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# Development of Safflower Protein

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## ABSTRACT

Development of safflower protein and safflower protein isolate (SPI) containing as high as 95% protein (N x 5.3) is described. SPI exhibits favorable nitrogen solubility, foaming, and bread-baking properties. Composition of SPI and select functional properties may be altered by the choice of pH used to precipitate the extracted protein (5 or 6). PER of SPI (1.26) was increased to as high as 2.13 by the addition of L-lysine at levels of 0.75% of the diet. Theoretical estimates of production costs for SPI are similar to estimates for soy protein isolate. SPI has been evaluated experimentally in pastas, baked products, and beverage systems. Nutritional and functional properties indicate that SPI has promising potential as either a protein fortificant and/or a functional ingredient in various foods.

## INTRODUCTION

Safflower *(Carthamus tinctorius* L.) is one of the oldest cultivated oilseed crops. Originally grown for the dyestuff carthamin, safflower has been cultivated more recently for its polyunsaturated oil. Once the oil has been extracted, the remaining high protein meal is the raw material from which flours, protein concentrates, and isolates are derived. The potential role of safflower as a human food has been reviewed (1,2).

Production of safflower increased sharply in the 1960s, but has since stabilized. Although safflower is a relatively drought tolerant crop, yields improve with irrigation. Yields range from 250 to more than 3000 Kg/ha with an average of ca. 2000 Kg/ha (1). World production of safflower seed and resultant oil and protein indicates that more than 100,000 metric tons of protein are available from this source. Major producers include countries such as India and Mexico where indigenous protein sources represent a valuable resource both for their nutritional value and their impact upon balance of payments. If average values of 40% and 15% are used for oil and protein, respectively, Mexico had the potential to produce 120,000 and 45,000 metric tons of safflower oil and protein, respectively, in 1976-1977.

Although safflower oil is consumed by humans, the press cake or meal is commonly used as an ingredient in animal rations. In the U.S. two commercially produced meal fractions are available; a high fiber and a low fiber fraction containing 20 and 42% crude protein (N x 6.25), respectively. The seed generally consists of 50% each kernel and hull, or pericarp. Average compositional values are 40% crude fat, 15-19% crude protein and 20-25% crude fiber (1). Earlier workers have suggested various methods for developing flours and protein concentrates (3,4). Flours are bitter and extraction of both bitter and cathartic substances with 70-80% ethanol was recommended to prepare an edible concentrate (5). Both bitter flavor and cathartic activity have been associated with lignan glycosides; bitterness with 1-matairesinol-mono-β-D-glucose, and cathartic activity with 2-hydroxy-arctiin, a flavorless compound (6,7). The removal of these glycosides is imperative for the preparation of acceptable, edible protein products. The preparation of safflower protein isolates (SPI) represents one approach to this problem.

Safflower protein isolates combine the advantages of high concentrations of true protein (≥ 90%, N x 5.3), favorable functionality including solubility, foaming capacity and baking quality, and absence of all but trace quantities of lignan glycosides (1,8,9). In addition, through alteration of extraction and precipitation conditions, functionality of SPI may be partially modified (10).

## SAFFLOWER PROTEIN ISOLATES

### *Nature of the Protein*

Safflower protein, nearly 80% of which is located in the kernel, was subjected to classical fractionation on the basis of solubility (11). Major protein fractions were soluble in 1N NaCl or 0.1N NaOH, with these fractions containing 41.5 and 39.1%, respectively, of the nondialyzable nitrogen (12). Amino acid composition of the fractions varied significantly with the water soluble protein containing lysine in quantities equivalent to 84% of the FAO provisional amino acid pattern (13).

