proportional to the total concentration of phenolics, except for spinach. Broccoli phenolics showed nearly 65% inhibition followed by peas, 48.5%; carrots, 30.4%; beans, 26.9%;, and spinach, 23.3%. In spite of the highest concentration of flavonols and acidic phenolic compounds among the vegetables studied, the phenolics of spinach inhibited carotene bleaching the least. This can be explained by the fact that flavones in spinach were mostly methylated compared to other vegetables (Hermann, 1988) and methylation led to a considerable reduction of antioxidant activity (Letan, 1966). In addition, the acidic phenolic compounds in spinach consisted mainly of *p*-coumaroyl derivatives (Tadera and Mitsuda, 1971; Hermann, 1978), compounds that showed very low inhibitory effects on carotene bleaching by lipoxygenase (Table I).

A careful examination of Table II reveals that neutral fraction A, which consisted mainly of flavans such as catechins and procyanidins and other polar phenolics, had a closer correlation ($r = 0.74$) to the inhibitory effect than neutral fraction B ($r = 0.18$) and the acidic phenolic compounds ($r = 0.01$). This agrees with the observations that were made with the standard phenolics in the model system. Therefore, it is concluded from this study that among the various phenolic groups, flavans and other polar phenolics are the major inhibitors of the lipoxygenase that bleaches carotene in vegetables.

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Proteins from Double-Zero Rapeseed

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Solubility profiles of protein, phytic acid, and glucosinolates in aqueous electrolytes of varying pH were found to be similar for defatted meals from two varieties of double-zero (low glucosinolate/low erucic acid) rapeseed (*Brassica napus*) and conventional Chinese rapeseed (*Brassica juncea*). All three meals yield upon extraction with aqueous electrolytes at pH 4–5 products that are enriched in protein to the extent of 20–35%. Simultaneously, the level of phytic acid in the extracted meals is reduced by about one-third while the glucosinolates are virtually eliminated. Extraction of the defatted meals at pH 12–13 results in dissolution of >90% of the protein, one-third of the phytic acid, and <10% of the glucosinolates. The resulting extract provides upon precipitation of the protein at pH 4–5 protein isolates (>90% protein) in yields of about 60% having phytic acid content of 2–2.5% without any detectable levels of glucosinolates. The amino acid compositions of the meals and protein isolates indicate favorable nutritive value of such products.

Rapeseed and related cruciferous oilseed crops are a rich source of protein (Finlayson, 1976). Technological processes have been suggested in the past for the removal of certain undesirable constituents of rapeseed, such as

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Table I. Composition of Rapeseed and Defatted Rapeseed Meal[a]

	00-Ceres		00-Cobra		conventional rapeseed	
	seed	meal	seed	meal	seed	meal
oil, % (w/w)	49.7		43.7		47.0	
protein, % (w/w)	20.2	40.2	20.9	37.2	23.7	43.5
glucosinolate						
total, μmol/g		16.2		15.6		
isothiocyanate, mg/g						17.1
oxazolidinethione, mg/g						2.0
phytic acid (w/w)		3.2		2.9		3.0

[a] All data given on a dry weight basis.

glucosinolates (Ballester et al., 1970; Bhatty and Sosulski, 1972; Kozlowska et al., 1972; Mukherjee et al., 1979) and preparation of protein concentrates (Eapen et al., 1969; Jones, 1979; Ohlson and Anjou, 1979; Thompson et al., 1976, 1982) and protein isolates (Owen et al., 1971; Kodagoda et al., 1973; Gillberg and Törnell, 1976a; Aman and Gillberg, 1977; El Nockrashy et al., 1977; Blaicher et al., 1983). Rapeseed meals and protein concentrates as well as isolates prepared therefrom contain unusually high levels of phytic acid (Jones, 1979; Blaicher et al., 1983), which can cause deficiency of certain minerals, especially zinc, that must be compensated by suitable supplementation (Jones, 1979). Protein isolates containing low levels of phytic acid have been prepared (Gillberg and Törnell, 1976a; Aman and Gillberg, 1977; Blaicher et al., 1983), and such isolates have been found to have desirable functional properties (Dev and Mukherjee, 1986).

Considerable progress in plant breeding has been achieved in the past two decades, yielding new commercial varieties of rapeseed that are not only almost free of erucic acid, the so-called zero-erucic strains, but also essentially free of both erucic acid and glucosinolates, the double-zero (00) varieties (Thompson and Hughes, 1985). The latter varieties of rapeseed have recently gained popularity due to their acceptability in feed, and such varieties are now widely cultivated in several countries (Larsen and Sørensen, 1985).

