In vitro relative protein digestibility and lipoxygenase activity used as parameters to test and compare quality in five varieties of soybean meals.

Elizabeth Maforimbo
Department of Research and Specialist Services, Chemistry and Soil Research Institute,
P. O. Box CY 550 Causeway, Harare, Zimbabwe.

Abstract

Proximate composition, In Vitro Relative Protein Digestibility of 5 varieties of soybean *(glycine max)*, was done. Meals from whole seeds and dehulled seeds were both investigated. Stability to photoxidation was also monitored on the oils over a period after sunlight exposure and finally the extent of oil oxidation was determined after 10 months of ambient storage.

There was an intervarietal similarity in proximate composition for the whole seeds as confirmed by the low coefficients of variation, i e, 4.73%; 2.23%; 5.09%; 3.87% and 11.76% for moisture, crude protein, ether extract, ash and fibre respectively. An intervarietal similarity was also shown in dehulled seeds with coefficients of variation at 11.9%, 3.49%, 2.3%, 4.0% for moisture, crude protein, ether extract and ash respectively, except for the fibre content which had a higher coefficient of variation of 31.62%.

Significant differences in relative protein digestibility were shown among varieties for both the whole and dehulled seeds. Dehulling significantly improved relative digestibility in all varieties as confirmed by the t-test at a 5% level. Nyala variety gave the highest relative protein digestibility both for the whole and dehulled seeds, at 78.03% and 87.02% respectively. The yield of oil after petroleum ether extraction was highest in the Roan and least in the Duiker seed variety at 20.70% and 17.8% respectively.

Stability to photoxidation (lipoxygenase activity) for the oils was least in the Duiker variety, giving an $E^{1\%}_{1cm}$ (UV absorbance at 232 nm) of 11.26 after 12 hours exposure to sunlight and 62,0 after 10 months of ambient storage. The most stable oil to photoxidation was from the Soma variety giving an $E^{1\%}_{1cm}$ of 6.23 after 12 hours of sunlight exposure and 15.63 after ambient storage of 10 months.

The t-test indicated that there was no significant difference *($P < 0.05$)* in the rate of oil oxidation between the Roan and the Soma varieties up to a period of 10 months at ambient storage. Ranking for the following quality parameters, protein, relative protein digestibility, oil content and its stability to oxidation were in the following order; Roan, Nyala, Soma, Nondo and Duiker.
Key words: relative digestibility, stability, photoxidation, lipoxygenase activity, soybean meals.

Introduction

The conventional protein sources, meat and dairy products are fast becoming unaffordable, forcing the greater population to seek other forms of protein. Oil seed meals are now available in large quantities and the press cake which remains after oil has been expressed from soy bean, cotton seed, peanut, etc contain about 60% protein of relatively good biological value (Milner 1966). The oil seed meal could help counteract the problem of Protein Energy Malnutrition, which is rampant in the rural areas.

Although proteins of plant origin offer a considerable protein source for alleviating shortage of food protein facing the greater segment of the population, many plants are known to contain antinutritional factors which affect the protein quality (Liener, 1982).

Inorder to predict the nutritional quality of food proteins for growth and maintenance, protein quality measurements are used. These tests are designed to reflect the amino acid content, bioavailability of amino acids present in a protein and protein digestibility of the food tested. Biological (in vivo) assays are costly and lengthly, so chemical or biochemical (in vitro) assays have been developed to predict how a protein will meet nutritional and growth requirements. These include enzyme assays that model mammalian digestion for estimation of protein digestibility (Barbara 1994).

While amino acid profile pattern is probably the most determinant of protein quality, digestibility of a protein and bioavailability of its constituent amino acids are the next important factors, (Oshodi et. al.,1995). This is true because proteins are digested, absorbed and utilised to different extents.

The differences in protein digestibility are brought about by the succeptibility of a protein to enzymatic hydrolysis in the digestive system and this is directly related to primary, secondary and tertiary structure of the protein (Barbara 1994). Processing and storage conditions alter the protein structure thus improving or lessening its succeptibility to enzymes. Chemical reactions, e.g.Maillard browning can significantly reduce the biological availability of essential amino acids, in particular lysine.

