Grape Seed: A Potential Source of Protein[1]

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ABSTRACT

Because grape seed is a potential source of edible protein, it is essential to remove the polyphenols to significantly improve protein digestibility. This study describes a procedure whereby grape seed protein concentrate is significantly purified and protein digestibility is improved. The procedure involves soaking whole seeds in an alkaline solution and subsequently extracting the protein with a concentrated salt solution.

INTRODUCTION

The importance of the use of grape seed as an alternative source of proteins is related to the high quantities of this byproduct in some grape-producing countries (1). Information on grape seed protein, including methods of extractions and isolation, as well as nutritional value, is limited (1-4).

Grape seed represents about 2-3% of the whole grape berry and protein content of the seed ranges from 10 to 13%. Grape seed also contains a very high concentration of fiber and polyphenols. These compounds react readily with protein under certain conditions. Thus, enzyme-catalyzed reactions can be inhibited, with possible modification of biological pathways (1,2,5,6).

Information on grape seed and grape seed protein, including general method of extraction and isolation were previously reported (1). This study discusses grape seed composition and suggests a method of protein extraction with minimal polyphenolic interaction.

EXPERIMENTAL PROCEDURES

Materials

Grape seeds were obtained from Beaulieu Vineyard, Rutherford CA (Pinot Noir, unfermented and blend from fermented red grapes) and from Cantina Sociale Passaggio di Bettona, Perugia, Italy (Sagrantino red, fermented). The composition of seeds dried at 105 C for 1 hr is presented in Table I.

All reagents used were analytical grade.

The centrifuges used were a continuous imperforate bowl centrifuge, 17-in. bowl diameter, 1,500 rpm, RCF_{max} = 540 g, Fletcher Works, Fletcher Standard, size 17, and a batch Sorvall ultracentrifuge SS. 34 Rotor, 4.25-in. diameter.

METHODS OF ANALYSIS

Proximate composition, including analyses for total solids, nitrogen, TCA, insoluble nitrogen, ether extractives, crude fiber and ash were determined by standard AOAC methods (7). Total phenolics (PFT) were determined using the Folin-Ciocalteu reagent. The results were expressed as gallic acid mg/L (8). Amino acids analyses were performed according to the procedure of Kohler and Palter (9).

Optimal pH of Protein Precipitation

The freeze-dried proteinaceous material was redissolved with 0.01 N NaOH and adjusted to the different pH with 0.01 N HCl. The amount of precipitate at each pH was determined by direct weighing. The protein was determined on the precipitate whereas the total phenolics were determined on the supernatant before and after precipitation. The phenolics content on the proteinaceous material was determined by difference.

Bioevaluation of Protein

The protein efficiency ratio (PER) was determined by a 14-day study with diets containing 10% protein according to standard AOAC procedures. Diets were fed ad libitum to groups of four 21-day-old, male weanling (Sprague-Dawley) rats. Rats were randomly divided into groups in which the mean initial weight was 54 g. All rats were individually housed in screen-bottomed cages with feed consumption and body weights of each rat recorded weekly.

Nitrogen digestibility was determined during the second week of the study and corrected for fecal nitrogen of rats fed a nitrogen-free diet. Statistically significant differences between mean PER values were determined by Duncan's Multiple Range test (10).

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TABLE I

Composition of Grape Seed and Grape Seed Protein Concentrate

Components (%)	Grape seed			Protein concentrate	
	Pinot Noir	Red Blend	Sagrantino	A*	B*
Total solids	94.15	90.04	93.02	98.00	97.60
Moisture	5.85	9.96	6.78	2.00	2.40
Nitrogen	1.89	1.62	1.80	5.47	6.10
Protein (N X 6.25)	11.81	10.12	11.25	34.17	38.13
Fat	12.04	16.34	15.20	16.29	16.82
Fiber	42.53	32.39	–	5.44	2.18
Ash	2.40	2.21	3.91	6.04	23.78
Total phenolics	–	–	–	17.60	15.12

*See Fig. 2 (B, pilot plant).

In vitro digestibility of protein was determined following the methodology of Akeson and Stahmann (11), using pepsin-pancreatin digestion. Digestibility was evaluated on the basis of disappearance of trichloroacetic acid (TCA)-insoluble nitrogen.

