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The Effect of Germination on the Oligosaccharides, Trypsin Inhibitors and Nutrient Content of Cowpea Milk

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ABSTRACT

Two cultivars of cowpeas, big white and small white, were germinated for 15 and 22 h respectively, before milk was extracted using a set of nylon netting sieves. Ungerminated seeds of the cowpea cultivars were similarly treated for milk extraction. The energy, protein, moisture, total solids, oligosaccharides, trypsin inhibitor activity, total sugars, minerals and digestibility were determined using appropriate methods.

The energy content of the milk from germinated seeds was 20·5 kcal for the small white and 16·5 kcal for the big white, while the corresponding figures for ungerminated beans were 29·1 and 42·4 kcal respectively. In all cases, germination reduced the nutrient content as well as the oligosaccharides when compared with the milk from ungerminated cowpeas. Trypsin inhibitor activity was, however, still high in both the germinated and ungerminated milk samples while in-vitro protein digestibility was not substantially changed in either variety.

Due to nutrient reduction, germination does not seem to be a good method of removing oligosaccharides which may produce flatulence in young children consuming cowpea milk.

INTRODUCTION

The problem of protein energy malnutrition (PEM) continues to be endemic in many developing countries and is most rampant among pre-school-age children. The most critical period of the infant's life is the weaning period when breast milk alone will not support growth and this period usually

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begins after 4–6 months (Belavady, 1980). To prevent the endemic nature of PEM, attempts have been made in many developing countries to provide substitutes for the expensive milk formulas used as supplements during this weaning period, with available local resources. A current effort being made in Nigeria in this direction is the extraction of milk from cowpeas, which is available to all mothers and relatively inexpensive when compared to other milk formulas. However, cowpeas contain various antinutritional factors which may affect their utilization by young children. These factors are associated with the presence of oligosaccharides and trypsin inhibitors which may contribute to the development of flatulence and possibly diarrhoea in children consuming cowpea-based products. (Steggarda, 1964; Steggarda & Dimmick, 1966). They have the effect of reducing cowpea utilization by mothers whose children would otherwise have benefited from the products. It has been reported that about 10% of young children showed some reaction to cowpea foods from a random sample survey of 1000 mothers (Hussain *et al.*, 1984). This figure becomes significant if translated into actual numbers of such children within the population.

Germination is known to reduce the amount of oligosaccharides in legumes (East *et al.*, 1972; Hsu *et al.*, 1980). Similarly, the protein quality of cowpeas is reduced by the presence of trypsin inhibitors. It is thus essential that products developed from legumes for consumption by infants must contain low levels of both the oligosaccharides and trypsin inhibitors while maintaining high nutrient levels.

The objective of this study was, therefore, to determine the effect of germination on the nutrient content, protein digestibility, trypsin inhibitor activity and oligosaccharide content of milk substitutes from two cultivars of cowpeas.

MATERIALS AND METHODS

Defective seeds and foreign bodies were removed from the dry cowpea seeds which were then germinated on two separate trays. Germination was carried out by soaking the seeds in distilled water for 10 min and spreading on moistened cotton wool on the individual trays before covering with another tray. These covered trays were placed in a cupboard in the laboratory and left at room temperature to germinate. After 15 and 22 h respectively, 90% of the big white and small white seeds had germinated. Percentage of germination was obtained through individual counting.

To obtain a mash, 500 g samples of germinated cowpeas were hulled by soaking in cold water for 1–4 h, and then soaked in cold water for 2–3 h before blending the beans using a 10-speed Osterizer blender with a ratio of one part beans to three parts water. This mash was sieved using three different sized nylon sieves to obtain the milk. To serve as the control for evaluating the effect of germination, 500 g samples of ungerminated cowpea seeds were similarly processed to extract milk. The milk was pasteurized at 60–70°C for 30–35 min followed by cooling and refrigeration. The process was repeated in duplicate on different days.

Samples of the cowpea milk were analysed for moisture, energy, protein, selected minerals and total sugars using appropriate AOAC (1980) methods. Trypsin inhibitor activity was determined using the method of Kakade *et al.* (1974), while in-vitro protein digestibility was determined using the method of Saterlee *et al.* (1979). All enzymes used were obtained from Sigma Chemical Co., St Louis, Missouri, USA. The values obtained were expressed per 100 ml of the milk extracts. Oligosaccharides were determined by paper chromatography (Onigbinde and Akinyele, 1983).

RESULTS AND DISCUSSION

The results showed that the cowpea milk yield (Table 1) of the big white cultivar was affected by germination. The yield from 500 g of the sample was reduced from 2850 ml to 2420 ml, while that of the small white variety was unaffected. Germination reduced the total solids, energy and protein contents of both cultivars. Protein digestibility (Table 2) of the big white cultivar milk was also reduced due to germination.

The oligosaccharide contents (Table 3) of the milk extracts were significantly reduced in both cultivars as a result of germination ($P < 0{\cdot}05$). The raffinose content was reduced from 2·6 to 1·07 g in the small white cultivar and from 1·29 to 0·84 g in the big white cultivar. The stachyose content was reduced from 3·5 to 2·34 g in the small white cultivar and from

TABLE 1
Nutrient Content and Yield of 100-ml Cowpea Milk

	Small white		*Big white*	
	Non-germinated	*Germinated*	*Non-germinated*	*Germinated*
Moisture (%)	92·9	94·9	89·3	95·7
Total solids (%)	7·1	5·1	10·7	4·3
Energy (kcal)	29·1	20·5	32·4	16·5
Protein (g)	2·6	1·4	3·0	1·2
Yield (ml) from 500 g	3 400	3 480	2 850	2 420

TABLE 2
Digestibility and Trypsin Inhibitor Activity of 100-ml Cowpea Milk

	Small white		*Big white*	
	Non-germinated	*Germinated*	*Non-germinated*	*Germinated*
Digestibility (%)	67·3	67·2	78·5	56·0
Trypsin inhibitor activity (Tiu/ml)	6·1	6·2	5·8	5·8

Tiu, trypsin inhibitor units.

