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): 534-537, 2007
ISSN 1680-5194
© Asian Network for Scientific Information, 2007

Effect of the Edible Mushroom, *Pleurotus tuberregium* on the Cyanide Level and Nutritional Contents of Rubber Seed Cake

J.E. Ukpebor[1], E.O. Akpaja[2], E.E. Ukpebor[1], O. Egharevba[3] and E. Efedue[1]
[1]Department of Chemistry, University of Benin, Benin City, Nigeria
[2]Department of Botany, University of Benin, Benin City, Nigeria
[3]Department of Rubber Technology, Rubber Research Institute of Nigeria, Iyanomo, Edo State, Nigeria

Abstract: In this study, Rubber Seed Cake (RSC) was treated with the mycelium of the mushroom (*Pleurotus tuberregium*) so as to assess the effect of its enzymatic ability on the cyanide level and nutritional value of the rubber seed cake and hence enhance its agricultural and industrial applications. Levels of cyanide in the RSC were determined with the use of the Picrate kit paper method. Results from this study indicates that treating the RSC with the mushroom mycelium yielded about 81% reduction in the total cyanogens content after 96 hours at room temperature. The initial cyanide concentration of 500ppm in the freshly obtained rubber seed cake was reduced to a level of 5ppm for the undefatted RSC and from 300ppm to 4ppm for the defatted RSC. The values obtained for the treated RSC were found to be below the limit of 10ppm as set by FAO/WHO regulatory bodies. The proximate analysis after fermentation with the mushroom mycelium revealed that the protein content of the RSC increased significantly ($p = 0.01$) from a value of 29.36% to a value of 39.27% further enhancing its use as feed for livestock.

Key words: Rubber Seed Cake, cyanide, edible mushroom, picrate paper kit

Introduction

Rubber Seed Cake (RSC) is used as livestock feed for animals in developed countries. However, in Nigeria, its use is highly limited because of the long storage period observed for its cyanide level to be reduced to an acceptable level fit for consumption. Natural rubber (*Hevea brasiliensis* Muell. Arg.) is a native of the tropical America and is an abundant tree in Nigeria. The importance of increased livestock production in developing countries like Nigeria cannot be overemphasized. The increase in population has greatly increased the need for comparable foods with high biological values such as are found in meat and eggs. Despite the great demand for protein of high biological values for human consumption, pigs and poultry in the tropics (developing countries) are scanty and inefficient as a rule.

The use of unconventional feed has been advanced as a way to improve animal production in the tropics (Iyayi and Tewe, 1994). Examples of the unconventional feed are sorghum, wheat, but of particular interest is the rubber seed cake. That rubber seed cake can be used as feed for animals has been reported (Ong and Yeong, 1977), its nutritional value as a possible source of highly nutritional feed for pigs and poultry has also been reported (Nwokolo, 1987). However, in the Netherlands, Braderman (Bredemann, 1931) analyzed rubber seed products and found that the may contain as much as 0.02% hydrocyanic acid which was unsuitable for livestock. This limitation of the use of rubber seed cake as livestock feed is as a result of the presence of cyanogenic glycosides linamarin and lotaustralin in this plant. Enzymatic hydrolysis of the glycosides by endogenous β-glucosidase, linamarinase occurs when the plant tissue is damaged either mechanically or otherwise, thereby liberating HCN, a deadly poison.

It is worthy to note that deleterious factors such as cyanogens when present in animal feed could result in growth depression and hamper hatchability of incubated eggs. In addition, they result in depletion of amino acids (Vitharauge Mallika *et al*., 1991) of the body when ingested. This is because sulphur-containing amino acids are needed for cyanide detoxification resulting in a condition akin to amino acid imbalance and hence reduced protein synthesis. Thus, proper processing, of these seeds to reduce the total cyanogens content will result in improved performance when used as animal feed.

A wide variety of different methods of processing the rubber seed cake to reduce their cyanogenic potential and hence their toxicity is known. These methods comprise a combination of boiling, drying and fermentation of whole seeds; all of which reduce the total cyanide of the seeds. A simple and more effective method could be achieved through enzymatic hydrolysis (Fermentation). Fermentation is a chemical change brought about in organic substances by living organisms (Yeast, bacteria, fungi, mould etc) by enzyme action. There is a dearth of information on the ability of edible mushrooms to perform this feat. Fungi such as *Gloeocercospora sorghi* and some species of *Gibberella* have been reported to have the ability to

