*Food Chemistry* **42** (1991) 129–134

# Effect of Process Method on the Energy and Protein Content, Antinutritional Factors and In-Vitro Protein Digestibility of Cowpea Milk (*Vigna unguiculata*)

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(Received 23 May 1990; revised version received and accepted 20 November 1990)

*ABSTRACT*

*Milk was obtained from two cultivars of cowpeas (*Vigna unguiculata*) by hot water extraction, cold water extraction or boiling for 10 min before cold water extraction. The energy content of the extracts for both cultivars and extraction methods ranged (per 100 g) from 25·0 to 31·0 kcal (1 cal = 4·2 J), protein from 1·0 to 2·0 g, sugars from 0·5 to 3·8 g and iron from 0·9 to 1·5 mg. In-vitro digestibility coefficient ranged from 60·5 to 81·8%, trypsin inhibiting units/ml from 4·5 to 6·1, while phytic acid ranged from 61·8 to 80·5 μg/100 g. Milk yield was higher in the small white cultivar than the big white. The beany taste was completely absent in the hot water extraction process while it was slightly perceptible in the pre-boiled process and distinct in the cold water extraction.*

## INTRODUCTION

Preparation of liquid supplements continue to be of interest in infant nutrition due to the Structural Adjustment Programme (SAP) in Nigeria which continues to make commercial infant formulas expensive and unavailable to many mothers.

The impact of SAP is felt most among the infants and children of poor families who are at risk of malnutrition when breast milk alone will not support growth after four months of age (Belavady, 1980).

Research efforts have been directed towards the provision of substitutes derived from local food materials which could provide the necessary nutrients to support the growth and development of infants and children of



*Food Chemistry* 0308-8146/91/$03·50 

poor families. It has been previously shown (Akinyele & Adesina, 1986; Akinyele & Abudu, 1990) that milk extraction from cowpeas was possible; however, a suitable extraction method, which would increase its acceptability, needs to be determined.

The objective of this study was to compare the effects of three processes on the energy and protein content, antinutritional factors, in-vitro protein digestibility and acceptability of cowpea milk substitute.

## MATERIALS AND METHODS

Two cultivars of cowpeas (*Vigna unguiculata*) were used for the study. The cultivars (local small white and local big white) were obtained from the market. Foreign bodies and defective seeds were removed from the bulk purchased cowpea seeds before being used in milk preparation.

Milk extraction was carried out using 500 g of each cultivar, soaked in cold water to remove seedcoats, with re-soaking of the dehulled beans in cold water for several hours before blending in a 10 speed blender (Osterizer, Milwaukee, Wisconsin, USA) with hot water, cold water or boiling the cotyledons for 10 min before blending with cold water. The mash obtained was sieved using three different sized nylon netting sieves to obtain the milk. The milk was pasteurized in a water bath at 60°C for 30 min followed by cooling and refrigeration. The process was repeated in triplicate for each cultivar on different days.

Samples of the cowpea milks were analysed in triplicate for moisture, energy, crude protein, total sugars, trypsin inhibitor activity, phytic acid, iron and in-vitro protein digestibility.

### Compositional analyses

Moisture was determined using the AOAC (1980) procedure in which 10 ml of the milk samples were weighed into clean moisture cans in triplicate; the cans were weighed before and after, then dried in a air oven (Gallenkamp, London, UK) initially at 60°C for 12 h then at 105°C until constant weight was obtained. The moisture content was then calculated by difference and expressed as a percentage.

The caloric values of the samples were determined using the ballistic bomb calorimeter (Gallenkamp (London, UK) model CB-370) with benzoic acid as standard (AOAC, 1980). The basic principle was to measure, by a galvanometer and thermocouple system, the temperature rise due to the combustion of a known weight of sample in excess oxygen. The maximum deflection on the galvanometer was read and used to calculate the energy content per gram of sample.