SAMPLE PREPARATION

Flours

Laboratory methods: after storage at -20 C, the seeds were washed and cleaned manually several times in cold water and then dried at 60 C for 2 hr in a ventilated oven. They were ground using a Wiley mill with screen no. 10 at +4 C; the temperature was maintained by grinding solid CO_2 in the mill before grinding the seed. Pilot plant method: the seeds, stored and washed as above, were dried in a pilot plant air dryer at 60 C for 2 hr and cleaned in an air classifier (air speed = 335 m/min). They were finally ground to the desired mesh with a laboratory single-disc refiner.

Proteinaceous Material

The protein extraction technique for the flours is given in the discussion.

RESULTS AND DISCUSSION

Location of Protein in the Seed

The structure of grape seed is atypical of most oilseeds. Macroscopically, the seeds are pear-shaped. A scanning electron micrograph (Fig. 1) illustrates the major morphological characteristics of the microscopic structure. The external epiderm (12) is supported by large porous cells, some of them containing raphides, crystals of calcium oxalate, and polyphenols. The internal epiderm is made up of stone cells, characterized by their radial elongation, brown color, very hard walls and high content of polyphenols. The endosperm is formed by polygonal cells containing proteins, fats, tannins, minerals and, mainly, calcium oxalate crystals.

Preliminary data on distribution of those constituents indicated protein and lipid were concentrated within the endosperm, whereas phenolics were located mainly in the internal and external epiderm (P. Fantozzi, unpublished data). Thus, to obtain protein and oil from grape seed, contents of the endosperm should be released with minimal disruption of the stone cells and outer epiderm.

PROTEIN EXTRACTION

Preliminary tests (P. Fantozzi, unpublished data) on protein extraction from the seed showed that air classification of grape seed flour did not effect any appreciable protein concentration. Previous defatting of the seed did not allow more effective extraction of protein, and total solid and polyphenol content of the protein extracts did not show important differences. While extraction by alkali allowed the highest protein recovery, the minimal amount of phenols was obtained using 20% (w/v) NaCl solutions as the extracting agent. The proteins seemed to be mainly glutelins, followed by globulins, prolamines and traces of albumins, according to the Osborne classification (13).

