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Surface Activities of Whey Protein Concentrates

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Abstrat: This experiment was conducted to study the surface activity of Whey Protein Concentrates (WPC). The Surface Tension (ST) of whey protein concentrates at various levels of ultrafiltration (1-4X) and at different protein loads (0.5, 1 and 2% w/v) were determined. Statistical analysis shows that there is no significant difference in the ST at 1 and 1.5X. However, at higher levels of ultrafiltration (2 up to 4X) the ST decrease with the protein load, except for WPC (3X) at 2% (w/v). At lower protein loads the ST decrease we observed a decrease in the ST while at higher protein loads the trend was different. The Interfacial Tensions (IT) reveal a different behaviour: the protein concentration does not significantly affect the surface tension except for WPC (1.5X) at 2% (w/v). Only at 2% w/v, the WPC IT was significantly affected. The surface free energy was not affected at protein loads varying between 0.5 and 1% for WPC concentrated to two times (X2). However, at 2%, we observed an increase in the (SE) except for WPC (X3). At a higher WPC (X4), the surface free energy increased with the protein load.

Key words: Interfacial tension, surface tension, contact angle, surface energy, whey protein

INTRODUCTION

Whey protein concentrates are commonly used as ingredients in numerous foods because of their excellent technological functionality and high nutritional value (Ronald, 1987; Hugunin and Nishikawa, 1977; Kinsella and Whitehead, 1989; Kowsi, 1979; Phillips *et al*., 1995). Whey protein food functionalities include thickening, gelation, water and shape retention, foam and emulsion stability and even creamy mouth-feel. Foam and emulsion require information about the behaviour of a particular preparation at the oil/water and air/water interface. Many applications in foods depend on such gelation because it makes an essential contribution to the texture of the final food products. The heat-induced gelation of whey protein-water systems has been described as a 2-stage process in which the denaturation of the native protein is followed by subsequent aggregation. If protein-protein interactions lead to the formation of a 3-dimensional network capable of entraining water molecules, a gel is likely to form (Mangino, 1992). The biggest single use of whey proteins is in infant formulae where the objective of the manufacturer is usually to try to produce an amino acid composition resembling that of mothers'milk as closely as possible (Tang *et al*., 1993). The high nutritional value of whey proteins together with their useful functional properties have created much interest by the food industry in their recovery as protein-rich powders, such as Whey Protein Concentrates (WPCs) and isolates (WPIs). They are produced from acid or sweet cheese whey using several techniques, including ion exchange chromatography, ultrafiltration and diafiltration (Fenton-May *et al*., 1971; Patocka *et al*., 2006). Whey protein preparations are quite heterogeneous and reveal marked differences in molecular and physicochemical properties especially, in gelling properties. These differences are mainly owing to the variability in whey composition and processing conditions during the manufacture, different conditions used in the gelation process, as well as various methods used to measure gel characteristics (Morr and Ha, 1993; Patocka *et al*., 2006). Intrinsic factors such as the composition and concentration of the proteins and extrinsic factors such as heating temperature, pH, salt content a nd the presence of other food components, such as lipids and sugars are of great importance (Brandenberg *et al*., 1992; Mangino, 1992; Patocka *et al*., 2006; Boye *et al*., 1995).

The aim of the present experiment is to study whey proteins adsorption at water/air interface (surface tension), oil/water interface (interfacial tension) and determine the surface free energy to find out how they are related to the interfacial behaviour of different whey protein concentrates.

MATERIALS AND METHODS

Whey protein concentration was obtained from Mississippi State University (MSU) dairy plant.

Metacryloxypropyltrimethoxysilane (silane) was obtained from Sigma Chemical company St. Louis MO. Peanut oil was purchased from Walmart.

Protein dispersions of WPC: Whey protein concentrates at different levels of ultrafiltration (WPC) from one fold to 4 folds (1-4X) were dispersed into 5 mM sodium phosphate buffer at pH 7. Whey protein concentrates dispersions were then diluted accordingly to obtain the following concentrations: 2, 1 and 0.5 (%w/v).

