1977). This significant increase reinforces the requirements for further studies on the interaction of microwave energy and gluten and starch before definite conclusions are possible. Although the increase in firmness is significant, the magnitude of the change should not be discernible. The increased firmness increases the cooking quality of the spaghetti. As expected (Doty and Baker, 1977), spaghetti color was not reduced by 60 s of microwave conditioning.

In summary, on the basis of 200-g sample data, microwave conditioning should be confined to samples of at least 16.0% tempered moisture. At this moisture level, samples are characterized by decreased starch damage, lipoxygenase activity, and processing pressure. Semolina color and speckiness are not reduced, while spaghetti color is maximum, the higher the moisture level. Overall spaghetti cooking quality is not decreased by microwave conditioning durum wheat at 16.0% moisture. In addition, minimal energy requirements of 0.19 and 1.12 kcal increase semolina extraction 2.7 and 5.8 percentage points, respectively. Application of 8.96 kcal (60 s at 625 W) of microwave energy to 2000-g samples increases semolina extraction and spaghetti firmness. Improvement of product yield and quality justifies the minimal alteration in tempering required for optimal results. In addition, the energy expenditures are minimal. The feasibility of conditioning durum wheat with microwave energy (Watkins, 1971) is confirmed.

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Acidic Butanol Removal of Color-Forming Phenols from Sunflower Meal

Giancarlo Sodini and Marco Canella*

This paper describes the ability of acidic 1-butanol to remove color-forming phenols (chlorogenic and caffeic acid) and oligosaccharides from sunflower meal without detectable protein denaturation. Defatted sunflower meal of Ala variety was repeatedly extracted with a solution of 1-butanol and 0.005 N HCl (92:8, v/v), giving a protein concentrate (70% protein) with a low chlorogenic residual content (≤0.05%). Protein extraction of this product at pH 9.5 and subsequent precipitation at pH 5.0 yielded a colorless protein isolate (93.5% protein). The amino acid compositions of sunflower meal, concentrate, and isolate were similar. The phenol-free isolate and the untreated isolate exhibited identical minimum solubility points (pH 5.0) but the former showed a higher protein extractability above pH 7.0 than the latter. No significant differences appeared among electrophoretic patterns of albumin and globulin fractions from meal and protein concentrate.

Sunflower (*Helianthus annuus* L.) is the second largest oilseed crop as a world source of vegetable oil. Because of its high content of protein having good nutritional quality (Clandinin, 1958), sunflower meal also represents a protein source of great interest as a human food. However, the preparation of sunflower protein isolates for food products is prevented by the presence of undesirable phenolic compounds in the seed (Pomenta and Burns, 1971), such as chlorogenic and caffeic acids (Sechet-Sirat et al., 1959; Brummett and Burns, 1972). These acids bind to polar groups of the proteins at alkaline pH values usually employed for protein extraction, giving dark-green or yellow products (Cater et al., 1970), thereby strongly reducing the available lysine content (Loomis and Battaile, 1966; Feeny, 1968).

Removal of these polyphenolic substances from sunflower meal has been attempted with organic solvents either in the presence or absence of reducing agents (Smith and Johnsen, 1948; Joubert, 1955; Gheyassudin et al., 1970; Girault et al., 1970). However these methods yield colored protein isolates, as color-forming phenols are only incompletely removed and cause denaturation of the proteins.

Colorless protein isolates were obtained from sunflower meals by Sosulski et al. (1972). These authors extracted the undesirable phenols by shaking whole kernels in 0.001 N HCl at different temperatures; however, partially denatured proteins were produced by this method (Kilara

Laboratori Ricerche di Base, Snamprogetti S.p.A., Monterotondo, Rome, Italy.