This report records the results of studies aimed at technological processing of 00-varieties of rapeseed for the production of protein-rich products. For comparison, similar products obtained from a conventional "double-high" variety of rapeseed were investigated as well.

EXPERIMENTAL SECTION

Materials. Seeds of double-zero rapeseed, *B. napus* cv. 00-Ceres and 00-Cobra, were obtained from Rapool-Ring, GmbH, 3000 Hannover, Federal Republic of Germany; conventional Chinese rapeseed, *B. juncea*, was provided by the Ministry of Commerce, Beijing, Peoples Republic of China. The seeds were ground, defatted by extraction with hexane at 55–60 °C in a Soxhlet apparatus, desolventized at ambient temperature, and sieved through a 0.2-mm screen. In order to deactivate the thioglucoside glucohydrolase, the conventional rapeseed was treated with boiling water for 3 min and air-dried prior to grinding and defatting.

Analytical Procedures. Moisture content of the samples was determined by drying in an air oven at 130 °C for 1 h, and nitrogen estimations were carried out according to a semimicro Kjeldahl procedure (AOAC, 1980). Protein content was calculated as % N × 6.25 except for the products from conventional rapeseed containing high levels of glucosinolates; in the latter case the protein content was calculated as % N × 5.5. Glucosinolate content of the 00-rapeseed meals was determined by hydrolysis using thioglucoside glucohydrolase followed by enzymatic measurement of the glucose released (Fiebig, 1988). In the conventional rapeseed meal and the products derived therefrom, the glucosinolate content was determined after their enzymatic hydrolysis to isothiocyanates and oxazolidinethione (Appelqvist and Josefsson, 1967). Phytic acid was determined by the procedure adopted by Blaicher et al. (1983). Protein contained in the various samples was hydrolyzed, and the composition of the resulting amino acids was determined in an amino acid analyzer.

Solubility of the Constituents of Rapeseed Meal at Various pH. Rapeseed meal (1 g) was extracted in centrifuge tubes for 20 min at ambient temperature by magnetic stirring with 20 mL of the electrolyte solution, i.e., water adjusted to a desired pH by the addition of HCl or NaOH. Subsequently, the mixture was centrifuged at 1600*g*, the supernatant was separated, and aliquots thereof were analyzed for nitrogen, phytic acid, and glucosinolates.

Extraction of Rapeseed Meal for Preparation of Protein-Rich Products. For successive extractions, rapeseed meal (1 g) was treated as described above with 6.7 mL of the electrolyte solution kept at pH 5 (00-rapeseeds) or pH 4 (conventional rapeseed), the extract was removed by centrifugation, and the extractions were repeated two more times under identical conditions (net solvent to meal ratio 20:1). The extracts were combined and aliquots analyzed.

Three 1-g portions of each rapeseed meal were subjected to three-stage countercurrent batch extractions at pH 5 (00-rapeseeds) or pH 4 (conventional rapeseed) with a meal to solvent ratio of 1:20 in a manner similar to that described earlier (El