Determination of surface and interfacial tensions: The surface tension (air/water) and interfacial tension (oil/water) of freshly made dispersions were measured using a surface tensiomat (semi-automatic, Fisher model 21). The semi-automatic drive mechanism actuates the torsion arm by exerting an upward force on the ring. The level of the surface or interface was preset. The platinum ring was placed in the liquid beneath the surface or interface so that the entire ring would be wetted (1/8 inch immersion). The IT measurements were determined immediately after pouring peanut oil on the surface of the aqueous dispersion. Oil was poured slowly to avoid contact with the ring.

Determination of contact angle: The contact angle was determined by applying a drop of amphiphiles dispersions on pre- silanated glass slips. A diamond ground syringe needle was used to generate drops of uniform size. The glass slips were coated by dipping them overnight in silane and drying them in vacuo. The contact angle of the drop of the aqueous surfactant dispersion with the slip was determined by taking the tangent of the drop with the silane coated glass slip that was placed in the path of a beam of light. An accurate measurement was performed with a small telescope with cross–hairs attached to a goniometer (model D-1060 kayeness Inc). The projection system was composed of a 40X magnification a semi circular viewing screen with a rotable protactor (360°) for reading the contact angle and a top focus system. At least three readings were recorded for each concentration of a given amphiphile and the means were tabulated.

Surface free energy of protein samples: The surface energy is calculated through the extended young Equation:

$$1+\cos 2 = (2/Y_L^{tot})[(Y_L^{LW}\times Yp)]^{LW}$$

where, 2 represents the contact angle Y_l and Y_P are the surface energy of the liquid used for contact angle measurements and the surface energy of the protein layer. LW and SR correspond respectively to the lifshitz-Van der walls and the short range hydrogen bonding components, while TOT refers to the sum of both contributions. For water Y^{SR} = 5.10 mjmG^2, y^{LW} = 21.8 mjmyG^{2}and Y^{tot} = 72.8 mjmG^2 for a "- bromonaphtalene Y^{SR} = 0.8 mjm^{-2}, $y^{lm} = y^{tot}$ = 44.4 mjmG^2, In the case of a "-bromonaphtalene, molecule do not interact with each other via hydrogen bonds to any significant degree. As a result, the surface energy does not include the SR contribution. Thus, with contact angles measured with two liquids of known YSR and y^{lw} and using equation twice, one can obtained two equations with two unknowns from which Y^{SR} and y^{lw} can be derived.

RESULTS

The Surface Tension (ST) of Whey Protein Concentrates (WPC) at various levels of ultrafiltration (1X to 4X) and at different protein loads (0.5, 1 and 2% w/v) was determined. The ST data were represented in a 3 dimensional plot (Fig. 1). Data were also analysed to find out if for a given protein load, the WPC surface tension was affected. At 0.5 and 1% of protein load, we observed a decrease in the ST up to WPC (3X) and then an increase (WPC 4X). At 2%, the trend was different from WPC (2X) and was contrary to what was expected.

The Interfacial Tensions (IT) were also measured to study the behaviour of the different WPC at varying protein loads (Fig. 2). For the different ultrafiltration levels used (1-4X), the protein concentration did not significantly affect the surface tension except for WPC (1.5X) at 2% (w/v) (Table 1). For a given protein concentration, the IT of different WPC were also compared (Table 2).