3·8 to 2·27 g in the big white cultivar. Germination reduced the mineral content of the cowpea milk. The magnesium and iron contents were reduced considerably (Table 4).

The first stage of germination involves the breakdown of seed reserves and their utilization by the growing shoot and root for respiration. Beevers and Guernsey (1966) and Beevers (1968) showed that in peas during the period of rapid axis growth, the nitrogen content of the cotyledons declined rapidly. According to Chen and Thacker (1978), there is probably a turnover of proteins and amino acids during germination, with the balances between synthetic and degradative processes determining the resultant pattern. Thus, the lower protein and total solids content of the milk from germinated seeds resulted from the effect of germination.

The concept of germination before milk extraction from cowpeas does not augur well for the nutrient content of the resultant milk product. This becomes a disadvantage in its use as a supplement for infants. The advantages of reduction in oligosaccharide content do not outweigh the reductions in nutrient content, since the concern is to meet nutrient requirements so as to prevent malnutrition.

TABLE 3
Total Sugars and Oligosaccharide Content of 100-ml Cowpea Milk

	Small white		*Big white*	
	Non-germinated	*Germinated*	*Non-germinated*	*Germinated*
Sugars (g)	0·3	0·01	0·6	0·4
Raffinose (g)	2·60	1·07	1·29	0·84
Stachyose (g)	3·50	2·34	3·80	2·27

TABLE 4
Mineral Content of 100-ml Cowpea Milk

	Small white		*Big white*	
	Non-germinated	*Germinated*	*Non-germinated*	*Germinated*
Magnesium (mg)	9·8	4·2	16·0	4·5
Manganese (mg)	0·5	0·3	0·5	0·2
Iron (mg)	1·2	0·8	1·5	0·7
Copper (mg)	0·04	0·03	0·07	0·02
Zinc (mg)	0·5	0·4	0·6	0·2

Primary considerations in developing cowpea milk are to assist low-income mothers to successfully feed their infants through supplementation of breast milk. The goal of a milk substitute is to have as close a chemical composition as possible to cow's milk. Thus the feeding value of that milk would be determined by the closeness of the milk substitute to cow's milk. The process of germination has further lowered the expected feeding value of cowpea milk based on chemical composition. The process should, therefore, not be recommended to mothers as a means of reducing the flatulence properties associated with the oligosaccharides of cowpeas.

It may be possible to ferment the cowpeas or use enzyme hydrolysis to reduce the oligosaccharide level before milk extraction for feeding infants. Use of enzymes may not be practicable at the village level, while fermentation may introduce a sour flavour which may be unacceptable to some consumers. There is a dearth of information in the literature on the effect of germination on nutritive value of milk substitutes processed from legumes. This makes difficult a comparison of the trend obtained in this study with others. However, it is clear that in this study, germination considerably reduced the nutrient content of cowpea milk produced, while considerably reducing the oligosaccharides which cause flatulence in children consuming cowpea products.

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REFERENCES

AOAC (1980). *Official Methods of Analysis* (13th edn). Association of Official Analytical Chemists, Washington, DC.

Beevers, L. (1968). Protein degradation and proteolytic activity in the cotyledon of germinating pea seeds (*Pisum sativum*). *Phytochem.*, **7**, 1837.

Beevers, L. & Guernsey, F. S. (1966). Changes in some nitrogenous components during the germination of pea seeds, *Plant Physiol.*, **41**, 1455.

Belavady, B. (1980). Dietary supplementation and improvements in the lactation performance of Indian women. In *Maternal Nutrition During Pregnancy and Lactation*, ed. H. Abei & R. Whitehead. Nestlé Foundation Publ. Series 1, Bern, Switzerland, p. 264.

Chen, L. H. & Thacker, R. (1978). Germination and nitrogenous constituent of pea seeds (*Pisum sativum*). *J. Food Sci.*, **43**, 1884.

East, J. W., Nakayama, T. & Parkman, S. B. (1972). Changes in stachyose, raffinose, sucrose and monosaccharides during germination of soybean. *Crop. Sci.*, **12**, 7.

Hsu, D., Leug, H. K., Finney, P. L. & Morad, M. M. (1980). Effect of germination on nutritive value and baking properties of dry peas, lentils and faba beans. *J. Food Sci.*, **45**, 87.

Hussain, M. A., Akinyele, I. O. & Omololu, A. (1984). Maternal perception of problems associated with consumption of cowpeas by children. Paper presented at World Cowpea Research Conference, IITA Ibadan, 5–9 Nov.

Kakade, M. L., Rackis, J. J., McGhee, J. E. & Puski, G. (1974). Determination of trypsin inhibitor activity of soy products. A collaborative analysis of an improved procedure. *Cereal Chemistry*, **51**(3) 376.

Onigbinde, A. O. & Akinyele, I. O. (1983). Oligosaccharide content of 20 varieties of cowpeas in Nigeria. *J. Food Sci.*, **48**(4), 1250.

Saterlee, L. D., Marshall, H. F. & Tennyson, J. M. (1979). Measuring protein quality. *J. Am. Oil Chem. Soc.*, **56**, 103.

Steggarda, F. R. (1964). Mechanism of gas production following the consumption of beans. In *Proceedings of the 7th Annual Conference on Dry Beans Research*, Ithaca and Geneva, NY, p. 1456.

Steggarda, F. R. & Dimmick, J. F. (1966). Effects of bean diets on concentration of CO_2 in flatus. *Am. J. Clin.*, **19**, 120.