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Food Chemistry 69 (2000) 153–160

Food Chemistry

www.elsevier.com/locate/foodchem

Functional properties and in vitro digestibility of almond (*Prunus dulcis* L.) protein isolate☆

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Received 28 May 1999; accepted 5 October 1999

Abstract

Almond protein isolate (API) solutions were less viscous than those of soy protein isolate (SPI). The foaming capacity of API at pH 5.0 and 6.46 was comparable to that of SPI at pH 4.42 and 5.0. At pH 8.2, SPI had better foam capacity and stability compared to that of API. API had better oil absorption capacity than that of SPI [3.56 and 2.93 g/g dry weight basis (dwb), respectively]. Emulsion activity index (EAI) of API was significantly higher than that of SPI. API was easily hydrolyzed by pepsin in vitro.

1. Introduction

Proteins are important in food processing and food product development because they are responsible for many functional properties that influence the consumer acceptance of food product(s). These functional properties include both physicochemical and nutritional properties. Protein hydration and hydration related properties such as the protein solubility, water holding capacity, oil binding properties, foaming capacity and stability, emulsion capacity and stability, viscosity, and gelation are some the functional properties that have a significant impact on product quality. Protein amino acid composition and the ease with which digestive enzymes can hydrolyze a food protein are two important determinants of food protein quality.

Since oilseeds are valuable sources of lipids as well as proteins, numerous studies on protein functionality of major and minor oilseeds such as soybean (Arrese, Sorgentini, Wagner & Añón, 1991; Carp, Wagner, Bartholomai & Pilosof, 1997; Elizalde, Pilosof & Bartholomai, 1991; Kinsella, 1979; Nir, Feldman, Aserin & Garti, 1994; Sorgentini, Wagner, Arrese & Añón, 1991; Yasumatsu et al., 1972; Wolf, 1970), peanut (Kim, Park & Rhee, 1992; Monteiro & Prakash, 1994), rapeseed (Sosulski, Humbert, Bui & Jones, 1976), sunflower (Fleming, Sosulski, Kilara & Humbert, 1974; Sanchez & Burgos, 1997), winged bean (Okezie & Bello, 1988; Sathe, Deshpande & Salunkhe, 1982), and groundnut (Ramanatham, Ran & Urs, 1978), have been reported.

Almonds are a good source of high quality protein and contain 16–22% protein on a dry weight basis (dwb) (Sathe, 1993). Almonds in various forms such as whole, chopped, sliced, or paste, are used in many bakery products such as cookies, cakes, and pies (Young & Cunningham, 1991). Almond paste is extensively used in many bakery as well as confectionery products (Campbell, 1991, 1992). The desirable texture of almonds and almond products is partly dependent on properties of almond proteins. Almond proteins contain one major (~65% of the total protein) and several minor proteins that are water soluble and with the exception of methionine, contain all essential amino acids in adequate amounts (Sathe, 1993; Wolf & Sathe, 1998). Almond nutrient composition has been investigated by several investigators (Rikhter & Pyzhov, 1987; Sathe, 1993; Seron, Garrigos, Poveda, Prats Moya, Martin Carratala, Beranguer, Navarro & Grane'-Tervel, 1998; Soler, Canellas & Saura-Calixto, 1989). However, functional properties of almond proteins have not been investigated. Since almonds are expensive, commercial production of almond protein isolate may not be economical. However, cull almonds may be used for

☆ Presented in part at the Annual Meeting of the Institute of Food Technologists, Atlanta, GA, 25–29 June 1994.

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0308-8146/00/$ - see front matter
PII: S0308-8146(99)00244-7

production of protein isolates. Almond protein isolates may be used in development of low fat almond based products. As a part of long term ongoing investigations on tree nuts, we recently reported disulfide exchange, ionic interactions, and hydrogen bonds promoted thermal gelation of almond proteins (Sathe & Sze, 1997). In this paper, we report some of the hydration related properties and in vitro pepsin digestibility of almond protein isolates.