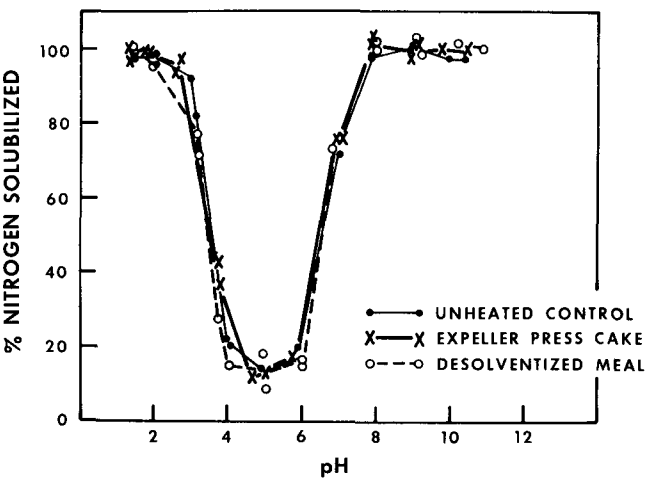


FIG. 1. Precipitation of extracted nitrogen from various safflower meals as a function of pH.

TABLE I

Composition[a] of Safflower Meal and Extracted Fractions

| | Nitrogen | Protein (N X 5.3) | Crude fat | Crude fiber | Ash |
|---|---|---|---|---|---|
| Fraction | % | % | % | % | % |
| Meal[b] | 8.88 | 47.06 | 1.74 | 9.95 | 8.82 |
| Protein Isolate[c] | 17.47 | 92.60 | 0.42 | 0.34 | 0.76 |
| Supernatant | 5.62 | 30.00 | 0.67 | 0.20 | 8.11 |
| High fiber fraction (Extracted meal) | 5.26 | 28.00 | 1.48 | 18.78 | 15.41 |

[a]Moisture-free basis.
[b]Meal prepared in the laboratory by hexane extraction (25 C) of expeller press cake (Betschart, 1975).
[c]Extraction pH 9, precipitation pH 6, neutralization to pH 7.

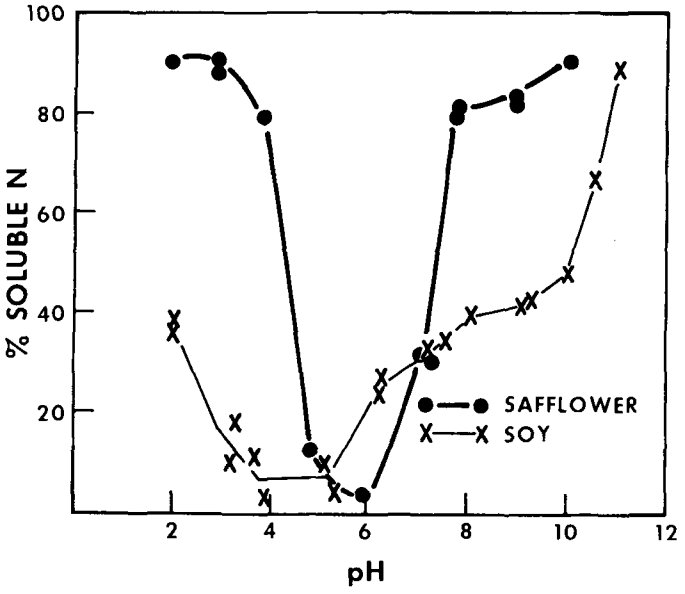


FIG. 2. Nitrogen solubility of soy protein isolate and safflower protein isolate extracted at pH 9, precipitated at pH 6, neutralized to pH 7, and freeze dried.

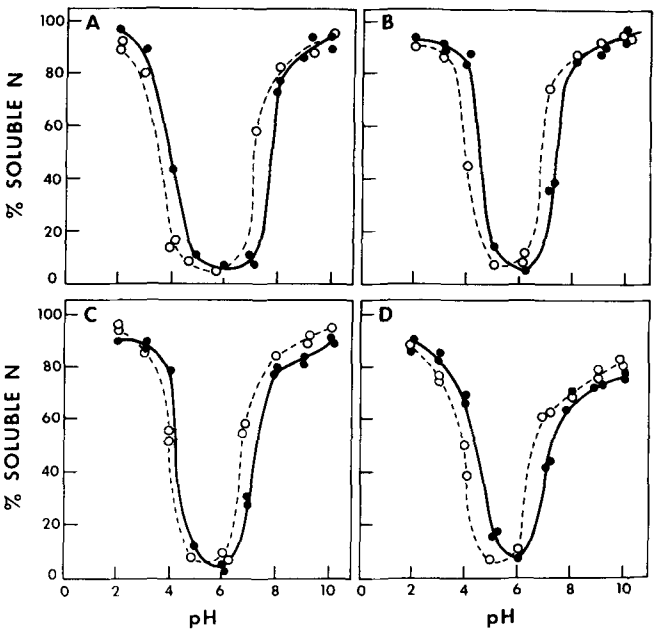


FIG. 3. Nitrogen solubility of safflower protein isolates precipitated at pH 5 (○—○) or 6 (●—●). A=extracted at pH 9, unneutralized; B, C, and D extracted at pH 8, 9, and 10, respectively, and neutralized to pH 7 prior to being freeze dried.

