Extraction of Seed Oils with Supercritical Carbon Dioxide: Effect on Residual Proteins

Egon Stahl, Karl W. Quirin, and Robert J. Blagrove*[1]

Vegetable oils can be extracted from seed meals with supercritical carbon dioxide at relatively low temperatures without the need to remove traces of solvent by heating. Despite the inert nature of carbon dioxide, its possible interaction with the proteins of the residual meal must be investigated. The isolation and characterization by gel electrophoresis of proteins from various seed meals of commercial interest exposed to supercritical CO_2 are described. No significant difference, compared with meals extracted with hexane, was observed by amino acid analysis. The major protein subunits were not cross-linked, while the degradation by trypsin of extracted lupineseed globulins was not enhanced by treatment with CO_2, indicating minimal denaturation.

Pressing of oilseeds, followed by extraction with organic solvents, is used widely in the production of vegetable fats and oils. Recent reports (Stahl et al., 1980, 1981, 1982; Friedrich and List, 1982) have demonstrated the feasibility of introducing extraction with supercritical carbon dioxide in place of hexane. While the parameters that influence oil yield and quality have been investigated, no attention has been paid to the residual protein-rich meal. Stahl et al. (1980) have postulated that extraction with carbon dioxide at ambient temperature will lead to less denaturation of the seed proteins than hot organic solvents; this would be an advantage if a soluble protein isolate is to be recovered from the meal, so long as a subsequent severe heat treatment is not required to inactivate antinutritional factors.

In order to determine the likely influence of supercritical CO_2 on food constituents, Weder (1980) studied its effect on the composition, structure, and properties of ribonuclease. This protein was chosen for testing because heat-induced alterations of its structure have been well documented. When commercial ribonuclease was treated with wet CO_2 at 300 bars, both at room temperature and at 80 °C, the amino acid composition was unaffected but sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis showed some cross-linking of the ribonuclease molecule through intermolecular disulfide bonds. At the same time, there had been partial cleavage of peptide bonds within the disulfide loops of the molecule. Wet nitrogen under the same conditions produced similar effects; Weder related the observed fragmentation to the presence of water.

Tryptic digestion of the ribonuclease samples (Weder, 1980) confirmed that unfolding had occurred to an extent influenced by the temperature and by the presence of water. While these studies indicate relatively minor effects of supercritical CO_2 on proteins, the introduction of disulfide cross-links and the cleavage of peptide bonds could negate the advantages foreseen (Stahl et al., 1980) in the use of CO_2 for extraction of vegetable fats and oils. Ribonuclease does contain significantly more cystine than most oilseed proteins and may behave differently as a consequence. The present report is concerned with the effect of supercritical CO_2 on the proteins in a number of oilseed meals of commercial interest.

EXPERIMENTAL SECTION

Materials. The soya flakes were from Lucas Meyer GmbH, Hamburg, West Germany. Seeds of soya, *Glycine max* var. Corsoy, were obtained from the American Soybean Council, St. Louis, MO, those of *Lupinus Mutabilis* (a bitter, white-seeded type, 1978 crop from Southern Peru) from Holtz and Willemsen, Krefeld, West Germany, those of cotton, *Gossypium barbardense* var. Giza 70, from the Bundesanstalt für Fettforschung, Münster, West Germany, and those of jojoba, *Simmondsia chinensis*, from the Jojoba Marketing Corp., Interagro GmbH, München, West Germany. The seeds were crushed for 30 s in a laboratory mill, Krups type 202, to a size of 20–70 mesh.

Methods. The solvent power of compressed gases is a well-known phenomenon that depends on the fact that, at temperatures just above the critical temperature of a gas, liquidlike densities are approached for pressures in the range of 1–3 times the critical pressure. Extraction using dense gases as the solvent is a simple procedure. In the present experiments, gaseous carbon dioxide from a storage tank was condensed by a diaphragm compressor at a pressure of 350 bars, brought to the desired temperature of 40 °C by passage through a heat exchanger and subsequently led into the extraction vessel containing the crushed seeds. The gas extracts a certain amount of the oil in such a sample depending on the pressure and the temperature (Stahl et al., 1980). The dissolved oil was transported to a receiving vessel and was recovered from the gaseous phase by lowering the pressure. The gas released was led off through a flow meter and condensed again by the compressor, thus completing the cycle. The soya flakes and the ground seeds were treated with supercritical CO_2 for 2 h. The residual oil was extracted with hexane in a Soxhlet apparatus for 6 h and the content determined gravimetrically; values for the seed meals were soya 7.5%, lupin 5.8%, cotton, 4.1%, and jojoba 5.1% compared with 0.6% for the soya flakes.