2. Materials and methods

Dr. Sam Cunningham of the Blue Diamond Growers (Sacramento, CA) provided whole almonds (Nonpareil). Dr. W. J. Wolf (USDA, NCAUR, Peoria, IL) provided soybeans (Century 82). Electrophoresis grade acrylamide, bis(*N,N′*-methylene bis-acrylamide), TEMED (*N,N,N′,N′*-tetramethylenediamine), Tris[tris-(hydroxymethyl) aminomethane], glycine, and ammonium persulfate were from Bio-Rad Laboratories, Richmond, CA. Bromphenol blue, Coomassie Brilliant Blue R, glycerol, β-mercaptoethanol (β-ME), and pepsin (porcine stomach mucosa, batch number 88F-8010) were from Sigma Chemical Company, St. Louis, MO. Low molecular weight (MW) marker kit (94 to 14.4 KDa) was from Pharmacia, Inc. Piscataway, NJ. All other chemicals were from Fisher Scientific Company, Orlando, FL and were of reagent or better grade. Wesson vegetable oil (Hunt-Wesson Food Inc., Fullerton, CA) was purchased from a local grocery store.

2.1. Defatted flour

Whole almonds (Nonpareil) were ground in an Osterizer Blender. The ground almond meal was defatted by multiple extractions with cold (4°C) acetone (meal to acetone ratio of 1:5 w/v) with constant magnetic stirring. After each extraction, the slurry was filtered through Whatman filter paper #4 and residue used for next extraction. Residue from final extraction and filtration step was dried in a fume hood, homogenized in the blender to obtain a 40 mesh almond defatted flour, and stored at −20°C until further use. Defatted soybean (*Glycine max* L.) flour was prepared in the same manner.

2.2. Preparation of protein isolate

2.2.1. Almond protein isolate

API was prepared by solubilizing proteins with 20 mM Tris–HCl pH 8.1 (defatted almond flour to solvent ratio of 1:10 w/v) for 1 h with constant magnetic stirring at 25°C. Samples were filtered through glass wool, and filtrate centrifuged (12,000 *g*, 20 min, 4°C). The supernatant was vacuum filtered to remove debris, dialyzed against 5 l distilled deionized water (48 h, 6 changes, 4°C), lyophilized, and stored at −20°C in air-tight plastic bottles. The average API yield ($n=4$) from 100 g defatted almond flour was 19.42 ± 1.88 g.

2.2.2. Soy protein isolate

Defatted soy flour was extracted with 30 mM Tris–HCl pH 8.1 (containing 0.01% β-mercaptoethanol, 0.02% NaN_3) for 1 h (flour to solvent ratio of 1:10 w/v) with constant magnetic stirring at 25°C. Samples were filtered through glass wool, filtrate centrifuged (12,000 g, 20 min, 4°C) and the supernatant was adjusted to pH 4.5 with 2 N HCl and centrifuged (12,000 g, 20 min, 4°C). The precipitated proteins were dialyzed against distilled deionized water, lyophilized, and stored at −20°C in airtight plastic bottle. Typically, the yield of soy protein isolate was ∼25% (w/w) of defatted soy flour.

We included β-ME in the buffer used for solubilizing soy proteins because reducing agent helps improve soy protein solubilization. We used isoelectric precipitation to prepare soy protein isolate since that is the normal procedure used in industrial production of soy protein isolate.

2.3. Functional properties of protein isolate

2.3.1. Viscosity

Stock protein solutions (10% w/v, pH adjusted to 8.2 with 0.1 N NaOH) were prepared for each protein isolate using distilled deionized water as the solvent. Working protein solutions of desired concentration (1, 2, 3, 4, 5, 7, and 10% w/v, 5 ml each) were prepared from appropriate stock solution. Viscosity was determined using the Ostwald type viscometer (Cannon Instrument Co., State College, PA; size #100).

