PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Mob: +92 300 3008585, Fax: +92 41 8815544

E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 6 (6): 660-664, 2007
ISSN 1680-5194

Improving the Nutritional Quality of Cowpea and Bambara Bean Flours for Use in Infant Feeding

Mune Mune Martin Alain[1], Mbome Lape Israël[2] and Minka Samuel René*[1]
[1]Department of Biochemistry, University of Yaounde I, PO Box 812, Yaounde, Cameroon
[2]Food and Nutrition Research Centre, PO Box 6163, Yaounde, Cameroon

Abstract: Ground cowpea [*Vigna unguiculata* (L.) walp] and Bambara bean [*Vigna subterranea* (L.) verde] were treated with 60% ethanol to eliminate anti-nutrients and improve their nutritional quality. Analytical results show that the resulting flour products had a yield of 81-82% and a protein content increase of 11.61 and 4.68% for cowpea and Bambara bean respectively compared to the untreated flours. Their fat content also increased by 10.74 and 15.24% in the same order, as well as their fibre content. *In vitro* protein digestibility studies showed an increase of 68.71 to 81.82% and 71.58 to 83.83% for alcohol-treated cowpea and Bambara bean flours, compared to the untreated flours, thus indicating the elimination of protease inhibitors. Likewise, gas chromatography of neutral sugars in the treated flour samples showed elimination of the major flatulence-causing sugars. Alcohol treatment caused loss of Fe and concentration of Ca and Zn. Semi-liquid gruels prepared from blends of the treated grain legume flours and fermented maize dough powder are adequate in energy and some nutrients to meet the requirements of 6-8-month-old infants, when fed three meals a day.

Key words: Bambara bean, cowpea, digestibility, energy density, flatulence, protease inhibitor

Introduction

To ensure proper infant nutrition during the first year of life remains a great challenge in developing countries. Rapid changes in the nutritional needs of young infants and the inadequacies of feeding practices place them in an infernal cycle of diarrhoea and malnutrition with negative implications in growth and psychomotor development. Poor food quality, insufficient food intake and repeated infections are often observed (Nnam, 2001). UNICEF (1995) estimated that as much as 50% of all children in tropical countries pass through a stage of serious malnutrition during growth.

The problem of providing nutritious, low cost protein supplements to the diet of young children has focused the attention of nutritionists and food scientists in several sub-Saharan African countries. Ideally, the ingredients for low cost complementary foods must be derived from dietary staples available and affordable in the region of interest.

Bambara bean [*Vigna subterranea* (L.) verde] and cowpea [*Vigna vinguiculata* (L.) walp], for example, are edible grain legumes widely cultivated in Africa (Pasquet and Fotso, 1991). In 2000, world production of cowpea was about 3.3 million tons (IITA, 2003). These grain legumes are used for human and animal nutrition (Borget, 1989; Goli, 1995). Like most grain legumes cultivated in Africa, cowpea and Bambara bean are important sources of proteins and carbohydrates (Borget, 1989; Minka and Bruneteau, 2000; Tharanathan and Mahadevamma, 2003). Their proteins are made up of over 32% of essential amino acids, with lysine and leucine being predominant (Minka and Bruneteau, 2000; Jipara *et al*., 2001). However, they contain indigestible oligosaccharides such as raffinose, stachyose and verbascose which cause flatulence and diarrhoea in adults and, particularly children (Onyenekwe *et al*., 2000; Besançon, 1999), as well as protease inhibitors, principally anti-trypsin and anti-chymotrypsin (Borget, 1989; Besançon, 1999).

Traditional processing methods such as boiling and soaking in water eliminate less than 50% of stachyose and raffinose from cowpea grains (Onyenekwe *et al*., 2000). Roasting and toasting of grain legumes denature protein inhibitors but also affect amino acids and essential fatty acids stability (Friedman, 1992).

This study was undertaken to improve the nutritional quality of these two grain legumes by eliminating anti-nutritional factors without affecting the overall nutrient content and to evaluate the suitability of the treated flour products for use in preparation of gruel for young infants, when blended with the traditional fermented maize dough.

Materials and Methods

Samples and sample preparation: Fermented maize dough, dried grains of two varieties of cowpea (white and brown) and one variety of Bambara bean were purchased in the local market. The maize dough was freeze dried, ground into flour and passed through a 500 µm sieve. Cowpea and Bambara bean grains were hand-picked, washed and rinsed in deionised water.