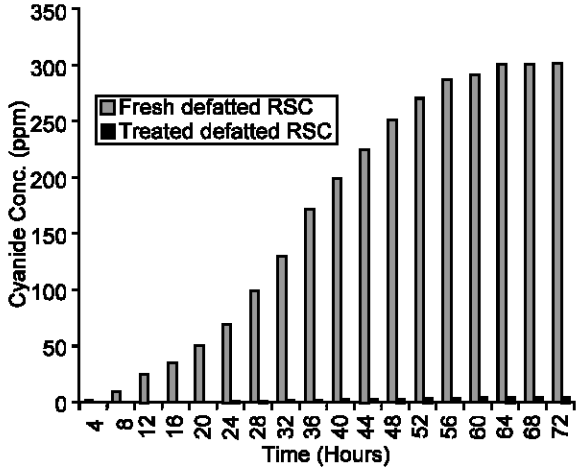

Fig. 1: Cyanide concentration of the fresh and treated defatted Rubber seed cake

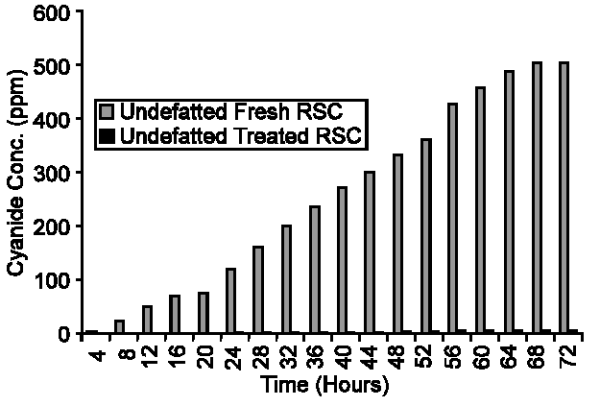

Fig. 2: Cyanide concentration of the fresh and treated undefatted rubber seed cake

convert hydrocyanic acid to a non-toxic formamide (Wainwright, 1992). Fermentation of food may also enhance the nutritional value of food and destruction of toxic substances present making it more digestible (Odunfa, 1985). The primary aim of this study is to examine critically the effect of fermenting rubber seed cake with the mycelium of the mushroom *Pleurotus tuberregium*.

Experimental: The *Hevea brasiliensis* seeds used in this experiment were collected from the Rubber Research Institute of Nigeria, Iyanomo, Edo State, Nigeria.

Collection and treatment of seeds: Fresh seeds were collected soon after falling from the parent plant; this was done because the seeds deteriorate very rapidly after falling on the ground as a result of its moisture content. The kernels were dried in the oven at 75°C for 24hours to reduce the moisture content. Shelled seeds were then grated and the endogenous β-glycosidase was allowed to interact with the cyanogenic glycosides for 60mins.

Extraction of oil: The oil of some of the macerated samples was extracted using a 5 litre Soxhlet extractor using n-hexane as the solvent. The oil extraction lasted for 6 hours to ensure maximum extraction and the n-hexane oil mixture was then distilled to recover the solvent. The meal obtained after extraction were designated "defatted samples" while those with their oil not extracted were designated "undefatted samples".

Determination of cyanogenic glucoside: The cyanogenic glycosides content of the seeds was determined using the picrate paper kit method as developed by Bradbury *et al.* (1999) with a slight modification for the determination of the cyanide level in rubber seed cake. This method was recently developed and the kits are available free of charge to health workers and agriculturists in developing countries for quantitative determination of cyanide (CN) in cyanogenic plants and food (AOAC, 1990).
100mg of the ground rubber seed cake was weighed using a weighing balance and placed on a round disc containing enzyme linamarinase at pH 6 buffer. The sample was then placed in plastic vial. 1 mL of distilled water was then added. The yellow picrate paper was introduced and the vial was then screwed shut. The vial was allowed to stand for 72hours at room temperature. The colour that developed on the picrate paper was read against a colour chart and the corresponding cyanide concentration was recorded.

Proximate analysis: Protein, fat and moisture contents were determined by methods described in AOAC (1990).

Fermentation of RSC: The spawn of the mushroom used for this experiment was obtained from the Mushroom Science Unit of Botany Department, University Of Benin, Benin City. The moisture content of five grams (5g) oven-dried coarsely ground rubber seed cake was adjusted to 70% by adding and mixing with sterile distilled water appropriately in 250 mL. Conical flast 5s. The mouth of the conical flask was then plugged with cotton wool and thereafter sterilized at 115°C and 18kg/cm for 30 minutes. Ten replicated RSC in each conical flask were prepared and sterilized.
After the sterilized RSC had cooled down to ambient temperature (26±2°C), each conical flask was inoculated with the spawn of the mushroom, *P. tuberregium* at the rate of 3% wet weight of the RSC. The inoculated RSC was thereafter properly agitated for the even mixing of the spawn with the substrate. Incubation of the material was done for 96 hours to ensure proper interaction between the RSC and the mushroom mycelium.