Legume seeds all contain proteins that inhibit the proteases of the mammalian digestive tract, notably trypsin and chymotrypsin (Bressani *et.al.*, 1982). Studies with experimental animals fed on diets of unheated soybean meal show reduced efficiency of protein utilisation. When these legumes are heated, the efficiency of utilisation increases, but prolonged heating does not allow theoretical efficiency to be reached. This is so because of significant losses of amino acids (Coultate, 1996).

During storage, legume seeds develop off flavours due to enzymic degradation of

unsaturated fatty acids. Linoleic and linolenic acids are the main precursors of these volatile short chain aldehydes and both volatile and nonvolatile longer chain aldehydes (Saskia *et. al.*,1995).

Conjugated dienes are the primary products of lipid oxidation followed by the formation of the hydroperoxides which later break down to these aldehydes (Coultate, 1996). The measurement of the conjugated dienes in these fatty acids will give an indication of the degree of unsaturation in the oil. The dienes absorb strongly in the region of 230-234 nm (Tsaknis, 1996).

Soybean, being a richful source of protein and essential fatty acids, needs to be protected. As quality measures, relative in vitro protein digestibility and lipoxygenase activity were monitored on five varieties of soybean meal.

Materials

Soybean bean *(Glycine max)* seeds of five varieties were collected from the farm at the Department of Research and Specialist Services with the help of Mr.H. Mushoriwa. The seeds had been stored in a cold room since harvest to minimise oxidation. During experiments, however, seeds were stored at ambient conditions.

Roan, Yellow seed coat and grey hilum
Soma light brown seed coat with black hilum
Duiker light yellow seed coat with a light yellow hilum
Nyala light brown seed coat with dark brown hilum
Nondo yellow seed coat with a brown hilum (fodder variety).

Part of the seeds were dehulled using a Hippo Grinding mill, model mark 2. The whole seeds and dehulled seeds were dry milled to a fine meal using Type SCIS-Allen West. Seperation of the testa and cotyledon for the dehulled seeds was done manually before milling.

Commercial papain, obtained by drying the fruit latex, digests protein and is used medicinally to aid digestion.

Crude papain was used as the proteolytic enzyme and was extracted from papaya stems, following the method from "The Wealth of India raw materials Vol 2", pp 79-81.This crystalline papain is most stable in the pH range 5-7 and its proteolytic activity is 3-4 times that of the commercial one. A multienzyme system could not be afforded.

For the albumin standard, egg albumin (80% protein), was used. The egg albumin was dried in an oven at 35°C and later stored in a tight container.

Methods

Proximate analysis, moisture, crude protein, ether extract, ash and fibre were estimated for all samples both whole and dehulled seeds following the methods from Association of Analytical Chemists, (AOAC) 1990. The protein analysis was done using the Auto Kjeltec 1030 analyser. All samples were analysed in triplicate.

For in Vitro Relative Digestibility, method followed was from CAM/Proc// Rel Digest/140193, University of Humberside.

0.5 g of meal was steamed for 1 hour and to this, 0.01g of crude papain (correct to 3 decimal places) and 10 mls of buffer, pH 6.0 (optimum for the enzyme),were added to the test tubes. The test tubes were shaken to distribute the enzyme and incubated at 50° C for 2 hours in an oven. After incubation, the tubes were centrifuged and the supernatant kept for protein analysis using the automated Kjeltec system.

The digestibility for each sample was calculated as a percentage of the protein in the albumin sample, (protein hydrolysate) after digestion. The rate of photosensitised oxidation of the oils from the 5 varieties was monitored over a period of 12 hours and finally after 10 months at ambient storage. This was done to assess lipoxygenase activity.

Oil was extracted from the whole seed meals using the cold extraction method with petroleum ether BP 40,60. 50g of soy meal was ground with anhydrous sodium sulphate using a mortor and pestle. The sample was later extracted twice with 200 ml. portions of petroleum ether B.P.40,60. The residue was filtered off using Whatman filter paper no.42. The solvent was evaporated off on a rotor vapour at a pressure of 7mm Hg and a water bath temperature of 35°C using a Buchi-RE model. Cold extraction of oils was done to prevent deactivation of lipoxygenase enzyme.

The oils extracted from whole seed were monitored for lipoxygenase activity over 12 hours under sunlight exposure.

