Food Chemistry **46** (1993) 159–162

In vitro protein digestibility of *Leucaena leucocephala* seed kernels and protein isolate

Poonam Sethi & Pushpa R. Kulkarni

Food and Fermentation Technology Division, Department of Chemical Technology, University of Bombay, Matunga, Bombay-400 019, India

(Received 12 February 1992; revised version received and accepted 8 May 1992)

The *in vitro* digestibility of *Leucaena* seed kernel meal was appreciably improved from 25·3% to 89·2% on autoclaving. There was an increase in digestibility of *Leucaena* protein isolate (LPI) as compared to the unautoclaved (raw) seed kernels, which could be attributed to the removal of a number of antinutritional factors, particularly mimosine, in the non-precipitable supernatant (whey) fraction. Autoclaving of the raw *Leucaena* seed meal, as well as converting it into protein isolate, was found to improve its in vitro digestibility appreciably.

INTRODUCTION

Although wild legume seeds are an important reservoir of protein in the vegetable kingdom, few of such seeds have been studied from the nutritional point of view (Giral *et al.*, 1978; Sotelo *et al.*, 1980; Lucas *et al.*, 1988). There are many reports on the nutritional evaluation of leaves of one such wild legume plant, *Leucaena* (Ter Meulen *et al.*, 1979; Akbar & Gupta, 1984, 1985; Hongo *et al.*, 1983, 1987; Kewalramani *et al.*, 1987; Yadav & Yadav, 1988). However, these high protein seeds have still remained unexplored in this respect. Besides mimosine (Matsumoto *et al.*, 1951; Ter Meulen *et al.*, 1979; Hongo *et al.*, 1983, 1987; Akbar & Gupta, 1984; Padmavathy Shobha, 1987; Yadav & Yadav, 1988), many other antinutritional factors (CSIR, 1962; Jones & Earle, 1966; Lesniak, 1977, 1983; Fukuda *et al.*, 1980; D'Mello & Fraser, 1981; Akbar & Gupta, 1985; Tangendjaja *et al.*, 1986) present in different parts of the *Leucaena* plant have been known to limit their utilization by non-ruminants.

Therefore, in the present work, a study was undertaken of the proteins from seeds of one of the unexplored species, *L. leucocephala*, and also of the protein isolate. The study was concerned with in vitro digestibility and with a view to utilizing *L. leucocephala* as an edible source of protein.

MATERIALS AND METHODS

The *L. leucocephala* seeds were a gift from Gloria Land, Dairy Farm, Sri Aurobindo Ashram, Pondicherry, India. The seeds were manually dehulled after soaking the whole seeds in hot water (initial temperature, 80°C) for 24 h.

Pepsin (Loba-Chemi Indo Austranal Co.) and pancreatin from M/s. B.D.H., London, were used. Soy protein isolate (SPI) procured from Tata Oil Mills Co. (India) was included for comparison.

Separate sets of five samples of *Leucaena* seed, weighing 50g each, were randomly selected from the bulk of *Leucaena* seeds for seed flour preparation, for autoclaved samples, and also for *Leucaena* protein isolate (LPI) preparation.

In all the experiments, mean ± SD values were calculated. However, for the purpose of plotting Figs 1 and 2 (see below) only the mean values were employed.

Flour preparation

The moist *Leucaena* seed kernels were dried in the sun for approximately 48 h, and ground to pass through a 40-mesh sieve.

Autoclaving

The seeds, after soaking in water (80°C, initial temperature) for 24 h, were autoclaved at 15 psi/g for 15 min (seed : water ratio, 1 : 2 v/v), manually dehulled, sun-dried and ground, as described above.

Isolate preparation

Leucaena protein isolate (LPI) was prepared using defatted *Leucaena* seed kernel flour, extracted in 5% sodium chloride and separated by precipitation at isoelectric pH (pI) of 2–2·5. The pI of *Leucaena* seed kernel proteins was determined by adding varying amounts of 0·1N HCl to a fixed volume of the seed extract and checking the pH of the sample at which maximum precipitation of protein took place. The yield of LPI was 20–21 g/100 g seed kernels.