Table 1: Proximate Analysis of the Defatted and Undefatted Rubber Seed Cake

	Undefatted		Defatted	
Properties (%)	Before Fermentation	After Fermentation	Before Fermentation	After Fermentation
Moisture Content	9.20±0.09	4.40±0.08	3.90±0.04	4.52±0.16
Ash Content	3.80±0.20	2.99±0.15	2.50±0.08	2.91±0.10
Crude Fiber	4.37±0.08	4.41±0.08	4.07±0.06	4.52±0.08
Lipid Content	37.30±0.06	18.50±0.65	2.00±0.02	1.32±0.09
Crude Protein	21.35±0.10	35.87±0.39	32.54±0.09	39.27±0.93
Total Carbohydrate	23.98±0.05	33.83±0.59	54.69±0.07	47.46±0.52

Results and Discussion

In this study the ability of the test fungus, *Pleurotus tuberregium* (Fr.) Sing. to detoxify and enrich Rubber Seed Cake (RSC) is demonstrated. The mushroom, a white rot fungus, was able to significantly ($p = 0.05$) lower the cyanide level of rubber seed cake while at the same time increasing the nutritional quality of the product. The ability of many fungi to perform this dual role as been earlier stressed by Carlie *et al.* (2001). The level of cyanide increased with up to 72 hours (Fig. 1 and 2). This may not be unconnected with the conversion of cyanogenic glycosides into cyanide (Okafor and Anyanwu, 2006) during the maceration process. The upward trend in the level cyanide (Fig. 1 and 2) suggests that the hydrolysis of the cyanogenic substance in the RSC continued unabated despite the initial drying at 75°C for 24 hours.

The concentration of the toxic substance was generally higher in undefatted RSC compared to the defatted one. This observation may be due to the removal of some amounts of cyanide along with the oil during the extraction of rubber seed oil as earlier indicated by Uzu *et al.* (1986).

Despite the significant rise in the level of cyanide in the undefatted and defatted RSC, as observed in this study, fermentation of the material with pure culture of the mushroom, *P. tuberregium* led to a significant reduction in the amount of this toxic substance. As shown in Fig. 1 and 2, in fresh undefatted RSC, the cyanide level reduced from 500 to 5.00ppm after fermentation. In the same vein, the cyanide level equally reduced significantly from an initial concentration of 300.00ppm to 4.00ppm, representing a 98% reduction.

Although the catabolic process involved in the significant reduction of cyanide in the RSC was not elucidated in this study, Wainwright (1992) had earlier on stated that some species of *Gibberella*, using the enzyme cyanide hydrates detoxifies cyanide by converting it to a non-toxic formamide. Harris *et al.* (1987) had also reported a cyanide treatment process involving fungi.

The ability of mushroom to thrive on RSC may have also been encouraged by the presence of oil lipid in it. The extensive utilization of lipids as partial carbon source by fermenting micro organism was also reported by Ugwuanyi and Njoku (1996). And, the ramification of the RSC by the mushroom mycelium may have led to the significant ($p = 0.05$) differences observed in the proximate composition of the RSC before and after fermentation. The protein content increased from 21.35±0.10 to 35.87±0.39 after fermentation for undefatted RSC. Equally, the protein increased from 32.54±0.09 to 39.27±0.93 after fermentation for defatted RSC. This increase in protein level is attributable to the utilization of the lipid and carbohydrate components of the RSC as carbon sources. The protein content of many mushrooms (and invariably, their mycelia) ranges from 25 to 40% on dry matter basis (Quimio *et al.*, 1990). As shown in Table 1, this trend of increase in food components was also true for crude fibre and total carbohydrate with an exception to this trend being that total carbohydrate value dropped after fermentation in the case of defatted RSC. This may not be unexpected since the major carbon source after the removal of the lipid was the carbohydrate component of the RSC. As shown in Table 1, there was reduction in the lipid content for both defatted and undefatted RSC, which suggests that the fungus, *P. tuberregium* can use rubber seed oil as carbon source.

The study has revealed the positive effect of fermenting RSC with the mycelium of an edible mushroom, *P. tuberregium*. The need to evaluate the ability of more edible mushrooms in this regard can not be over-emphasized. The enhancement of the nutritional value coupled with its ability to detoxify RSC by the reduction of the cyanide is a welcome development.

Although, other methods such as storage for over 6 months and heating /drying at 105°C, as indicated by Uzu *et al.* (1986), can equally lower the cyanide level, they can as well lead to the loss of some essential volatiles such as vitamins. Moreover, the above stated methods do not improve on the protein content in contrast to the results from this study. The need to conduct further studies into the various aspects of fermenting RSC with edible fungi is therefore underscored?.