Table 1: Solids yield, proximate composition some mineral contents of alcohol-treated and untreated cowpea and Bambara bean flours

	Red cowpea		White cowpea		Bambara bean	
Nutrients (100g sample)	Untreated	Treated	Untreated	Treated	Untreated	Treated
Solids yield (%)	100.00	81.59^{a}	100.00	81.14^{a}	100.00	81.07^{a}
Crude protein (%)	28.80^{e}	32.11^{f}	22.66^{a}	25.29^{d}	23.07^{b}	24.15^{c}
Lipids (%)	1.19^{a}	1.34^{ab}	1.21^{a}	1.34^{ab}	5.90^{c}	6.80^{d}
Fibre (NDF) [%]	5.97^{c}	6.72^{d}	5.75^{bc}	6.69^{d}	5.35^{ab}	6.25^{cd}
Ash (%)	4.61^{d}	2.07^{b}	4.67^{d}	1.89^{b}	3.88^{c}	0.86^{a}
Carbohydrate (%)	59.43^{a}	57.76^{a}	65.71^{c}	64.79^{c}	61.80^{b}	61.94^{b}
Ca (mg)	6.83^{a}	7.17^{a}	7.35^{a}	7.71^{a}	4.38^{b}	6.25^{a}
Fe (mg)	12.38^{bc}	7.51^{cd}	19.39^{a}	11.76^{bc}	16.66^{ab}	4.24^{d}
Zn (mg)	8.09^{a}	8.15^{a}	9.93^{a}	10.00^{a}	6.78^{a}	8.24^{a}

Figures in the same line with different following letters are significantly different at the 5% level

Bambara bean grains were dried in an air-convection oven at 50°C for 72 h and then cracked and dehulled. White and brown cowpea grains were soaked for 1 and 3 h respectively, dehulled and then dried as above. The dried grains were ground into flour and sieved as above.

Sample treatment: Flour samples were treated with 60% ethanol as described by Cuq (1991). About 250 g of flour were homogenized with 2500 mL of 60% ethanol. The mixture was adjusted to pH 5 with 5mol/L H_2SO_4, stirred for 30 min and filtered. The resulting residue was treated twice in like manner and the final residue was dried in an air convection oven at 45°C for 48h, ground, sieved and stored until analysis.

Chemical analyses: Total simple sugars were determined by the anthrone method (Montreuil *et al.*, 1981) and reducing sugars by the 3, 5-dinitrosalisylic method (Montreuil *et al.*, 1981), following the sugars extraction in hot 80% ethanol (Cerning and Guilhot, 1973). Minerals (ash) and fat were determined by standard AOAC (1984) methods and crude protein (N×6.25) by Kjeldhal's method. Dietary fibre was analyzed using neutral acid detergent (Goering and Van Soest, 1970) and starch+maltodextrins were obtained by difference. Energy content was calculated from the proximate composition using the Atwater factors (4 kcal/g of protein or available carbohydrate, 9 kcal/g of lipids). Dry matter content of flours and gruels were determined by oven drying at 105°C to constant weight. Quantitative analysis of neutral sugars was done by gas chromatography after HCl hydrolysis of samples and conversion of resulting monosaccharides into alditol acetate according to Sawardeker *et al.* (1965). Likewise, fatty acids were determined by gas chromatography following hydrolysis of samples with 4 mol/L HCl at 100°C for 48 h and methylation with diazomethane.

In vitro protein digestibility of samples was determined by the three-enzyme method of Hsu *et al.* (1978), involving treatment with trypsin (from porcine pancreas), α-chymotrypsin and peptidase (from porcine intestinal mucosa). Percentage digestibility was computed from the formula 210.464-18.1x, where x is the pH change occurring after incubation of the substrate-enzyme mixture at 37°C for 10 min.

Some minerals (Ca, Fe and Zn) were determined by atomic absorption spectrophotometry, after digestion with concentrated nitric acid under heat according to Laurent (1981).

Preparation of gruels: Gruels were prepared from a 30%:70% mixture of each treated grain legume flour and ground fermented maize dough by stirring a predetermined flour mass with cold water into a slurry, taking into consideration the required final solid concentration of gruel and the rate of water evaporation during cooking. The slurry was cooked by stirring over low heat for 5 min, once the mixture had started to boil.