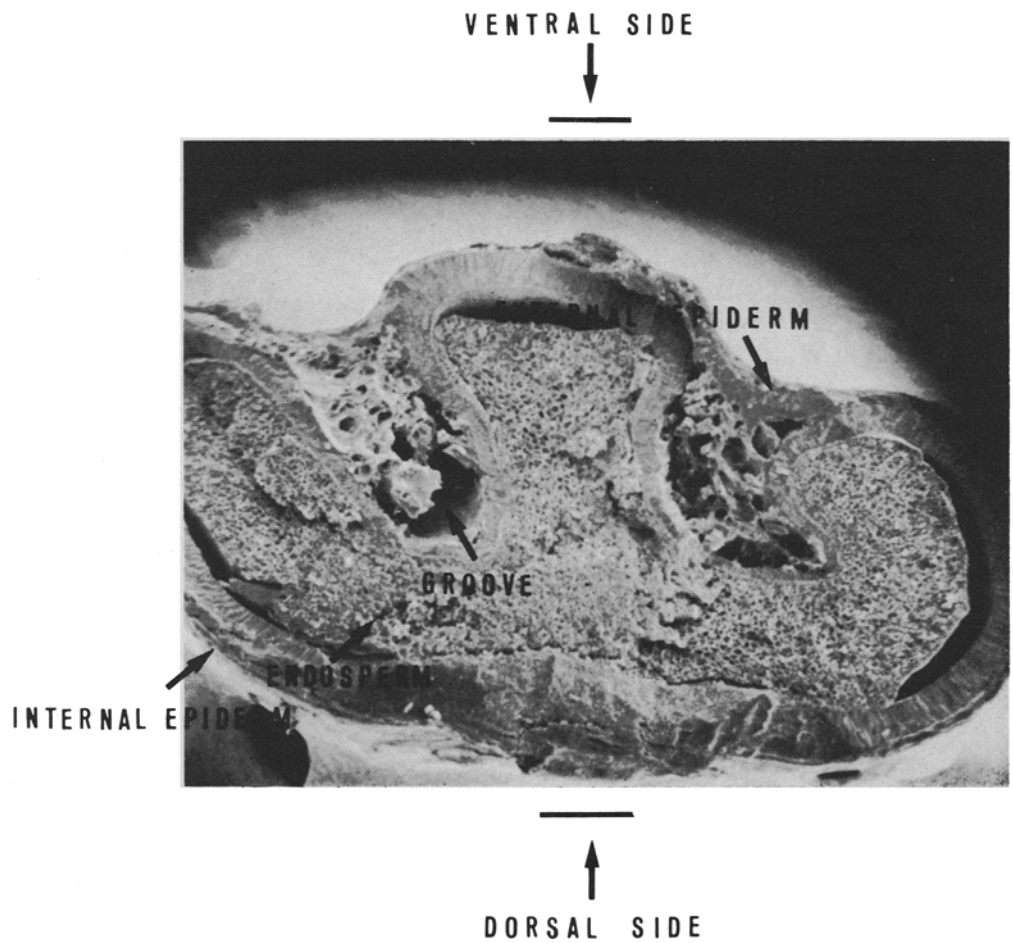

FIG. 1. Scanning electron micrograph of cross section of unfermented Pinot Noir grape seed.

Thus, the laboratory and pilot plant protein extraction procedures were based on the use of NaCl solutions (20%, w/v) and are summarized in Figure 2.

Laboratory Procedures

To improve the separation of protein from polyphenols, insoluble polyvinylpyrrolidone (Polyclar AT, 20%, w/v) alone and with antioxidants (SO_2, 0.5%, w/v) were added to the NaCl solution during extraction (14,15). Large amounts of polyphenols were extracted from the whole seed with 0.05 M NaOH for 20 min at 100 C (1:25, w/v) before the seeds were dried and milled, according to the method of Chavau et al. (16).

Pilot Plant Procedure

We had difficulty separating the fibrous solid residue from the denatured protein by means of the continuous centrifugation because this technique did not bring about separa-