Method for lipoxygenase activity: (adapted from Powers, 1994).
0.2g $\pm$ 0.001g of oil was dissolved into 25 mls volumetric flasks with iso-octane, spectroscopic grade. This was further diluted x 5 to obtain measurements of conjugated dienes using UV absorbance at 232nm. Iso-octane was used in the reference cell. UV spec. model, DMS-100, Varian was used for spectroscopic readings.

$$\text{Absorbance } E^{1\%}_{1CM} = \frac{A}{C \times d}$$

where A = Absorbance at 232 nm.
C = conc. of solution analysed in g/100 mls.
d = length of cell in cm.

Results and Discussion

All data in 4 tables represent 3 replicates. Parametric and non Parametric statistics done from the methods of .Breen, (1997),

Table 1 Proximate composition (g%) of soybean meal varieties (whole seeds) A1-A5. Results calculated on dry matter basis.

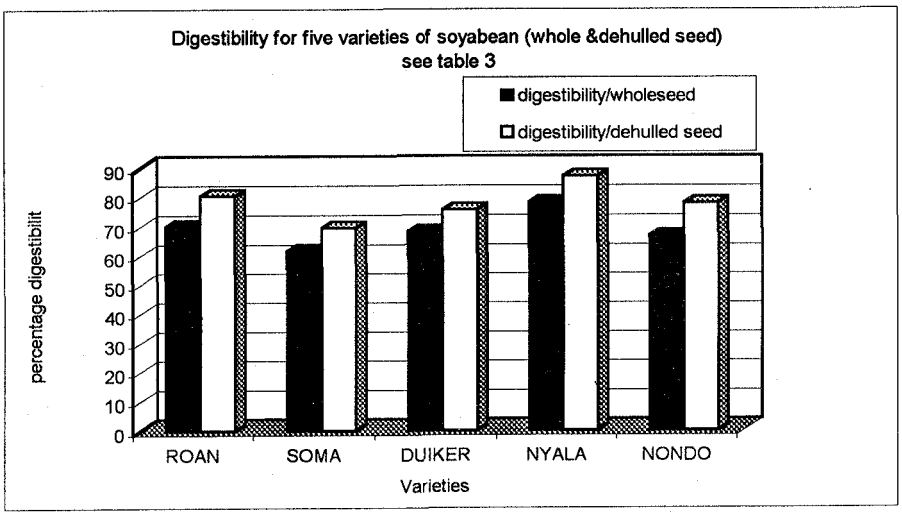
Variety	moisture	crude protein	ether extract	ash	fibre
A1	9.2	45.5	20.7	4.9	6.0
A2	9.7	45.5	19.4	5.2	6.9
A3	9.3	43.8	17.8	4.6	7.0
A4	8.4	45.7	19.1	4.9	5.2
A5	8.9	45.0	20.2	4.9	5.5
Mean	9.1	45.1	19.44	4.9	6.12
S.D	0.43	0.69	0.99	0.19	0.72
C.V%	4.73	2.22	5.09	3.87	11.86

Humberside University. The proximate composition of the samples varied according to varieties. There was generally an intervarietal similarity in, moisture, crude protein, ether extract, fibre and ash. This was attested to by the low coefficients of variation as shown in table 1, ie; 4.73%; 2.22%;5,09%; 3.87%; and 11.86% for moisture,crude protein, ether extract, ash and fibre respectively. For the dehulled seeds, the proximate composition also shows a similar pattern for moisture, crude protein, ether extract, ash as also confirmed by the low coefficients of variation, 11.9%; 3.49%; 2.30%;4.10%; respectively. The fibre content show a higher coeff. of variation at 31.62%, ranging from 2.3% for Nondo B5 up to 5.2% fibre for Roan B1, (**Table 2**).

Table 2 Proximate composition (g %) of Dehulled seeds, B1- B5. Results on dry matter basis.

Variety	moisture	crude protein	ether extract	ash	fibre
B1	8.1	49.9	22.6	4.9	5.2
B2	7.0	46.4	22.3	5.3	3.0
B3	9.6	45.9	21.5	4.7	2.5
B4	7.8	49.8	21.3	5.1	4.0
B5	9.5	48.7	22.3	5.0	2.3
Mean	8.40	48.14	22.0	5.0	3.4
S.D.	1.00	1.68	0.51	0.20	1.08
C.V.%	11.90	3.49	2.30	4.10	31.62

Key: A = whole seeds; B = dehulled seeds
1- Roan; 2 - Soma; 3- Duiker; 4- Nyala; 5- Nondo



The fibre contents for the dehulled seeds are significantly lower than those for the whole seeds as seen from the mean values, **Table 1** and **2**, suggesting that fibre is more concentrated on the outer seed coat (testa) than in the endosperm. The dehulled seeds have higher protein and fat values than the whole seeds as shown by the mean values. This suggests that protein and fat are more concentrated in the endosperm than in the outer coat.