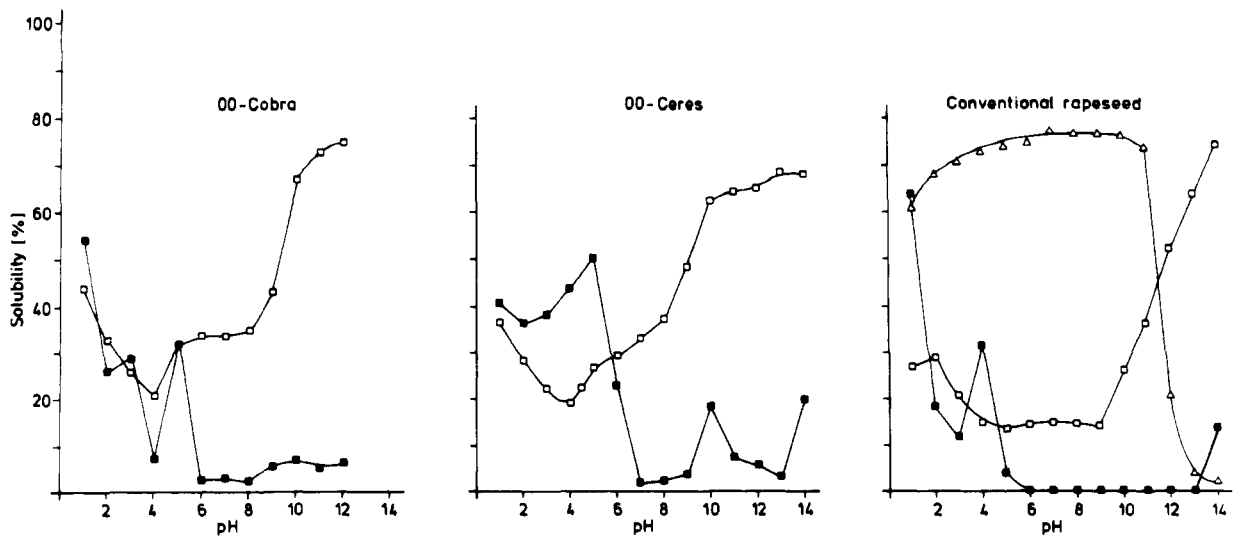

Figure 1. Effect of pH on the solubility of constituents of rapeseed meal; □, protein; ■, phytic acid; △, glucosinolates.

Table II. Composition and Yield of Products Formed by Removal of Soluble Constituents of Defatted Rapeseed Meals by Extraction at Acidic pH[a]

	successive extractions			countercurrent extractions		
	00-Ceres	00-Cobra	conventional rapeseed	00-Ceres	00-Cobra	conventional rapeseed
protein content, % (w/w)	47.9	45.6	59.8	48.3	44.9	59.0
protein yield, % (w/w)	82.7	82.4	79.6	82.3	82.0	81.3
glucosinolate content						
isothiocyanate, mg/g			1.1			1.9
oxazolidinethione, mg/g			0.1			0.0
glucosinolate yield, % (w/w)						
isothiocyanates			6.3			10.9
oxazolidinethiones			5.1			0.0
phytic acid content, % (w/w)	2.2	2.0	2.7	2.3	2.2	2.4
phytic acid yield, % (w/w)	45.3	44.9	54.9	49.1	50.9	48.5

[a] Meals from double-zero rapeseed were extracted at pH 5, and those from conventional rapeseed, at pH 4. All compositional data are reported on a dry weight basis.

Table III. Dissolution of Constituents of Defatted Rapeseed Meal by Three-Stage Countercurrent Extraction at Alkaline pH[a] (Yield, % (w/w))

	00-Ceres	00-Cobra	conventional rapeseed
protein	92.8	92.9	94.1
glucosinolate			7.7
phytic acid	30.0	39.1	30.6

[a] Meals from 00-rapeseed were extracted at pH 12, and those from conventional rapeseed, at pH 13.

Nockrashy et al., 1977). The resulting extracts were combined and aliquots analyzed.

Effect of pH on the Precipitation of Constituents of Rapeseed Meal from Alkaline Extracts. Three-stage countercurrent extraction of the rapeseed meals at pH 12 (00-rapeseeds) or pH 13 (conventional rapeseed) was carried out with a meal to solvent ratio of 1:20 as described above and the extent of dissolution of the various constituents determined. Aliquots of the extracts were adjusted to various pHs ranging from pH 4 to pH 9, each of the resulting precipitates was separated by centrifugation, and the supernatants were analyzed in order to measure the extent of precipitation of protein, phytic acid, and glucosinolates.

Preparation of Rapeseed Protein Isolates. Three 5-g portions of each rapeseed meal were subjected to countercurrent extraction at pH 12 (00-rapeseeds) or pH 13 (conventional rapeseed) with a meal to solvent ratio of 1:20 in a manner similar to that described above. Each extraction was carried out by initial homogenization for 5 min with an Ultra-Turrax apparatus (Janke & Kunkel, 7813 Staufen, i. Br., Federal Republic of Germany) followed by magnetic stirring for 15 min. The combined extracts from each experiment were adjusted to pH 5 (00-rapeseeds) or pH 4 (conventional rapeseed), and the precipitated protein was separated from the supernatant by centrifugation at 3000*g*. The protein was washed with 50 mL of water followed by 100 mL of acetone and air-dried to yield the protein isolates.

RESULTS AND DISCUSSION

Compositions of the two varieties of 00-rapeseed, the conventional rapeseed, and the corresponding defatted meals obtained by extraction with hexane are given in Table I. All three varieties of rapeseed contain similar levels of oil, protein, and phytic acid. The meals derived from the 00-varieties of rapeseed have very low glucosinolate contents compared to conventional rapeseed.

