Proteins from *Crambe abyssinica* Oilseed. I. Isolation Procedure

E. Massoura*, J.M. Vereijken, P. Kolster, and J.T.P. Derksen

Agrotechnological Research Institute (ATO-DLO), 6700 AA Wageningen, The Netherlands

ABSTRACT: *Crambe abyssinica* is a promising new oil crop because of the specific properties of its oil. However, little information is available concerning the properties of the proteins, which constitute a major component of the seed. Therefore, a method for the isolation of proteins from *Crambe* seeds was developed. Protein extractability for whole and dehulled *Crambe* meal was studied as a function of pH. Highest extractability was obtained with dehulled meal at pH 11. Double extraction at this pH increased the extractability to about 66%. Protein precipitation from the above-mentioned extract was studied as a function of pH with and without addition of a precipitating agent, i.e., carboxymethylcellulose (CMC). Addition of CMC resulted in a protein recovery of about 75% at pH 4.4. Without CMC, about half of the protein was recovered by isoelectric precipitation. The remaining soluble protein could be concentrated by ultrafiltration with a recovery of about 65%. The developed process, not including CMC addition, results in two protein fractions, i.e., an isoelectric precipitate (protein content 75%) and a retentate (protein content 87%), which together account for about 50% of the protein present in *Crambe* meal. Application of heat decreased protein extractability, but the protein contents of the resulting fractions were comparable to those from non-heat-treated meal.

JAOCS 75, 323–327 (1998).

KEY WORDS: *Crambe abyssinica,* heat treatment, oilseeds, protein, protein isolation.

Several new crops have been considered for introduction in agriculture to broaden the current narrow crop rotation schedule and to supply industry with new resources. Furthermore, new crops could contribute to a reduction in agricultural surpluses of the traditional arable crops. *Crambe abyssinica* particularly is a promising new oil crop because of both its agronomic performance and the specific properties of its oil (1–3). Important in this respect is that *Crambe* oil is one of the richest known sources of erucic acid, which makes up 55 to 60% of the oil glycerides (4,5). High-erucic acid oils and derivatives of erucic acid have a broad range of applications. They can be used, for instance, in existing markets for polymer additives, plasticizers, lubricants, coatings, and as raw material for the synthesis of nylon (6,7). However, for profitable cultivation of this crop, applications of other major components of the seed, such as the protein fraction, could well be of major importance.

In contrast to the oil, little is known about the proteins of *Crambe*. Crude protein levels range from 20% in the whole seed to nearly 50% in the dehulled, defatted meal (8). *Crambe* protein, from a nutritional point of view, has a well-balanced amino acid pattern (5,9,10) and, therefore, the meal could be used as feed. However, the feeding value of the meal is greatly impaired by the presence of antinutritional factors, known as glucosinolates. When hydrolyzed by an endogenous enzyme, thioglucosidase, glucosinolates form compounds that limit palatability and/or render the meal toxic, particularly to nonruminants (8). Glucosinolates may reach a level up to 10% in the dehulled, defatted meal (8,9). Therefore, so far most research has been concerned with the removal of these compounds to render the meal suitable for feed (8,11–13).

The suitability of *Crambe* proteins for food and nonfood applications with higher added value is not yet known. Therefore, the primary aim of this work was the development of a protein isolation procedure to study the intrinsic properties of these proteins on biochemical and functional levels (14). During industrial extraction of the oil, heat is applied to the seed. Therefore, a secondary aim of this paper was to obtain an indication of the effect of heat treatment of the meal on the isolation efficiency of the proteins.

EXPERIMENTAL PROCEDURES

Materials. Seed of *C. abyssinica* Hochst Ex. R.E. Fries was provided by Cebeco-Handelsraad B.V. (Rotterdam, The Netherlands). The Bio-Rad *DC* protein assay kit was supplied by Pharmacia (Uppsala, Sweden). Carboxymethylcellulose (CMC) of low DP (degree of polymerization) was obtained from Akzo (Amsterdam, The Netherlands).

Preparation of meal. Crambe seeds were dehulled by cracking the seeds in roller mills. Removal of the hulls was accomplished by air classification. The ratio of hulls to meal was 35:65. For milling, whole or dehulled seeds were mixed with liquid nitrogen in a laboratory grinder (A10; Janke and Kunkel

*To whom correspondence should be addressed at present address: Lomvardou 81, 11474 Athens, Greece.

