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CHARACTERIZATION OF WHEY PROTEIN EMULSION FILMS

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Abstract - Stearic acid was incorporated into whey protein through emulsification to produce films. Whey protein films were prepared by dispersing 6.5% protein in distilled water. Glycerol was the plasticizer agent. Stearic acid was added at different levels (0.0 to 1.0%) and the films were analyzed at different pHs (5.0, 6.0, 7.0 and 9.0). The emulsion films were evaluated for mechanical properties, water vapor permeability and protein solubility. It was observed that water vapor permeability and protein solubility values for the film decreased with increasing fatty acid content in the film, but the mechanical properties also decreased.

Keywords: whey protein, edible films, emulsion, water vapor permeability, mechanical properties.

INTRODUCTION

Edible films of protein offer an efficient alternative for packaging which provides barriers to moisture, gas or solute, improve mechanical integrity of foods, transports food ingredients and is completely biodegradable, reducing thus environment pollution.

Films consist of a polymeric material as a forming agent; however films formed only of polymeric materials such as proteins or polysaccharides are brittle, mainly at a low relative humidity, requiring the use of plasticizers to increase flexibility. Plasticizer molecules reduce the attractive forces between the protein chains, increasing the mobility and flexibility of the filmogenic the matrix (Coupland et al., 2000). A plasticizer is defined as a nonvolatile molecule that is added to polymeric materials to change their tridimensional structure, decreasing intermolecular forces along protein chains and creating free volume and film mobility (Banker, 1966). Polyols, such as glycerol and sorbitol, were extensively used for this purpose.

The characteristics required of edible films are to control mass transfer (moisture, gases and solutes) to improve food quality and to extend food shelf life. Moisture is an important variable in maintaining food quality, freshness and crispness and avoiding microbial growth, shrinkage, loss of firmness and wilting (Krochta and De-Mulder Johnston, 1997).

Whey protein films produce transparent and flexible films with an excellent oxygen barrier and good mechanical properties (Chen, 1995). However, they have poor moisture barrier properties due to their hydrophilicity and/or to the level of plasticizer added to filmogenic solutions. Incorporation of hydrophobic compound, such as fatty acids, monoglycerides and waxes, to a film solution to reduce the water vapor permeability of protein films has been studied. The efficiency of the barrier properties of films depends on the polarity and uniform distribution of lipid particles in the protein

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*To whom correspondence should be addressed matrix (Kamper and Fennema, 1984; Debeaufort et al., 1993).

Lipid films are good moisture barrier, but they usually require solvents and high temperatures to form films and exhibit poor mechanical properties (McHugh and Krochta, 1994).

Emulsions are heterogeneous systems containing at least one immiscible liquid dispersed in another that stabilizes in droplet particles. Emulsifying agents or surfactants are often necessary to improve the stability of lipid particles. In lipid-protein films protein acts as an emulsifier agent, lowering the interfacial tension between protein and lipid phases (Baldwin et al., 1997). Emulsion stability is affected by film morphology, by continuous phase characteristics (pH, viscosity, ionic strength) and by the dispersed phase (size, density of lipid droplet) (Pérez-Gago and Krochta, 1999). Protein and lipid interactions at the interfacial surface depend on homogenization and droplet size (Jost et al., 1986).

Fatty acid type and concentration have an effect on film properties such as permeability characteristics, degree of reaction velocity, color retention and mechanical properties (Santosa and Padua, 1999). The effect of pH on whey emulsion film formation was studied by Pérez-Gago and Krochta (1999). At pHs above or below the isoelectric point (pI) of proteins, water vapor permeability of emulsion films decreased.

The aim of this work was to study the effect of adding lipid to film-forming solution on water vapor permeability, protein solubility and the mechanical properties of whey protein/stearic acid emulsion films.

MATERIALS AND METHODS

Raw Materials

Whey protein concentrate (WPC) was supplied by CALPRO Ingredients Ltd. Glycerol (Merck) was used as the plasticizer. Stearic acid (Riedel-de-Haën) as lipid incorporated compound. Sodium chloride (Merck) was used for formation of a saturated salt solution (75% relative moisture).

