Production of Soluble Enzymatic Protein Hydrolysate from Industrially Defatted Nondehulled Sunflower Meal

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Industrially defatted nondehulled sunflower meal (SFM) with a protein content of 27.7% and polyphenol and soluble carbohydrate contents of 6.1% and 4.0%, respectively, can be separated by a flotation/sedimentation process into three fractions: proteinaceous (PF), soluble (SF), and lignocellulosic (LCF). The PF is washed at pH 4.5 with water in a three-step extraction process. This enhances production of a protein concentrate (PC) containing 58.3% protein and significantly reduced polyphenol and soluble carbohydrate contents of 1.5% and 0.6%, respectively. Hydrolysis of this concentrate by the neutral protease Kerase to 18.8% degree of hydrolysis transforms the concentrate into a protein hydrolysate containing 77.6% protein and low polyphenol and soluble carbohydrate contents 1.1% and 2.2%, respectively. Due to its composition and solubility at practically all pH values, this product has high potential (1) for dietetic purposes as a food ingredient or whipping agent in the food industry and (2) as a nitrogen source for media formulation in the fermentation industry.

INTRODUCTION

Sunflower is cultivated in Spain for industrial processing of seeds into edible oils (M.A., 1988). During processing, defatted nondehulled sunflower meal (SFM) containing 27.7% protein is generated, with relatively high ratios of polyphenols/protein (0.22) and soluble carbohydrates/protein (0.14). SFM presently is used on animal feed supplement (Kercher et al., 1974; Lusas, 1985). However, protein of this byproduct may be used in additional applications if appropriate processes are developed.

SFM is high in insoluble protein content, originated during extraction process with organic solvents at high temperature (Cheftel, 1985), which greatly limits its applications. Its relatively high content of soluble carbohydrates (4.0%) and polyphenols (6.1%) could cause oxidative problems during processing and storage which limit its use in the food industry (Lusas, 1985); the polyphenols also inhibit proteases (Sastry, 1984) so they must be removed from sunflower meal before their use as substrate for production of enzymatic protein hydrolysates. In addition, the high polyphenol content might inhibit growth of some microorganisms (Parrado et al., 1990a,b), including genetically engineered microorganisms, which limits its use as a nitrogen source for formulation of fermentation media. These problems could be overcome by different pretreatments.

While soluble carbohydrate and polyphenol contents can be reduced with physicochemical treatments, the insoluble proteins require chemical or enzymatic methods for their improvement. Enzymatic hydrolysis of proteins generally results in profound changes in functional properties of the proteins treated. Protein hydrolysates of protein concentrates low in soluble carbohydrates and polyphenols may be expected to fulfil certain of the food industry's demands for proteins with particular well-defined functional properties (Kinsella, 1976).

In the present paper we describe a process for elaboration of sunflower soluble protein hydrolysates low in polyphenols and soluble carbohydrates, using Kerase as a protease and a protein concentrate (PC) obtained in situ directly from SFM as a protein source.

MATERIALS AND METHODS

Enzyme. Kerase (CEPA, S.A., Aranjuez, Madrid) is a microbial protease (food grade preparation) of *Streptomyces fradiae* supplied as powder. Biochemical and electrophoretic analyses show the presence of various endo- and exopeptidases. It is nontoxic and free of antibiotics and bacterial contamination (Posada et al., 1979). Enzyme with an activity of 5000 AU/mg was resuspended (1:9 w/v) in water to facilitate addition to the reactor.

Choice of Raw Material. SFM, industrially produced by Ardelsa (Sevilla, Spain) and obtained by the prepress solvent extraction method, was used as the protein source. The SFM was fractionated by a flotation/sedimentation process, indicated schematically in Figure 1, according to procedures described previously (Bautista et al., 1990). PC was obtained by repeated in situ wash as described under Results and Discussion.