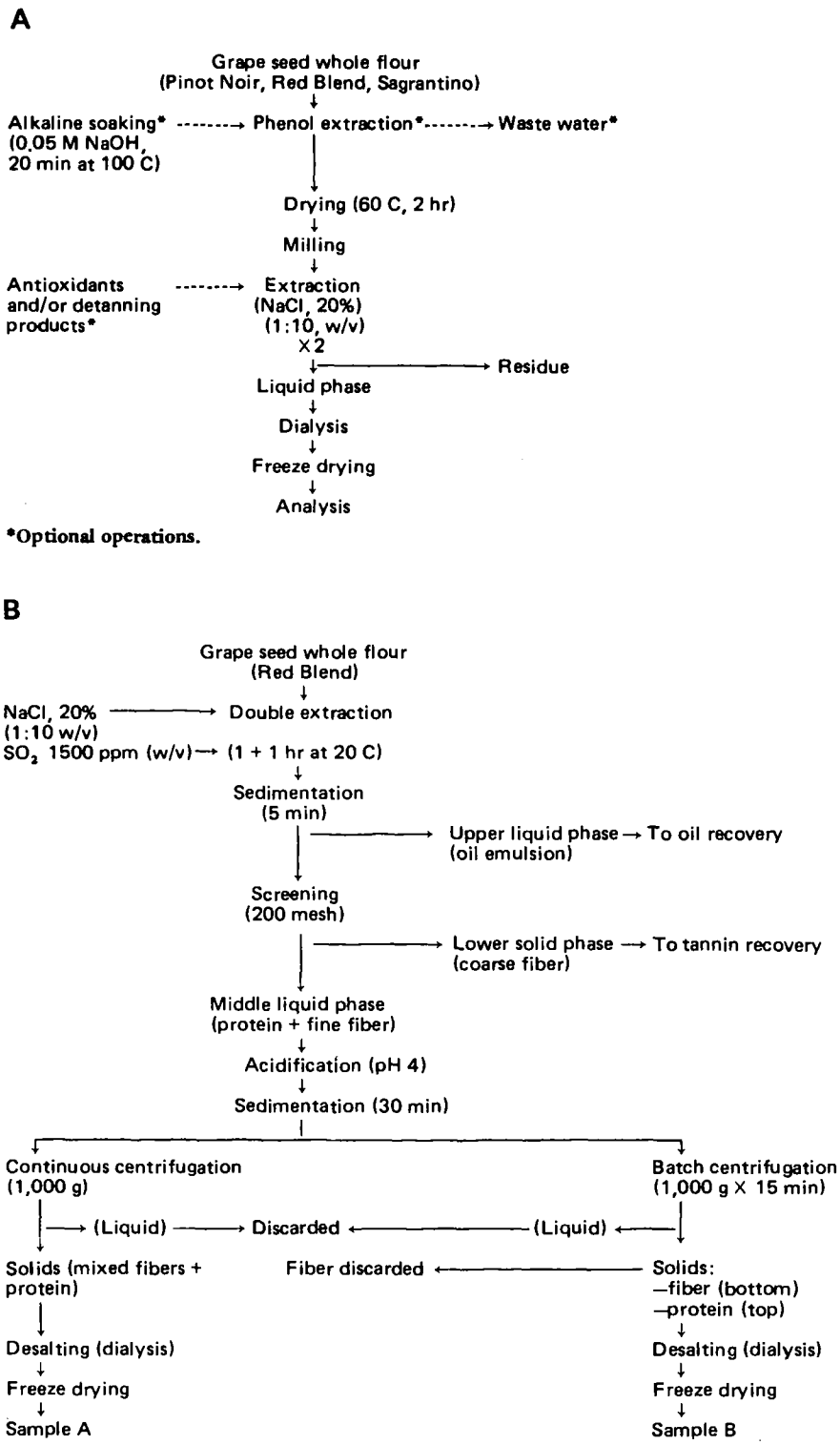

FIG. 2. Grape seed protein extraction in: (a) laboratory; (b) pilot plant.

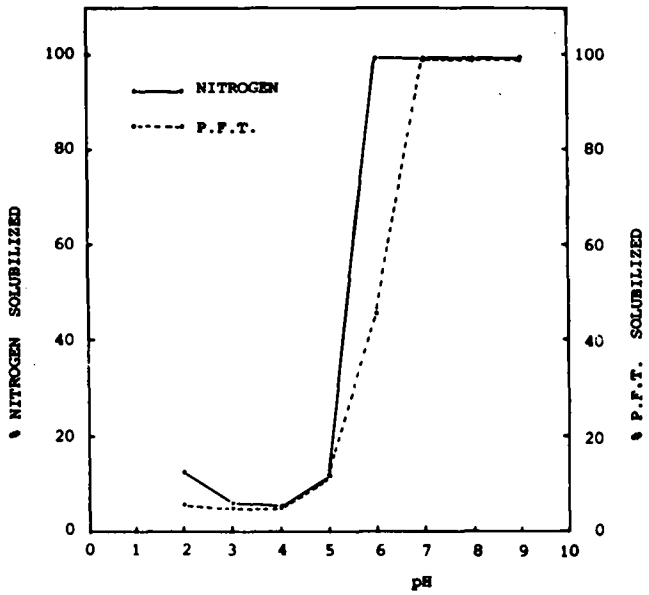

FIG. 3. Precipitation of extracted nitrogen and total phenolics (P.F.T.) from the proteinaceous material obtained from grape seed by the salting-out method.

TABLE II

Grape Seed Protein Amino Acid Composition (g/100 g of protein)

Amino acid	Grape seed protein	FAO 1973 Provisional pattern
Tyrosine	3.02	–
Phenylalanine[a]	3.81	–
Tyr + Phen	6.83	5.60
Histidine	2.62	–
Lysine[a]	3.18	5.44
Arginine	7.54	–
Aspartate	8.02	–
Glutamate	21.47	–
Threonine[a]	3.22	4.00
Serine	4.98	–
Proline	3.84	–
Alanine	4.34	–
Glycine	8.58	–
Valine[a]	5.47	4.96
Cystine[a]	1.87	–
Methionine[a]	1.49	–
Cys + Meth[a]	3.36	3.52
Isoleucine[a]	4.22	4.00
Leucine[a]	6.59	7.04
Tryptophane[a]	ND[b]	–

[a]Essential amino acid.
[b]ND = none detected.

