractions during malting and brewing, e.g. as being coupled with reaction kinetics in thermal processes. However, dilutions required for surface hydrophobicity measurements should consistently be made up in appropriate, buffered systems of continuous ionic strength. Thus, measurement of surface hydrophobicity could be a means to understand the contribution of modifications of these fractions to beer quality, e.g. by proteolysis and Maillard reaction.

9 References

1. Achouri, A., Boye, J.I., Yaylayan, V.A., Yeboah, F.K. (2005). Functional properties of glycated soy 11S glycinin. Journal of Food Science **70** (4), pp. 269-274.
2. Alizadeh-Pasdar, N., Li-Chan, E. (2001). Application of PRODAN fluorescent probe to measure surface hydrophobicity of proteins interacting with k-carrageenan. Food Hydrocolloids **15**, pp. 285-294.
3. Alizadeh-Pasdar, N., Li-Chan, E.C.Y. (2000). Comparison of protein surface hydrophobicity measured at various pH values using three different fluorescent probes. Journal of Agricultural and Food Chemistry **48**, pp. 328-334.
4. Antipova, A., Semenova, M. (1997). Effect of neutral carbohydrate structure in the set glucose/sucrose/maltodextrin/dextran on protein surface activity at air/water interface. Food Hydrocolloids **11** (1), pp. 71-77.
5. Antipova, A.S., Semenova, M.G., Belyakova, L.E. (1999). Effect of sucrose on thermodynamic properties of ovalbumin and sodium caseinate in bulk solution and at air-water interface. Colloids and Surfaces B – Biointerfaces **12** (3-6), pp. 261-270.
6. Bamforth, C.W., Kapp, G.R., Smythe, J.E. (2001). The measurement of hydrophobic polypeptides in beer using the fluorochrome 1-anilino-8-naphtalenesulfonate. Food Chemistry **75**, pp. 377-383.
7. Benichou, A., Aserin, A., Garti, N. (2002). Protein-polysaccharide interactions for stabilization of food emulsions. Journal of Dispersion Science and Technology **23** (1-3), pp. 93-123.
8. Bramanti, E., Ferri, F., Sortino, C., Onor, M., Raspi, G., Venturini, M. (2003). Characterization of denatured proteins by hydrophobic interaction chromatography: a preliminary study. Biopolymers **69** (3), pp. 293-300.
9. Caessen, P.W., Gruppen, H., Slangen, C.J., Visser, S., Voragen, A.G. (1999). Functionality of β-Casein peptides: Importance of amphipathicity for emulsion-stabilizing properties. Journal of Agricultural and Food Chemistry **47**, pp. 1856-1862.
10. Chevalier, F., Chobert, J.M., Popineau, Y., Nicolas, M.G., Haertle, T. (2001). Improvement of functional propoerties of β-lactoglobulin glycated through Maillard reaction is related to the nature of the sugar. International Dairy Journal **11**, pp. 145-152.
11. Cornec, M., Kim, D.A., Narsimhan, G. (2001). Adsorption dynamics and interfacial properties of a-lactalbumin in native and molten globule state conformation at air-water interface. Food Hydrocolloids **15**, pp. 303-313.
12. Damodaran, S. (2005). Protein stabilization of emulsions and foams. Journal of Food Science **70** (3), pp. 54-66.
13. Faunagh, J.L., Kennedy, L.A., Regnier, F.E. (1984). Comparison of hydrophobic interaction and reversed phase chromatography of proteins. Journal of Chromatography 317, pp. 141-155.
14. Foegeding, E.A., Luck, P.J., Davis, J.P. (2006). Factors determining the physical properties of protein foams. Food Hydrocolloids **20**, pp. 284-292.
15. Galazka, V.B., Dickinson, E., Ledward, D.A. (2000). Influence of high pressure on interactions of 11S globulin Vicia faba with i-carrageenan in bulk solution and at interfaces. Food Hydrocolloids **14**, pp. 551-560.
16. Galazka, V.B., Sumner, I.G., Ledward, D.A. (1996). Changes in protein-protein and protein-polysaccharide interactions induced by high hydrostatic pressure. Food Chemistry **57** (3), pp. 393-398.

17. Haskard, C.A., Li-Chan, E.C.Y. (1998). Hydrophobicity of bovine serum albumin and ovalbumin determined using unharged (PRODAN) and anionic (ANS) fluorescent probes. Journal of Agricultural and Food Chemistry **46**, pp. 2671-2677.

18. Ju, Z.Y., Hettiarachchy, N.S., Rath, N. (2001). Extraction, denaturation and hydrophobic properties of rice fluor proteins. Journal of Food Science **66** (2), pp. 229-232.