2.3.2. Foaming properties

Foaming capacity and stability were determined according to Sathe and Salunkhe (1981). Briefly, 50 ml of 1% (w/v) protein isolate solution of appropriate pH was whipped for 3 min in an Osterizer blender at "stir" setting and then poured into a 100 ml graduated cylinder. When needed, pH of protein solution was adjusted (prior to whipping) to the desired pH value with 0.1 N HCl and or 0.1 N NaOH. We used "natural pH" of the reconstituted protein isolate solution (pH 6.46 for API and pH 4.42 for SPI), and pH 5 and pH 8.2. The "natural pH" is the pH of the protein solution when protein isolate was dispersed in distilled deionized water. We chose to include "natural pH" because in food applications, one is likely to encounter this pH when the protein isolate is reconstituted in water prior to its intended food use. We chose pH 5 because it is closer to the isoelectric pH (pI) of almond as well as soy proteins (for both API and SPI, pI range is pH 4–5). Choice of pH

8.2 was based on the fact that both almond and soy proteins are effectively solubilized at this pH. The total sample volume was monitored at 0 min for foam capacity and up to 120 min for foam stability. Volume increase (%) was calculated according the following equation:

$$\text{Volume increase (\%)} = \frac{(\text{Volume after whipping} - \text{Volume before whipping})\,\text{ml}}{\text{Volume before whipping (ml)}} \times 100$$

2.3.3. Oil absorption

Oil absorption was determined by vortex mixing 0.1 g of protein and 1 ml of Wesson vegetable oil, density = 0.9239 g/ml, for 30 s and allowed to stand for 30 min. The mixture was centrifuged (13,600 *g*, 10 min, 25°C) and the weight of the supernatant was obtained. The weight (g) of oil absorbed per g of protein on a dwb was reported.

2.3.4. Emulsion properties

Emulsion activity and emulsion stability were determined by the method of Pearce and Kinsella (1978) and Beuschel, Culbertson, Partridge and Smith (1992). Five ml of protein isolate solution of desired concentration and pH was blended with 15 ml vegetable oil for 45 s with a Sorval Omnimixer (Model 17150, Dupont Instruments, Newton, CT) at speed setting 4. The emulsions (80 μl) were diluted to 10 ml with 0.1% sodium dodecyl sulfate and sample absorbance was read up to 90 min at 500 nm, using Perkin–Elmer Lambda 3 UV/VIS Spectrophotometer. Emulsion activity index (EAI) was calculated according to Pearce and Kinsella (1978).

2.4. API in vitro digestibility

API was dissolved in distilled deionized water (pH adjusted to 8.2 with 0.1 N NaOH) and aggregates were removed by centrifuging the solution at 13,600 *g* for 5 min at 25°C. API digestions were done in plastic microcentrifuge tubes in a total final volume of 0.5 ml (for monitoring absorbance at 280 nm) or 0.2 ml (for electrophoretic characterization). Final API concentration was 5 mg/ml and the digestion buffer was 0.1 M HCl (final concentration) All digestions were done at 37°C in a controlled temperature water bath. Protein to enzyme ratio was 50:1, 100:1, 500:1, and 1000:1 (w/w) and the digestion time ranged from 0 to 60 min for each ratio. All digestions were initiated by adding appropriate enzyme amount. At the end of the digestion, 0.5 ml of 20% TCA (4°C, 10% w/v final concentration) was added to the digestion mixture and the mixture was centrifuged (13,600 *g*, 10 min, 25°C). The supernatant was collected and absorbance of the supernatant was determined at 280 nm to monitor production of soluble amino acids and peptides. For samples to be used for electrophoresis, 20 μl of 0.1 N NaOH and 280 μl of SDS-PAGE buffer with 2% β-ME was added to the digestion mixture at the end of digestion and samples were immediately heated for 2 min to inactivate the enzyme. Appropriate substrate and enzyme controls were run simultaneously.

2.5. Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done by the method of Fling and Gregerson (1986). Details have been described in Sathe (1991). The samples were electrophoresed on 8–25% linear acrylamide gradient gels (14.5×15.5×1.5 mm separating gel and 1.0×15.5×1.5 mm stacking gel). The gels were run at a constant current (8 mA/gel) with running tap water cooling (15°C) until the tracking dye reached the gel edge.

2.6. Protein determination

Total protein in seed samples was determined using micro-Kjeldahl method (N×5.18 for almond and N×6.25 for soybean). Soluble protein content of samples was determined by the Lowry procedure as described earlier (Sathe, 1993).