Determination of in vitro protein digestibility

This was carried out using a modification of the standard reported method (Akeson & Stahman, 1964; Ramasubramanian, 1983) with 3 h pepsin digestion followed by 6 h pancreatin digestion.

Estimation of α-amino nitrogen

α-Amino nitrogen was estimated at 0 h, 1 h, 2 h and 3 h periods during pepsin digestion and at 0 h, 1 h, 2 h, 3·5 h 5 h and 6 h periods during the course of pancreatin digestion, by the formol titration method (Levy, 1957).

Estimation of TCA-soluble nitrogen

Ten millilitres of 10% TCA (trichloroacetic acid) was mixed with 10 ml of digestion slurry at 0 h and 3 h of peptic digestion and at 0 h and 6 h of pancreatic digestion. The mixture was allowed to stand overnight and filtered. TCA filtrate (10 ml) was analyzed for nitrogen by the Microkjeldahl Nesslerization method (ISO, 1981). The net increase in TCA-soluble nitrogen was calculated after taking into account the corresponding blank values.

Protein digestibility was defined as the net increase in TCA-soluble nitrogen, obtained by the pepsin-pancreatin action as a percentage of total nitrogen.

RESULTS AND DISCUSSION

The in vitro protein digestion was followed in terms of the increase in α-amino nitrogen, and the percentage digestibility was calculated by expressing total TCA-soluble nitrogen as a percentage of total nitrogen. The percentage digestibility during the initial pepsin digestion for 3 h followed by pancreatin digestion for 6 h more is depicted in Fig. 1, where an appreciable increase from 25·3 to 89·2% is shown following autoclaving *Leucaena* seed meal. The percentage digestibility of *Leucaena* seed meal on autoclaving (89·2%) was in very close agreement with values obtained for wheat protein concentrate (90·4%) (Hsu *et al.*, 1977), safflower protein isolate (88·8–90·9%) (Paredes–Lopez & Ordorica–Falomir, 1986), and casein (89·2–90·9%) (Ramasubramanian, 1983; Hsu *et al.*, 1977).

Also, the amount of α-amino nitrogen released at the end of the 9 h pepsin—pancreatin digestion (Fig. 2) was much greater in the case of the autoclaved sample, than the unautoclaved sample. The difference in the release of α-amino nitrogen between the two samples was more pronounced at the pancreatin digestion stage, which could in turn be probably attributed to the heat-inactivation of certain toxic constituents interfering in the protein digestion, on autoclaving. Similar increases in the enzymatic release of amino acids have been reported in the case of soybean meal after steaming (Sheffner, 1967; Stahmann & Woldegiorgis, 1975).

In the present work, both soy and *Leucaena* protein isolates showed a fairly good percent digestibility (71–76%), but the *Leucaena* isolate appeared to be slightly more digestible than the soy protein sample (Fig. 1). Also, in the case of both protein isolate samples studied, the release of TCA-soluble nitrogen