Viscosity measurements and nutrient density estimation: After preparation, gruels were cooled and maintained at 45°C in a thermostatic water bath and aliquots were taken for viscosity measurements (at the same temperature) using a Haake VT-02 viscotester. For each flour mixture 5-7 gruels were prepared at different solids concentration and the viscosities were recorded in Pa.s. The best-fit plots of viscosity versus dry matter were made for each using polynomial regression equations of the experimental values. Solids content, energy and nutrient densities were then estimated by corresponding regression equations, for the viscosity value of 3 Pa.s proposed by Nout (1993) as the upper limit for easy-to-swallow semi-liquid gruels suitable for young infants.

Statistical analyses: All experiments were conducted in triplicates. Differences in analytical results were determined using the Student-Newman-Keuls test, while correlations between them were computed by Kendall's tau-B test. The SPSS software (version 10.1) was used.

Results and Discussion

Proximate composition: Analytical results of samples show that treatment of cowpea and Bambara bean flours with 60% ethanol gave a product yield of 81-82% (Table 1). Crude protein, lipids and fibre contents of treated red cowpea, white cowpea and Bambara bean

Table 2: Some fatty acids content of cowpea and Bambara bean flour (% total fatty acids) before and after treatment with 60% alcohol

	Red cowpea		White cowpea		Bambara bean	
Fatty acids	Untreated	Treated	Untreated	Treated	Untreated	Treated
Myristic (14:0)	3.1	2.6	1.6	1.3	Nd	Nd
Palmitic (16:0)	46.7	44.6	65.1	60.4	39.4	41.2
Stéaric (18:0)	9.5	11.5	11.9	15.1	12.5	14.1
Oleic (18:1)	15.3	17.3	8.2	12.9	20.4	21.0
Linoleic (18:2)	21.0	20.2	13.2	10.3	25.1	21.8
Linolenic (18:3)	4.4	3.8	Nd	Nd	2.6	1.9

Nd: not detected

Table 3: Available carbohydrate content and *in vitro* protein digestibility of cowpea and Bambara bean flours before and after treatment with 60% ethanol

	Red cowpea		White cowpea		Bambara bean	
Parameter (100g sample)	Untreated	Treated	Untreated	Treated	Untreated	Treated
Total sugars (%)	8.77^{d}	0.98^{ab}	9.41^{e}	1.16^{ab}	7.15^{c}	1.35^{b}
Reducing sugars (%)	0.37^{abc}	0.48^{ab}	0.30^{a}	0.51^{bc}	0.36^{ab}	0.56^{c}
Glucose (%)	48.92^{ab}	52.35^{b}	46.83^{a}	52.45^{b}	47.36^{a}	53.17^{b}
Galactose (%)	6.62^{c}	Nd	5.13^{b}	Nd	7.38^{d}	2.80^{a}
SM (%)	50.66^{a}	56.79^{a}	56.29^{a}	63.63^{b}	54.50^{a}	60.54^{b}
IVPD (%)	68.71^{d}	81.82^{b}	68.53^{d}	81.53^{b}	71.58^{c}	83.83^{a}

IVPD: *in vitro* protein digestibility, Nd: not detected, SM: Starch+malto-dextrins, Figures in the same line with different following letters are significantly different at the 5% level

flours were higher than those of corresponding untreated flours. But for lipids content in red and white cowpeas, increases for the other components in treated samples were significant ($p<0.05$) and varied from 4.68 to 11.1%, for crude protein, 12.56 to 16.84% for NDF fibre and 10.74 to 15.27% for lipids. Increase in lipids content could be due mostly to non polar lipids which are insoluble in 60% ethanol and are concentrated during treatment of flours. As for NDF fibre increase, it could be mainly as a result of the non elimination of cellulose and hemi-cellulose which are insoluble in 60% ethanol (Leske and Coon, 1999).

Mineral content: On the contrary, ethanol treatment of flours caused significant overall mineral (ash) loss varying from 55.10 to 77.84%. Of the three minerals analyzed, the loss concerned only Fe (in all 3 treated flours). As observed for this mineral in cereals (Jacobsen and Slotfeld-Ellingsen, 1983), the minerals most concerned by the loss could be those forming ethanol-soluble complexes with sulphates, nitrates, phytates or amino acids. For Ca and Zn, one observes instead an increase in contents (Table 1). Positive correlations between ash and protein content of ethanol treated flours (R^2 = 0.95-0.99, $p<0.05$), indicate that the minerals retained are closely associated with proteins.