Relative in Vitro Protein Digestibility, as illustrated in **Table 3**, varied in both whole and dehulled seed, ranging from 61.54% for Soma up to 78.03% for Nyala. The average relative digestibility for the whole seed was 68.88% with a coefficient of variation at 7.78%, whilst that for the dehulled seed was 78.03% and 7.37% respectively.

The higher mean value for the dehulled seed indicates that dehulled seeds had better digestibilities, and this was also confirmed by the t-test which indicated that dehulling significantly improved digestibility ($P < 0.05$). The coefficients of variation were close for both sets as also attested by the F-test which showed that sets have the same underlying variance. Although these sets were shown to have the same underlying variance, they were not assumed to come from the same normal distribution according to ANOVA. This suggests that digestibility for the whole and dehulled seeds were not uniform.

From the grain varieties, Nyala, (4), gave the highest digestibility for the whole and dehulled seeds, ie; 78.03% and 87.02% respectively. Roan (1), gave the second highest value of 69.88% and 80.44% for the whole and dehulled seeds respectively. Soma (2) variety was the least digestible both for the whole and dehulled seed. Rank correlation test showed that no correlation existed between the fibre contents and the digestibilities although dehulling significantly improved digestibility. There was also no correlation

Table 3 In vitro relative digestibility for soybean meal varieties (whole A; and dehulled B; seeds), heat treated (steamed) for 1 hour.

Whole seeds	% Relative Digestibility	Dehulled seeds	% Relative Digestibility
A1	69.88	B1	80.44
A2	61.54	B2	69.51
A3	68.21	B3	75.53
A4	78.03	B4	87.02
A5	66.73	B5	77.66
Mean	68.88	Mean	78.03
S.D,	5.36	S.D.	5.75
CV%	7.78	CV%	7.37

Key, on table 2

between the protein values and digestibility according to the same test. Both heat treatment and dehulling have improved in vitro digestibilities for African yam bean in Oshodi et. al; 1995, (using a multienzyme system). This current work has also agreed with Oshodi et. al;1995. Dehulling is the only parameter used in this current work because of the assumption that beans are consumed after cooking. Although this phenomenon is different for Nondo, which is a fodder variety.

Poor protein digestibility has been reported in developing countries due to the use of less refined cereals and pulses, Oshodi, (1995). If soybean and other legumes must meet the protein needs for the target groups, dehulling should be encouraged for other recipes in order to improve protein utilisation efficiency.

Intervarietal differences are shown for oil stability to photoxidation at all stages (**Table 4**), as attested by the big coefficients of variation. This difference is even magnified after 10 months of ambient storage, with a coefficient of variation at 57.24 %. UV absorbance at 232 nm, $E^{1\%}_{1cm}$ was smallest in the Soma variety, ie; 1.66 at 0 time up to 6.23 after 12 hours of exposure to sunlight and 15.63 after 10 months of ambient storage.

The $E^{1\%}_{1cm}$ for the Duiker variety was highest, giving 3.25 at time 0, up to 11.26 after 12 hours of sunlight exposure and 62.00 after 10 months of ambient storage. This suggests that Duiker seed oil is the most unstable to photoxidation and Soma seed oil being the most stable. The Soma and Roan varieties however, did not have significant difference in oil deterioration up to 10 months storage as confirmed by the t-test ($P < 0.05$), suggesting that these two are equally stable.

There was a slight correlation existing between the oil content and oil stability, $E^{1\%}_{1cm}$, giving a correlation coefficient, r_s of 0.7. This correlation (not close to 1), however cannot be conclusive for anything.

According to Kordylas, (1990), seed oils are rich sources of Vitamin E and extracted oils should remain quite stable unless conditions allowing photoxidation or autoxidation of the polyunsaturated fatty acids arise during processing or storage. Vitamin E delays the onset of rancidity in oils but the inevitable build-up of peroxides in the oil will eventually overcome the tocopherols (Vitamin.E in particular) and cause their oxidation to compounds lacking antioxidant activity (Coultate, 1996). This was evidenced in this work where photoxidation of the oils was allowed to test stability and the extent to which Vitamin E protected the oils.