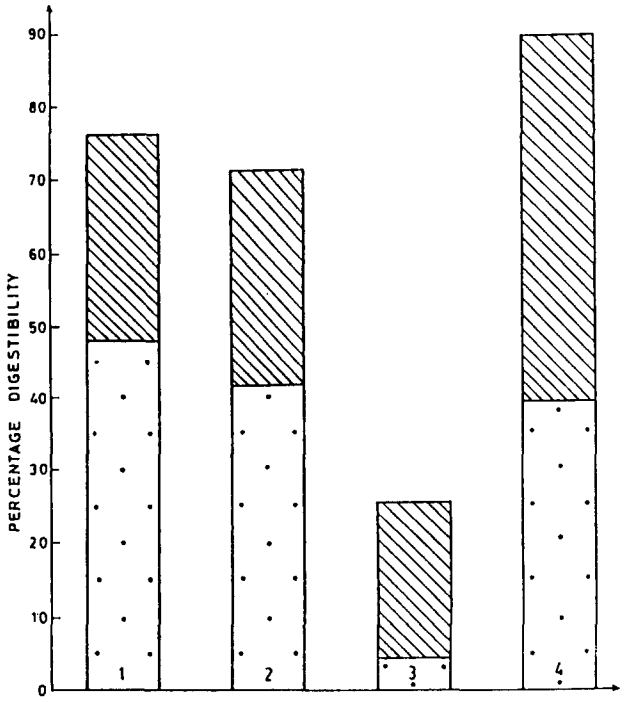

Fig. 1. In vitro protein digestibility of *Leucaena* and soy protein isolates (LPI and SPI) and untreated and autoclaved *Leucaena* seed kernels using pepsin and pancreatin. (1, LPI; 2, SPI; 3, untreated: 4, autoclaved; ⊡, peptic digestion (3h); ▨, pancreatic digestion (6h)).

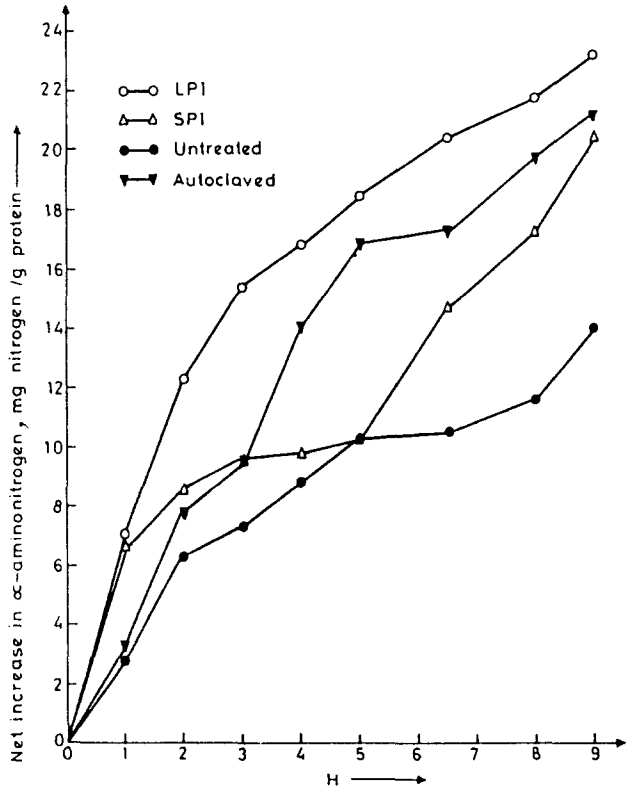

Fig. 2. Release of α-amino nitrogen during in vitro digestibility of *Leucaena* and soy protein isolates (LPI and SPI) and untreated and autoclaved *Leucaena* seed kernels.

was greater at the end of pepsin digestion than during pancreatin digestion.

The percentage digestibility of LPI (76·1%) was found to be approximately three times that of untreated raw kernels (25·3%). There was also an increase in the pepsin digestibility of LPI by almost ten times the values for unautoclaved (raw) seed kernels (48·1% and 44%, respectively) (Fig. 1). This could be attributed to the easier action of the enzyme on isolated protein compared to the native form.

When the digestibility of LPI was compared with the digestibility of the autoclaved *Leucaena* seed kernel samples, the latter was found to have a higher percent digestibility (89·2%) than the former (76·1%) (Fig. 1). Also, autoclaving seemed to have a very significant effect on pancreatin digestion, with the percentage digestibility (on pancreatin digestion of autoclaved kernels) being almost double (50·0%) that with LPI (28·0%) (Fig. 1). The rise in pancreatin digestion in autoclaved samples could be attributed to the inactivation of the heat-labile toxic and antinutritional factors. On the other hand, a partial removal of soluble antinutritional factors during protein isolation preparation can explain the relatively lower digestibility of LPI.

