

The reversible nature of binding of gossypol to these proteins strongly suggests that only noncovalent interactions are involved. The low binding constants (Table I) also suggest that the binding is of a weak type and involves noncovalent interactions. However, covalent interaction between gossypol and cottonseed proteins was observed by earlier workers (Clark, 1928; Markman and Rzhikhin, 1965; Damaty and Hudson, 1979), where more drastic conditions such as high temperatures (and pressures) were used. Also estimation of "available" lysine was used to follow the interaction. We are unable to comment on the sensitivity of this method. Possibly the drastic conditions facilitate covalent interaction.

The higher binding constant ( $4.17 \times 10^3 \text{ M}^{-1}$ ) in the case of glycinin indicates that the affinity of the protein for gossypol is greater than that of gossypin and congossypin, whose binding constants are almost the same. This may not be due to any gossypol bound to cottonseed proteins in situ.

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Registry No. Gossypol, 303-45-7.

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## Characterization of Sweet Potato Stillage and Recovery of Stillage Solubles by Ultrafiltration and Reverse Osmosis

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Sweet potatoes were fermented to ethanol. After ethanol was distilled, residual stillage was separated into filter cake, centrifuged solids, and stillage solubles. The protein in filter cake was much less soluble than that in sweet potato. Of the nitrogen in stillage solubles, 91% passed through a 10 000 molecular weight cutoff membrane. Permeate from stillage solubles processed by combined ultrafiltration and reverse osmosis had much lower nitrogen, solids, and ash contents than that of stillage solubles. Thus, ultrafiltration combined with reverse osmosis can be used to recover sweet potato stillage solubles for potential food or feed uses while providing a permeate that can be reused for water or safely discarded.

Sweet potato (*Ipomoea batatas*) is one of the most promising crops for energy production; Jones et al. (1983) estimated yields of 570-760 and 712-1140 gal of ethanol/acre for Jewel and HiDry sweet potatoes, respectively. Matsuoka et al. (1982) reported alcohol fermentation of

raw sweet potato in a one-step process. Chua et al. (1984) used no heating or low-temperature heating to convert sweet potato starch for ethanol fermentation. Wu and Bagby (1987) reported effects of commercial pectinases on viscosities of sweet potato slurries before fermentation and on maximum ethanol concentrations and presented proximate and amino acid compositions of fermentation products from sweet potatoes with normal (18-24%), relatively high (27-30%), and very high (35% and up) dry-matter contents.

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Sweet potato stillage solubles contain 4–6% dry matter, and evaporation of water to recover solids is expensive. Ultrafiltration (UF) and reverse osmosis (RO) do not evaporate water; therefore, large savings of energy and cost can be achieved by these methods. UF and RO can separate a large volume of dilute solution into a small volume of concentrated solution and a large volume of permeate that can be reused as water or safely disposed. RO of stillage solubles from corn, without prior UF to remove the larger molecules, resulted in leakage of the RO column (Wu et al., 1983). UF combined with RO was used to process stillage solubles from dry-milled corn fractions (Wu and Sexson, 1985), sorghum (Wu and Sexson, 1984), and barley (Wu, 1986). This paper reports the protein solubility and molecular size of fermented products from sweet potatoes and the use of UF combined with RO to recover sweet potato stillage solubles for potential food or feed uses.