Conclusion: This study, designed to enhance the nutritional value of RSC has revealed the following:

By the use of mushroom, a natural product, the cyanide level of RSC can be reduced significantly to a level that makes it readily applicable in livestock feed. Furthermore, the protein content of RSC can also be increased by treatment with mushroom, a highly proteinous substance.

Acknowledgement

We wish to acknowledge the assistance of Mr. F. Efosa of Rubber Research Institute for providing the RSC. We are indebted to Mr. Anyaesere of Biochemistry Department of the University of Benin for assisting in the proximate analysis. Lastly, we wish to thank immensely Dr. Howard Bradbury of the Botany Department, Australia National University for supplying the test kit for cyanide analysis.

References

AOAC, 1990. Official Method of Analysis, protein, fat, moisture content. 15th Edition Association of Official Analytical Chemistry, Arlington USA, pp: 777-781.

Bradbury, J.H., M.G. Bradbury and S.V. Egan, 1994. Comparison of Method of Analysis of Cyanogens and Cassava, Acta Hort., 375: 87-96.

Bradbury, M.G., S.V. Egan and J.H. Bradbury, 1999. Determination of all Forms of Cyanogens in Cassava Roots and Cassava Products Using Picrate Paper Kits. J. Sci. Food Agri., 17: 61-66.

Bredemann, G., 1931. Danger of Hydrocyanic acid content of Hevea seed products used for fodder. Tropenfl., 34: 249.

Carlie, M.J., S.C. Watkinson and G.W. Gooday, 2001. The Fungi. 2nd Edition, Academic Press, New York, pp: 588.

Harris, R.E., A.W. Bunch and C.J. Knowles, 1987. Microbial Cyanide and nitrite metabolism. Sci. Prog. Oxford, 71: 293-304.

Iyayi, E.A. and O.O. Tewe, 1994. Cassava feeding in a small holder livestock units Acta Horticulturae, 375: 261-269.

Nwokolo, E., 1987. Biochemical and nutritional qualities of rubber seed meal. Tropical Agriculture (Trinidad), 64: 170-171.

Odunfa, S.A. *et al*., 1985. African Fermented Food in Microbiology of Fermented food Ed. 2. (Ed B.J. Wood).

Okafor, P.N. and N.O. Anyanwu, 2006. Enzymatic and Oven-drying Method of Processing Rubber Seeds for Animal Feed and the Evaluation of the Toxicity of Such Feed in Rats. J. Anim. Vet. Advances, 5: 45-48.

Okafor, P.N., 2000. Goitre, Konzo and Cassava consumption in Nigeria. Cassava Cyanide Diseases Network, 6: 2-3.

Ong, H.K. and S.W. Yeong, 1977. Prospect of rubber seed meal for feeding swine and poultry. In: Proceedings of the symposium of Foodstuff for livestock in South East Asia. C.K. Lumpur, pp: 337-344.

Quimio, T.H., S.T. Chang and D.J. Royce, 1990. Technical guideline for mushroom growing in the tropics. Plant production paper 106. Food and Agriculture organization, Rome, pp: 155.

Thomas, V., V.C. Mercykutty and C.K. Saraswarthyama, 1998. Rubber seeds: Its biological and industrial applications-A Rreview: The Planta, 74: 437-443.

Ugwuanyi, J.O. and O.U. Njoku, 1996. Effect of fermentation of seed on the chemical properties and fatty acid profile of Rubber seed Oil. Indian J. Natural Rubber, 9: 75-81.

Uzu, F.O., G.A. Ihenyen, F. Chukwuma and S.O. Imoebe, 1986. Processing, analysis and utilization of rubber (*Hevea brasiliensis*) seed oil and cake. In: Industrial utilization of Natural rubber (*Hevea brasiliensis)* seed, latex and wood. Proceeding of National Conference on Industrial utilization of Natural Rubber (*Hevea brasiliensis)* seed, latex and wood [E.E. Enabor (Editor)]; Rubber Research Institute of Nigeria, Iyanomo, Benin City, 22-24 January, (1985).

Vitharauge Mallika, G., M.P. Jansz, M.P. Nirmala and A.M. Abeyoskara, 1991. Some studies on controlling the action of lipase linamarase during rubber seed kernel processing. J. Natn. Science Coun. Sri-lanka, 19: 143-150.

Wainwright, W., 1992. An introduction to Fungal Biotechnology. J.A. Bryant and J.F. Kennedy (Eds). John Wiley and Sons, New York, pp: 2002.