The semi-micro-Kjeldahl procedure (AOAC, 1980) was used to determine the crude protein content. Digestion was carried out on a Techne Dri-block DB 4 system and distillation was with the Markam apparatus. Hydrochloric acid was used to titrate the distillate and the percentage of nitrogen calculated. This was multiplied by 6·25 to obtain the protein content.

The amount of sugars was estimated using a combination of the methods of Dubois *et al.* (1956) and Southgate (1969). Samples were extracted with 80% (v/v) ethanol in 250 ml conical flasks. The suspensions were heated until boiling before being filtered using Whatman filter paper No. 1 followed by rinsing with 40 ml 80% ethanol. The filtrate was then evaporated to about 20 ml and then made up to 100 ml with distilled water. Six drops of saturated lead acetate were added and the solution filtered until a clear colour solution was obtained. Ethanol (0·5 ml of 80%) followed by 5 ml conc. $H_2SO_4$ were added to 2 ml of the filtrate in test tubes and allowed to stand for 10 min for colour development. The absorbance was read at 490 nm on a spectronic 20 (Bausch and Lomb) spectrophotometer and the amount of total sugars calculated using the standard curve of glucose. Phytic acid was determined by the method of Young and Greaves (1936).

Protein digestibility was estimated in vitro by the multi-enzyme technique of Satterlee *et al.* (1979). All enzymes were obtained from the Sigma Chemical Co. (St Louis, MO). The antitryptic activity of the samples was estimated using the method of Kakade *et al.* (1969). Trypsin inhibitor was expressed in TIU/ml.

The samples of the milk substitutes used for sensory analysis were prepared as described earlier. Samples were prepared in the morning and a fourteen member laboratory panel evaluated the products in mid-afternoon using the multiple comparison test (Larmond, 1982) to evaluate the flavour since the beany taste was a major reason for the non-use of cowpea milk by some urban mothers.

## RESULTS AND DISCUSSION

The results of the study are presented in Tables 1 and 2. These results show that milk from the hot water process for the local small white contained 1·6% protein, 3·8 g total sugars and 72·1 $\mu$g phytic acid. In-vitro digestibility was 81·8% while yield was 3250 ml of milk per 500 g starting material. The values obtained for the other two processes were significantly different ($P < 0{\cdot}05$) for energy, protein, sugars and in-vitro protein digestibility. The energy content for the hot water process was similar to the cold water extract but different from the pre-boiling process before cold water extraction. Difference in digestibility was due to differences in level of trypsin inhibitor (Table 1).

**TABLE 1**
Energy, Protein, Antinutritional Factors and In-vitro Protein Digestibility of Milk from Small White Cowpea Cultivar

| | *Hot water extraction* | *Boil for 10 min then cold water extraction* | *Cold water extraction* |
|---|---|---|---|
| Moisture (g/100 g) | 92·5 | 95·4 | 92·4 |
| Energy (kcal/100 g) | 29·5 | 26·9 | 31·0 |
| Crude protein (g/100 g) | 1·6 | 1·0 | 2·0 |
| Sugars (g/100 g) | 3·8 | 2·9 | 0·5 |
| Iron (mg/100 g) | 1·2 | 1·2 | 0·9 |
| Digestibility coefficient (%) | 81·8 | 60·5 | 66·0 |
| TIU/ml | 4·5 | 6·7 | 6·1 |
| Phytic acid (μg/100 g) | 72·1 | 77·2 | 80·5 |
| Yield of milk (ml) from 500 g sample | 3 250 | 3 400 | 3 400 |

The result from the big white cultivar shows that energy, protein, iron, and in-vitro protein digestibility were not significantly different for the three processes. Similarly, the yield and level of phytic acid were also not significantly different (Table 2).

The result of sensory evaluation showed that all panellists were in agreement in choosing the hot water extracted milk sample as having a bland taste, the sample boiled for 10 min before cold water extraction still having a slightly beany taste, while the cold water extracted product was considered unacceptable due to the strong beany taste of the milk.