TABLE III

Protein Efficiency Ratio (PER) and Digestibility of Grape Seed Protein

Sample	PER[a,b]	Digestibility	
		Diet[c]	Nitrogen[d]
Casein	2.50	95	94
Grape seed protein sample A[e]	-1.04	69	24
Grape seed protein sample B[e]	-0.33	79	49
Nonprotein control	–	95	–

[a]PER (protein efficiency ratio) = weight gain/protein intake.
[b]Duncan's Multiple Range Test: Data are highly significantly different ($p<0.01$).
[c]Digestibility of diet = feed intake - fecal weight/feed intake X 100.
[d]Nitrogen digestibility = N intake - (fecal N - endogenous fecal N)/N intake X 100.
[e]See Fig. 2 (B, pilot plant).

TABLE IV

Total Phenolics Content and in vitro digestibility of Grape Seed Proteins Obtained by Different Methods

Laboratory extraction method[a]	in vitro digestibility[c] (%)	Phenols content[d] (%)	Color of flour
Pinot Noir grape seed			
0.1M NaOH (pH 8)[b]	4	23.4	Dark brown
20% NaCl	41	5.4	Gray-white
20% NaCl + Polyclar AT (20%, w/v)	60	4.2	Off-white
20% NaCl + Polyclar AT + SO_2 (0.5%, w/v)	ND[e]	6.0	Gray-white
Sagrantino grape seed			
20% NaCl	58	4.7	Gray-white
20% NaCl + previous whole seed alkaline soaking (16)	77	0.2	White
Casein	95	–	–
B.S.A.[f]	95	–	–

[a]See Fig. 2A (laboratory).
[b]See Fig. 2A (laboratory); 0.1M. NaOH used instead of 20% NaCl.
[c]% of disappearance of TCA insoluble nitrogen.
[d]% of seed initial concentration.
[e]Not determined.
[f]Bovine serum albumin.

tion of these 2 solid phases (sample A). To alleviate the problem, we tried a discontinuous centrifugation technique to obtain 2 different solid fractions; the protein-rich fraction was less concentrated in fiber and phenols (sample B). Proximate analysis of those 2 materials is also reported in Table I.

Optimal pH for precipitation of the protein is shown in Figure 3. Analysis of the graph shows that all the polyphenols present in the solution appeared to be found in combination with the protein. Amino acid composition of the precipitated proteins is reported in Table II. Lysine seems to be a limiting amino acid, as is common in most vegetable proteins. Sulfur amino acids, on the contrary, are in good agreement with the FAO provisional amino acid reference pattern.

DIGESTIBILITY AND POLYPHENOL PURIFICATION

The A and B proteinaceous materials prepared in the pilot plant were examined for PER and protein digestibility (7,11). Results are reported in Table III. Both samples have very low nitrogen digestibility and are incapable of supporting growth in the rat. The sample with lower concentration of polyphenols was less detrimental than the other. Also, the apparent N digestibility is higher in sample B.

Table IV reports the improvement of the in vitro digestibility related to reduction of the phenolics content of the proteinaceous material. The data show that the digestibility of grape seed protein was increased from 4 to 77%, by modification of the extraction procedure, in a system where casein and bovine serum albumin were 95% digestible.

It is apparent that extraction of protein with 20% NaCl resulted in better protein digestibility than with 0.1 M NaOH. Polyclar AT partially protected the protein from polyphenols and enhanced digestibility. The presence of antioxidants, such as SO_2, apparently acted as a solvent for polyphenols, rather than preventing the protein-phenols reactions. Alkaline extraction brought about a drastic removal of polyphenols in the whole seed and resulted in enhanced in vitro protein digestibility. However, this procedure generated a large amount of wastewater pollutants because of the high content of phenols.

Studies are in progress in our laboratory on the topic of phenol-rich wastewater sanitation (G. Montedoro, unpublished data).

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