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Nutritive Value and Functional Properties of Protein Concentrate Fractionated from Chrysanthemum Flowers

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Abstract

Some functional properties and nutritive value were determined for the protein concentrate fractionated from chrysanthemum flower in order to renew interest in the flowers as food. Proximate components of chrysanthemum flower protein concentrate (FPC) showed 61.2% protein, 2.0% fat and 35.2% carbohydrate on a dry basis. In amino acid composition of FPC, glutamic acid was the highest in the content, followed by aspartic acid, leucine and lysine. The ratio of essential/ total amino acids (E/T) was 0.42, showing a higher level of essential amino acids compared to the FAO reference protein. Digestibility of chrysanthemum FPC by pepsin and trypsin was lower than that of casein with minimum solubility at around pH 4. Bulk density of FPC was higher than that of milk casein and was negatively correlative to both water and fat absorptions. Similar characteristics were determined between chrysanthemum FPC and milk casein in their emulsifying activity and emulsion stability. This results indicate that flowers or petals of chrysanthemum might be developed as a good source of protein.

Key words : chrysanthemum flower protein concentrate (FPC), amino acids, digestibility, functional properties

INTRODUCTION

Proteins are essential components of the diet needed for survival of animals and humans. The basic function of proteins in nutrition is to supply adequate amounts of required amino acids. The protein quality, also known as the nutritional or nutritive value of a food, depends on its amno acid content and on the physiological utilization of specific amino acids after digestion, absorption, and minimal obligatory rates of oxidation. As population growth continues to increase, and as the main sources of food may be approaching maximum per capita output, the demand seems likely to outpace food production (1).

Chrysanthemum was native to China but now is grown over the world. Chrysanthemum has been used for a natural medicine or food as well as a gardening flower from the ancient times. Chrysanthemums are divided into two groups, wild mums (*Chrysanthemum boreale*) and horticultured mums (*Chrysanthemum morifolium*) (2). *Chrysanthemum* spp. has yellow petals in flowering season and the harvested petals have several uses as a flavoring material for traditional foods as well as a natural medicine. A lot of studies were made on petals of *Chrysanthemum* spp. in terms of pharmacological aspects.

On the other hand, associated with the flower-eating culture in the oriental world, Konta (3) reviewed flowers as a food and their chemical composition, nutritive value, edibility and utilization. Kwon and Yoon (4) investigated into the functional properties of acacia flower protein concentrate (AFPC) and its nutritive value by comparing with other plant protein concentrates. This work was intended to determine the nutritive value and some functional properties of the protein concentrates from chrysanthemum flowers with a view to renewing interest in the flowers as a food.

MATERIALS AND METHODS

Material

Flowers of chrysanthemum (*Chrysanthemum molifolium* R.) were collected in the suburbs of Youngcheon in October, 1996. Fresh flowers picked immediately before the fullbloom stage, including petal and calyx, were frozen until use.

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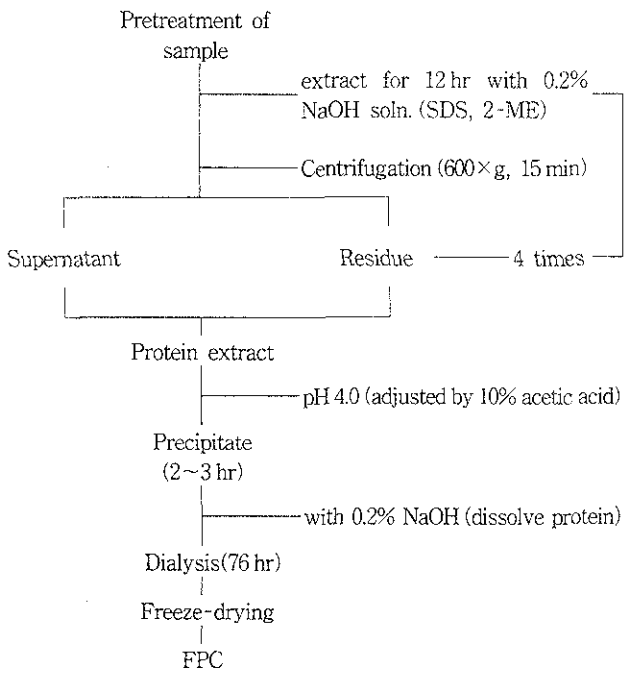
Preparation of flower protein concentrate

Flower protein concentrate (FPC) of chrysanthemum was prepared using the pretreated petals according to the method of Yasui and Yoshizawa (5) with some modifications (Fig. 1). Frozen petals were macerated several times with cold acetone (-20°C) using a Waring blender to remove pigments. The filtrated residue was air-dried at below 50°C and then ground to be used for the protein extraction. Five hundred grams of the pretreated sample were extracted for 12 hrs with 0.2% sodium hydroxide solution, containing 0.5% sodium dodecyl sulfate (SDS) and 0.5% 2-mercaptoethanol (2-Me). The protein extract was obtained by centrifugation of the suspension at 3,000 rpm for 15 min. The same procedures were repeated four times for the residue. The protein was precipitated from the combined supernatant at around pH 4.0 with 10% acetic acid. The precipitated protein was dissolved in a 0.2% sodium hydroxide solution and dialyzed against water for 76 hr. The remaining protein fractions were directly freeze-dried following washing with diethyl ether.