2.7. Statistical analysis

All experiments were done in triplicate. Data are reported as mean ± standard deviation. When appropriate, data were analyzed for significant differences using Fisher's LSD (protected test, $P = 0.05$) as described in Ott (1977). Correlation coefficient (r) was calculated for appropriate data.

3. Results and discussions

3.1. Functional properties

3.1.1. Viscosity

As expected, viscosity was concentration dependent (Fig. 1). SPI viscosity was higher than that of API at comparable concentrations. Viscosity is partly dependent on protein concentration as well as type of protein (Catsimpoolas & Meyer, 1970; Fleming et al., 1974; Kinsella, 1979; Sathe & Salunkhe, 1981). It has been reported that SPI contains an insoluble fraction that contributes significantly to swelling ability. Swelling ability has been suggested to be a significant contributory factor to viscosity of protein solutions (Kinsella, 1979). Almond proteins are highly soluble in water (Sathe, 1993) and therefore may have less swelling ability and

therefore less viscosity compared to soy proteins. The low viscosity of API even at high concentrations may be useful in development of high protein drinks without suffering the adverse consequences of high viscosity.

3.1.2. Foaming properties

The foaming capacity (total foam volume between 58 and 64 ml) of both API and SPI was comparable (Fig. 2). At pH 8.2, foam stability of SPI was higher than that

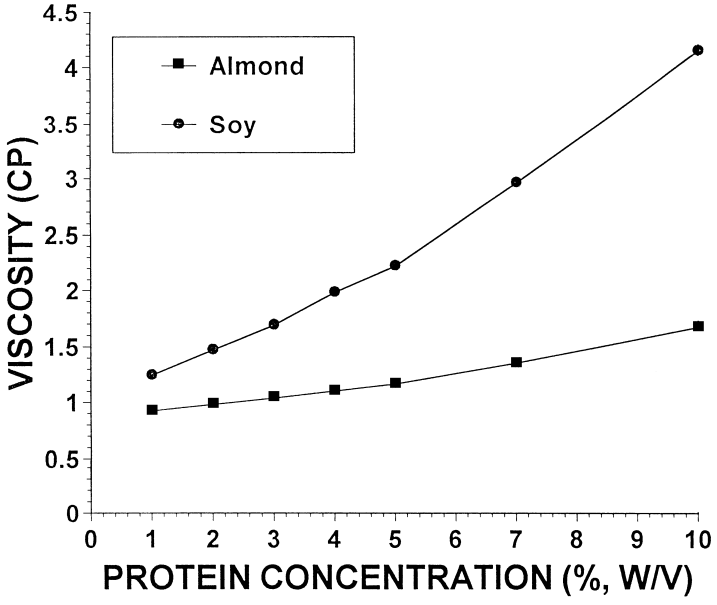

Fig. 1. Apparent viscosity of API and SPI. Each data point represents average of three determinations.