Fatty acids content: Gas chromatography analysis of six fatty acids following HCl hydrolysis of samples and methylation with diazomethane, show that alcohol treatment caused loss of three of the acids (myristic, linoleic and linolenic) in all three samples (Table 2). Loss of palmitic acid was also observed, except in treated Bambara bean where there was an increase instead. Likewise, there was increase in the concentrations of stearic and oleic acids in all treated samples. It should be noted that, because of its relatively high lipids content, Bambara bean constitutes a potential source of essential fatty acids, especially linoleic.

Sugar content: Anthron analysis of 80% ethanol extract of the grain legume flours showed great reduction of total simple sugars ranging from 81.12 to 88.82% in the 60% ethanol-treated flours and an increase in reducing sugars varying between 30 and 70% (Table 3). On the other hand, quantitative analysis of neutral sugars by gas chromatography after HCl hydrolysis of flour samples and conversion of resulting monosaccharides into alditol acetate, showed complete elimination of galactose (flatus-causing sugar) after treatment with 60% ethanol, for the cowpea flours. Only about two-thirds of this sugar was eliminated from the Bambara bean flour. The elimination of galactose is indicative of the elimination of neutral flatus-causing sugars such as raffinose, stachyose and verbascose, of which galactose is a constituent (Peterbauer and Richter, 1998) and which are abundant in cowpea and Bambara bean (Minka and Bruneteau, 2000). The incomplete elimination of galactose from Bambara bean flour could be due to its relatively high fat content. In this connection, it may be mentioned that the 60% ethanol treatment was used to obtain complete elimination of galactose and other flatus-causing sugars in defatted soybean (Ohren, 1981).

Table 4: Nutrient composition of blends of ethanol-treated grain legume flours and fermented maize dough (30:70 dry weight basis) and gruels prepared thereof

	Content in blends (/100g dry weight)			Content/100 g gruel at viscosity of 3 Pa.s			Content/100 Kcal in gruel at viscosity of 3 Pa.s		
Nutrients	RCF	WCF	BBF	RCF	WCF	BBF	RCF	WCF	BBF
Dry matter	100	100	100	8.17	8.43	8.18	24.66	24.65	24.06
Crude protein (g)	15.02	12.98	12.64	1.23	1.09	1.03	3.71	3.19	3.03
Lipid (g)	3.76	3.76	5.40	0.31	0.32	0.44	0.94	0.94	1.29
Carbohydrate (g)	77.88	79.99	79.13	6.36	6.75	6.47	19.20	19.73	19.03
Ash (g)	1.04	0.99	0.68	0.08	0.08	0.06	0.24	0.23	0.18
Fibre (g)	2.30	2.29	2.16	0.19	0.19	0.18	0.57	0.56	0.53
Ca (mg)	2.84	3.00	2.56	0.23	0.25	0.21	0.69	0.73	0.62
Fe (mg)	7.23	8.51	6.25	0.59	0.72	0.51	1.78	2.10	1.50
Zn (mg)	8.90	9.45	8.92	0.73	0.80	0.73	2.20	2.34	2.15
Energy (Kcal)	405.46	405.71	415.67	33.13	34.20	34.00	100	100	100

BBF = bambara bean based composite flour, RCF = red cowpea based composite flour, WCF = white cowpea based composite flour

***In vitro* digestibility:** *In vitro* protein digestibility of the untreated flours was 68.71, 68.53 and 71.58% for red cowpea, white cowpea and Bambara bean respectively (Table 3). Ethanol-treated flours showed significantly higher ($p<0.01$) digestibility (81.82, 81.53 and 83.83%). These results demonstrate the elimination of protease inhibitors, particularly anti-trypsin and anti-chymotrypsin. These inhibitors are generally of low molecular weights (Tsukamoto *et al*., 1983) and are soluble in 60% ethanol (Guegen *et al*., 1999).