Proximate composition and amino acid analysis

Moisture, crude protein, crude fat and crude ash were determined by the standard AOAC methods (6). Analysis of the total amino acids was carried out after HCl hydrolysis. Two-ml of 6N HCl was added to 2 mg of sample protein in a pyrex tube that was then sealed *in vacuo* and was heated at 110°C for 24 hr to allow for a complete hydrolysis. After cooling, the solution was filtered and evaporated to dryness under reduced pressure. The volume was adjusted to 4 ml by the addition of 0.1 M citrate buffer solution (pH 2.2). The final solution was then injected into an amino acid analyzer (Biochrom 20, Parmacia) (7).



Solubility of chrysanthemum flower protein concentrate

Solubility of protein concentrate was determined according to the modified method of Wang and Kinsella (8). Protein samples (50 mg) were suspended in 5 ml water. The solution was shaken on a incubation shaker at room temperature for 30 min, and then centrifugated at 10,000 rpm for 10 min. The protein concentrations of clear supernatants were determined by Kjeldahl method (6). The solubility was calculated as the percent of proteins present in the supernatant per whole solution. Effects of pH on solubility of FPC were determined by replacing water with appropriate amounts of 0.1N HCl and 0.1N NaOH solution in order to obtain specific pH values over a range of pH 3~12.

In vitro digestibility

Digestion of chrysanthemum FPC by pepsin (1:10,000, w/v, Junsei Co.) and trypsin (2,000 E/G, Junsei Co.) was performed with the modified method of Tashiro and Maki (9). The protein samples were dissolved in 0.12 M NaCl-HCl solution (pH 2.0) and 0.12 M phosphate buffer (pH 8.0), respectively, before enzymatic reactions performed by pepsin and trypsin. Trichloroacetic acid (10%) was added to stop the reaction and milk casein (Junsei Co.) was used as the reference protein. The digestibility of FPC was measured by the following equation using pre-and post-digestion nitrogen contents in the reactants, that were determined by Kjeldahl method (6).

$$\text{Digestibility} = \frac{P-(A+B)}{N-B} \times 100$$

N : total nitrogen of the sample
P : total nitrogen of the reactant
A : blank of the enzyme
B : blank of the sample

Functional properties

Bulk density of FPC was determined as reported by Rahma and Narasinga Rao (10). Protein sample was placed in a 10 ml-graduated cylinder, packed by gently tapping the cylinder on the bench top 10 times from a height of 5 cm, and its volume was measured. Bulk density was calculated as g/ml of the sample. Water and fat absorptions were determined by the method described by Sathe and Salunkhe (11). Emulsifying activity and emulsion stability were estimated according to the method of Wang and Kinsella (8). All procedure was repeated three times at least and each value was represented as the mean.

RESULTS AND DISCUSSION

Proximate composition

Proximate composition of chrysanthemum petals and its protein concentrate is shown in Table 1. Chrysanthemum petals macerated several times with cold acetone (-20°C), contained 26.9% of crude protein and 59.5% of carbohydrate. From 4 kg of fresh petals, the process yields 500 g of pretreated sample, accounting for approximately 25 g of dry FPC. Chrysanthemum FPC showed a relatively high content of crude protein (61.2%), even though that is lower than that of radish LPC (Leaf Protein Concentrate, 87.2%) (5) and acacia FPC (77.3%). But it was shown similar to that of most other LPC's, such from pumpkin (62.0%), arrowroot (59~67%) and sunflower (57.4%) (4,12).

Compared to chrysanthemum petals, its FPC showed lower contents of other components except for crude protein, as was explained during the protein extraction, insoluble components and soluble components, such as fiber, ash and carbohydrates which were eliminated by acid precipitation, thereby resulting in the reduction of its nutritive value (13).

Table 1. Proximate composition (%) of chrysanthemum flower and its protein concentrate (FPC)

Composition (%)	*C. morifolium*	Pretreatment *C. morifolium*	Chrysanthemum FPC[1)]
Moisture	11.0	5.8	0
Crude protein	11.7	26.9	61.2
Crude fat	2.6	2.0	2.0
Ash	5.2	5.8	1.6
Carbohydrate	69.5	59.5	35.2

[1)]FPC: flower protein concentrate as dry basis

Table 2. Total amino acid composition of chrysanthemum flower and its protein concentrate[1)] (FPC) (%, dry basis)