A series of control meals for amino acid analysis and protein extraction under nondenaturing conditions were prepared by extraction with hexane (25 mL/g × 2) at 20 °C for 18 h. For comparison, a sample of lupineseed meal was extracted with hexane in a Soxhlet for 16 h. The major storage proteins were extracted from each of the CO_2-treated and control meals under a variety of conditions and were compared by using various SDS–polyacrylamide gel electrophoresis systems (Weber and Osborn, 1975). (1) The proteins were dissociated and denatured by extraction

University of Saarland, Department of Pharmacognosy and Analytical Phytochemistry, D-6600 Saarbrücken, West Germany (E.S. and K.W.Q.), and Federal Center for Lipid Research, Institute for Biochemistry and Technology, H. P. Kaufmann—Institute, D-4400 Münster, West Germany (R.J.B.).

[1]Present address: CSIRO Division of Protein Chemistry, 3052 Victoria, Australia.

0021-8561/84/1432-0938$01.50/0 © 1984 American Chemical Society

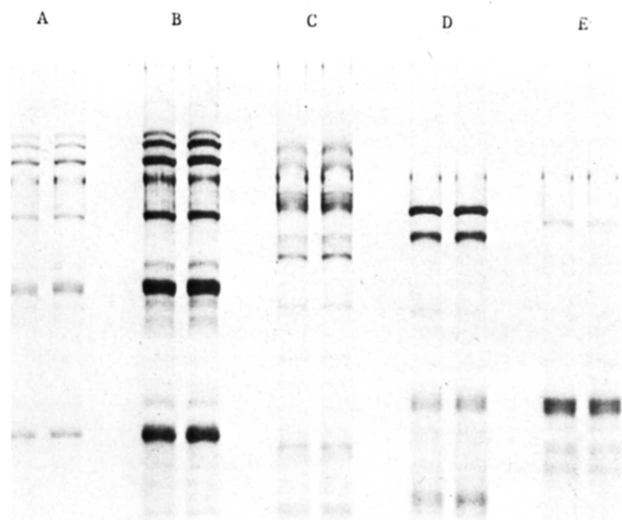

Figure 1. SDS-polyacrylamide gel electrophoresis [see Methods, (2)] of reduced proteins extracted from (A) soybean, (B) soya flakes, (C) lupineseed, (D) cottonseed, and (E) jojoba. Left-hand side: control. Right-hand side: treated with supercritical CO_2.

in 2% SDS-0.025 M sodium phosphate buffer, pH 7.2 (50 mL/g, 75 °C, 15 min), the residue was filtered off, and the protein subunit composition was compared by electrophoresis in a continuous buffer system (0.05% SDS-0.025 M sodium phosphate buffer, pH 7.2) using both homogeneous 8% polyacrylamide gels and 4-30% polyacrylamide gradient gels (Pharmicia). (2) The proteins were dissociated and denatured by extraction in 2% SDS-0.06 M Tris-HCl, pH 6.8 (50 mL/g, 75 °C, 15 min), the residue was filtered off, and the protein subunit composition was compared by electrophoresis in a discontinuous buffer system (0.1% SDS-0.037 M Tris-glycine, pH 8.9) using 15% polyacrylamide gels, before and after reduction of disulfide bonds (5% 2-mercaptoethanol). (3) The denatured and reduced polypeptide chains were extracted directly into 10% 2-mercaptoethanol-2% SDS-0.06 M Tris-HCl, pH 6.8 (50 mL/g, 75 °C, 15 min), the residue was filtered off, and the polypeptides were compared as in (2) above. (4) The proteins from lupineseed were recovered under nondenaturing conditions; the meals were first extracted with distilled water (40 mL/g, 20 °C, 1 h) and sequentially with 1 M NaCl-0.2M Tris-HCl, pH 8.0 (40 mL/g, 20 °C, 1 H). The proteins in each fraction were compared as in (2) above. (5) The salt-soluble lupineseed globulins were extracted at low ionic strength (0.1 M NaCl-0.1 M Tris-HCl, pH 8.0) from a water-washed meal and digested for 5 or 50 min with trypsin at 20 °C (protein-enzyme, 30:1). To minimize destruction of amino acids by other constituents of the meals, the samples for amino acid analysis were hydrolyzed at high dilution (Pusztai and Morgan, 1963) before analysis on a modified Beckman 120C analyzer.

RESULTS AND DISCUSSION

The treatment with supercritical CO_2 used in this study did not completely remove the oils and waxes from the seed meals (see Methods). This was evident from the turbidity of the oil in water emulsions formed during direct extraction of the samples with the various SDS solutions. However, these components did not interfere with the electrophoretic separation of the extracted proteins.

