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The effect of enzymatic treatment of a sunflower protein isolate on the rate of adsorption at the air–water interface

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Abstract

The adsorption kinetic at the air–water interface of a sunflower protein isolate (SPI) and its hydrolysates at different degrees of hydrolysis (DH = 5.62%, 23.5% and 46.3%) adsorbed from aqueous solutions, was studied. Data are presented and analyzed for short adsorption time, taking into account the diffusion of the protein (SPI and its hydrolysates) from the bulk phase to the interface, and the long-term adsorption, taking into account the adsorption and penetration of the protein at the interface. The adsorption kinetics were determined by surface tension measurements as a function of time and protein concentration in the bulk phase (within the range of 1 and 1×10^{-5} %, w/w). The ionic strength (0.05 M) and the temperature (20 °C) were maintained constant. The adsorption of SPI and its hydrolysates to the interface increases with the protein concentration in the bulk phase, depending on the protein and, especially, on the degree of hydrolysis. The adsorption kinetic at short adsorption time is diffusion controlled. However, the mechanism that controls the adsorption at long-term adsorption is the penetration of the protein at the interface. The optimum functionality of SPI hydrolysates occurs at low degrees of hydrolysis and high protein concentrations in solution.

Keywords: Sunflower protein isolate; Protein hydrolysis; Food emulsifier; Food dispersions; Air–water interface; Adsorption; Surface pressure

1. Introduction

The rate of emulsifier (proteins, low molecular weigh emulsifiers and their mixtures) adsorption at fluid-fluid interfaces is considered (Damodaran, 1990; Dickinson, 1992; Halling, 1981) to play an important role in the formation and stabilization of food dispersions (emulsions and foams). During the formation of a dispersed system the emulsifier must be adsorbed at the interface to prevent the recoalescence of the initially formed bubbles or droplets. In addition, during the emulsifier adsorption the surface or interfacial tension of fluid interfaces lowers, which is an important factor both in optimizing the input of energy involved in the emulsification or foaming process (Walstra, 1993) and, finally, in achieving smaller droplet and bubble size—which is an important factor for the stability of the dispersed system (Damodaran, 1990; Dickinson, 1992; Halling, 1981). In addition, emulsification and foaming involve interfacial deformation and the response of the adsorbed layer to such deformations is crucial for understanding the role of emulsifiers in food systems (Benjamins, 2000; Bos & van Vliet, 2001; Dickinson, 1998a, 2001; Wilde, 2000).

The aim of this work is to systematically study the effect of the protein concentration in the bulk phase on the adsorption kinetics of a sunflower protein isolate (SPI) and its hydrolysates at different degrees of hydrolysis (DH = 5.62%, 23.5% and 46.3%) at the air–water interface. Although systematic studies dealing with protein adsorption at fluid interfaces have been published recently (Benjamins, 2000; Carrera, Rodríguez Niño, Lucero, &

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Rodríguez Patino, 2005; Horne & Rodríguez Patino, 2003; Miller et al., 2000; Rodríguez Niño, Carrera, Pizones, & Rodríguez Patino, 2005, 2003), the kinetics of adsorption of (SPI) and its hydrolysates at the air–water interface have not been studied so far.