Feeding livestock with large amounts of highly oxidised foods induces a wide range of symptoms related to Vitamin E defficiency. It is also assumed that the high intake of oxidised fats overwhelms the body's natural antioxidant systems (Coultate 1996). This applies to Nondo which is a fodder variety. Improper processing and storage practices, eg. exposure of the meal to sunlight for long periods could offer a basis for such problems. This experiment showed that with poor processing and storage practices, products from soybean are inevitably exposed to both photoxidation and thermal autoxidation and this reduces their shelf life. Duiker has shown its succeptibility to this phenomenon. Because lipoxygenase is abundant in soybean seeds, physical damage to the seeds, milling,or any process involving breakage of the seeds will expose the lipids to attack by this enzyme. This enzyme catalyses the oxidation of unsaturated fatty acids into hydroperoxides which in turn oxidise carotenes and other oxylabile compounds. This reduces the nutrient value of the foods prepared.

Table 5, shows how post-harvest handling affects oil quality. According to Mounts (1980), studies were done on the effects of post harvest handling on soybens. Crude oil was extracted from whole soybeans, split soybeans and composites. Oil from split beans showed significant differences in oil quality, as reflected by increased levels of Fe, (a catalyst for oxidation of soybean oil), FFA (free fatty acids) and PVs (peroxide values).

Non parametric test for the following quality parameters; protein content, relative protein digestibility, oil yield and

Table 4 Lipoxygenase activity; $E^{1\%}_{1cm}$, for oils extracted from the 5 varieties, exposed to sunlight over a period of 12 hours, and later stored at ambient temperature for 10 months.

Variety	$E^{1\%}_{1cm}$ at time 0	$E^{1\%}_{1cm}$ after 4 hr	$E^{1\%}_{1cm}$ after 8 hrs	$E^{1\%}_{1cm}$ after 12 hr	$E^{1\%}_{1cm}$ 10 months*
A1	1.84	3.91	4.52	6.78	18.75
A2	1.66	3.35	4.06	6.23	15.63
A3	3.25	6.06	8.52	11.26	62.00
A4	2.71	5.01	6.34	8.53	32.15
A5	3.03	4.81	5.92	7.25	20.40
Mean	2.498	4.628	5.872	8.01	29.78
SD	0.637	0.935	1.571	1.793	17.05
CV%	25.5	20.22	26.75	22.38	57.24

* Same oil stored at room temperature for 10 months.

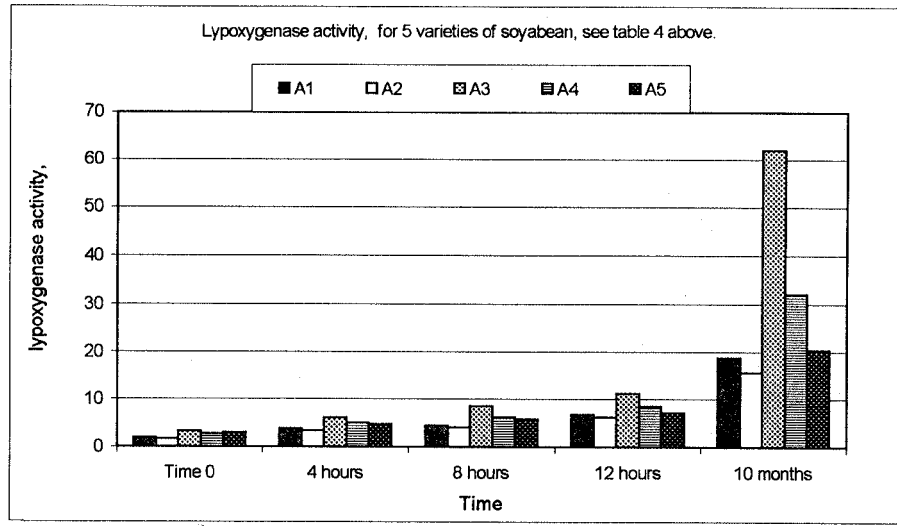


Table 5 Soybean oil quality evaluation[a] during post harvest handling.