The defatted meals were extracted with aqueous electrolytes at various pHs in order to determine the pH region at which maximum dissolution of the undesirable constituents, e.g., glucosinolates and phytic acid, occurred at a minimal loss of protein. The data given in Figure 1 show for the three rapeseed meals that the protein has minimum solubility between pH 4 and 5 and high solubility at pH 12 and higher values. Phytic acid, on the other hand, has a high solubility in range of pH 4–6 and low solubility between pH 12 and 13. The glucosinolates in the conventional rapeseed meal are freely soluble between pH 3 and 11, but they have low solubility between pH 12 and 13. The solubility profiles of protein and phytic acid (Figure 1) are similar to those reported in an earlier study carried out with meals from low-erucic rapeseed varieties containing high levels of glucosinolates as in conventional rapeseed (Gillberg and Törnell, 1976b).

Table IV. Composition and Yield of Protein Isolates from Defatted Rapeseed Meals by Countercurrent Extraction and Isoelectric Precipitation[a]

	00-Ceres	00-Cobra	conventional rapeseed
protein content, % (w/w)	93.0	93.8	92.3
protein yield,[b] % (w/w)	62.9	61.3	56.8
phytic acid content, % (w/w)	2.2	2.6	2.0
glucosinolate	nd[c]	nd	nd

[a] Defatted rapeseed meals were extracted as given in Table III and the extracts adjusted to pH 5 (00 varieties) or pH 4 (conventional) for isoelectric precipitation of protein. All data are given on a dry weight basis. [b] With respect to protein contained in defatted meal. [c] Not detectable.

On the basis of solubility data given in Figure 1, pH 5 and pH 4, respectively, were chosen for the extraction of defatted meals of 00-rapeseed and conventional rapeseed, in order to attain a high degree of removal of phytic acid and glucosinolates (especially from conventional rapeseed) with the least loss of protein. The results of both successive extractions and countercurrent extractions are given in Table II. They show that extraction at acidic pH results in substantial enrichment of protein at a loss of about 20% of protein contained in the meals. The protein content of the products obtained by acidic extraction is, however, below the levels (65–70%) set for protein concentrates. It is conceivable though that acidic extraction of defatted meals from dehulled rapeseed would provide products with substantially higher protein contents (55–65%). Acidic extractions also eliminate approximately 90% of the glucosinolates contained in the conventional rapeseed meal (Table II); the corresponding figures for the 00-rapeseed meals could not be determined due to the extremely low levels of glucosinolates in the extracted meals. More than 50% of the phytic acid contained in the three rapeseed meals is removed by acidic extraction, resulting in the reduction of phytic acid content by about one-third (Table II).

The following experiments were designed to establish the conditions that would provide high yields of protein

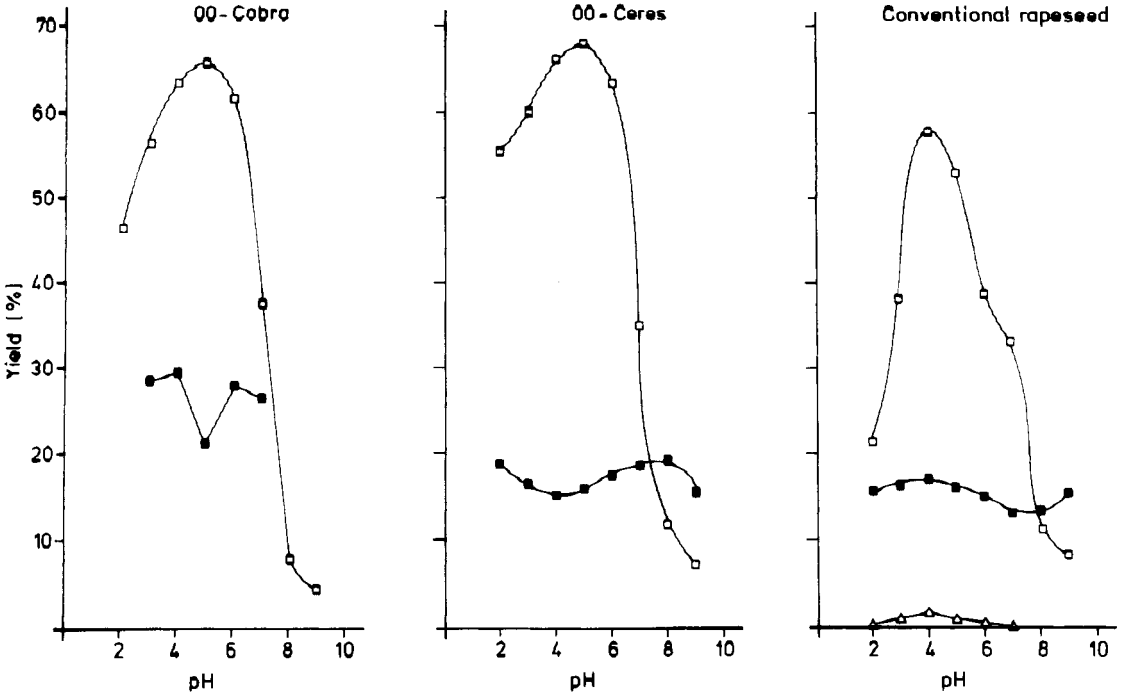

Figure 2. Yield of protein (□), phytic acid (■), and glucosinolates (△) by precipitation at various pHs from countercurrent extracts of rapeseed meal.