Suitability of treated flours for infant feeding: Traditional complementary foods in developing countries are mainly semi-liquid starch-based gruels of low energy and nutrient density, prepared from fermented cereals, especially fermented maize dough (Trèche and Mbome, 1999; Ayebo and Mutasa, 1987). Hence, blends of each of the 60% ethanol-treated grain legume flours and ground freeze-dried fermented maize dough were made in the usual 30%:70% (dry weight basis) and their suitability for infant feeding was evaluated when prepared as gruels. Composition of the blends and gruels thereof are presented in Table 4. According to current recommendations, a complementary food should contain at least 15 g/100g (dry wt. basis) protein and as much as 21% of energy as fat (FAO/WHO/Codex, 1994). On this, only treated red cowpea-based flour has an adequate protein content, the others having between 12.6 and 13.0 g/100g. Treated Bambara bean blend is highest (11.7%) but inadequate in fat energy, while the rest have about 9.3% each. The fibre contents of the blends are like those of similar products used as complementary foods in developing countries. When easy-to-swallow semi-liquid gruels suitable for young infants (viscosity 3 Pa. s) are prepared from the blends, they contain only 8.17-8.43 g of solids/100 g. Their energy densities (33.13-34.20 Kcal/100 g) are in conformity with FAO recommendations (WHO, 1998) for feeding of 6-8 months infants with high breast milk intakes, 3 gruel meals a day. With respect to minerals, Zn density values of all gruels are much higher than the above recommendations of 0.5 mg/100 Kcal, while those of Fe are 12.5 to 37.5% lower than the 2.4 mg/100 Kcal recommended. The gruels are seriously deficient in Ca.

Conclusion: From the above results, it is evident that treatment of cowpea and Bambara bean flours with 60% ethanol improves their nutritional quality by eliminating anti-nutrients and concentrating most essential nutrients. Blends of the treated flours with freeze-dried fermented maize dough are good sources of various nutrients for young infants, even though in lower quantities for many of them than currently recommended. When prepared as easy-to-swallow semi-liquid gruels suitable for feeding infants, they have sufficient energy density, Zn density and, to a lesser extent Fe density, to meet the requirements of 6-8 month-old infants with high breast-milk intake. The actual use of these blends and gruels in developing countries will require supplementation with minerals and vitamins as well as addition of an amylase source to reduce bulk (Trèche and Mbome, 1999) and allow incorporation of larger quantities of flour blends in the gruels, without changing their semi-liquid consistency.

Acknowledgements

This work was done with the resources of the Food and Nutrition Research Centre (IMPM), Ministry of Scientific Research and Innovation, Yaounde Cameroon. The authors are grateful to Drs. M. T. Pommier and M. Bruneteau of Claude Bernard University-Lyon I France, for doing the gas chromatography analyses of sugars and fatty acids.

References

AOAC, 1984. Official Methods of Analysis. 14th edition. Association of Official Analytical Chemists, Washington DC.

Ayebo and M.P. Mutasa, 1987. Fermented maize-based 'Mahewa'. Household level food technology. In proceeding of the workshop held in Nairobi. 12-16 October 1987. Edited by Alnwick, Moses and Schmidt. IDRC: Ottawa, pp: 174-180.

Besançon, 1999. Safety of complementary foods and Bioavailability of nutrients. In Dop, M.C., Benbouzid, D., Tréche, S., B. de Benoist, Verster, A. and F. Delpeuch. Complementary feeding of young children in Africa and the middle East. World Health Organisation, Geneva, pp: 59-73.

Borget, M., 1989. Les légumineuses vivrières tropicales. France, Maison neuve et Larose et ACCT.

Cerning, J. and J. Guilhot, 1973. Change in carbohydrate composition during maturation of wheat and barley kernel. Cer. Chem., 50: 220-225.

Cuq, J.L., 1991. Technologie des protéines végétales. Université de Montpellier/Institut des Sciences de l'Ingénieur, Montpellier.

FAO/WHO/Codex, 1994. Aliments diététiques ou de régime y compris les aliments destinés aux nourrissons et enfants de bas âge. CODEX STAN 74-1981. Commission du Codex Alimentarius, vol. 4, 2nd edition. FAO/WHO, Rome.

Friedman, 1992. Dietary impact of food processing. Ann. Rev. Nutr., 56: 203-216.

Goering, H.K. and P.J. Van Soest, 1970. Forage fiber analysis (apparatus, procedures and some applications). Agriculture hand-book, agricultural research service, USA.