### Extraction and Precipitation of Protein

Earlier workers examined the extraction of nitrogen from select safflower kernel meal (14). More recently, parameters including extraction time, temperature, and concentration of safflower meal (w/w) were reported to have little influence upon protein extractability. In contrast, extractability was effected by both pH and previous high temperature treatment of safflower meal. Extraction of protein increased at alkaline pH values with 83, 80, and 68% of safflower nitrogen extracted at pH 9 from control, expeller press cake, and desolventized meals, respectively (12). Temperatures reached during oil extraction processes were ca. 25, 85-93, and 107-110 C for control, expeller, and desolventized meals, respectively. Temperatures of 107 C and above appear to impair protein extraction under mild alkaline conditions. Expeller press cake, from which residual oil was extracted at 25 C, was subsequently used since protein extraction was similar to the unheated control.

Recovery of protein from aqueous extracts by acid precipitation (HCl) was similar for the three types of meal (Figure 1). At pH $\leq$ 2 and $\geq$ 8, 95-100% of the extracted nitrogen was soluble. Minimum solubility, or maximum protein precipitation, occurred at pH 5 to 6 (12). At pH values of minimum solubility, 10-15% of the nitrogen was not recovered. Data suggest that either pH 5 or 6 would be suitable for protein recovery.

Extraction of safflower protein at pH values of 8 to 10 and precipitation at pH 5 or 6 seemed to be the appropriate conditions to investigate. Thus, laboratory and pilot plant experiments were conducted using these variables as well as evaluating the effect of neutralizing SPI to pH 7 prior to drying (8). Resultant SPI were subsequently evaluated to determine nutritional and functional properties. During extraction and precipitation, analysis of variance indicated that yields of SPI, expressed as either weight or nitrogen recovered, increased from pH 8 through 10 (8). Precipitation pH of 5 vs. 6, however, only caused significant increases in weight yields with nitrogen recoveries being not significantly different.

Proximate analyses (15) indicate that composition of SPI was influenced by precipitation pH. SPI precipitated at pH 6 contained significantly more nitrogen than did those precipitated at pH 5 (17.6 vs. 16.7% mfb) (8). Composition of safflower meal, SPI, and other by-products illustrate the concentration of protein within SPI (Table I). Crude fiber remains with the extracted meal which is similar in composition to commercially available high fiber meal which contains 20% crude protein.

### Protein Quality

SPI was evaluated by chemical and biological methods (8). Amino acid scores of SPI, when compared with the FAO provisional amino acid pattern (13), ranged from 39 to 46. The scores were not consistently influenced by either extraction or precipitation pH. With the addition of the

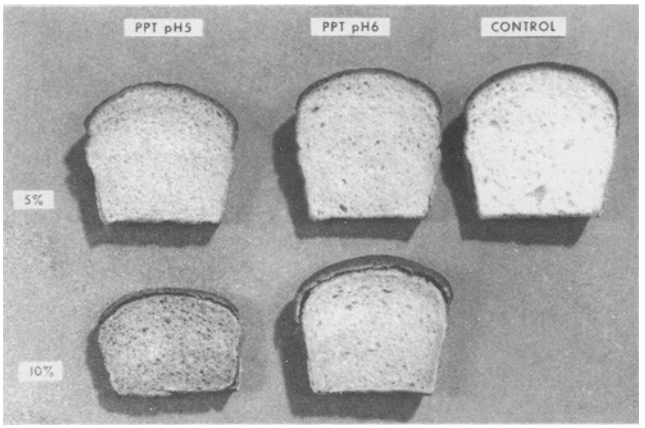


FIG. 4. Laboratory pup loaves including 5 and 10% safflower protein isolate, precipitated at pH 5 and 6, and wheat flour control.