Film Formation

Whey protein film solutions were prepared by dispersing 6.5% protein (WPC) in distilled water; 3.0% glycerol (plasticizer agent) was added under stirring. The solution was heated in a water bath at 90ºC for 30 min, and 1.0% stearic acid was added. After lipid melting, the solution was immediately homogenized for 10 minutes in a mechanical mixer on a hot plate and then cooled at room temperature. The solution was degassed with a vacuum pump to remove air bubbles. The pH was adjusted (7.0) with 1M CH_3COOH or 1M NaOH. The solution was poured onto plastic (polyethylene) plates and dried overnight at room temperature.

Water Vapor Permeability

Water vapor permeability (WVP) of films was determined using the ASTM gravimetric standard method E96-95 (ASTM, 1995). The films were fixed on the top of test cells containing a desiccant (silica gel). Test cells were placed in a chamber with controlled temperature and relative humidity (25ºC and 75%rh). Permeation cells were weighed before and after five days of incubation, and then the acquired weight was used to calculate the water vapor permeability. There were at least five repetitions per experiment.

Mechanical Properties

Tensile testing for determining tensile strength (TS) and percentage elongation (E) at break was performed according to ASTM standard method D882 (ASTM, 1995). Films were cut into 25.4 × 100.0 mm strips and then were preconditioned at 75% relative humidity and 25ºC for 48h. The TS and E were measured using a texturometer TATX2 Instrument (Stable Microsystems SMS). The initial grip separation was set at 50mm and crosshead speed was set at 1mm/min. The TS was calculated by dividing maximum force at break by thickness, and percentage elongation was calculated by the difference in distance between grips holding the film specimen before and after the break. There were at least five repetitions per experiment.

Protein Solubility

Samples of films were cut into small pieces (10mm) and an amount containing 100mg protein was weighed and then immersed in Mcllvaine buffer and shaken in a vortex for 1 minute. The mixture was kept without shaking for 48 hours and then shaken again in a Vortex for 1 minute, followed by filtration through filter paper. Protein in supernatant was measured by semimicro Kjedhal (AOAC, 1997). The results were calculated as % of soluble protein, according to Motoki et al. (1984).

Film Thickness

Film thickness was measured using a micrometer (Mitutoyo Mfg Co. Ltd., Japan) and measurements were taken at five random positions on the film. Average values were used for determinations of properties

RESULTS

Whey protein emulsion films were obtained at different pHs: acidic (5.0, 6.0), neutral (7.0) and alkaline (9.0). Stearic acid was incorporated into emulsion film solution at different levels (0.0-1.0%). At pH 5.0 and 6.0, no intact films were formed, they were brittle, nonhomogeneous and difficult to handle. Thus not allowing measure the final properties. Moreover, at these pHs, the film-forming solution had a high viscosity that made it difficult to remove air bubbles prior to film casting. One possible explanation is that at pHs near to the protein isoeletric point, its net charge approaches zero, electrostatic repulsions become weak and attractive interactions increase, causing protein aggregate and as a consequence a highly viscous film solution was formed. The effect of pH on emulsifying and film-forming capacity was attributed to conformational changes in the whey protein structures (Shimizu et al., 1985).

Similar results were obtained by Pérez-Gago and Krochta (1999) for whey protein/beeswax emulsion films at pH 4.0-5.0; under these conditions the films were brittle and developed cracks, resulting in relatively high water vapor permeability. The higher viscosity of filmogenic solution occurred due to protein-protein aggregation that probably decreased lipid mobility and interchange, reducing the film barrier properties.

Protein Solubility

Whey protein films maintained their integrity throughout the film immersion treatment (48h). This indicated that the protein polymer network was highly stable and that only small molecules such as small peptides, monomers and nonprotein material were soluble.

Protein solubility decreased with an increasing stearic acid concentration in the film formulation, confirming that a higher hydrophobic compound concentration resulted in a partial mobilization of proteins at the protein-lipid interface (Figure 1).