Goli, A.E., 1995. Bambara groundnut. In. Proceedings of the workshop on conservation and improvement of Bambara groundnut. 14-16 November 1995. Edited by Heller, J., Begemann and Mushonga. Harare, Zimbabwe. International Plant Genetic Resources Institute.

Guegen, J., R. Sanchez-Vioque and C. Malabat, 1999. Les protéines végétales: un réservoir de macromolécules multifonctionnelles. Oléag. Corps Gras, Lip., 6: 498-504.

Hsu, H.W., N.E. Sutton, Banjo, L.D. Satterlee and J.G. Kendrick, 1978. The C-PER and T-PER assays for protein quality. Food Tech., 32: 69-73.

International Institute of Tropical Agriculture [Internet], 2003. Cowpea [cited on 2006 Jan 12]; Available from www. iita.org.

Jacobsen, T. and D. Slotfeld-Ellingsen, 1983. Phytic acid and metal availability: a study of Ca and Cu binding. Cer. Chem., 60: 392-395.

Jipara, P., H. Normah, M.M. Zamaliah, R. Asmah and K. Mohamad, 2001. Nutritional quality of germinated cowpea flour (*Vigna unguiculata*) and its application in home prepared powdered weaning foods. Plant Food. Hum. Nutr., 56: 203-216.

Laurent, L., 1981. Elements minéraux. In Deymié, B., Multon, J.-L. and Simon, D. Techniques d'analyse et de contrôle dans les industries agro-alimentaires. Vol.4. Technique et Documentation, Paris, pp: 61-82.

Leske, K.L. and Coon, 1999. Nutrient content and protein and energy digestibilities of ethanol-extracted low alpha-galactoside soybean meal as compared to intact meal. Poult. Sci., 78: 1177-1183.

Minka, S.R. and M. Bruneteau, 2000. Partial chemical composition of Bambara pea [*Vigna subterranea* (L.) Verde]. Food Chem., 68: 273-276.

Montreuil, J., G. Spik and M.T. Tollier, 1981. Dosages colorimétriques des glucides. In Deymié, B., Multon, J.-L. and Simon, D. Techniques d'analyse et de contrôle dans les industries agro-alimentaires. Vol. 4. Technique et Documentation, Paris, pp: 85-143.

Nnam, N.M., 2001. Chemical, sensory and rheological properties of porridges from processed sorghum (*Sorghum bicolor*), Bambara groundnut (*Vigna subterranea L. Verde*) and sweet potato (Ipomoea batatas) flours. Plant Food. Hum. Nutr., 56: 251-264.

Nout, M.J.R., 1993. Processed weaning foods for tropical climates. Int. J. Food Sci., 43: 213-221.

Ohren, J.A., 1981. Process and product characteristics for soy concentrates and isolates. J. Am. Oil Chem. Soc., 58: 333.

Onyenekwe, P.C., G.C. Njoku and D.A. Ameh, 2000. Effect of cowpea processing methods on flatus causing oligosaccharides. Nutr. Res., 20: 349-358.

Pasquet, R. and M. Fotso, 1991. Les légumineuses alimentaires au Cameroun: premiers résultats. Du politique à l'économique, France, pp: 317-360.

Peterbauer, T. and A. Richter, 1998. Galactosyl ononitol and stachyose synthesis in seeds of adzuki bean; purification and characterisation of stachyose synthase. Plant Phys., 117: 165-172.

Sawardeker, J.J., J.H. Sloneker and A.R. Jeanes, 1965. Quantitative determination of monosaccharides as their alditol acetates by gas-liquid chromatography. Anal. Bioch., 12: 1602-1604.

Tharanathan, R.N. and S. Mahadevamma, 2003. Grain legumes-a boon to human nutrition. Trend. Food Sci. Tech., 14: 507-518.

Trèche, S. and I.L. Mbome, 1999. Viscosity, energy density and osmolality of gruels for infants prepared from locally produced commercial flours in some developing countries. Int. J. Food Sci. Nutr., 50: 117-125.

Tsukamoto, L., M. Miyoshi and Y. Hamaguchi, 1983. Purification and characterisation of three trypsin inhibitors from beans, phaseolus vulgaris 'kintoki'. Cereal Chem., 60: 281-285.

UNICEF, 1995. The state of the world's children 1992. Oxford University Press, Oxford.

WHO, 1998. Complementary feeding of young children in developing countries-a review of current scientific knowledge. WHO/NUT/98.1, Geneva.