Hydrolysis. The PC was hydrolyzed batchwise by treatment with Kerase (CEPA) in a pH stat until a predetermined degree of hydrolysis was achieved. The production of hydrolysate was conducted according to Figure 2. The hydrolysis curve was obtained by the pH-stat technique using the following hydrolysis parameters: substrate concentration (S) = 10%, enzyme–substrate ratio (E/S) = 2%, pH 7.5, temperature (T) = 55 °C, with 0.15% $CaCl_2$ used as stabilizer for the enzyme. The hydrolysis was conducted in a 1000-mL reaction vessel, equipped with a stirrer, thermometer, pH electrode, a tube for admission of 1.0 N NaOH, and an on/off regulated pump for pH regulation. Hydrolysates were centrifuged at 8000g for 30 min. To increase the protein yield of the process, the sludge was resuspended in water and recentrifuged. The supernatants were combined and dryed in a spray dryer.

The degree of hydrolysis was calculated from the consumption of base according to the method of Adler-Nissen (1977).

Analysis of Amino Acids. Peptides were hydrolyzed under vacuum at 110 °C in 6 M HCl containing 0.05% phenol for 24 h. Hydrolysates were derivatized with PITC, and the phenyl isothiocyanate (PTC) amino acids were analyzed with a Waters HPLC system and a C_{18} reverse-phase column (Waters Millipore Corp.) as described by Bidlingmeyer et al. (1984).

Soluble Carbohydrates. Soluble carbohydrates were determined by a modification of the method proposed by the *Journal Officiel des Communantes Européennes* (JOCE, 1980). Briefly, carbohydrates were extracted by a mixture of ethanol/water (2:3) and inverted with 4 M HCl for 30 min, and the sugars produced were determined according to the method of Miller (1959).

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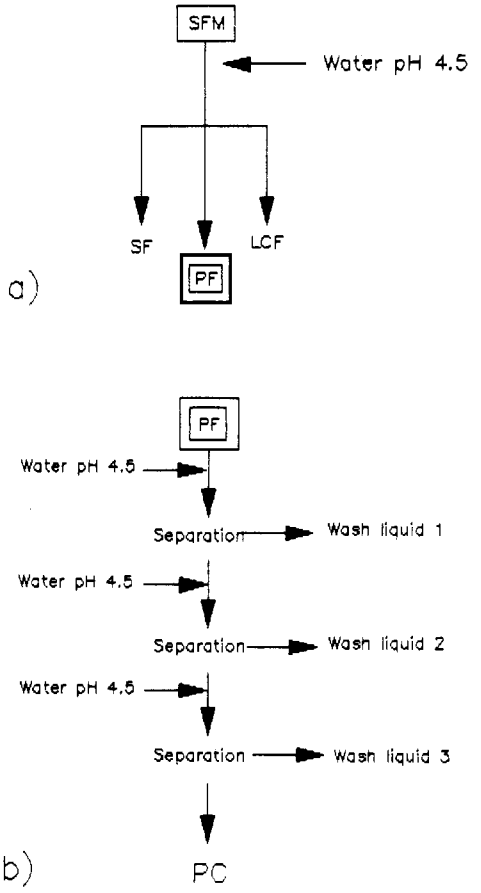

Figure 1. Production of sunflower PC from sunflower meal. (a) Sunflower meal fractionation by flotation/sedimentation. (b) Sunflower PC production by stepwise wash.