Amino acid	*C. morifolium*	Chrysanthemum FPC	FAO Ref.[14)]
Isoleucine	2.5	6.3	4.2
Leucine	6.9	11.3	4.8
Lysine	2.5	10.7	4.2
Methionine	0.9	2.7	2.2
Phenylalanine	2.0	6.6	2.8
Threonine	1.8	7.5	2.8
Valine	6.0	7.1	4.2
Aspartic acid	7.4	14.3	
Serine	1.5	8.5	
Glutamic acid	5.1	14.8	
Proline	38.8	1.9	
Glycine	1.1	7.7	
Alanine	3.9	7.6	
Cystine	2.3	2.0	
Tyrosine	1.8	6.0	
Histidine	0.9	3.2	
Arginine	0.3	7.1	
TEAA[2)]	22.6	52.2	
NEAA[3)]	63.1	73.1	
TEAA/ NEAA	0.36	0.71	

[1)]Values are g of amino acid per 16 g of nitrogen
[2)]Total essential amino acid
[3)]Total nonessential amino acid

Amino acid composition

Amino acid composition of chrysanthemum petals and its protein concentrate were given in Table 2 over comparison with the amino acid of reference proteins. Proline was the highest in the petals, but glutamic acid was the highest, followed by aspartic acid, leucine and lysine in FPC. In contrast to other leaf protein concentrates which are usually low in methionine contents, the FPC samples showed an apparently higher contents of methionine. In general, amino acid patterns of chrysanthemum FPC were comparable to other results on plant protein concentrates (8-12). Moreover, the higher levels of essential amino acids were observed in chrysanthemum FPC than in the FAO reference (14). Ratio of essential to nonessential amino acids in chrysanthemum FPC was 0.71, which was similar to that of alfalfa LPC (12). Kohler and Palter (15) indicated that several factors can be involved in the analysis of amino acids depending on the samples. Choi et al. (16) also noted that the greater the protein content of plant sources, the higher

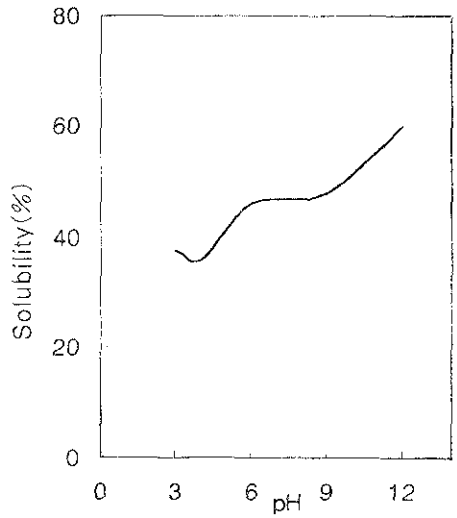


Fig. 2. Effect of pH on the solubility of chrysanthemum flower protein concentrate.

is the yield of LPC, and their average protein contents in LPC were about 58%, that were mainly composed of glutamic acid and leucine. The amino acid patterns of chrysanthemum FPC were found similar to those of acacia FPC as reported by Kwon and Yoon (4). The results suggested that chrysanthemum flower (petals) could be a good resource for the production of plant protein concentrates.

Effect of pH on the solubility

Effect of pH on the solubility of chrysanthemum FPC is illustrated in Fig. 2. The protein concentrate showed a minimum solubility at around pH 4, whereas the solubility increased as the pH changed to the alkaline range. Kim et al. (17) reported that positive correlation between solubility and fat absorption of acacia LPC was found, showing the difference in their levels between chloroplastic and cytoplasmic proteins. The low solubility of chrysanthemum FPC at around pH 4 was similar to that of acacia FPC and to LPC of other plant sources (4,18).

Digestibility and functional properties

The *in vitro* digestion test showed that chrysanthemum FPC was easily digested by pepsin and trypsin (Table 3). The digestablity was lower than that of milk casein, which was lower than that of acacia by pepsin (82.2%) and trypsin (80.4%) and was higher than that of ginseng leaf by pepsin (32%) and trypsin (42.4%) (19). Some functional properties of chrysanthemum FPC were estimated as compared to milk casein (Table 4). The protein samples showed higher bulk density and lower water and fat absorption than the corresponding values of milk casein. The negative correlation between bulk density and absorption capacity of protein concentrates was confirmed by Wang and Kinsella (8), which was observed for chrysanthemum FPC. The various factors influencing emulsifying capacity of chrysanthemum FPC might be the addition speed of oil, temperature, pH, form of protein, solubility, concentration, salts, sugar and water content, which were discussed by Saffle (20). The emulsifying capacity and emulsion stability of chrysanthemum FPC were not greatly different from those of milk casein, and were lower than LPC of arrowroot leaf protein (1).

Table 3. Digestibility of casein and chrysanthemum flower protein concentrate (FPC) by pepsin and trypsin[1)]

Sample	Digestibility(%)	
	Pepsin	Trypsin
Casein	96.1	84.4
Chrysanthemum FPC	73.7	68.7

[1)]Reaction was made at 37°C for 24 hrs.

Table 4. Functional properties of chrysanthemum flower *protein concentrate* (FPC)

Protein	Bulk density (g/ml)	Water absorption (ml H_2O/g)	Fat absorption (ml oil/g)	Emulsifying activity(%)	Emulsion stability (%)
Casein	0.61	3.2	3.0	47.5	49.1
FPC	0.85	2.2	1.7	45.1	44.0

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