GmbH, Staufen, Germany) and ground for 30 s. Defatting was performed by stirring a suspension of whole or dehulled meal in petroleum ether (ratio meal to solvent, 1:10 wt/vol) for 45 min at room temperature. After phase separation, the supernatant was decanted, the pellet was resuspended in solvent, and the same procedure was repeated. The remaining pellet was left to dry overnight at room temperature.

Extractability. Protein extractability was studied as a function of pH. A suspension of defatted meal in water (1:10 wt/vol) was stirred for 5 min. Then, the pH was adjusted to the desired value by adding drops of dilute sodium hydroxide or hydrochloric acid solution. Stirring was continued for 1 h, while the pH was checked every 10 min and readjusted, if necessary. After centrifugation in a Beckman J-21C centrifuge (Palo Alto, CA) at $10,960 \times g$ for 45 min, the supernatant was filtered to remove floating particles. Aliquots of the supernatant were freeze-dried, and their protein content was determined by Kjeldahl analysis.

Precipitation. Protein precipitation with and without the addition of CMC was studied as a function of pH. First, proteins were extracted at pH 11 from defatted–dehulled meal. Two aliquots of this extract were diluted with water to give a concentration of approximately 1.5 mg protein per mL. The pH was then readjusted to 11. In one of the two solutions, CMC was added to a ratio of 0.166 CMC/protein (w/w). This ratio yielded the highest precipitation of rapeseed proteins (15). Samples of varying pH were prepared from both solutions and kept at ambient temperature for 1 h, after which they were centrifuged for 15 min at $16,000 \times g$. The protein content of the supernatants was determined with the Bio-Rad *DC* protein assay kit. Solubility at pH 11 was assumed to be 100%, and the results were expressed relative to this value. For larger-scale experiments, the pH 11 extract was acidified to the optimal pH for precipitation, maintained at this pH for 1 h, and then centrifuged at $10,960 \times g$ for 30 min.

Ultrafiltration. The supernatant that remained after isoelectric precipitation was subjected to ultrafiltration by circulation through a membrane (Nephross Andante HF/Organon Technica BV, Boxtel, The Netherlands) of molecular weight cut-off 5000 Da at a pressure of 0.5 Bar.

Preparation of heat-treated material. Dehulled meal was heated to 93°C in glass-stoppered flasks in an oven. As soon as this temperature was reached, water of 93°C was added, the flasks were shaken gently and placed back in the oven for 30 min. Water was added in such amount that the meal had 14% moisture content. After this incubation period, the meal was dried at the same temperature for 1 h and then defatted as previously described. Protein extraction, precipitation, and ultrafiltration were carried at the optimal conditions found for the nonheated material.

Chemical analyses. The moisture content was determined gravimetrically according to AACC Method 44-15A (16). Fat content was determined with a Soxtec system HT 1043 extraction unit (Tecator, Höganäs, Sweden). Crude protein content of seeds, meal, and freeze-dried materials was determined by the Kjeldahl method, AACC Method 46-12 (16).

RESULTS AND DISCUSSION

Table 1 shows the crude fat and crude protein contents of *Crambe* meals. Crude protein levels ranged from 19.2% in the whole seed to nearly 40% in the dehulled, defatted meal. The values obtained for crude protein in the defatted meals were lower than those quoted by Carlson and Tookey (8). This is partly due to the presence of higher residual fat content in our materials. Our results for crude protein in whole and dehulled meals were similar to those reported by Carlson and Tookey (8).

The efficiency of oil extraction was about 82 and 83% for the whole and dehulled meal, respectively. This result indicates that the presence of hulls did not affect oil extraction, at least at a laboratory scale. Despite two extractions, defatting was not complete, probably owing to the fact that the procedure was performed at ambient temperature. No additional oil was removed, even when a third extraction was performed. For higher oil extraction efficiency, heat is often applied to the meal prior to and/or during oil extraction (1,12,17). This is also evident from our results with respect to the effect of heat (see below). Although insufficient oil extraction may affect protein extractability, extraction at room temperature was applied to avoid possible heat-induced protein denaturation.