The effect of pH on protein solubility is shown in Figure 1. Solubility was lower at alkaline pHs, indicating a high cohesion of emulsion film matrix at pH 9.0. Alkalinity exposure of the SH groups. Pérez-Gago and Krochta (1999) observed that at pH>8.0, SH group reactivity led to disulfide interchange reactions which could have caused entrapment of the lipid particles in the protein network, preventing phase separation.

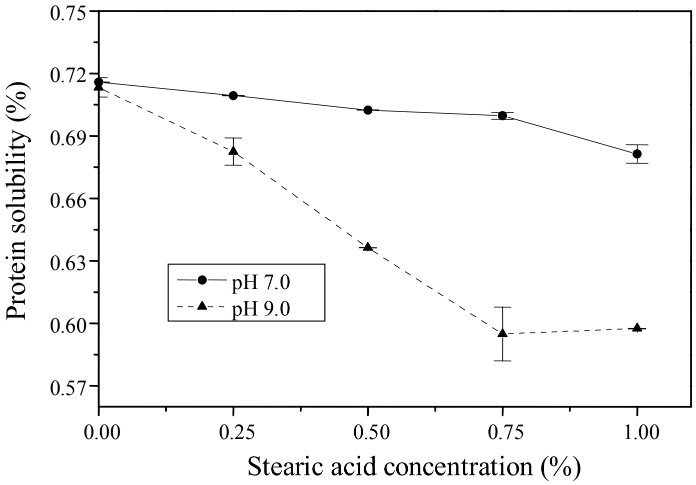

Figure 1: Protein solubility of whey protein emulsion films obtained at pH 7.0 and 9.0.

Water Vapor Permeability

Protein films are hydrophilic and as a consequence they provide a high water vapor permeability. Incorporation of lipid into a film solution to improve this barrier efficiency has been studied. Thus, lipids have poor mechanical properties making lipid concentration an important variable in water vapor permeability and mechanical properties of the emulsion films.

The effect of stearic acid concentration on water vapor permeability of whey protein emulsion films is illustrated in Figure 2. The WVP was reduced at high levels of lipid added, mainly at alkaline pH (9.0). Fatty acid provides a lower mobility, forming viscoelastic films at the oil-water interface and reducing water vapor diffusivity. Pérez-Gago and Krochta (1999) obtained similar results with beeswax/whey protein isolate and Shellhammer and Krochta (1997), with whey protein emulsion films with different levels of lipids.

Mechanical Properties

Incorporation of lipid into protein films resulted in lower tensile strength at break, indicating that they became weaker and nonhomogeneous. This could be explained by the fact that the protein phase has higher tensile strength than the lipid phase. TS was reduced with the higher stearic acid concentration added at pH 7.0 and 9.0, but at pH 7.0 films were more stable and stronger, requiring a stronger force to break their structures (Figure 3).

Fatty acids containing carboxyl groups in their molecules that could compete with the protein chains, reduce the polymer interchanges and as a consequence decrease the tensile strength of edible films.

Homogenization and lipid particle size affected the mechanical properties. The diameter and distribution of lipid particles throughout the polymeric network have an effect on the tensile strength and the percentage elongation of protein films. As particle size decreases and homogeneous distribution occurs, continuous and regular film matrix is formed.

Percentage elongation at break markedly decreased as stearic acid concentration increased (Figure 4), thus the flexibility of whey protein emulsion films was reduced. This may be attributed to noncontinuous film matrix formation probably due to presence of lipid globules. At pH 7.0, the reduction in elongation was higher than at pH 9.0. The protein phase provided a higher elasticity than did the lipid phase compared to the results of whey protein films with and without incorporation of stearic acid.

The elasticity of protein emulsion films decreased with the low structural integrity of the matrix obtained. Similar results were reported by Yang and Paulson (2000) for the addition of stearic acid, palmitic acid and beeswax to gellan emulsion films.

Homogeneity of emulsion films is important to obtain good mechanical properties, since cracks, pinholes and insoluble particles could give rise to weak regions in the film matrix.