The Surface free Energy (SE) as function of different WPC at the same protein loads was determined and the

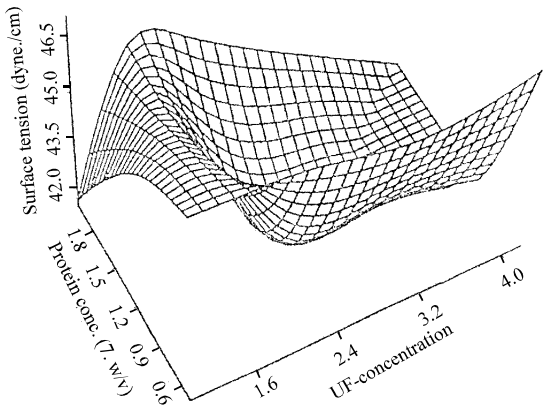

Fig. 1: Effect of ultrafiltration on surface tension of whey protein concentrates

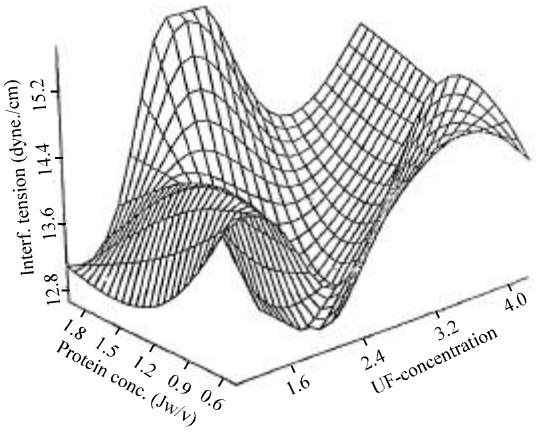

Fig. 2: Effect of ultrafiltration on interfacial tension on whey protein concentrates

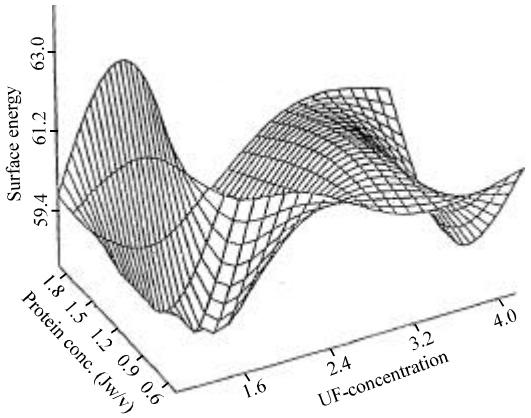

Fig. 3: Effect of ultrafiltration on surface energy of whey protein concentrates

Table 1: Effect of Whey protein concentrates on Surface tension (dyne cmG^{1})

Level of Ultrafiltration	Concentration (%w/v) 0.5	1	2
UF 150	47.06^{Aa}	47.15^{Aa}	47.13^{Aa}
UF 100	46.4^{ABa}	46.87^{Aa}	46.36^{Ba}
UF 75	46.30^{Ba}	43.40^{Bb}	42.40^{Dc}
UF 50	41.40^{Da}	41.60^{Ca}	45.40^{Cb}
UF 35	45.30^{Ca}	43.40^{Bb}	41.40^{Ec}

A, B, C, D, E: Means value in the column not followed by the letter(s) different significantly (p<0.01), a, b, c: Means value in the column not followed by the letter(s) different significantly (p<0.01)

Table 2: Effect of Whey protein concentrates on Interfacial tension (dyne/cm)

Level of Ultrafiltration	Concentration (%w/v) 0.5	1	2
UF 150	14.60^{Aa}	14.30^{Aa}	13.35^{Ba}
UF 100	14.70^{Aa}	14.36^{Aa}	14.03^{ABb}
UF 75	14.50^{Aa}	12.90^{Aa}	13.10^{Ba}
UF 50	14.47^{Aa}	13.05^{Aa}	13.50^{Ba}
UF 35	15.80^{Aa}	14.50^{Aa}	15.06^{Aa}

A, B, C, D, E: Means value in the column not followed by the letter(s) different significantly (p<0.01), a, b, c: Means value in the column not followed by the letter(s) different significantly (p<0.01)

values were plotted (Fig. 3). When the WPC was concentrated up to two times (WPC X2), the surface free energy was not affected at protein loads varying between 0.5 and 1% (Table 3). However, at 2%, we observed an increase in the (SE) except for WPC (X3). At a higher WPC (X4), the surface free energy increased with the protein load. For specific protein loads we have also determined the SE of different WPC. WPC had the basically, the same behaviour for protein loads of 0.5% and 1%, the SE decreased up to WPC (X2) and remained constant. At 2% the trend was different a decrease in the SE was only observed between WPC (1.5X) and WPC (3X).