Protein extractability. Figure 1 shows protein extractability as a function of pH for dehulled and nondehulled meal. The shape of both curves was similar with no pronounced minimum in the range of pH 3 to 8. This could be an indication of the presence of proteins with a wide range of isoelectric points. In terms of absolute values, the protein extracted may be lower than that shown in Figure 1 owing to the presence of nonprotein nitrogenous compounds. VanEtten *et al.* (18) reported that 12% of *Crambe* meal nitrogen was derived from nonprotein nitrogenous compounds. The shape of the curves resembles that obtained from hexane-treated canola meal (19) and rapeseed meal (20) and is unlike the shape obtained from other oilseeds, such as soybean (21) or sunflower (22), which exhibits a clear minimum. For both types of *Crambe* meal, the extraction efficiency was higher at alkaline than at acid pH values. In comparison with whole meal, the extraction efficiency for dehulled meal was higher at all pH values tested. It may well be that the hulls contain compounds, such as tannins, that interact with proteins to form insoluble complexes.

Generally, protein extractability was lower than that reported by VanEtten *et al.* (18), possibly owing to the presence of fat in our material and/or to differences in cultivar, agro-

TABLE 1
Crude Fat and Protein Contents, Dry Basis, of *Crambe* Meals[a]

Constituents (%)	Whole meal	Dehulled meal	Defatted whole meal	Defatted, dehulled meal
Crude fat	32.7	43.1	7.9	11.2
Protein (N × 6.25)	19.2	24.5	26.0	38.2

[a]Means of duplicate determinations.

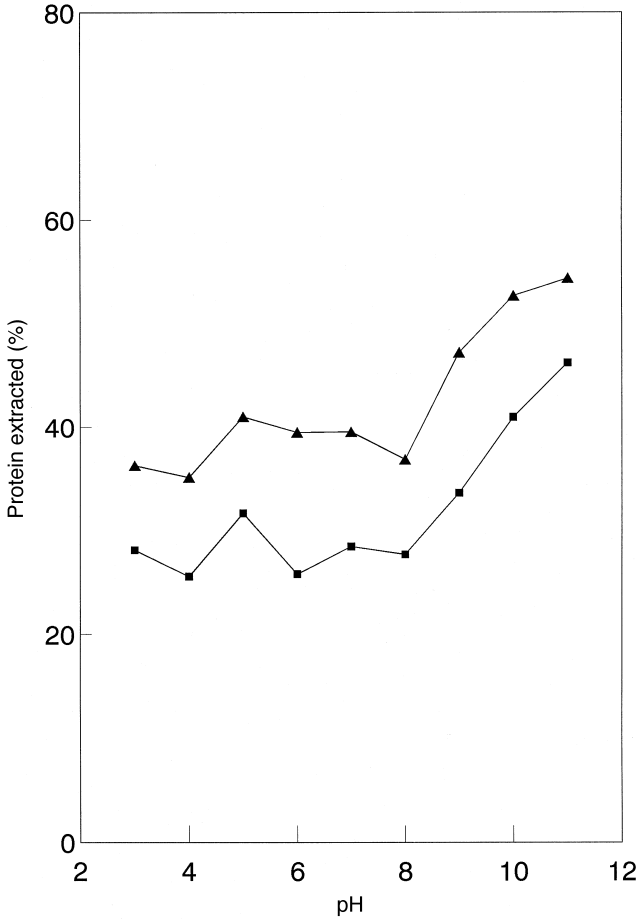

FIG. 1. Protein extractability as a function of pH: (■) defatted whole meal; (▲) defatted dehulled meal.