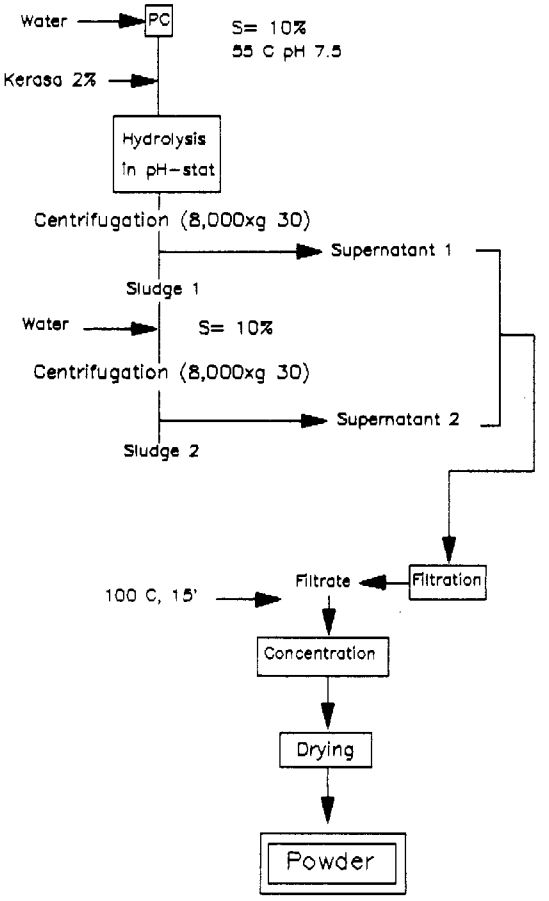

Figure 2. Flow sheet: production of soluble sunflower protein hydrolysate.

Total Carbohydrates. Total carbohydrates were determined according to the method of Lane-Eynon as recommended by the AOAC (1980).

Total Polyphenols. Total polyphenols were estimated by the Folin–Ciocalteu method (Singleton and Rossi, 1965) after proteins were eliminated with trichloroacetic acid.

Proteins. Total and mineral nitrogen contents were determined by the Kjeldahl procedure. The crude protein content value was calculated by subtracting mineral nitrogen from total nitrogen and multiplying the results by 5.5 (Gassmann, 1983; Bautista et al., 1990). The protein content in soluble samples also was calculated by the Biuret method (Gornall et al., 1949).

Trichloroacetic Acid Solubility Index. Trichloroacetic acid (TCA) solubility index was determined by the method described by Adler-Nissen (1986). Briefly, a 5-mL portion of 2.4 M TCA was added to 10 mL of hydrolysate, the precipitate removed by centrifugation (8000*g*, 10 min), and the nitrogen concentration (grams per liter) of the supernatant determined. The TCA index was calculated as

$$\text{TCA index} = (15 \times \%\ \text{N (g/L) in supernatant}) / \%\ \text{total N before TCA}$$

Chemicals. All chemicals used were of analytical grade.

Protein, soluble carbohydrate, and polyphenol contents are expressed as percent on a dry matter basis.

RESULTS AND DISCUSSION

SFM was fractionated by a sedimentation/flotation process at several pH values to produce proteinaceous, soluble, and lignocellulosic fractions (PF, SF, and LCF, respectively). The PF settled as sediment to the bottom of the tank, while the LCF floated to the top of the tank. After the LCF was mechanically removed, the SF and PF were separated by vacuum filtration. The results obtained are shown in Figure 3. No differences were observed in

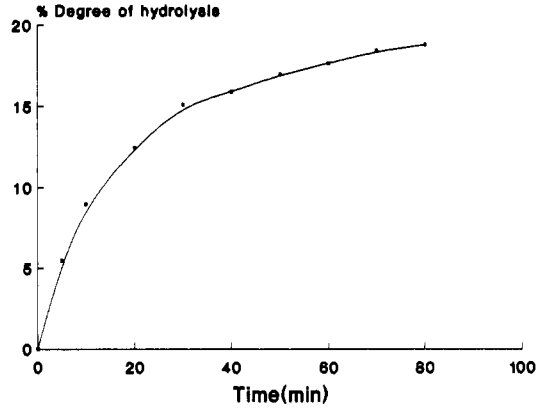

Figure 3. Yield of sunflower meal fractionation at several pH values (PF + SF + LCF = 100%). Results are expressed as percentage on solid matter basis.

protein recovery between the fractionation at acid, neutral, or isoelectric pH. This might be due to loss of solubility of these proteins acquired during the extraction process with organic solvents (Cheftel, 1985). We selected pH 4.5 for further study, due to the fact that at low acidic pH (approximating the isoelectric point) oxidation processes of polyphenols to *o*-quinones (which then quickly convert into brown polymerics) are minimized (Pierpoint, 1966, 1969; Sosulski et al., 1972).