Table 3: Effect of Whey protein concentrates on surface energy (mJ mG^{2})

Level of Ultrafiltration	Concentration (%w/v) 0.5	1	2
UF 150	62.96^{Aa}	61.92^{Aa}	61.76^{Ba}
UF 100	60.56^{Aa}	60.83^{ABa}	64.31^{Ab}
UF 75	57.97^{Ca}	58.36^{Ca}	60.96^{BCb}
UF 50	58.06^{Ca}	59.57^{BCb}	57.14^{Dc}
UF 35	57.88^{Ca}	60.08^{Bb}	60.13^{Cb}

A, B, C, D, E: Means value in the column not followed by the letter(s) different significantly (p<0.01), a, b, c: Means value in the column not followed by the letter(s) different significantly (p<0.01)

DISCUSSION

Statistical analysis showed no significant difference in the ST at 1 and 1.5X (Table 1). However, at higher levels of ultrafiltration (2 up to 4X), the ST decreased with the protein load, except for WPC (3X) at 2% (w/v). Data were also analysed to find out if for a given protein load, the WPC surface tension was affected. We observed a decrease in the ST which may be due to the effect of the protein interaction with each other to form a network structure and decreasing thereby the surface tension (Adams *et al*., 1978). The functional properties such as: foaming, emulsifying, gelling and water binding properties were enhanced (Phillips *et al*., 1995). The network formation also involves calcium or other ionic bridges, hydrophobic interaction, disulfide bonds and others. During ultrafiltration, calcium and other ions are lost. This lost may affect protein-protein association. Calcium has been shown to be an effective protein cross linking agent in casein system as well as in mediating interaction between whey protein and casein. Furthermore, calcium ions concentration could affect both the rate and solubility of whey protein denaturation. Besides ions, small amphipathic peptides were also lost during the ultrafiltration processing steps could decrease the surface

tension at the interface by interacting between each other to form structures or films. This could explain the reason for higher values even when the protein concentration was high (Dickinson and Woskett, 1989).

For the interfacial tensions, there was no significant difference in the IT of WPC at 0.5 and 1% (w/v). However at a higher protein concentration (2% w/v) the WPC IT was significantly affected. For a given protein concentration, the IT of different WPC were also compared. There was no significant difference in the IT of WPC at 0.5 and 1% (w/v). However, at a higher protein concentration (2% w/v) the WPC IT was significantly affected. It was reported that an increase in the whey protein concentration facilitates the adsorption of more protein at the oil/water interface. The adsorbed proteins reduce the interfacial tension and for a thicker and stronger film (Kinsella, 1984).

Statistical analysis was also conducted on WPC. The analysis revealed that for WPC concentrated up to 2 times (WPC X2), the surface free energy was not affected at protein loads varying between 0.5 and 1% (Table 3). At higher WPC levels, we noticed an increase in the SE value. It was suggested an increase in the SE was correlated to the wetting power of protein dispersion resulting thus in a higher spread ability. The other components such as calcium and peptides which contribute to the stability of the system were lost during the ultrafiltration affecting the change in the SE (Dickinson and Woskett, 1989). Furthermore, Tang *et al*. (1993) have found that at high WPC concentrations, rheological behaviour changed from time-independent to time-dependent (thixotropic) shear-thinning relationship.

CONCLUSION

The surface tension of whey protein concentration was affected by the increasing level of ultrafiltration, especially from WPC (3X). The interfacial tension was not significantly influenced by the WPC, except at a protein load of 2% (w/v). For different WPC, the SE increased with the protein load except for WPC (X3). The effect of the ultrafiltration on the SE was the same for protein loads of 0.5 and 1% (w/v), however the behaviour was different at higher protein loads.

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