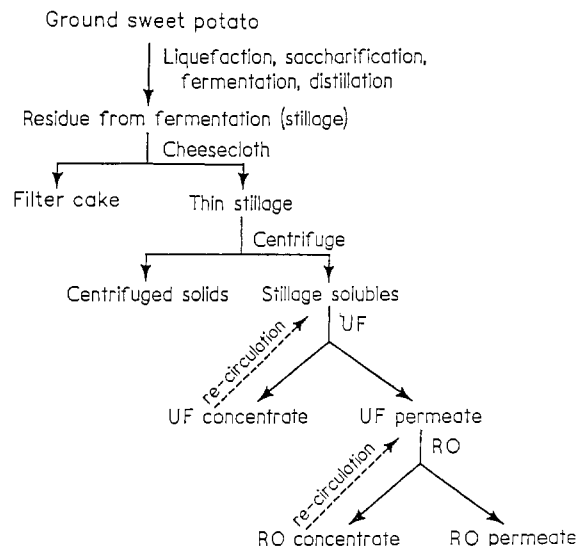
#### MATERIALS AND METHODS

**Fermentation.** Jewel is the normal commercial sweet potato for table use. Sumor has relatively high dry-matter and HiDry has very high dry-matter content and appears most desirable for ethanol fermentation (Wu and Bagby, 1987). Sweet potatoes were ground in a food processor upon arrival from South Carolina and stored at  $-18^{\circ}\text{C}$ . For fermentation without pectinase, tap water was added to ground wet sweet potatoes to form a slurry that can be stirred in a stainless-steel, temperature-controlled, jacketed fermentor equipped with stirrers. HCl (6 M) and NaOH (12.5 M) were used for pH adjustment. The slurry was adjusted to pH 6.2, Taka-Therm  $\alpha$ -amylase (Miles Laboratories, Elkhart, IN) was added, and the temperature was raised to  $90^{\circ}\text{C}$ . The mixture was stirred for 1 h. The slurry was cooled to  $60^{\circ}\text{C}$ , slurry pH adjusted to 4.0, and Miles Diazyme L-100 glucoamylase was added and stirred for 2 h. The mixture was cooled to  $30^{\circ}\text{C}$ , slurry pH adjusted to 4.5, and 500 mL of yeast (*Saccharomyces cerevisiae*) containing 5 million cells/mL added. Samples were withdrawn at 0, 24, 48, and 66 h, when fermentation was stopped. Nitrogen from yeast, amylase, and glucoamylase accounted for 8–10% of total sweet potato nitrogen.

For substrate preparation with Clarex L pectinase (Miles Laboratories), tap water was added to ground, wet sweet potatoes, to get a slurry with about 20% dry-matter content. The slurry was adjusted to pH 3.5, and 3 mL of Clarex L/1000 g of wet sweet potatoes was added to decrease the slurry viscosity during 2 h at  $50^{\circ}\text{C}$  with stirring. Then, Taka-Therm  $\alpha$ -amylase, Diazyme L-100, and yeast were added as described above. Nitrogen from pectinase, amylase, glucoamylase, and yeast accounted for 4–9% of total sweet potato nitrogen. Additional details of the fermentation procedure were reported previously (Wu and Bagby, 1987; Wu and Sexson, 1984). Figure 1 is a schematic diagram of the whole process including the fermentation, fractionation of stillage, and UF and RO recovery.

**Fractionation of Stillage.** After distillation of alcohol, fermentation residue (stillage) was filtered through cheesecloth under suction. Material that remained on the cheesecloth was filter cake, and the thin stillage passing through the cheesecloth was centrifuged in a continuous centrifuge to yield centrifuged solids and stillage solubles. More details were reported previously (Wu and Bagby, 1987; Wu and Sexson, 1984).

**Protein Extraction.** Nonprotein nitrogen of fresh Jewel sweet potato was determined by blending with 13% trichloroacetic acid and analyzing nitrogen in the supernatant after centrifugation and filtering as described by Purcell et al. (1978). Nonprotein nitrogen of the water



**Figure 1.** Schematic diagram of sweet potato fermentation, fractionation of stillage, and UF and RO recovery.

extract of sweet potato was determined by mixing equal volumes of 20% trichloroacetic acid with water extract, centrifuging, and analyzing nitrogen in the supernatant. Fresh Jewel sweet potato (20 g) was put in a stainless-steel cup with 50 mL of solvent and blended for 5 min in a Waring Blender. The slurry was then centrifuged at  $10400g$  for 10 min, the supernatant decanted, and the residue extracted with the next solvent. Two sequential extraction methods were used. Solvents in method 1 were water (2 $\times$ ), 1% sodium chloride, 70% ethanol, and borate + 0.1% dithiothreitol (DTT) + 0.5% sodium dodecyl sulfate (SDS) at pH 10.5. The borate solution was made from 500 mL of 0.05 M sodium tetraborate, 430 mL of 0.2 N sodium hydroxide, and 49.66 g of sodium chloride without any pH adjustment. For Sumor sweet potato, 2 g of freeze-dried material and 20 mL solvent were used. For sweet potato filter cake, 1.1 g of oven-dried ( $80^{\circ}\text{C}$ ) sample and 100 mL solvent were used. Solvents used in sequential extraction method 2 were water, 1% sodium chloride, 70% ethanol, 0.1 N sodium hydroxide + 0.1% DTT at pH 12.5, and 0.1 N sodium hydroxide + 0.5% SDS + 0.1% DTT, pH 12.5. Each supernatant and the final residue were analyzed for nitrogen, and the amount and percent of nitrogen for each fraction were calculated.