19. Kapp, G.R., Bamforth, C.W. (2002). The foaming properties of proteins isolated from barley. Journal of the Science of Food and Agriculture **82**, pp. 1276-1281.

20. Kato, A., Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. Biochimica et Biophysica Acta 624, pp. 13-20.

21. Kim, D.A., Cornec, M., Narsimhan, G. (2005). Effect of thermal treatment on interfacial properties of β-lactoglobulin. Journal of Colloid and Interface Science 285 (1), pp. 100-109.

22. Konrad, G., Kleinschmidt, T., Rohenkohl, H., Reimerdes, E.H. (2005). Peptic partial hydrolysis of whey protein concentrate for modifying the surface properties of whey protein. II. Effects on the emusifying and foaming properties. Milchwissenschaft **60** (2). pp. 195-198.

23. López, L.M.I., Brullo, A., Natalucci, C.L., Caffini, N.O., Sorgenti, D.A., Wagner, J.R. (1998). Thermal behaviour, solubility and structural properties of soy concentrate hydrolyzed by new plant proteases. Journal of Food Biochemistry **22** (2), pp. 125-141.

24. Mohamed, A., Peterson, S.C., Hojilla-Evangelista, M.P., Sessa, D.J., Rayas-Duarte, P., Biresaw, G. (2005). Effect of heat treatment and pH on thermal, surface and rheological properties of Lupinus albus protein. Journal of the American Oil Chemists' Society **82** (2), pp. 135-140.

25. Molina, E., Papadopoulou, A., Ledward, D.A. (2001). Emulsifying properties of high pressure treated soy protein isolate and 7S and 11S globulins. Food Hydrocolloids **15**, pp. 263-269.

26. Mutilangi, W.A.M., Panyam, D., Kilara, A. (1996). Functional properties of hydrolysates from proteolysis of heat.deantured whey protein isolate. Journal of Food Science **61** (2), pp. 270-274, p. 303.

27. Nakai, S., Li-Chan, E. (1988). Hydrophobic interactions in food systems. CRC Press Inc., Boca Raton, FL, USA.

28. Palazolo, G.G., Mitidieri, F.E., Wagner, J.R. (2003). Relationship between interfacial behaviour of native and denatured soy bean isolated and microstructure and coalescence of oil in water emulsions – effect of salt and protein concentration. Food Science and Technology International **9** (6), pp. 409-419.

29. Petrucelli, S., Anón, M.C. (1996). pH-induced modifications in the thermal stability of soybean protein isolates. Journal of Agricultural and Food Chemistry **44** (10). pp. 3005-3009.

30. Puppo, C., Chapleau, N., Speroni, F., de Lamballerie-Anton, M., Michel, F., Anón, C., Anton, M. (2004). Physicochemical modifications of high-pressure treated soybean protein isolates. Journal of Agricultural and Food Chemistry **52**, pp. 1564-1571.

31. Raymundo, A., Franco, J., Gallegos, C., Empis, J., Sousa, I. (1998). Effect of thermal denaturation of lupin protein on its emulsifying properties. Nahrung – Food **42** (3-4), pp. 220-224.

32. Semenova, M., Belyakova, L., Antipova, A., Jubanova, M. (1999). Influence of maltodextrins with different dextrose equivalent on the thermodynamic properties of legumin in a bulk and at the air-water interface. Colloids and Surfaces B - Biointerfaces **12**, pp. 287-297.

33. Sorgentini, D.A., Wagner, J.R., Anón, M.C. (1995). Effects of thermal treatment of soy protien isolate on the characterisitcs and structure-function relationship of soluble and insoluble fractions. Journal of Agricultural and Food Chemistry **43**, pp. 2471-2479.

34. Uruakpa, F., Arntfield, S. (2006). Surface hydrophobicity of commercial canola grain proteins mixed with k-carrageenan or guar gum. Food Chemistry **95**, pp. 255-263.

35. Voutsinas, L., Cheung, E., Nakai, S. (1983). Relationships of hydrophobicity to emulsifying properties of heat denatured Proteins. Journal of Food Science **48**, pp. 26-32.

36. Were, L., Hettiarachchy, N.S., Kalapathy, U. (1997). Modified soy proteins with improved foaming and water hydration properties. Journal of Food Science **62** (4), pp. 821-823, p. 850.

37. Wu, W.U., Hettiarachchy, N.S., Qi, M. (1998). Hydrophobicity, solubility and emulsifying properties of soy protein peptides prepared by papain modification and ultrafiltration. Journal of the American Oil Chemists' Society **75**(7), pp. 845-850.

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