Extraction of the proteins from soya flakes and soybean, lupine, cotton, and jojoba seed meals under denaturing and dissociating conditions, viz., 2% SDS solutions at 75 °C, with or without reduction of disulfide bonds, showed no significant difference in the relative proportions of the subunits or reduced polypeptides recovered from CO_2-treated meals compared with their respective controls (see, for example, Figure 1). Nor was there any evidence of protein cross-linking, which would result in the appearance of higher molecular weight, lower mobility, bands on the SDS gels. This is in contrast to the report by Weder (1980), which showed that ribonuclease was partially cross-linked when exposed to carbon dioxide at high pressure and elevated temperature.

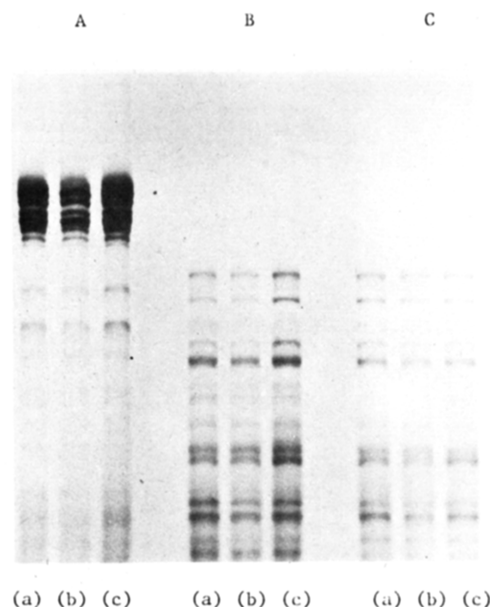

Figure 2. SDS-polyacrylamide gel electrophoresis [see Methods, (2)] of lupineseed proteins: (A) unreduced proteins; (B) trypsin, 5 min; (C) trypsin, 50 min. (a) Extracted with hexane, 20 °C; (b) treated with supercritical CO_2; (c) extracted with hexane in a Soxhlet.

The proteins recovered from lupineseed meal under nondenaturing conditions, both the water and subsequent salt extracts, were very similar in subunit composition for material prepared by room temperature or Soxhlet hexane extraction and CO_2 treatment. Since the relative amounts of protein extracted and the gel banding patterns observed were nearly identical, one can conclude that treatment with supercritical CO_2 caused little denaturation (evidenced by loss of solubility) and no significant cross-linking. The slight decrease in the amount of protein extracted from the CO_2-treated meal could indicate that the protein body membranes have been damaged less by this treatment than by washing with hexane.

The pattern of tryptic digestion at 5 and 50 min for the storage globulins isolated from CO_2-treated lupineseed meal was identical with that for hexane-treated meals (Figure 2). This result reinforces the conclusion reached above that little denaturation has occurred and that the proteins are in their native conformation. Fukushima (1968) has shown a linear increase in the initial rate of enzymic digestion of a structurally related soybean globulin, glycinin, with increasing alkali denaturation. Likewise, a dramatic difference in the extent of tryptic digestion of soybean glycinin in the time scale used above has been found (Lynch et al., 1977) for both acid- and alkali-denatured material. On the other hand, extensive heat denaturation (Richardson and Catsimpoolas, 1979) slows the initial rate of tryptic degradation of glycinin by a factor of 8, apparently due to the effect of heat-induced aggregation, which hinders enzymic attack. Nevertheless, denaturation has always been associated with significant

Table I. Amino Acid Analyses (Percent Residues) of Supercritical CO_2- and Hexane-Extracted Seed Meals

amino acid	soybean		lupine		jojoba	
	hexane	CO_2	hexane	CO_2	hexane	CO_2
Lys	5.7	5.7	5.2	5.2	4.3	4.4
His	2.2	2.2	2.4	2.4	1.7	1.7
Arg	5.7	5.8	8.0	7.9	5.6	5.5
Asp	12.0	12.0	10.4	10.3	10.0	9.8
Thr	4.5	4.5	4.4	4.3	6.0	5.9
Ser	6.9	7.0	7.0	7.0	6.9	6.8
Glu	16.5	16.4	21.6	21.5	10.4	10.5
Pro	6.2	6.2	5.0	4.9	6.0	5.9
Gly	7.7	7.7	7.5	7.5	16.2	16.2
Ala	6.6	6.6	5.5	5.5	6.3	6.3
$^1/_2$-Cys	1.5	1.4	1.4	1.6	3.5	3.5
Val	4.7	4.7	3.9	4.0	5.4	5.5
Met	1.1	1.1	0.5	0.5	0.9	0.9
Ile	4.1	4.1	4.0	4.1	3.1	3.3
Leu	7.8	7.7	7.0	7.0	6.8	6.9
Tyr	2.8	2.8	3.2	3.3	3.6	3.6
Phe	4.0	4.0	3.0	3.0	3.3	3.3

changes in the initial rate of enzymic hydrolysis of seed globulins. Hence, we conclude that treatment with supercritical CO_2 at 350 bars and 40 °C for 2 h did not denature the lupineseed proteins.