**Fractionation of Stillage Solubles.** An Amicon Model 52 ultrafiltration cell with 43-mm-diameter membranes under 50 psi of nitrogen pressure was used. UMO5 and PM10 membranes were used, having nominal molecular weight cutoffs (MWCO) of 500 and 10000, respectively (Amicon, 1972). Stillage solubles (15 mL) were introduced above each membrane, nitrogen pressure was applied, and 60 mL of permeate (solution below the membrane) was collected by adding distilled water above the membrane continuously or batchwise.

**UF and RO.** An OSMO Econo Pure RO unit (Osmonics, Inc., Minnetonka, MN) equipped with OSMO-112 Separators (1.0- $\text{m}^2$  membrane, hold-up volume about 600 mL) was used for UF at 100 psi with a SEPA-O cellulose acetate (CA) membrane, which has a MWCO of 1000 for organic compounds. The solution that passed through the membrane is permeate, and the solution retained by the membrane is concentrate. The concentrate stream was recirculated back to the initial solution. The flow rate of UF permeate was 20 L/ $\text{m}^2$  per h.

A Model UHPROLA-100 RO system (Village Marine Tec., Gardena, CA) equipped with a SW 30-2521 module with 1.1- $\text{m}^2$  polyamide (PA) membrane (Filmtec Corp.,

**Table I. Protein Fractions of Sweet Potato and Sweet Potato Filter Cake<sup>a</sup>**

fraction	% of total N			
	Jewel	Sumor	Jewel FC	Sumor FC
method 1				
water extr	64	80	13	11
1% NaCl extr	4	2	4	4
70% ethanol extr	1	1	3	1
borate + 0.5% SDS + 0.1% DTT extr, pH 10.5	9	2	32	21
residue	13	9	58	54
method 2				
water extr			13	
1% NaCl extr			4	
70% ethanol extr			3	
0.1 N NaOH + 0.1% DTT extr, pH 12.5			37	
0.1 N NaOH + 0.5% SDS + 0.1% DTT extr, pH 12.5			10	
residue			20	

<sup>a</sup>Nonprotein nitrogen of Jewel sweet potato was 31% of total nitrogen. Key: FC = filter cake; DTT = dithiothreitol; SDS = sodium dodecyl sulfate.

**Table II. Nitrogen Distribution and Content of Jewel Sweet Potato Stillage Solubles**

membrane	approx MW	fraction	% of total N	N content, % dry basis
UM05	<500	permeate	77	6.61
	>500	concentrate	23	1.96
PM10	<10000	permeate	91	4.11
	>10000	concentrate	9	2.16

Minneapolis, MN) was used for RO at 800 psi at room temperature. The hold-up volume of the membrane module is 605 mL. UF permeates from sweet potato stillage solubles were used as feed solutions for RO. The concentrate stream was recirculated back to the initial solution. Samples of concentrate plus initial solution (termed concentrate subsequently) and permeate were taken for analyses. The RO permeate flow rate was 13.5 L/m<sup>2</sup> per h for the first 7 fractions of Jewel and 15.5 L/m<sup>2</sup> per h for HiDry, averaged over 10 fractions. Additional details of UF and RO were reported previously (Wu et al., 1983).

For each UF and RO experiment, amounts of nitrogen, solids, and ash in permeate, concentrate, hold-up, and wash fractions were determined, and percent recovery was calculated on the basis of stillage solubles for UF and on the UF permeate for RO. For UF and RO, average percent recoveries of nitrogen, solids, and ash were 98–100.

**Analyses.** Nitrogen in quadruplicate and ash contents in duplicate were determined by AACC *Approved Methods* (1983). Solids content (dry matter) of solution was determined in duplicate by pipeting a known volume into a previously weighed crucible, drying overnight in an air oven at 100 °C and then for 3 days in a vacuum oven at 100 °C, and weighing. Conductivity of stillage fractions

was measured with a Radiometer type CDM 2e conductivity meter with a CDC 104 NS cell.