Plant proteins are increasingly being used as an alternative to proteins from animal sources in human nutrition. Enzymatic hydrolysis is frequently used to improve the functional and nutritional properties of food plant proteins. Protein hydrolysates can be classified into three major groups depending on the degree of hydrolysis, which determines their applications (Damodaran & Paraf, 1997; Kilara & Panyam, 2003; Pedroche et al., 2004; Vioque, Clemente, Pedroche, Yust, & Millán, 2001, 1999): hydrolysates with a low degree of hydrolysis with improved functional properties (mainly foaming and emulsifying capacity), hydrolysates with a variable degree of hydrolysis that are used as flavourings, and extensive hydrolysates that are used as nutritional supplements and in special medical diets (functional foods). As the protein fraction with lower molecular mass increases at higher degrees of hydrolysis (Miñones Conde, Yust, Pedroche, Millán, & Rodríguez Patino, 2005), the reduction of molecular masses in SPI hydrolysates might promote foam and emulsion formation due to the faster diffusion of molecules to fluid interfaces (air–water and oil–water, respectively) (Horne & Rodríguez Patino, 2003; Rodríguez Niño, Wilde, Clark, Husband, & Rodríguez Patino, 1997a, Rodríguez Niño, Wilde, Clark, Husband, & Rodríguez Patino, 1997b; Rodríguez Niño & Rodríguez Patino, 2002; Rodríguez Niño, Rodríguez Patino, Carrera, Cejudo, & Navarro, 2003). However, peptides formed during hydrolysis may be too small, especially at higher degrees of hydrolysis, to stabilize fluid interfaces, which is essential for the formation and stability of the dispersed system (Damodaran & Paraf, 1997; Dickinson, 1992; Halling, 1981). Thus, research is necessary to counterbalance the positive effect of the degree of hydrolysis on the solubility and protein fractions with lower molecular mass, and the negative effect of lower protein–protein interactions in protein fractions with lower molecular masses. A minimum degree of protein–protein interaction is necessary for the stabilization of the interfacial film.

2. Materials and methods

2.1. Materials

The isolation of sunflower proteins (SPI) from defatted sunflower meal, the preparation of sunflower protein hydrolysates with low (5.62%), medium (23.5%) and high (46.3%) degrees of hydrolysis (DH), the determination of solubility, and chemical characterization (including the determination of the molecular masses by gel filtration chromatography, amino-acid analysis by high-performance liquid-chromatography and chemical composition) have been described elsewhere (Miñones Conde et al., 2005).

Table 1
Physico-chemical properties of sunflower protein isolate

Component	Composition (%)
Moisture	5.10 ± 0.83
Ash	3.00 ± 0.08
Protein content	82.81 ± 0.45
Soluble sugars	0.22 ± 0.02
Polyphenols	0.45 ± 0.08
Others	8.60

Table 2
Solubility at pH 7 and chemical composition of sunflower protein isolate and protein hydrolysates from sunflower protein isolate at different degrees of hydrolysis (HD)

Degree of hydrolysis (%)	Protein	Modified protein	Solubility (%) at pH 7.0
Isolate	82.1	–	27.9
HD 5.62	93.9	60.5	70.8
HD 23.3	80.8	83.9	86.2
HD 46.3	74.5	94.7	92.8

The physico-chemical properties of sunflower protein isolate and the solubility and chemical composition of sunflower protein isolate and its hydrolysates are included in Tables 1 and 2, respectively (Miñones Conde et al., 2005). Proteins represent the main component of sunflower protein isolate. Soluble sugars (0.22%) and polyphenols (0.45%) represent a minor components. The protein concentration in the isolate and in protein hydrolysates is higher than in the original sunflower meal (33.3%, w/w), but decreases as the degree of hydrolysis increases. The modified protein also increases with the degree of hydrolysis due to the action of the enzyme on native sunflower proteins.

The water used as subphase was purified by means of a Millipore (Milford, MA) filtration device (Milli-Q). To adjust subphase pH, a commercial buffer solution called *trizma* $[(CH_2OH)_3CNH_2/(CH_2OH)_3CNH_3Cl]$ for pH 7 was used as supplied by Sigma (St. Louis, MO, >99.5%) without further purification. Sodium azide (Sigma) was added (0.05 wt%) as an anti-microbial agent. Ionic strength was 0.05 M in all experiments.

2.2. Surface pressure measurements

Surface pressure measurements were used to determine the rate of protein adsorption at the air–water interface. Surface pressure was deduced from the surface tension, $\pi = \sigma_0 - \sigma$, where σ_0 and σ are the aqueous subphase surface tension and the surface tension of the interface covered by protein, respectively. A range of techniques has been development for the measurement of the dynamic surface tension (Adamson, 1990; Dukhin, Kretzschmar, & Miller, 1995). For dynamic surface tension measurements of adsorbed protein films at the air–water interface, at short and long-term adsorption time, an automatic drop