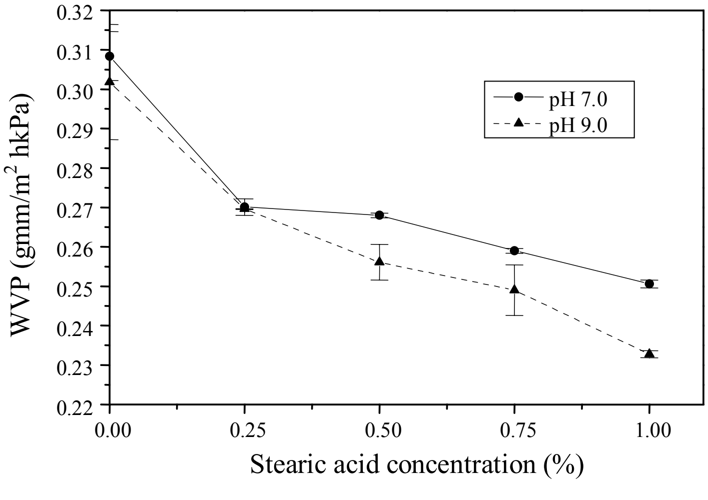

Figure 2: Water vapor permeability in whey protein emulsion films obtained at pH 7.0 and 9.0.

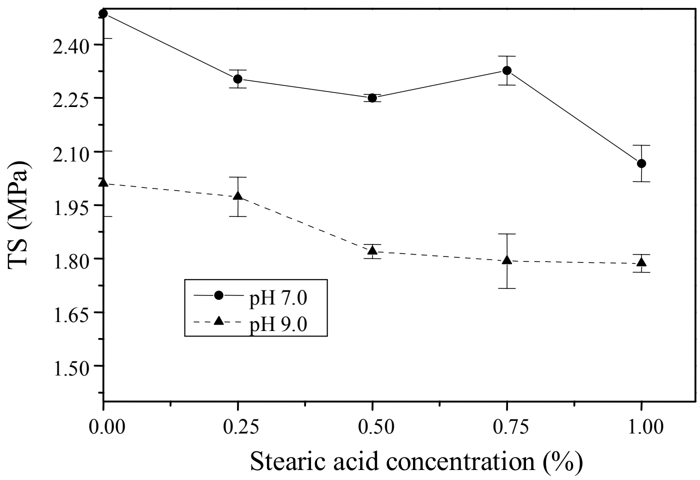

Figure 3: Tensile strength at break in whey protein emulsion films obtained at pH 7.0 and 9.0.

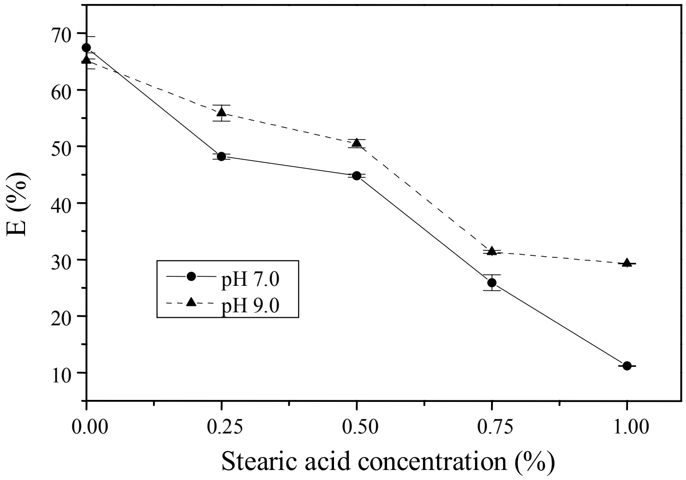

Figure 4: Percentage elongation at break in whey protein emulsion films obtained at pH 7.0 and 9.0.

CONCLUSIONS

The potential for altering the whey protein films through addition of fatty acid was observed. Addition of stearic acid to whey protein films improved the final properties, decreasing the protein solubility and water vapor permeability of whey protein films. However, the film's mechanical properties were lowered. At alkaline pHs, water vapor permeability and protein solubility were more effective than at pH 7.0 and tensile strength was lowered and percentage elongation increased.

ACKNOWLEDGEMENTS

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NOMENCLATURE

WVP	water vapor permeability	gmm/m^{2}hkPa
TS	tensile strength at break	MPa

E	percentage elongation at break	%

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