## RESULTS AND DISCUSSION

**Protein Fractions of Sweet Potato and Sweet Potato Filter Cake.** The largest nitrogen fraction for both Jewel and Sumor sweet potatoes was water extract (Table I). Nonprotein nitrogen accounted for 31% of total Jewel sweet potato nitrogen and 41% of total nitrogen of Jewel water extract. In Jewel sweet potato, 85% of all the nonprotein nitrogen was in the water extract. Walter et al. (1984) reported that sweet potatoes at harvest contain from 15 to 35% nonprotein nitrogen. The main components of the nonprotein nitrogen fraction for Jewel sweet potato after 107 days of storage were asparagine, aspartic acid, glutamic acid, serine, and threonine (Purcell and Walter, 1980); the remaining nonprotein nitrogen fraction contained small amounts of the other amino acids and ammonia. Since the amino acids, which accounted for most of the nonprotein nitrogen of Jewel sweet potato, are soluble in water, most of the nonprotein nitrogen in Jewel is soluble in water and will be in the water extract.

Water, 1% sodium chloride, 70% ethanol, and borate + SDS + DTT extracted albumins, globulins, prolamins, and glutelin, respectively (Table I). Albumin is the largest protein fraction for Jewel sweet potatoes after nonprotein nitrogen was subtracted from water extract. Sweet potato filter cakes had much lower albumin but higher glutelin contents; more than half the nitrogen remained unextracted in method 1. More alkaline conditions (method 2) were needed to extract most of the protein from Jewel sweet potato filter cake (Table I). Both sweet potatoes and sweet potato filter cakes were low in prolamin. The low protein solubility of sweet potato filter cake suggested that protein was denatured during fermentation or by heating.

**Nitrogen Distribution and Content of Sweet Potato Stillage Solubles.** Jewel sweet potato stillage solubles were fractionated by two membranes according to molecular weight (Table II). With the UM05 membrane, permeate accounted for 77% of the total nitrogen. Also, permeate had considerably higher nitrogen content than concentrate for both membranes. With PM10 membrane, only 9% of total nitrogen was in the concentrate; this small percentage indicated that most nitrogenous materials in stillage solubles were amino acids and peptides, since Walter et al. (1984) showed that about 90% of all nonprotein nitrogen of Jewel sweet potato was from amino acids. About the same MWCO results were obtained for corn stillage solubles when PM10 membrane, Millipore membrane (Millipore Corp., Bedford, MA) with MWCO of 10000, and dialysis tubing were compared (Wu et al., 1981).

**UF and RO of Jewel Sweet Potato Stillage Solubles.** Concentrations of nitrogen, solids, and ash in UF permeate were about two-thirds those of stillage solubles (Table III). The nitrogen, solids, and ash concentrations of RO permeate decreased to 0.17, 0.45, and 0.48%, re-

**Table III. Ultrafiltration and Reverse Osmosis of Jewel Sweet Potato Stillage Solubles<sup>a</sup>**

	vol, mL	concentration, mg/mL		
		N	solids	ash
stillage solubles	4720	1.41	60.1	23.1
permeate (UF)	4536	0.936	40.0	17.4
concentrate (UF)	156	2.00	88.5	21.4
permeate (RO), range, 10 fractions	3344	0.0016	0.182	0.084
	330–340	0.00063–0.0062	0.036–0.575	0.0021–0.325
concentrate (RO), range, 9 fractions	898	1.05	45.0	20.4
	98–100	0.743–1.59	31.9–63.6	13.9–34.5

<sup>a</sup>In addition to permeate and concentrate, hold-up and wash fractions were also collected for both UF and RO.

Table IV. Ultrafiltration and Reverse Osmosis of HiDry Sweet Potato Stillage Solubles<sup>a</sup>

	vol, mL	concentration, mg/mL		
		N	solids	ash
stillage solubles	4290	0.500	39.8	13.9
permeate (UF)	4256	0.305	25.1	10.4
concentrate (UF)	237	0.785	61.7	12.8
permeate (RO), range, 10 fractions	3128	0.0021	0.0882	0.0194
	275-340	0.0013-0.0028	0.031-0.121	0.0057-0.0294
concentrate (RO), range, 9 fractions	810	0.344	28.3	11.9
	90	0.241-0.530	19.2-42.1	7.67-17.7

<sup>a</sup>In addition to permeate and concentrate, hold-up and wash fractions were also collected for both UF and RO.