Fraction	Fe ppm	FFA	PV, meq/kg oil
New Oleans(barge train)			
Composite	0.4± 0.04[b]	0.7±0.3[b]	0.9±0.0[b]
wholes	0.7± 0.4	1.0± 0.3	1.2±0.1
splits	1.2± 0.5	1.1± 0.6	2.2± 1.0
New Orleans (vessel)			
composite	0.5± 0.4	1.1± 0.5	0.7± 0.1
wholes	0.5± 0.2	0.7±0.5	1.3± 0.5
splits	1.9± 0.4	1.4± 0.0	1.7± 0.9

a all values are the average of triplicate analysis
b Standard deviation
Source: Mounts 1980.

Table 6 Soyabean oil and protein quality evaluation.

Variety	Score	Rank
Roan	7	1
Nyala	10	2
Soma	12	3
Nondo	13	4
Duiker	18	5

stability of the oil to oxidation indicate that scores and ranking were in the following order; Roan, Nyala, Soma, Nondo and lastly Duiker

These results suggest the following recommendations:

Roan, Soma and Nyala seem to be good for infant formulas, soy cereal diets, roasting, bakery products, milk and milk products. Nyala being highest in protein content and digestibility would be best for infant formulas. Soma and Roan produce quality, stable oils and products which can last if stored well. Duiker is unsuitable for oil and the preserved products would likely to have a short shelf life, giving off flavours and taste. Nondo is adequate as a livestock feed but needs proper handling, storage and destruction of antinutritional factors before it is used.

Conclusions

An intervarietal similarity in proximate composition was shown for both the whole and dehulled seeds. The dehulled seeds gavè better Relative Protein Digestibility than the whole seeds and this was confirmed by the t- test at a 5% level of significance. Nyala seed gave the highest digestibility for both the whole and dehulled samples among the other 5 varieties.

Oil yield after petroleum ether extraction was highest in the Roan seed variety and lowest in the Duiker seed variety. The oil from the Duiker seed also seemed to have the least stability to photoxidation. The most stable oil to photoxidation was from the Soma seed, although the Soma and the Roan seed varieties gave no significant differences in oil stability at a 5% level of significance.

Acknowledgements

The support from the Chemistry and Soil Research Institute, Department of Research and Specialist Services, Harare, Zimbabwe, is acknowledged in this work. Special thanks are extended to H. Mushoriwa, Crop Breeding Institute, for the supply of soybean seeds.

References

Coultate T.P., (1996). "Food, The Chemistry of its components", 3rd edition, pub. The Royal Society of Chemistry,

Kordylas J. Maud, (1991), Processing Grain Legumes and Nuts, in: "Processing and Preservation of Tropical and Subtropical Foods", 1st edition, pub. ELBS,

Rasco A. Barbara, (1994), Protein Quality Tests, in "Chemical Analysis of Foods," edited by Susainne Nielsen, pub. Chapman and Hall.

Powers J.R, (1994), Application of Enzymes in Food Analysis in, "Chemical Analysis of Foods"edited by Susainne Neilsen, pub.Chapman and Hall.

David E. Phillips *et al.*, (1981) 'Protein quality in seed meals of Phaseolus Vulgaris and Heat-stable factors affecting Utilisation of Protein,' in J.Sc. Food and Agric. (32) 423-432.

A.A. Oshodi et. al., (1995) "Multi enzyme Protein Digestibility of African yam bean," in J.Sc. Food and Agriculture, (69), 373-377.

Mahammad Al-Wesali, et. al., (1995), " Pea seed Trypsin Inhibitors and Caseign digestibility", in the J. Sc. of Food and Agriculture, (68) 431-437.

Assosciation of Analytical Chemists, (AOAC), (1990), Official Methods of analysis.

Piero Mauro Spanghero, Stefano Marchetti & Stefano Moscardini, (1995), "Trypsin Inhibitory Activity of Raw Soya bean after incubation & rumen fluid", in the J. Sc. Food Agric, (67), 441-445.

Bressani R, Braham J.E., Elias L.G., (1982), "Effects of Nutritional Quality of Food Legumes", in, Chemistry and World Food Supplies, The new frontiers, chemrawn II. ed. Shemilt L.W. pp 491-500.

Irvin E. Liener, (1982), "Removal by Processing of Naturally occurring Toxicants and Antinutrients," in Chemistry and World Food Supplies, ed. Shemilt L.W., pp 453-461.

Mounts T. L., (1980), "Raw material and Soybean Quality", in World Soybean Research Conference 11, ed. Fred. T. Corbia, pp 662-663.