limiting amino acid, lysine, at levels of 0.25-0.75% of the diet, the typical Protein Efficiency Ratio (PER) of SPI (1.26) increased to as high as 2.13. All PER values were corrected to 2.5 for casein, and were obtained with 28 day studies using diets containing 10% protein (8,15). True nitrogen digestibility of SPI, alone or when supplemented with L-lysine, was 95-96%. The PER of SPI in combination with other digestible proteins with complementary amino acid patterns was superior to that of SPI alone. PER of combinations of SPI/Rice Protein Concentrate in ratios of 1:3.1 (protein/protein) was 2.19, whereas that of a SPI/Baked Bean diet (2:4, protein/protein) was similar to that of SPI. The latter result was influenced by the poor digestibility and resultant PER of the baked bean sample (8).

## Functional Properties

*Model systems.* Although precipitation conditions had little influence upon protein quality of SPI, it effectively altered functional properties, especially nitrogen solubility. The major effects of precipitating SPI at pH 6 rather than 5, the point of maximum weight yield, have been summarized elsewhere (16). Functional properties of SPI, included water and fat absorption capacities, emulsification activity and stability, together with suggested mechanisms responsible for functionality (10). This discussion will be limited to nitrogen solubility, foaming capacity, and stability, and bread-baking properties.

Nitrogen solubility profiles, as a function of pH, were determined for a commercial soy protein isolate and SPI extracted at pH 9, precipitated at pH 6, neutralized to pH 7, and freeze dried (10). The nitrogen solubility of SPI was greater than that of the soy at pH 2-4 and 8-9 (Figure 2). Favorable solubility properties at pH 2-4 suggest potential solubility in acidic systems including citrus and carbonated beverages.

Extraction and precipitation pH altered solubility profiles with precipitation conditions having the major influence (10). Solubility at pH $\geq$ 8 was diminished in the SPI extracted at pH 10 (Figure 3). Solubility profiles were generally shifted one pH unit toward alkalinity when SPI was precipitated at pH 6 as opposed to 5. The pH 6 precipitates were more soluble at pH 2-4, generally $\geq$ 80%, whereas those precipitated at pH 5 exhibited greater solubility at pH 7 (60-75%). Depending upon constraints and desired properties of food products to be supplemented, nitrogen solubility properties of SPI may be partially controlled by choice of precipitation pH. This effect would be expected to have application to various plant proteins prepared by acid precipitation from aqueous systems.

Foaming capacity of SPI was greater than that of the commercial soy protein concentrate or isolate evaluated. The methods of Lawhon et al. (17) and Lin et al. (18) were used according to modifications described by Betschart et al. (10). With one exception, SPI foam volumes were more than three times the original volume of the protein/water mixture, whereas the soy protein products produced foams approximately twice that of the original volume (10). Within unneutralized samples, those precipitated at pH 6 produced larger foam volumes than did those precipitated at pH 5. The pH of the foam is apparently critical since the stability of neutralized SPI foams was equivalent or superior to soy. In contrast, the foams of unneutralized SPI collapsed shortly after they were formed.

*Baked products and beverages.* SPI was incorporated into wheat flour breads at levels of 5 and 10%, replacement of flour, according to procedures previously described (10). Formulation included 3% hydrogenated vegetable oil with no additional dough improvers. Loaf volumes of breads containing 5 and 10% SPI precipitated at pH 6 were as high as 95 and 85%, respectively, of the wheat flour control. Protein content of the breads increased to $\geq$25 and 50% with the incorporation of 5 and 10% SPI, respectively. Due to higher concentration of protein, protein isolates have a greater impact upon protein quantity in breads than do protein concentrates or flours. Grain, texture, and volume of SPI-fortified breads may be compared to the control in Figure 4. SPI compared favorably with soy protein isolate as a fortificant of wheat breads; 10% SPI and soy protein isolate resulted in loaf volumes equivalent to 86 and 72%

