THE ROLE OF FERMENTATION AND SPROUTING IN THE REDUCTION OF PROTEASE INHIBITORS IN RAW SOYABEANS

P. T. Banda, F. Rumosa-Gwaze, I. D. T. Mpofu and S. M. Makuza

Department of Animal Science, University of Zimbabwe, P. O. Box M. P. 167, Mount Pleasant, Harare, Zimbabwe
ptbanda@agric.uz.ac.zw

INTRODUCTION

Cereal grains contribute substantially to total daily energy and protein requirements for humans and livestock. They are however low in protein levels as well as protein quality, especially the essential amino acid lysine. Soyabeans are rich sources of lysine and have higher amounts of proteins compared to cereals (Wang and Hesseltine, 1983). Soyabeans contain antinutritional factors which may reduce the efficiency of utilisation of protein, interfere with digestion and can also lead to ammonia toxicity (Leiner, 1994). Methods of eliminating antinutritional factors in Zimbabwe involve the use of elevated temperatures during oil extraction and roasting. The energy to generate the heat may not always be available especially at small scale levels of production. Fermentation and sprouting of leguminous seeds for human and livestock consumption are widely practised techniques in many African countries Prinyawiwatkul et al. (1996) and to destroy potential toxins such as phytate and protease inhibitors (Steinkraus, 1994). The objective of this study was to determine the effect of sprouting and fermentation on protease inhibitors factors in raw soyabeans as measured by the urease activity test and on the proximate composition of the soyabeans.

MATERIALS AND METHODS

Sample preparation; sprouting

Two treatments were investigated and they were: sprouting and the control in which beans were not sprouted. A sample of 400 g of soyabeans was soaked for 24 hr in excess water. The water was drained. For the control, the sample was not soaked. The samples were left under ambient conditions for 5 days after which period they were air dried to constant weight

Sample preparation; fermentation

A sample of 250 g of ground soyabeans was thoroughly mixed with 250 g of maize meal. Three treatments were investigated as follows: 50 ml of water were added to 50 ml of molasses (10% v/v) and the mixture stirred. The molasses contained 45 % sucrose. The molasses was thoroughly mixed with the soyabean-maize mix. 22.5 g of brown sugar (98 % g/g) was dissolved in 50 ml of water and mixed thoroughly with a soyabean-maize mix. No fermentable substrate was added to the third treatment (control). The samples were placed in plastic bags, compressed and sealed. The bags were left to ferment naturally at ambient conditions for 21 days.

The urease activity test
The Rasmussen's method (Analytichem, 2003) was used on bean samples that were ground using a Wiley Mill (1 mm screen) .

Proximate Analysis
Dry matter, ether extract and crude protein were determined using AOAC (1980) procedures on ground samples (Wiley Mill, 3 mm screen).

RESULTS AND DISCUSSION

Table 1. The effect of sprouting on mean dry matter content, ether extract (lipid) content, crude protein content and urease activity of raw soyabeans

Treatment	Dry Matter (%)	Ether Extract (%)	Crude Protein (%)	Urease activity (meq/ g)*
Sprouted seed	94.15 ± 0.19	16.84 ± 0.31	42.55 ± 0.3	0.59 ± 0.19
Unsprouted seed (control)	85.00 ± 0.43	22.68 ± 0.69	39 ± 0.66	6.42 ± 0.12

- *Correctly processed soyabean shows values between 0.5-1.0 meq/ g (Analytichem, 2003)*

A decrease of 26 % in ether extract was observed with sprouting (Table 1). This is supported by work done by Njoku and Okemadu (1989).

There was a significant decrease in dry matter content with sprouting. During germination, carbohydrate reserves are broken down to provide energy for the growing embryo (Opoku et al., 1983). Some nutrients are lost through leaching during soaking (Chavan and Kadam, 1989).

The increase in protein content with sprouting is consistent with work done by Opoku et al. (1983). The breakdown of tannin-protein complexes can increase the protein content during sprouting (Ikemefuna et al., 1991).

Table 2. The effect of fermentation of soyabean-maize mix on dry matter loss, crude protein loss and urease activity

	Dry Matter loss (%)		**Crude Protein loss (%)**		**Urease value (meq/ g)**	
	Fermented	***Unfermented***	***Fermented***	***Unfermented***	***Fermented***	***Unfermented***
Brown	3.88	1.6	14.9	3.0	0.46	4.3
sugar	± 0.07	± 1.64	± 0.34	± 0.76	± 0.04	± 0.13
Molasses	3.65	1.85	9.5	2.5	0.41	4.2
	± 0.07	± 1.64	± 0.34	± 0.76	± 0.04	± 0.13

The use of molasses and brown sugar significantly affected crude protein loss of the fermented soyabean-maize meal mix but not the dry matter loss and the urease activity (Table 2). Sugars and sugar rich materials such as brown sugar and molasses can be added during fermentation to encourage the growth of lactic acid bacteria (McDonald et al., 1984)

The loss of protein that was observed for soyabean-maize meal samples that were fermented with the molasses and brown sugar (Table 2) is consistent with work that was done by Njoku and Okemadu (1989). Protein can be released as volatile compounds such as ammonia and volatile fatty acids into the environment as a result of the activity of proteolytic microorganisms such as *Clostridia spp* (Collar et al. (1991); Umoh and Fields (1981)). High levels of protein breakdown are of concern because they can lead to the spoilage of the product and formation of biogenic amines (Wang and Hesseltine, 1981).

There was a loss of dry matter with fermentation for soyabean-maize meal samples that were treated with molasses and brown sugar (Table 2). This is consistent with work done by Umoh and Fields (1981). The decrease in dry matter may be partly explained by a corresponding decrease in protein content.

The urease activity of the fermented soyabean-maize meal mix was lower than that of the unfermented mix (Table 2). This is supported by work done by Reddy and Pierson (1994). Raw soyabeans contain the enzyme urease which breakdown urea to ammonia which is toxic (Bondi 1987). Urea is destroyed by heat and its presence can

be used as an indicator for other protease inhibitors which are also destroyed by heat such as trypsin and chymotrypsin inhibitors (McDonald et al., 1984).

CONCLUSIONS

Sprouting reduced the level of urease activity in raw soyabeans. There was a decrease in dry matter and ether extract (lipid) contents while the protein content increased.

Fermentation led to a decrease in protein content, dry matter content and urease activity. The addition of molasses or brown sugar affected the loss of protein but not loss of dry matter and urease activity. Further research is required to quantify and characterise the products of proteolysis and lipolysis which are known to affect the sensory properties of the product, its shelf life and safety.

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