Table I. Removal of Color-Forming Phenols and Oligosaccharides from Defatted Sunflower Meals of Four Italian Cultivars with Acidic 1-Butanol (Percent Moisture-Free Basis)

Description	Ala		Albinia		Amiata		Argentario	
	Meal	Concentrate	Meal	Concentrate	Meal	Concentrate	Meal	Concentrate
Protein (N × 6.25)	60.75	67.62	62.80	70.81	61.18	71.87	60.93	72.25
Chlorogenic acid	2.59	≤0.05	1.57	≤0.05	1.56	≤0.05	2.46	≤0.05
Caffeic acid	0.11	0.04	0.12	0.04	0.14	0.05	0.15	0.05
Sucrose	5.16	0.31	4.90	0.23	4.70	0.19	5.45	0.35
Raffinose	3.75	3.27	3.09	2.44	3.32	2.69	3.72	2.93
Crude fiber	4.33	4.87	3.87	4.61	4.15	4.80	4.09	4.65

et al., 1972). Recently Fan et al. (1976) compared several procedures for the extraction of chlorogenic acid and the preparation of protein concentrates from sunflower flour or seed.

In this paper the use of acidic 1-butanol for the exhaustive extraction of color-forming phenols and oligosaccharides from sunflower meal is presented as a method which does not cause detectable protein denaturation under the conditions used. The procedure yields a protein concentrate which is practically free from phenolic constituents (chlorogenic and caffeic acids).

EXPERIMENTAL SECTION

Analytical Methods. Dehulled seeds of sunflower Italian cultivars (Ala, Albinia, Amiata, and Argentario) were ground at 4 °C in a Sorvall omnimixer. Oil extraction was carried out by shaking the flour with *n*-hexane (1:3, w/v) for 16 h at 25 °C. The suspension was filtered under vacuum through Whatman No. 3 paper. The residue was dried under nitrogen, and ground to a 100 mesh size with a Bubler mill Model ML1-204.

Moisture, protein, lipid, crude fiber, and ash contents of sunflower meal and of protein concentrate and isolate were determined according to AOAC procedures (1975). Total sugars were measured following the phenol-sulfuric acid procedure of Dubois et al. (1956).

Amino acid analyses were performed according to Spackman et al. (1958), using a Beckman Model 120 C amino acid analyzer. Cystine and methionine were determined according to Moore (1963).

Anodic and cathodic disc electrophoresis on albumin fractions was carried out on polyacrilamide gels in different buffer systems by the procedure of Ornstein (1964). Globulin and albumin fractions were also submitted to gel electrophoresis in 0.016 M aluminum–lactate buffer at pH 3.1 containing 5 M urea according to Schwenke and Raab (1973).

pH–nitrogen profiles of sunflower protein isolates were obtained by the procedure of Gheyassudin et al. (1970).

Acidic 1-Butanol Extraction. For the preparation of protein concentrates, samples (20 g) of defatted meal were suspended in 400 mL of 1-butanol–0.005 N HCl (92:8, v/v) and the pH was adjusted to 5.0 with 0.5 N HCl. The extraction was carried out for 15 min at room temperature under magnetic stirring; the pH of the slurry was kept constant by the addition of 0.5 N HCl. At the end of extraction, the suspension was filtered through Whatman No. 3 paper under suction and the residue was extracted seven times under identical experimental conditions. The absorbance at 328 nm of the extracts obtained by this procedure was determined with a Beckman 25 spectrophotometer and the final extraction residue (to which we will refer as protein concentrate) was used for the chemical analyses.

GLC Technique. In order to evaluate the effectiveness of the acidic 1-butanol extraction of phenols and oligosaccharides, the residue from the acidic butanol extraction was extracted with methanol–water (80:20, v/v) (with sunflower meal as control) according to the method by Mikolajczak et al. (1970). After refluxing for 5 h, the extract was dissolved in HCl at pH 2.0 and then the pH was adjusted to 6.0 with diluted NaOH. Phenols and oligosaccharides were submitted to gas chromatography using an HP Model 7620 A gas chromatograph equipped with a hydrogen flame detector and a 3380 A integrator, after drying at 40 °C and silylation according to Sabir et al. (1974).

Extraction of Protein for Electrophoretic Analyses. Defatted sunflower meal or protein concentrate (10 g) was ground in a Buhler mill and suspended in 0.2 M phosphate buffer (pH 7.0) containing 0.5 M NaCl and 0.02% NaN_3. The mixture was stirred for 45 min at room temperature and centrifuged at 27 000*g* for 30 min at 4 °C. The extraction was repeated twice. The two extracts were pooled and dialyzed against distilled water for 72 h at 4 °C. The precipitate formed during dialysis was removed from the supernatant by centrifugation (27 000*g*, 30 min, 4 °C). The supernatant was freeze-dried without further treatment (albumin fraction), whereas the precipitate was twice washed with distilled water and freeze-dried (globulin fraction).