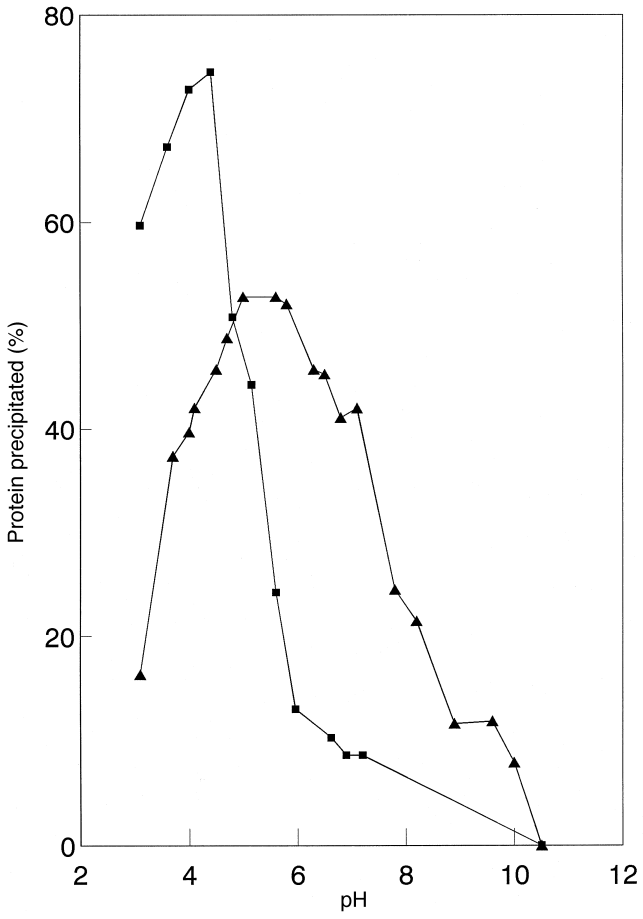

FIG. 2. Protein precipitation as a function of pH: (■) with carboxymethylcellulose (CMC); (▲) without CMC.

nomic, or climatic conditions. Highest protein extractability was obtained from dehulled meal at pH 11. Double extraction at this pH increased protein yield to about 66%. This extract, which had about 50% protein content [dry matter basis (d.m. basis)], was used in further experiments. To decrease the amount of nonprotein components present in the extract, further treatments were performed.

Precipitation efficiency. Precipitation of proteins, with or without addition of a precipitating agent, from alkaline extracts by acidification is a procedure that is often applied for the preparation of protein concentrates/isolates from oilseeds (15,20,23,24). To determine the optimal pH value for precipitation, protein precipitation efficiency was studied as a function of pH with and without CMC (Fig. 2). Addition of CMC clearly affected protein precipitation. Without CMC, a maximal precipitation of about 50% occurred at pH 5–6. Addition of CMC resulted in a more narrow curve, with maximal precipitation of about 75% at pH 4.4. This shift to more acidic pH values was also observed by Gillberg and Törnell (15). They attributed this to the formation of complexes between CMC and proteins, caused by interactions between carboxylic groups of CMC and positively charged amino acid residues of the proteins. The protein contents of the precipitates (d.m. basis), recovered at pH 4.4 (with CMC) and pH 5.5 (without CMC), were 64 and 58.8%, respectively. Although CMC causes higher precipitation efficiency, its addition resulted in only slightly higher protein content of the precipitate. Furthermore, CMC might affect the functional properties of proteins. Therefore, precipitation was subsequently performed at pH 5.5 without CMC. As mentioned earlier, a proportion of the oil originally present in the dehulled meal was not extracted. Most, if not all, of the unextracted oil was present in the precipitate. Removal of this oil by solvent extraction at room temperature increased the protein content of the precipitate to approximately 75% (d.m. basis).

Ultrafiltration. About half of the proteins remained soluble after isoelectric precipitation at pH 5.5. On a d.m. basis, the protein content of this supernatant was about 42%. To concentrate the supernatant, ultrafiltration was used. The membrane used had a molecular weight cut-off of 5,000 Da; therefore, materials with lower molecular weights would be removed. About 65% of the protein could be recovered by this procedure. The protein content of the freeze-dried retentate was about 87% protein (d.m. basis).

Protein recovery. The whole process developed integrates a series of steps: double extraction at pH 11, isoelectric pre-

cipitation at pH 5.5, ultrafiltration of the supernatant, and drying of the precipitate and the retentate. Table 2 summarizes the solids and protein balance of our isolation procedure for defatted *Crambe* meal. The yields are expressed relative to the starting meal. About 50% of the proteins present in the starting material was recovered in two fractions, namely, the defatted isoelectric precipitate and the retentate. These fractions have a protein content of about 75 and 87%, respectively, and together they constitute about 25% of the initial solids.