The direct use of SFM or PF as raw material for production of soluble protein hydrolysates presents problems, resulting from relatively high ratios of soluble carbohydrates/protein (0.14 and 0.017) and polyphenols/protein (0.22 and 0.086) (see Table I and Figure 4). This leads to browning problems of proteins and peptides (Chef-

Table I. Chemical Composition of Sunflower Meal (SFM), Protein Fraction (PF), Protein Concentrate (PC), and Hydrolysate[a]

	protein, %	carbohydrates, % soluble	carbohydrates, % total	polyphenols, %	ratio SC[b]/Pr[c]	ratio Pph[d]/Pr
SFM	27.7 ± 2.9	4.00 ± 0.2	19.70 ± 1.8	6.07 ± 0.6	0.140	0.220
PF	45.1 ± 5.2	0.76 ± 0.1	16.00 ± 1.7	3.90 ± 0.2	0.017	0.086
PC	58.3 ± 4.1	0.65 ± 0.1	9.45 ± 1.4	1.60 ± 0.3	0.011	0.027
hydrol	77.7 ± 5.5	2.20 ± 0.2	2.20 ± 0.2	1.10 ± 0.1	0.028	0.014

[a] All results are expressed on dry matter, and each value represents the mean ± SD of three experiments. [b] SC are the soluble carbohydrates. [c] Pr is the total protein. [d] Pph are the total polyphenols.

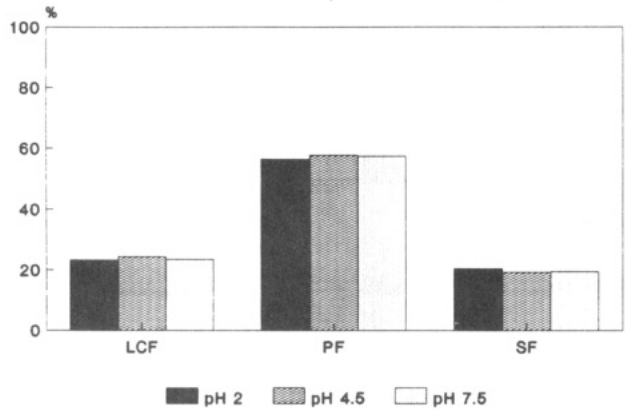

Figure 4. Reduction of soluble sugar and polyphenol content in sunflower meal (SFM), protein fraction (PF), and protein concentrate (PC) during PC production.

tel, 1985), due to both the Maillard reaction (Dworschak, 1980); Erickson, 1981) and the oxidation of polyphenols (Pierpoint, 1966, 1969) during drying and storage of the hydrolysates. This process (known as "aging of protein") results in generation of products with low or nonnutritive values with limited applications in the food industry. Also, a high content of polyphenols limits its application as nitrogen source for formulation of fermentation media, because polyphenols at concentrations higher than 2 mM inhibit the growth of most microorganisms (Parrado et al., 1990a,b).

Reduction of soluble carbohydrates and polyphenols is essential for production of commercially viable sunflower protein hydrolysates (Lusas, 1985). The separation of the PF by vacuum filtration reduces the content of soluble carbohydates by 80% (from 4.0% to 0.8%), while concentration of polyphenols remains high (see Table I and Figure 4), reaching only a reduction of 36.1% (from 6.1% to 3.9%) of its content. To further reduce contents of sugars and polyphenolic substances, we have applied an in situ stepwise aqueous/ethanol washing procedure, similar to that described by Olsen and Adler-Nissen (1979) for preparation of soy protein concentrates. This process yields a PC with protein, soluble carbohydrate, and polyphenol concentrations of 58.3%, 0.65%, and 1.6%, respectively, with low ratios of soluble carbohydrates/protein and polyphenols/protein (0.011 and 0.027, respectively).

This PC is the preferred raw material for the production of protein hydrolysates, instead of SFM, PF, or other commercially available sunflower protein concentrates. The in situ production of PC has the further advantage of reducing down costs because a drying step is bypassed. The procedure for this stepwise washing process is shown in Figure 2, where the liquid and the solid phases are separated by centrifugation.