Preparation of Protein Isolate. Samples (10 g) were extracted twice with 150 mL of 0.015 N NaOH for 15 min; the pH was kept constant at 9.5 by adding 0.02 N NaOH. The extracts were pooled, centrifuged at 27 000*g* for 20 min, and filtered through Whatman No. 3 paper. Proteins were precipitated from clear extracts with 0.5 N HCl at pH 5.0. The precipitate was collected by centrifugation, washed twice with diluted HCl (pH 5.0), suspended in water, and freeze-dried. The protein fraction obtained was coded protein isolate.

RESULTS

Table I shows chemical data of sunflower meals from four different cultivars and the corresponding protein concentrates obtained after acidic 1-butanol treatment. The contents of phenolic constituents reported in Table I are quite typical for the Italian sunflower varieties (Canella et al., 1976), although differences could be demonstrated among some cultivars. Only minor differences were evident in the protein and oligosaccharides contents, whereas the level of color-forming phenols significantly varied with the type tested. Chlorogenic acid was the major phenolic compound while caffeic acid was present in a much lower amount. The most abundant disaccharide was sucrose; the trisaccharide present was identified as raffinose.

The preparation of the protein concentrate (70% protein) from defatted sunflower meals of Table I was carried out by means of eight consecutive extractions of the meals with acidic 1-butanol (1:20, w/v) for 15 min at room temperature.

Regardless of the cultivar tested, this procedure yielded protein concentrates containing very low amounts of chlorogenic and caffeic acids and slightly higher amounts of crude fiber. The method was very effective in removing

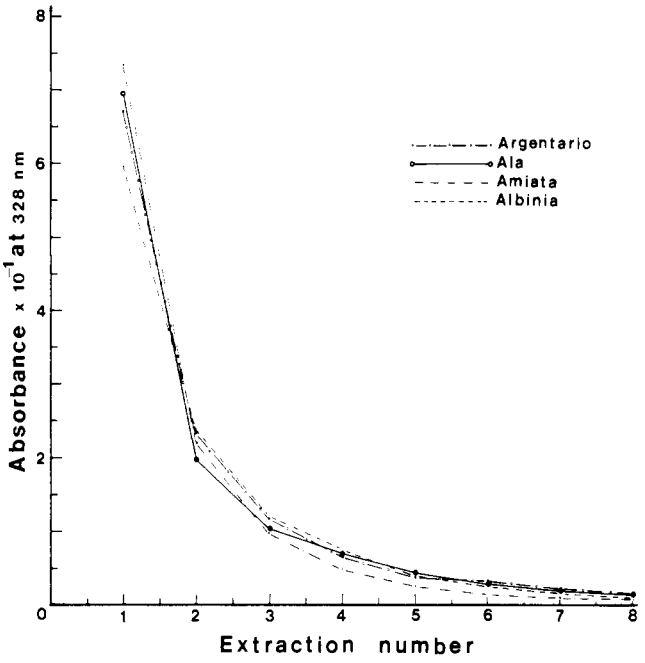

Figure 1. Acidic butanol removal of color-forming phenols from four different sunflower meal cultivars. Extraction conditions of each step were as follows: meal to solvent ratio of 1:20 (w/v), pH 5.0, 15 min at room temperature.

Table II. Proximate Composition of Ala Sunflower Meal and of the Corresponding Protein Concentrate and Isolate Obtained after Acidic 1-Butanol Treatment (Percent Moisture-Free Basis)

Description	Meal	Concentrate	Isolate
Moisture	10.0	10.4	2.9
Protein (N × 6.25)	60.75	67.62	93.52
Lipid	1.47	0.92	0.18
Chlorogenic acid	2.59	≤0.05	≤0.05
Total sugar	9.12	4.28	0.71
Crude fiber	4.33	4.87	0.19
Ash	7.25	8.07	1.36

sucrose, whereas raffinose was poorly extracted (Table I). Less than eight extractions with acidic 1-butanol did not allow the effective removal of chlorogenic and caffeic acid from the meal (Figure 1).