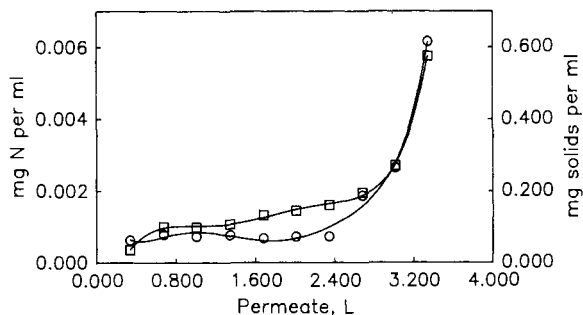


Figure 2. Nitrogen (O) and solids (□) concentrations of permeate during RO (800 psi) of UF permeate from Jewel stillage solubles.

spectively, of concentrations in UF permeate.

Nitrogen and solids concentrations of the RO permeate of UF permeate from Jewel stillage solubles (Figure 2) indicated slow increases in nitrogen and solids concentrations during the first two-thirds of the RO process, but then increased more rapidly. Nitrogen and solids concentrations of RO concentrate, however, increased at a relatively constant rate during the entire RO process (data not shown). For this and subsequent experiments (Tables III and IV), the lower number in each range of nitrogen, solids, or ash concentration in Table III was the value of the first RO fraction, and the higher number was that of the last fraction. The RO permeate contained 76% of the total volume, 0.13% of total nitrogen, 0.34% of total solids, and 0.36% of total ash of the UF permeate from Jewel stillage solubles (Table III). Similarly, the RO permeate contained 73% of the total volume, 0.083% of total nitrogen, 0.22% of total solids, and 0.26% of total ash of Jewel stillage solubles.

**UF and RO of HiDry Sweet Potato Stillage Solubles.** Concentrations of nitrogen, solids, and ash in UF permeate were approximately two-thirds those of stillage solubles (Table IV). The nitrogen, solids, and ash concentrations of RO permeate decreased to 0.69, 0.35, and 0.19%, respectively, of concentrations in UF permeate.

Nitrogen and solids concentrations of RO concentrate during RO of UF permeate from HiDry stillage solubles (Figure 3) showed that nitrogen and solids concentrations near the end of RO increased somewhat faster than during the first two-thirds of RO. Nitrogen and solids concentrations of RO permeate during RO of UF permeate from HiDry stillage solubles increased at a relatively constant rate during RO (data not shown). The RO permeate contained 76% of the total volume, 0.52% of total nitrogen, 0.27% of total solids, and 0.14% of total ash of the UF permeate.

**Conductivity of RO Permeate and Concentrate.** Solids and ash concentrations of RO permeate and concentrate are linearly related to conductivity for Jewel and HiDry sweet potatoes. Correlation coefficients of conductivity versus milligrams of ash or solids/milliliter of permeate or concentrate ranged from 0.992 to 0.998, except that of conductivity versus milligrams of solids/milliliter

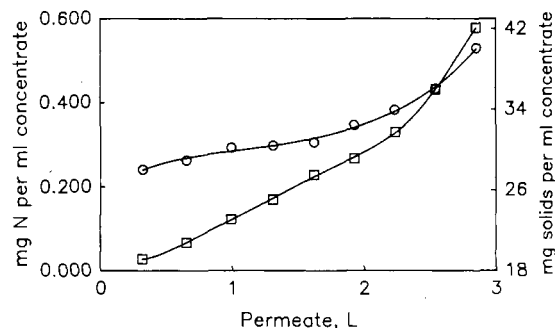


Figure 3. Nitrogen (O) and solids (□) concentrations of concentrate during RO at 800 psi of UF permeate from HiDry stillage solubles.

of HiDry permeate was 0.887 and that of conductivity versus milligrams of ash/milliliter of HiDry permeate was 0.937. Thus, conductivity measurements, which are more rapid than solids and ash determinations, can monitor concentrations of RO permeates and concentrates. Conductivities for Jewel RO permeates (0.043-0.67 mS/cm at 28 °C) and HiDry RO permeates (0.025-0.22 mS/cm at 25 °C) are lower than that of tap water (0.92 mS/cm).