Table V. Amino Acid Composition of Defatted Rapeseed Meals and Rapeseed Protein Isolates[a]

amino acid	rapeseed meal			protein isolate			human requirement
	00-Ceres	00-Cobra	conventional	00-Ceres	00-Cobra	conventional	
lysine	7.2	6.6	6.1	6.0	5.9	7.2	4.2
histidine	3.8	3.5	3.3	3.6	3.4	3.3	2.4
arginine	8.3	7.5	7.7	8.6	8.2	8.6	2.0
threonine	5.2	5.0	4.6	5.7	5.3	5.0	3.3
valine	5.2	5.5	4.9	5.0	5.5	5.1	4.2
methionine	2.2	2.0	2.4	2.6	2.6	2.6	1.7
							3.4[b]
cystine	1.3	2.0	1.7	1.7	1.5	0.4	
isoleucine	4.5	4.1	4.1	5.1	4.5	4.7	4.2
leucine	8.1	7.5	7.5	8.8	8.5	8.3	4.8
phenylalanine	4.0	3.6	4.1	5.2	4.5	5.0	
							6.0[c]
tyrosine	3.8	3.3	2.7	5.1	4.0	4.1	

[a] Grams/16 g of N. [b] Total sulfur-containing amino acids. [c] Phenylalanine + tyrosine.

isolates containing little phytic acid and virtually no glucosinolates. An established approach for the preparation of protein isolates involves exhaustive extraction of protein from the defatted meal and its subsequent recovery by isoelectric precipitation (Gillberg and Törnell, 1976a,b; El Nockrashy et al., 1977; Blaicher et al., 1983). In order to separate the protein from the meal, the defatted rapeseed meals were subjected to three-stage countercurrent extraction using aqueous sodium hydroxide. The pH of the solvent during extraction was maintained at 12 for the 00-rapeseed meals and 13 for the conventional rapeseed meal, because in this pH region the solubility of protein is high and that of phytic acid and glucosinolates is rather low (Figure 1). The results of countercurrent extraction, given in Table III, show that well over 90% of the protein is extracted from each of the three rapeseed meals. Simultaneously, very little glucosinolate (<10% of that present in conventional rapeseed) and about one-third of the phytic acid present in each of the three meals are extracted (Table III).

Aliquots of the extracts obtained in the experiments given in Table III were adjusted to different pH values, and the amounts of protein and phytic acid as well as glucosinolates (only for conventional rapeseed meal) precipitated at each pH were determined in order to establish the optimal conditions for preparation of protein isolates. The results, given in Figure 2, show for the defatted meals from both varieties of 00-rapeseed that the maximum precipitation of protein (65–70%) occurs at pH 5; for conventional rapeseed the maximum precipitation of protein occurs at pH 4. The data presented in Figure 2 also show that the pH has only a minor effect on the extent of coprecipitation of either phytic acid or glucosinolates.

On the basis of the data presented in Table III and Figure 2, protein isolates were prepared from defatted meals of 00-rapeseed or conventional rapeseed, by countercurrent extraction at pH 12 or 13, respectively, followed by precipitation from the extract at pH 5 or 4. The data given in Table IV show that protein isolates containing over 90% protein are obtained in good yields (about 60%) from both 00-rapeseed and conventional rapeseed. These protein isolates contain substantially lower proportions of phytic acid as compared to protein isolates described elsewhere (Blaicher et al., 1983). Glucosinolates are not detectable in the protein isolates.

The amino acid compositions of the rapeseed meals and the rapeseed protein isolates prepared therefrom are given in Table V. These data show that all three rapeseed meals and the corresponding protein isolates have more than adequate levels of the essential amino acids with the exception of cystine in the protein isolate prepared from conventional rapeseed.

The data presented in this study show that the new 00-varieties of rapeseed are suitable for the production of protein-rich products with desirable composition for dietary use. Similar products are already permitted in foods in a number of countries (Wolf, 1989).

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