TABLE II

Estimated Production Costs

| Product | Costs attributed to raw materials | Estimated production costs | Total cost of product |
|---|---|---|---|
| | | Dollars per pound | |
| Soy[a] | | | |
| Textured soy protein | 0.120 | 0.011 | 0.131 |
| Soy protein concentrate (68-70% protein) | 0.175 | 0.076 | 0.251 |
| Soy protein isolate (92-93% protein) | 0.387 | 0.062 | 0.449 |
| Safflower[b] | | | |
| Safflower protein isolate (93% protein) | 0.358 | 0.062 | 0.420 |
| No credit allowed for extracted meal | | | |
| Value credited for extracted meal | 0.270 | 0.062 | 0.332 |

[a]Mustakas and Sohns (1976).
[b]See text for methods of calculation and assumptions.

of wheat flour controls, respectively (10,19). In terms of specific loaf volumes (cc/g), values of 5.4 and 4.5 were obtained when SPI and soy protein isolate were included, as compared to 6.3 for the control.

Exploratory research and development of foods incorporating SPI developed at the Western Regional Research Center has been and/or is being conducted in academic and commercial laboratories, in the U.S. and abroad. Fortification of pastas with SPI has been studied incorporating levels of 5 through 25%. Calculated protein content of pastas increased to from 16 to 27% moisture free basis. Commercial research efforts are in progress examining the functionality of SPI in various bread and beverage formulations.

*Cost estimates.* Recent economic pressures within the oilseed processing industry have prompted processors of safflower seed to critically examine the returns obtained from their by-products, including meal. As a result, the feasability of producing SPI is currently receiving attention by some within the U.S. Estimates on costs of producing soy protein isolates (20) serve as a general guide for production costs for SPL. Processes for preparating both protein isolates are sufficiently similar to assume that major production costs would also be somewhat similar. During the past two years, commercially available, 42% crude protein meal has ranged in price from $150-205/metric tons with an average of ca. $190 (21). Costs of producing SPI were calculated as the sum of production costs plus costs of raw materials, i.e., safflower meal. Costs of safflower meal, per pound of SPI, were calculated as follows:

Estimated cost of producing SPI is compared with estimated costs for various soy protein products (Table II).

$$\text{Costs of safflower meal} = \frac{\text{Cost of 2,204 lbs Meal}}{\text{lbs SPI in 2,204 lbs Meal}} = \frac{\$190.00}{\frac{\%\text{ protein in meal}}{100} \times \frac{\%\text{ protein recovered in SPI}}{100} \times 2{,}204 \text{ lbs}}$$

$$\frac{\%\text{ protein in SPI}}{100}$$

Estimated cost of producing SPI is compared with estimated costs for various soy protein products (Table II). On a relative basis, SPI costs are similar to those for soy protein isolate. The cost of SPI, assuming that extracted meal would be sold as a by-product, was calculated on the basis of a weight yield of 50% for SPI and a sale price for the by-product comparable to 20% crude protein meal, i.e., ca. $95/metric ton.

Those regions of the world in which significant quantities of safflower are produced and processed are encouraged to explore this crop as a source of edible protein. This is especially appropriate for those countries which consume diets deficient in protein and calories.

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# Development of Grapeseed Protein


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## ABSTRACT

The potential for grapeseed oil and protein in regions where grape production is significant is discussed. Extraction and concentration procedures which improve the nutritional value of grapeseed protein and problems related to protein digestibility are presented.


Grapeseeds have been explored and used as a source of oil, both experimentally and by industrial processors. Information on grapeseed protein including methods of extraction and isolation, as well as nutrition value, is limited. Grapeseeds become a part of pomace, accounting for 20-26% of this residue which results from the process of winemaking (1). In the U.S. little use is made of pomace; occasionally it has been used as a soil conditioner or source of nondigestible fiber. In Europe, however, pomace is viewed as a potentially valuable by-product. The products which may be obtained from 100 Kg of grapes are shown in Figure 1 (2,3). In addition to oil, grapeseeds represent a viable source of protein and tannins.

Grape production varies widely in various regions of the world. Production of grapes and wine by major regions with estimated production of seeds, protein, and oil are shown in Table I (4). Grapeseeds account for an average of 2.5% of the grape with values ranging from 2.2 to 6.3%. This variability is attributed to differences in variety and maturity of the grape. Europe produces nearly 60% of the world's grapes and is responsible for almost 70% of the world wine production. In addition to Europe, sizable

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