The extent of α-amino nitrogen released at the end of the 9 h pepsin–pancreatin digestion in the case of LPI was comparable to, though slightly higher than, that for SPI and autoclaved seed kernels (Fig. 2). However, the values were very much lower for unautoclaved (raw) seed kernels, as was also seen in the case of TCA-soluble nitrogen. This was due to the lower digestibility of the latter sample. The greater release of α-amino nitrogen in the LPI sample as compared to the untreated (raw) seed kernels could be due to the removal of a number of aninutritional factors in the supernatant (whey) fraction, including mimosine. There was also a relatively higher rate of release of α-amino nitrogen at the pepsin digestion step for the LPI sample, as compared to the other three samples (Fig. 2). The only explanation possible for this phenomenon could be the unfolding of the *Leucaena* proteins during the isolation process, thus resulting in a greater release of α-amino nitrogen in this sample during the pepsin digestion step.

CONCLUSION

The results of the in vitro studies revealed that untreated (unautoclaved, raw) *Leucaena* flour had low protein digestibility (25·3%) which, however, increased dramatically to 89·2%, on autoclaving the seed samples. The in vitro digestibility of the *Leucaena* protein isolate (LPI) was found to be close to and slightly better than the soy protein isolate (SPI) sample in the present study. On comparing the digestibility of LPI with the untreated and autoclaved *Leucaena* seed kernel flours, it was apparent that the digestibility was greatly improved during the protein isolation process as compared to the untreated seed kernels, though the autoclaved sample had an even greater digestibility than the LPI.

ACKNOWLEDGEMENTS

The authors are grateful to Dyuman Bhai, Trustee, Sri Aurobindo Ashram, Pondicherry, India, and Mr. Manindra Pal of Gloria Land, Dairy Farm, Sri Aurobindo Ashram, Pondicherry, India, for gifts of the *L. leucocephala* seed samples. This research was supported in part by the R. D. Birla Smarak Kosh, Bombay Hospital, Bombay, India.

REFERENCES

Akbar, M. A. & Gupta, P. C. (1984). Mimosine in subabul (*Leucaena leucocephala*). *Indian J. Dairy Sci.*, **37**, 287–9.

Akbar, M. A. & Gupta, P. C. (1985). Proximate composition and tannin and mineral contents of various plant parts of subabul (*Leucaena leucocephala*). *Indian J. Anim. Sci.*, **55**, 808–12.

Akeson, W. R. & Stahmann, M. A. (1964). A pepsin pancreatin digest index of protein quality evaluation. *J. Nutrition*, **83**, 257–61.

CSIR. (1962). *Leucaena* Benth. (*Leguminosae*). In *The Wealth of India—Raw Materials*, Vol. 6. Council of Scientific and Industrial Research, New Delhi, p. 77–9.

D'Mello, J. P. F. & Fraser, K. W. (1981). The composition of leaf meal from *Leucaena leucocephala*. *Trop. Sci.* **23**, 75–8.

Fukuda, N., Koja, T., Chinen, I., Hongo, F., Shiroma, S. & Yomo, H. (1980). Studies on protease inhibitors in *Leucaena leucocephala* de Wit seed. *Nippon Nogeikagaku Kaishi*, **54**, 1015–19.

Giral, F., Sotelo, A., Lucas, B. & De La Vega, A. (1978). Chemical composition and toxic factors content in fifteen leguminous seeds. *Quart. J. Crude Drug Res.*, **16**, 143–9.

Hongo, F., Kawashima, Y., Shiroma, S. & Fukazawa, T. (1983). Purification of mimosine from *Leucaena leucocephala* de Wit and its physiological activity for mice. *Jpn. J. Zootech Sci.*, **54**, 217–23.

Hongo, F., Kawashima, Y., Tawata, S., Sunagawa, K. & Moromizato, S. (1987). Studies on chemical composition and mimosine content of *Leucaena leucocephala* de Wit. *Ryukyu Daigaku Nogakubu Gokujutsu Hokoku*, **34**, 51–7.

Hsu, H. W., Vavak, D. L., Satterlee, L. D. & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *J. Food Sci.*, **42**, 1269–73.