Amino acid analysis (Table I) showed no significant differences between the control meals and their CO_2-treated counterparts. The jojoba meal has an unusual composition; it is relatively low in glutamic acid plus glutamine and high in cystine and glycine compared with the other seed meals. These differences are presumably related to the virtual absence of salt-soluble globulins; the major subunits of the jojoba proteins extracted with water or salt solutions are of low molecular weight (see Figure 1) compared with other oilseed proteins.

The constant amino acid composition, coupled with the evidence above that there has been a negligible influence on protein solubility and enzymic digestibility, leads us to conclude that treatment with supercritical CO_2 at 40 °C will have no deleterious effects on the nutritional value of oilseed meals.

ACKNOWLEDGMENT

We thank Dr. C. M. Roxburgh, CSIRO Division of Protein Chemistry, for the amino acid analyses and Professor H. K. Mangold for providing research facilities.

LITERATURE CITED

Friedrich, J. P.; List, G. R. *J. Agric. Food Chem.* **1982**, *30*, 192.
Fukushima, D. *Cereal Chem.* **1968**, *45*, 203.
Lynch, C. J.; Rha, C. K.; Catsimpoolas, N. *J. Sci. Food Agric.* **1977**, *28*, 971.
Pusztai, A.; Morgan, W. T. J. *Biochem. J.* **1963**, *88*, 546.
Richardson, D. P.; Catsimpoolas, N. *J. Sci. Food Agric.* **1979**, *30*, 463.
Stahl, E.; Quirin, K. W.; Mangold, H. K. *Fette, Seifen, Anstrichm.* **1981**, *83*, 472.
Stahl, E.; Quirin, K. W.; Mangold, H. K. *Chem. Phys. Lipids* **1982**, *31*, 219.
Stahl, E.; Schütz, E.; Mangold, H. K. *J. Agric. Food Chem.* **1980**, *28*, 1153.
Weber, K.; Osborn, M. In "The Proteins"; Neurath, H.; Hill, R. L., Eds.; Academic Press: New York, 1975; Vol. 1, p 180.
Weder, J. K. P. *Z. Lebensm.-Unters.-Forsch.* **1980**, *171*, 95.

Received for review August 4, 1983. Accepted November 21, 1983.

Chemical Constitution of Starch and Oligosaccharide Components of "Desi" and "Kabuli" Chickpea (*Cicer arietinum*) Seed Types

Harpal S. Saini* and Edward J. Knights

Investigations on the chemical constitution of the chickpea seed types "desi" and "kabuli" have been performed. The breeding lines of the desi types contain higher levels of acid detergent fibers (9.4–14.7%; cf. 3.8–7.6% for kabuli) and higher average seed coat weights, but there appeared to be no difference in the total protein content. Variations have been noted in the total starch and percent amylose contents of desi and kabuli type seeds. The levels of the raffinose-series oligosaccharides were higher in kabuli than in desi types. Soluble sugar profiles revealed a substantial difference in the distribution of sucrose in the two types. Stachyose was the predominant sugar in desi types, but the majority of the kabuli types indicated sucrose as the main component. On the average, oligosaccharides did not show any differences in the two types although the sucrose content of kabuli types was 46.9% higher than the desi types. Verbascose represented only a small fraction (range 0.09–0.41 g/100 g of seed weight) of the total soluble sugars. Quantification of oligosaccharides and amylose offers a useful criteria for identification of genotypes of high nutritional quality.

Grain legumes are an important component of both human and livestock diets. In addition to complementing cereal protein, grain legumes also make a significant contribution to total energy intake. The quality as well as the quantity of grain legume carbohydrates is thus a major consideration in the development of new cultivars that have desirable nutritional properties.

Starch is often the major component of many grain legumes (Naivikul and D'Appolonia, 1979; Lineback and Ke, 1975). On ingestion, salivary α-amylase splits the starch molecules to liberate glucose to provide energy. However, the digestibility and hence the energy value of starch are often determined by the branching of the starch molecule and its ability to interact with the hydrolytic enzymes (Geervani and Theophilus, 1981).

New South Wales Department of Agriculture, Agricultural Research Institute, Wagga Wagga, New South Wales 2650, Australia.

0021-8561/84/1432-0940$01.50/0 © 1984 American Chemical Society