Kerase, a microbial protease preparation obtained from

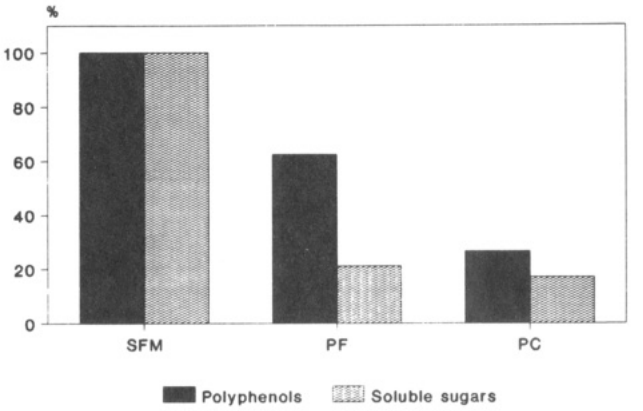

Figure 5. Hydrolysis of sunflower PC with Kerase by the pH-stat method.

Table II. Protein Recovery in Sunflower PC Hydrolysate[a]

PC	supernatant 1	supernatant 2	supernatant 3	sludge
100%	62.16 ± 1.45%	19.7 ± 0.76%	2.3 ± 0.13%	15.9 ± 2.34%

[a] Each value represents the mean ± SD of three experiments.

S. fradiae produced by CEPA S. A. (Aranjuez, Madrid), was used for hydrolysis of sunflower proteins. As shown in Figure 5 it has proven to be efficient in solubilizing sunflower proteins, reaching a 18.8% degree of hydrolysis in 80 min. The hydrolysates were centrifuged at 8000*g* for 30 min, and supernatant 1 was separated. To increase the protein yield of the process, the sludge was resuspended in water (1:10 v/v) and recentrifuged. The combined supernatants contained about 92% of the hydrolyzed protein and were filtered and heated at 90 °C for 15 min to inactivation of the protease. The hydrolysate may be concentrated either by evaporation (failing film) to a dry matter content of 40% or by reverse osmosis to 20% (Olsen and Adler-Nisse, 1981; Parrado et al., 1990a), followed by spray dryer. However, direct application of spray-drying after protease inactivation has proven sufficient for our purposes and has resulted in a reduction in processing energy.

With the described process, at a degree of hydrolysis of 18.8% we recovered 81.9% of the protein present in the PC (Table II). Also, 62.2% was recovered in supernatant 1 and 19.7% in supernatant 2; these results show that further washings are not economically advantageous.

The hydrolysate has a protein concentration of 77.7% and soluble carbohydrate and polyphenol concentrations of 2.2% and 1.1%, respectively. The soluble carbohydrates/protein and polyphenols/protein ratios are 0.028 and 0.014, respectively, indicating significant reductions in content and polyphenols/protein ratio. The soluble carbohydrate content and soluble carbohydrates/protein ratio are greater than in PC and PF but lower than in SFM. The increase in soluble carbohydrates may be explained by the presence of carbohydratases in the protease preparation (Parrado et al., 1990a,b).

The obtained hydrolysate is a slightly bitter, yellow-white powder that is highly soluble at acidic pH as shown by its high TCA index (0.85). This result indicates that the hydrolysates are primarily peptides of low molecular weight in the range 300 and 1500 and has been confirmed by electrophoretic and chromatographic results not shown here. The overall amino acid composition of the hydrolysate is similar to that of the raw material (Parrado et al., 1990b).

Another important functional property of our sunflower

protein hydrolysate is its complete solubility at the isoelectric pH of the protein in concentrations of 0–40 g/L (Parrado et al., 1990b). Complete solubility is maintained at pH values as low as 2.0, which includes the pH range of virtually all beverages. At higher pH, until above pH 10, the solubility is also 100% (Parrado et al., 1990b). This and other features suggest important applications for the product in the food industry and as a nitrogen source for formulation of fermentation media (Parrado et al., 1990a).

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