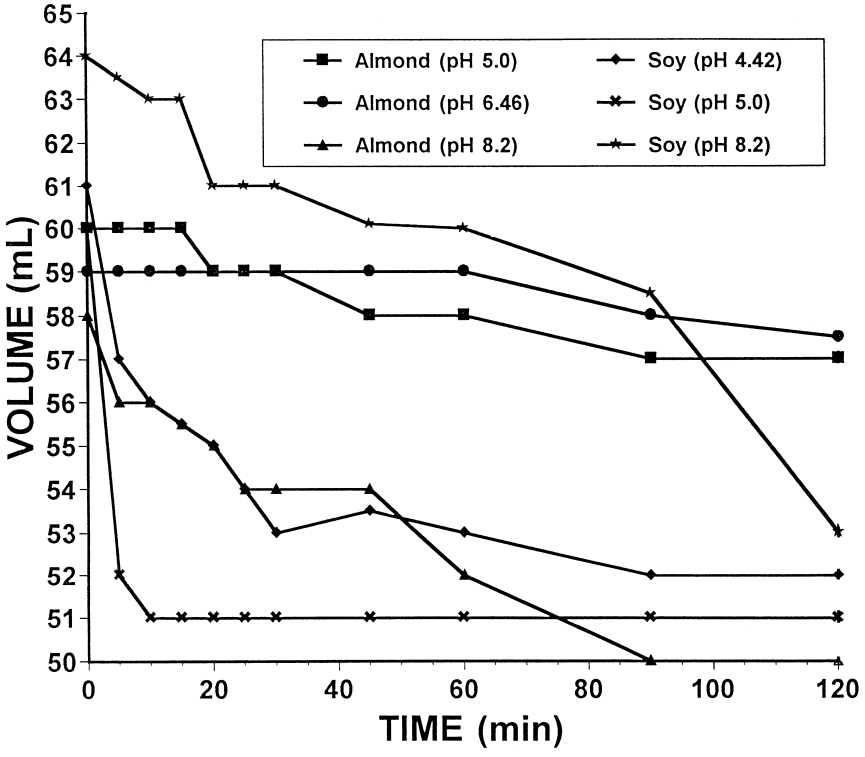

Fig. 2. Effect of pH on foaming capacity and stability of API and SPI.

Table 1
Emulsion activity index (EAI) of almond and soy protein isolates

Protein concentration (% w/v)	EAI (m^2/g)	
	Almond	Soy
0.1, pH 5	44.78 ± 1.13	11.51 ± 0.76
0.1, pH 8.2	51.77 ± 1.26	11.61 ± 0.25
0.2, pH 8.2	24.71 ± 0.82	10.32 ± 0.94
0.5, pH 8.2	12.68 ± 0.77	8.50 ± 0.07
1.0, pH 8.2	7.88 ± 0.10	4.86 ± 0.22
LSD[a]	1.17	0.72

[a] Difference between two means in the same column exceeding this value are significant.

of the API. However, at acid pH (pH 5.0 and 6.46) API had better foam stability than SPI. Foam stability of API at its natural pH of reconstitution (6.46) and at pH 5 was comparable to or better than the foam stability of several protein isolates including rapeseed, sunflower, and winged bean protein isolates (Lin, Humbert & Sosulski, 1974; Okezie & Bello, 1988; Sosulski et al., 1976). At pH 5 and 4.42, foam stability of SPI was minimal which was in agreement with similar observations of Deshpande, Sathe, Cornforth and Salunkhe (1982) for several dry bean proteins. The foam stability data suggest that API may be better suited for acidic foods than the SPI.

3.1.3. Oil absorption

API registered a higher oil absorption capacity when compared to that of SPI (3.555 ± 0.055 and 2.926 ± 0.098 g of oil/g protein isolate, dwb; respectively). The high oil absorption capacity of API, despite its high solubility in water, suggests the presence of an appreciable number of hydrophobic residues on protein surface. Further studies on API using hydrophobic probes will be required to verify the presence of hydrophobic residues on protein surface. Several other protein isolates such as those from sunflower, winged bean, and rapeseed have been reported to have higher oil binding capacity when compared to commercial SPIs or other soy protein products (Lin et al., 1974; Okezie & Bello, 1988; Sosulski et al., 1976).

3.1.4. Emulsion properties

Emulsifying activity index (EAI) and absorbance at 500 nm for API was significantly higher than that of the SPI (Table 1 and Fig. 3, respectively). EAI of 0.1% API at pH 5 and 8.2 were comparable to cowpea protein isolate (Aluko & Yada, 1993). EAI decreased with increased protein concentration which is consistent with similar reported observations on winged bean protein concentrate (Sathe et al., 1982) and sunflower protein isolates (Lin et al., 1974). At low protein concentrations, protein adsorption at the oil–water interface is diffusion