#### CONCLUSIONS

Jewel stillage solubles had 6.0% solids and accounted for 39% of the dry stillage, whereas HiDry stillage solubles had 4.0% solids and accounted for 46% of the dry stillage (Tables III and IV; Wu and Bagby, 1987). In each case 6.4 L of stillage solubles was produced/kg of ethanol. Gregor and Jeffries (1979) reported that the total cost for equipment, power, and labor for combined UF and RO was \$3.53/1000 gal of stillage treated, compared to \$8.33 for fuel alone by the evaporative route. UF in combination with RO thus appears to be a practical and economical method to process sweet potato stillage solubles. A large volume of dilute solution can be separated into a small volume of concentrated solution and a large volume of permeate that can be reused as water or safely disposed.

Combined UF and RO can recover more than 99.7% of the nitrogen and solids of sweet potato stillage solubles, assuming that only RO permeate is discarded. Combined UF and RO may also provide valuable food-grade products, since sweet potatoes are traditionally used for food, and they have good amino acid compositions (Wu and Bagby, 1987). These methods may also encourage increased food use of sweet potato filter cake, while the total cost of the sweet potato alcohol process is reduced.

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## Metabolism of Selenium from Soybean and Egg Products in Rats<sup>1,2</sup>

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The utilization of selenium (Se) from soy flour and eggs was evaluated by comparing whole-body absorption, retention, and tissue accumulation of <sup>75</sup>Se in rats from radiolabeled test meals. Selenium-depleted male Sprague-Dawley rats were fed selenium-adequate repletion diets containing either egg, soy, combined egg/soy, or sodium selenite supplemented torula yeast as the protein sources. The first meal of the repletion period was radiolabeled. Intrinsically labeled egg protein was obtained by gavaging hens with [<sup>75</sup>Se]selenomethionine or sodium [<sup>75</sup>Se]selenite. Soy was intrinsically labeled via nutrient culture with sodium [<sup>75</sup>Se]selenite (Na<sub>2</sub><sup>75</sup>SeO<sub>3</sub>) or sodium [<sup>75</sup>Se]selenate (Na<sub>2</sub><sup>75</sup>SeO<sub>4</sub>). The combined egg/soy diet contained egg from hens gavaged with Na<sub>2</sub><sup>75</sup>SeO<sub>3</sub> and soy grown with Na<sub>2</sub><sup>75</sup>SeO<sub>3</sub>. Torula yeast was extrinsically labeled by adding Na<sub>2</sub><sup>75</sup>SeO<sub>3</sub>. A significantly greater amount of <sup>75</sup>Se was absorbed and retained from egg than from soy protein. The <sup>75</sup>Se from mixed egg/soy protein diets was absorbed and retained at a level intermediate to that of the egg or soy protein diets alone, suggesting the formation of a common pool of selenium within the intestinal tract.

Many foods of animal origin (e.g., meat, fish, and egg) contain high levels of selenium. Plant foods, on the other hand, normally contain less selenium than foods of animal origin. However, the selenium content of food does not necessarily reflect the utilization of the mineral from the food.

Many studies have compared the utilization of selenium from different foods. Soybean meal has been shown to be more effective than fish in preventing exudative diathesis in chicks (Cantor et al., 1975a), but less effective in restoring glutathione peroxidase activity (Gabrielsen and Opstvedt, 1980). Selenium from wheat was more effective than selenium from tuna in preventing pancreatic fibrosis in chicks (Cantor et al., 1975b) and in restoring tissue glutathione peroxidase activities in rats (Douglass et al.,

1981; Alexander et al., 1983). No clear distinction can be made at this time as to the utilization of selenium from foods of animal versus plant origin.

The Food and Nutrition Board has established a 50-200 µg/day safe and adequate range of selenium intake for healthy individuals (Food and Nutrition Board, 1980). This range of selenium intake is not difficult to obtain in a varied American diet but may present problems for individuals on restricted nutritional regimens. Examples of such individuals are infants and enterally or parenterally fed patients. Selenium deficiency symptoms have been shown in persons receiving total parenteral nutrition. Symptoms of selenium deficiency reported are cardiomyopathy and muscle weakness (Quercia et al., 1984; Kien and Ganther, 1983). Selenium status of these individuals has been reported as very low with erythrocyte levels of glutathione peroxidase only 6-7% of normal (Baker et al., 1983).

Selenium in food sources is concentrated in protein. A protein source being used commercially in the formulation of infant and enteral nutrition is soy protein. Soy is used because of its high protein quality and content and because it is generally less allergenic than other vegetable proteins.

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