Protein isolate (93.5% protein) obtained by extracting proteins from Ala concentrate was colorless. The chemical compositions of Ala sunflower meal, concentrate, and isolate are reported in Table II. The residual content of chlorogenic acid in concentrate and in protein isolate is negligible (≤0.05%) and does not impart appreciable discoloration to the alkaline solutions of the two products; therefore, this value can be regarded as a possible guideline in the preparation of sunflower concentrates and isolates acceptable for human consumption. As expected, the contents of crude fiber and ash in the protein concentrate are slightly higher than those found for the meal, whereas they strongly decrease in the isolate. The residual level of 1-butanol measured by the GLC technique was 12 ppm in the concentrate and ≤2 ppm in the isolate.

In Figure 2 nitrogen solubility profiles are shown of Ala sunflower protein isolates before and after acidic 1-butanol treatment. The phenol-free isolate exhibited the same minimum solubility point at pH 5.0 of the authentic isolate but a higher nitrogen solubility above pH 7.0. Defatted Ala sunflower meal and the corresponding concentrate and isolate exhibit similar amino acid compositions (Table III).

Albumin fractions extracted from the Ala sunflower meal and the protein concentrate were analyzed by means

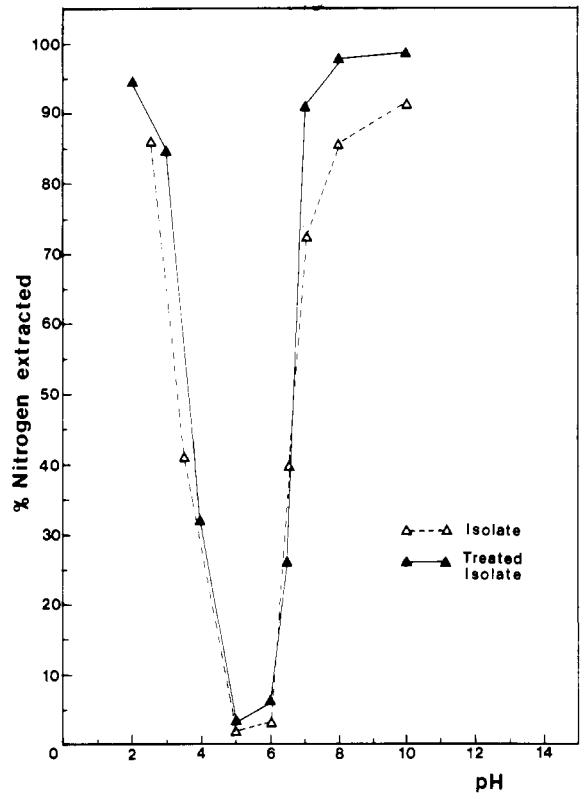

Figure 2. Nitrogen extractability profiles of Ala sunflower protein isolate before and after acidic butanol treatment.

Table III. Amino Acid Composition of Ala Sunflower Meal and of the Corresponding Protein Concentrate and Isolate Obtained after Acidic 1-Butanol Treatment (g/16 g of Nitrogen)

Amino acid	Meal	Concentrate	Isolate
Lysine	3.5	3.0	2.9
Methionine + cystine	3.7	3.4	3.2
Phenylalanine	4.2	4.2	4.4
Tyrosine	2.3	2.3	2.0
Isoleucine	3.6	3.5	3.6
Leucine	5.4	5.2	5.4
Threonine	3.5	3.0	3.6
Valine	4.3	4.2	4.1
Histidine	2.3	2.0	2.3
Arginine	8.0	7.0	7.0
Glycine	5.7	5.7	4.3
Serine	3.5	3.8	4.1
Alanine	4.0	3.7	3.8
Aspartic acid	8.2	8.1	10.8
Glutamic acid	21.1	21.6	21.8
Proline	4.2	4.1	3.7

of polyacrylamide gel electrophoresis in two buffer systems (pH 8.5 and 4.3). Only minor differences were observed among the electrophoretic patterns of albumin fractions (Figure 3). The globulin fraction could not be analyzed under such experimental conditions because of its low solubility in the buffer system used for this investigation. Therefore, globulin and albumin fractions were submitted to gel electrophoresis in aluminum–lactate buffer (pH 3.1) according to Schwenke and Raab (1973) (Figure 4). Under these conditions no differences could be detected among globulins extracted from the defatted flour and the protein concentrate.