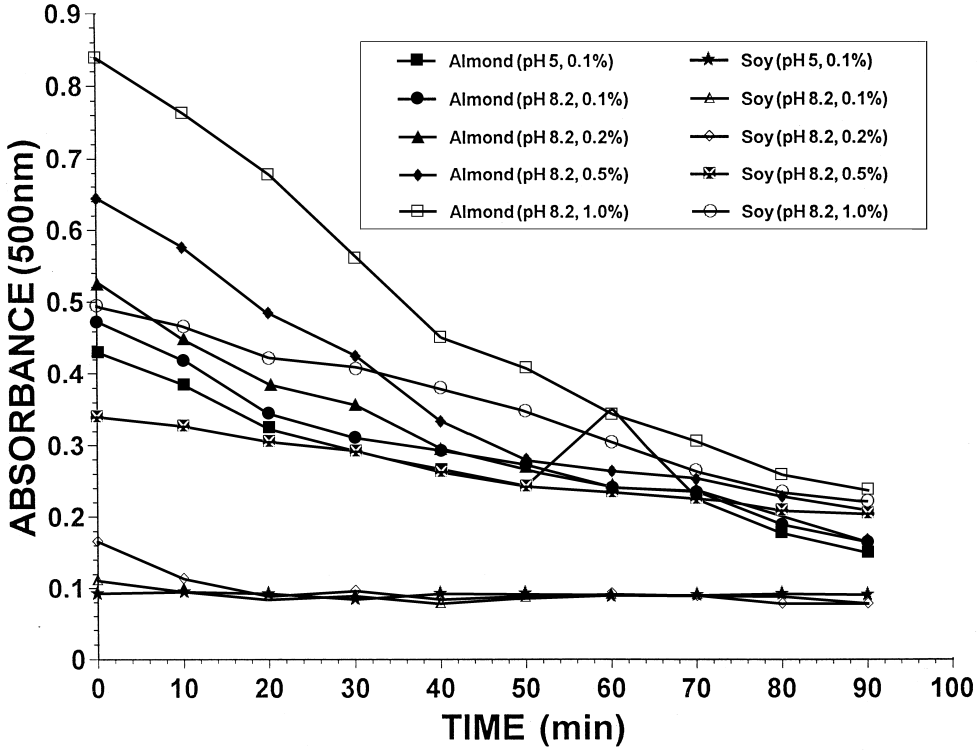

Fig. 3. Effect of pH and concentration on emulsion activity and stability of API and SPI.

controlled. At high protein concentration, activation energy barrier does not allow protein migration to take place in a diffusion dependent manner (Phillips, 1981) which may partly explain why the EAI decreases with increased protein concentration.

Both API and SPI emulsions were more stable at pH 5 than at pH 8.2 (Fig. 3). At or near isoelectric pH, protein adsorption and viscoelasticity at the oil–water interface has been reported to be maximum (Kamat, Graham & Davis, 1978; Kinsella, 1979) which may have contributed to the better emulsion stability of SPI and API at pH 5. At pH 8.2 the API and SPI proteins may have the hydrophobic amino acid residues buried in the interior of the protein molecules compared to at pH near their isoelectric pH. The higher exposure of hydrophobic amino acid residues at pH value near the isoelectric pH of protein may in turn improve emulsion properties of protein. The surface hydrophobicity measurements need to be done to verify this possibility. The high oil binding capacity and good foam and emulsion stability of API at acid pH may be a valuable trait of API that may be useful in development of food products that require acid pH as well as protein surface activity.

3.2. API in vitro digestibility

Among the proteases used in assessing almond protein in vitro digestibility, pepsin was found to be the most efficient in hydrolyzing almond proteins (Sathe, 1993). We therefore investigated API susceptibility to pepsin in vitro. API hydrolysis by pepsin remained linear for up to 5 min (Fig. 4) regardless of protein to enzyme ratio (r values were in the range 0.930–0.997) indicating a rapid hydrolysis of API. Electrophoretic