Influence of heat treatment. These experiments were carried out to obtain an indication of the effect of heat on protein isolation by using the procedure mentioned previously. This was considered important because, in the oilseed processing industry, the meal is subjected to elevated temperatures. Heat treatment has a positive effect on *Crambe* meal because of thioglucosidase inactivation. On the other hand, high temperatures (>105°C) may cause thermal degradation of glucosinolates, which has a negative effect on the quality of both oil and meal (17). These factors were taken into account by Carlson *et al*. (17), who developed a procedure for commercial processing of *Crambe* seed. The choice of our heating conditions was based on their suggestions.

The heat-treated dehulled, defatted meal had a protein content of 44%. The efficiency of oil removal was 92%, somewhat higher than that for the nonheat-treated material. As a result, the fat content of the meal dropped from 11.2 to 5.7%. The effect of heat treatment on the solids and protein balance is shown in Table 3. The isolation procedure permits about 35% recovery of protein from the meal, while giving rise to two fractions of 78 and 80% protein content, which together constitute 18.5% of the initial solids. Not all data presented in Tables 2 and 3 can be directly compared, owing to the different fat content in the initial material. However, for both the defatted isoelectric precipitate and the retentate, the difference in fat content between the heat-treated and the nonheat-treated materials is expected to be small. Heat treatment lowers protein recovery and solid matter. Lower protein extractability might result from protein denaturation and subsequent insolubilization. Heat treatment also decreases the ratio of defatted isoelectric precipitate to soluble protein, indicating that relatively less protein can be precipitated. On the other hand, heat treatment does not affect significantly the protein contents of the two fractions. The meal residue from heat-treated material has a high protein content (32%); therefore, by suitable treatment for removing glucosinolates, of which most are presumably removed during the alkaline extraction step, the meal residue may be useful for feed purposes.

This research has shown that it is possible to isolate proteins from defatted *Crambe* meal by a relatively simple procedure. The protein fractions obtained show interesting functional properties (14). Heat treatment affects protein recovery, yet significant amounts can be isolated. In addition, the meal residue remaining after alkaline extraction of the proteins may be useful for feed purposes.

TABLE 2
Effect of Extraction, Precipitation, and Ultrafiltration on Defatted *Crambe* Meal

	Protein (N × 6.25)[b] (%)	Yield[a] Solids[b] (%)	Yield[a] Protein (N × 6.25)[b] (%)
Starting meal	38.2 ± 1.5	100	100
Meal residue	22.7 ± 1.4	44.9 ± 3.8	25.5 ± 4.4
Extract at pH 11	48.1 ± 1.9	55.2 ± 1.3	66.0 ± 4.3
Isoelectric precipitate	58.8 ± 5.6	20.2 ± 1.0	29.5 ± 0.2
Defatted	75.0 ± 2.6	15.0 ± 0.9	29.0 ± 0.4
Soluble protein	41.6 ± 0.8	34.0 ± 2.2	35.0 ± 1.9
Retentate	87.0 ± 4.1	10.4 ± 0.7	22.6 ± 1.1
Ultrafiltrate	18.6 ± 0.6	23.4 ± 1.8	11.0 ± 0.7

[a]Values relative to the starting meal.
[b]Mean ± standard deviation, $n = 4$.

TABLE 3
Effect of Extraction, Precipitation, and Ultrafiltration on Heat-Treated Defatted *Crambe* Meal

	Protein (N × 6.25)[b] (%)	Yield[a] Solids[b] (%)	Yield[a] Protein (N × 6.25)[b] (%)
Starting meal	44.0 ± 1.0	100	100
Meal residue	32.2 ± 1.0	57.8 ± 1.3	42.2 ± 2.5
Extract at pH 11	48.4 ± 1.5	44.4 ± 0.5	48.9 ± 2.0
Isoelectric precipitate	75.2 ± 0.9	10.2 ± 0.6	17.4 ± 0.7
Defatted	78.6 ± 0.3	9.5 ± 0.3	17.0 ± 0.1
Soluble protein	40.1 ± 0.2	31.4 ± 0.4	28.7 ± 1.2
Retentate	80.6 ± 1.2	9.0 ± 0.6	17.2 ± 0.9
Ultrafiltrate	17.9 ± 0.6	22.2 ± 0.9	9.5 ± 0.8

[a]Values relative to the starting meal.
[b]Mean ± standard deviation, $n = 3$.

ACKNOWLEDGMENT

Financial support by the Commission of the European Communities (DG XII) is gratefully acknowledged.

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[Received April 7, 1997; accepted September 9, 1997]