DISCUSSION

In the preparation of a protein isolate from sunflower meal it is necessary to carry out exhaustive extraction of the color-forming phenols in order to prevent the devel-

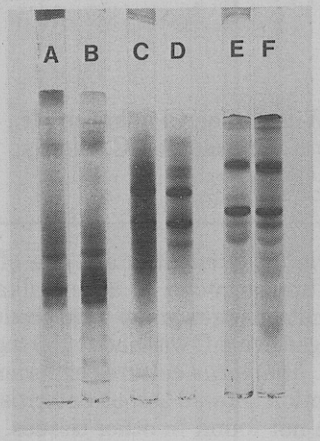

Figure 3. Polyacrylamide disc gel electrophoretograms of albumin fractions from Ala sunflower meal and protein concentrate in different buffer systems according to Ornstein (1964). Cathodic traces: (A) meal, (B) protein concentrate, at pH 4.3; (E) meal, (F) protein concentrate at pH 8.5. Anodic traces: (C) meal, (D) protein concentrate at pH 8.5.

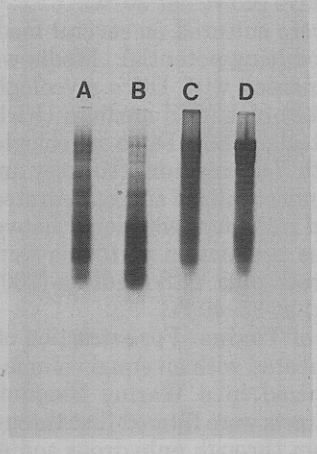

Figure 4. Polyacrylamide gel electrophoretograms of cathodic albumin and globulin fractions from Ala sunflower meal and protein concentrate in 0.016 M aluminum–lactate and 5 M urea buffer system at pH 3.1 according to Schwenke and Raab (1973). Albumins: (A) meal, (B) protein concentrate. Globulins: (C) meal, (D) protein concentrate.

opment of discoloration, the main drawback for the utilization of sunflower products in human diets. It is worthwhile to note that chlorogenic acid is found either free or bound to the sunflower seed macromolecular constituents (Loomis and Battaile, 1966; Pierpoint, 1969). During protein extraction in alkaline medium chlorogenic acid and other phenolic compounds are oxidized to *o*-quinones and form covalent linkages with proteins. Procedures proposed to the present for the removal of these undesirable substances generally alter proteins, whereas the extraction conditions should not lead to denaturation and the solvent system employed should not appreciably solubilize proteins in order to reduce the losses.

The solvent system used by us, acidic 1-butanol, meets all these requirements and can be considered as a valid alternative to obtain improved sunflower products. The choice of the solvent and of the extraction conditions comes from a systematic study of several mixtures of alcohols and acids at different pH values. 1-Butanol and HCl (92:8, v/v) at pH 5.0 constitute the extraction mixture giving the best results in the removal of chlorogenic acid. The ability of butanol to solubilize phenols is enhanced by the presence of a dilute acidic solution at pH levels corresponding to the maximum solubility of chlorogenic acid. The nondenaturing conditions of the solvent system are due to the presence of a high butanol concentration which assures a negligible protein solubility, preventing the hydration of proteins and the binding of chlorogenic acid. Moreover, acidic 1-butanol does not affect the properties of the protein, as is shown by the nitrogen solubility profiles (Figure 2). Further evidence that the proteins have not undergone appreciable denaturation comes from the electrophoretic patterns of albumins and globulins, isolated in different buffer systems, before and after removal of color-forming phenols (Figures 3 and 4).

Results obtained by Sosulski et al. (1972) showed that by hydrolysis of intact kernels at different temperature levels it was possible to remove chlorogenic acid by means of a diffusive process through the membranes of the plant cells, but the removal of phenols was complete only under denaturing conditions. Acidic 1-butanol treatment probably acts through an analogous phenomenon; if such assumption is correct, the efficiency of chlorogenic acid extraction with acidic butanol could be improved by controlling the diffusion parameters. Economic evaluations of the commercial feasibility of the present process are in progress in our laboratory.

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