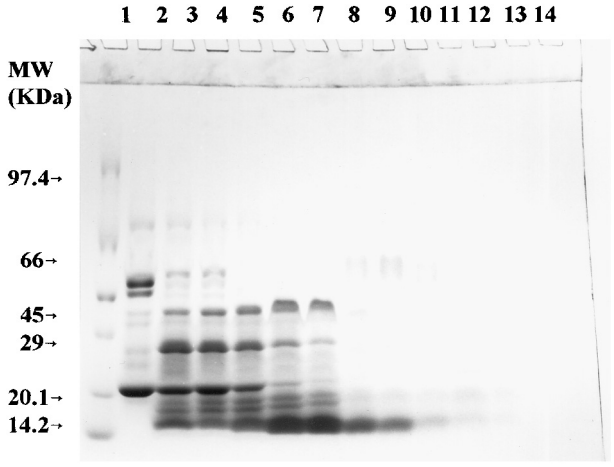

Fig. 5. SDS-PAGE, in the presence of 2% (v/v) β-ME, analysis of API pepsin hydrolysis in vitro. API:pepsin ratio was 500:1 (w/w). Lane: 1. Pharmacia low MW standards phosphorylase b (97.4 KDa), bovine serum albumin (66 KDa), ovalbumin (45 KDa), soybean Kunitz trypsin inhibitor (20.1 KDa), and α-lactoglobulin (14.2 KDa); lanes 2–13: 0, 0.5, 1, 2, 3, 5, 10, 15, 30, 45, and 60 min digestion, respectively (50 μg protein load in each lane); 14. enzyme control (0.1 μg).

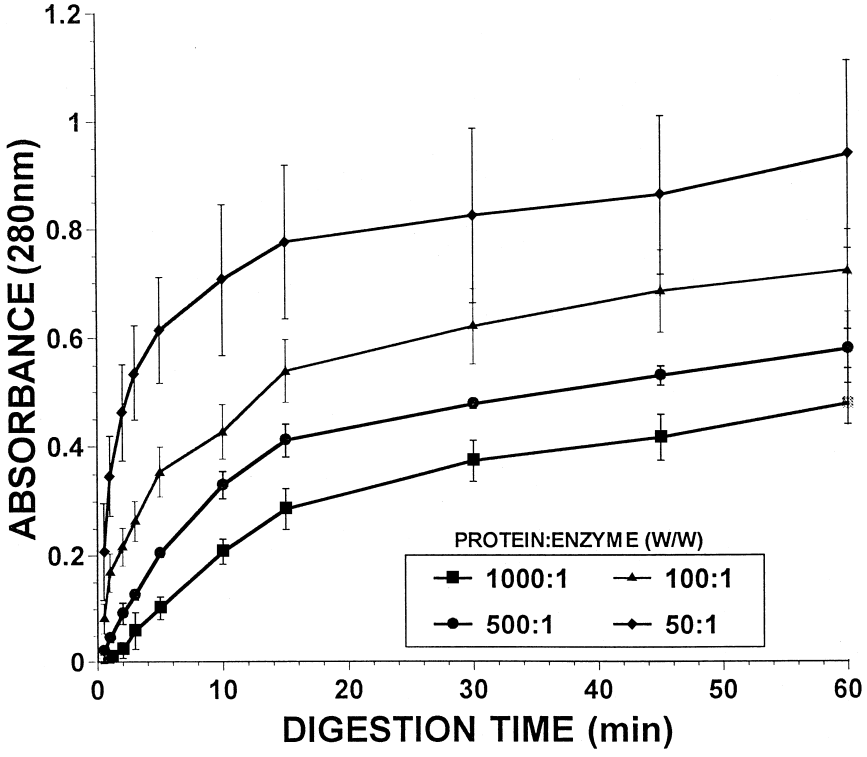

Fig. 4. Effect of digestion time and protein:enzyme (w/v) ratio on API pepsin hydrolysis.

analyses of pepsin hydrolysis of API further confirmed the high susceptibility of API to pepsin hydrolysis (Fig. 5). As can be seen from Fig. 5, major polypeptides with estimated MWs of 38–42 KDa were completely hydrolyzed within first two min of hydrolysis. The major polypeptides with estimated MWs 20–22 KDa were also efficiently hydrolyzed within the first 5 min of hydrolysis. Typically, pepsin hydrolysis initially produced polypeptides in the MW 15–36 KDa (first 5 min) followed by several small MW polypeptides ($\subseteq$15–20 KDa) and some in MW range 20–40 KDa. The 20–40 KDa polypeptides are generated due to hydrolysis of high MW polypeptides (MW >60 KDa) that are present in the API (these high MW polypeptides are visible only at high protein loads on gels, see Sathe, 1993). API may therefore be useful in production of food protein hydrolyzates.

Acknowledgements

The authors are grateful to Dr. S. Cunningham of Blue Diamond Growers (Sacramento, CA) for providing almonds and W.W. Wolf of USDA (Peoria, IL) for supplying soybeans used in these studies.

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