ISO. (1981). ISO 5378 : 1978 (BS 5697 : Part 2 : 1979). In *Pearson's Chemical Analysis of Foods*, 8th edn., ed. H. Egan, R. S. Kirk & R. Sawyer. Churchill Livingstone, Edinburg.

Jones, Q. & Earle, F. R. (1966). Chemical analysis of seeds II: Oil and protein content of 759 species. *Economic Botany*, **20**, 127–55.

Kewalramani, N., Ramchandra, K. S., Upadhyay, V. S. & Gupta, V. K. (1987). Proximate composition, mimosine and mineral contents of *Leucaena* sp. and hybrids. *Indian J. Anim. Sci.*, **57**, 1117–20.

Lesniak, A. P. (1977). Purification and characterisation of a hemagglutinin isolated from *Leucaena leucocephala*. *Plant Physiology Lancaster*, **59**, Supplement 67. Quoted in Ter Meulen, U., Struck, S., Schulke, E. and El-Harith, E. A. (1979). A review on the nutritive and toxic aspects of *Leucaena leucocephala*. *Trop. Anim. Production*, **4**, 113–26.

Lesniak, A. P. (1983). Purification and characterization of hemagglutinins in *Leucaena leucocephala* and *leptogorgia virgulata*. *Diss. Abstr. Int. B*, **44**, 985 (In *Chemical Abstracts* (1984) **100**: 33149s).

Levy, M. (1957). Titrimetric procedures for amino acids (Formol, acetone and alcohol titrations). In *Methods in Enzymology*, Vol. 3, ed. S. P. Colowick & N. O. Kaplan. Academic Press, New York, p. 457.

Lucas, B., Guerrero, A., Sigales, L. & Sotelo, A. (1988). True protein content and non-protein amino acids present in legume seeds. *Nutrition Reports International*, **37**, 545–53.

Matsumoto, H., Smith, E. G. & Sherman, G. D. (1951). The effect of elevated temperatures on the mimosine content and toxicity of Koa Haole (*Leucaena glauca*). *Arch. Biochem. Biophys.*, **33**, 201–11.

Padmavathy, P. & Shobha, S. (1987). Effect of processing on protein quality and mimosine content of Soobabul (*Leucaena leucocephala*). *J. Food Sci. Technol.*, **24**, 180–2.

Paredes-López, O. & Ordorica-Falomir, C. (1986). Production of safflower protein isolates: composition, yield and protein quality. *J. Sci. Food Agric.* **37**, 1097–103.

Ramasubramanian, N. (1983). Studies on seed proteins. PhD. (Tech.) Thesis, Dept. of Chemical Technology, University of Bombay, Bombay.

Sheffner, A. L. (1967). In vitro protein evaluation. In *Newer Methods of Nutritional Biochemistry—With Applications and Interpretations*, Vol. 3, ed. A. A. Albanese. Academic Press Inc., New York, p. 125–95.

Sotelo, A., Lucas, B., Uvalle, A. & Giral, F. (1980). Chemical composition and toxic factors content of sixteen leguminous seeds (II). *Quart. J. Crude Drug Res.*, **18**, 9–16.

Stahmann, M. A. & Woldegiorgis, G. (1975). Enzymatic methods for protein quality determination. In *Protein Nutritional Quality of Foods and Feeds, Pt. I. Assay Methods—Biological, Biochemical, and Chemical*, ed. M. Friedman. Marcel Dekker Inc., New York, p. 211.

Tangendjaja, B., Lowry, J. B. & Wills, R. B. H. (1986). Changes in mimosine, phenol, protein and fibre content of *Leucaena leucocephala* leaf during growth and development. *Aust. J. Exp. Agric.* **26**, 315–17.

Ter Meulen, U., Struck, S., Schulke, E. & El-Harith, E. A. (1979). A review on the nutritive value and toxic aspects of *Leucaena leucocephala*. *Trop. Anim. Production*, **4**, 113–26.

Yadav, P. S. & Yadav, I. S. (1988). Proximate composition, tannin and mimosine content in different parts of cultivars of subabul. *Indian J. Anim. Sci.*, **58**, 953–8.