The presence of considerable amounts of phytic acid would tend to

**TABLE 2**
Energy, Protein, Antinutritional Factors and In-vitro Protein Digestibility of Milk from White Cowpea Cultivar

| | *Hot water extraction* | *Boil for 10 min then cold water extraction* | *Cold water extraction* |
|---|---|---|---|
| Moisture (g/100 g) | 93·2 | 94·1 | 93·1 |
| Energy (kcal/100 g) | 26·5 | 25·0 | 25·9 |
| Crude protein (g/100 g) | 1·5 | 1·4 | 1·8 |
| Sugars (g/100 g) | 2·4 | 1·5 | 1·5 |
| Iron (mg/100 g) | 1·5 | 1·2 | 1·8 |
| Digestibility coefficient (%) | 69·4 | 61·6 | 65·0 |
| TIU/ml | 5·9 | 6·0 | 6·1 |
| Phytic acid (μg/100 g) | 61·8 | 66·9 | 71·7 |
| Yield of milk (ml) from 500 g sample | 2 950 | 3 000 | 3 000 |

indicate that bioavailability of minerals especially iron may be a problem due to its binding with phytic acid. This becomes of importance in the utilization of the products by infants and children.

The data reported in this study agree with previous reports (Akinyele & Adesina, 1986; Akinyele & Abudu, 1990) and further confirm that milk of acceptable quality can be produced from cowpeas. It is, however, essential that a hot water extraction process be used to eliminate the beany taste. It is noted that the energy values of the milk substitutes are still much lower than the energy content of cows milk, human milk or soya milk. Therefore, large volumes of the milks would need to be consumed to meet nutrient requirements especially for energy.

It is necessary for further studies to seek different sources of fat that could increase the energy content of the milk while maintaining acceptability. Alternatively, it might be interesting to determine whether enzymatic hydrolysis of the higher oligosaccharides before milk extraction would raise the level of sugars extracted into the milk to increase the energy content. However, such a process may not be acceptable for home use, especially by illiterate mothers.

## ACKNOWLEDGEMENT

The author is grateful to Ms I. A. Abudu and Mr Lekan Akinyemi for technical assistance, the Department of Human Nutrition for facilities, and Ms Y. F. Oyebamiji for typing the manuscript.

## REFERENCES

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

Akinyele, I. O. & Abudu, I. A. (1990). Acceptability and nutrient content of milk, substitutes from four cultivars of cowpeas (*Vigna unguiculata*). *J. Food Sci.*, **55**, 701–3.

Akinyele, I. O. & Adesina, A. (1986). Infant food preparations from cowpea, coconut and soybeans. *J. Food Tech.*, **21**(6), 711–15.

Belavady, B. (1980). Dietary supplementation and improvements in the lactation performance of Indian women. In *Maternal Nutrition during Pregnancy and Lactation*. Publication Series 1, Nestlé, Bern, Switzerland, 264–73.

Dubois, M., Gilles, K. A., Hamilton, S. K., Rebers, P. A. & Smith, F. (1956). Colorimetric determination of sugars and related substances. *Anal. Chem.*, **28**, 350–4.

Kakade, M. L., Simons, N. & Liener, I. E. (1969). An evaluation of natural versus synthetic substrates for measuring the antitryptic activity of soybean samples. *Cereal Chem.*, **46**, 518.

Larmond, E. (1982). Laboratory methods for sensory evaluation of food. Food Research Institute, Ottawa, Canada.

Satterlee, L. D., Marshall, H. F. & Tennyson, J. M. (1979). Measuring protein quality. *J. Amer. Oil Chem. Soc.*, **56**, 103–9.

Southgate, D. A. T. (1969). Determination of carbohydrates in foods. 1. Available carbohydrates. *J. Sci. Food Agric.*, **20**, 326–32.

Young, S. M. & Greaves, J. E. (1936). Influence of variety and treatment on phytic acid content of wheat. *Food Res.*, **5**, 103–5.