Thiamin, Riboflavin and Niacin Content of Solid State Fermented Lathyrus sativus Seed

A.B.M Moslehuddin[1], Khaleda Islam[2], Mein K.Mahmud[3], Dulal K. Saha[4] and Md. Nizamul Hoque Bhuiyan[5]

1, 2, 5 Institute of Nutrition and Food Science, University of Dhaka, Bangladesh
3. The Nutrition Research and Development Centre, Bogor, Indonesia
4. Department of Applied Chemistry & Chemical Technology, University of Dhaka, Bangladesh.

Introduction

The nutritional importan food legumes as an economic source of protein, carbohydrates, minerals and vitamins has been recognised throughout the world (Moslehuddin, A.B.M 1990)[1,2]. It is better in terms of economic consideration to consume legumes directly, instead of converting them into animal protein. Legumes have been considered as the main protein supply of the malnourished world as it is difficult for the developing countries to increase the animal protein sources due to increasing costs. Fifteen to thirty percent of the protein-calories are being met in the foods of the African, Asian and Central American people through the use of high protein leguminous seeds (Tannous and Ullah, 1969)[3]. Again legumes may easily be adopted to grow under a wide variety of climatic conditions which are within the purchasing capability of the low income people of the developing countries. Lathyrus sativus seeds are one of the leguminous foods generally cultivated in different parts of Bangladesh and India. These seeds are principally used in the preparation of bread called chapatis. The powder of the seeds are also used in the preparations of different fried and cooked products for consumption.

Lathyrus sativus seeds contain about 10% moisture, 28.2% protein, 0.6% fat, 38.2% carbohydrate and 3% mineral matter (Sastri,B.N 1962)[3,4]. Thi satisfactory proximate composition encouraged Scientists to further analysis on the amino acid level of the seeds. After analysis, the seeds were found to be rich in lysine and to contain good amount of other essential amino acids except methionine (Ramnchandran and Phansalker, 1956)[4,5]. But inspite of its fair amount of protein and essential amino acids, this seed contains undesirable toxic substances which cause lathyrism when consumed for long time(Gonopathy and Dwivedi, 1961)[6]. There are also some methods to remove the toxicity of the seeds (Mohan, Nagarjan and Gopalan)[7]. Despite the presence of the toxins in the seeds, people continue to grow it as a hardy crop and it survives in adverse condition.

Considerable efforts and resources have been diverted to improve the nutritional quality and detoxification of different legumes by using the modern processing technology available like solid state fermentation. Solid state fermentation of soybean by using **Rhizopus oligosporus** is one of the traditional fermented food in the Orient. The product of fermentation is called Tempeh which is a popular food in Indonesia (Hermana, 1972)[8]. The nutritional quality of the Tempeh made from soybean is better than the raw soybean itself. Moreover, this solid state fermented product is safer from food borne disease and antinutritional factors (Wang H.L. and Hesseltine, C.W 1969)[9]. An investigation of the nutritional qualities of the Tempeh has led to suggest that it can be used as possible source of low cost protein food for child feeding programme in the developing countries (Autret M. and Van Veen, A.G. 1955)[10] . The task of solid state fermentation of **Lathyrus sativus** seed was taken to improve the nutritional quality of the seeds which is cheap in Bangladesh.

Materials and Mathods

Seeds of **Lathyrus sativus** were collected from the market of Dhaka, Bangladesh. After receiving the seeds, undesirable matter were removed manually. The seeds were then dehulled by burr mill in dry condition so that molds cannot grow on the dehulled seeds. After removing the seed coat, the seeds were kept at room temperature. At the regular periods the seeds were examined for the presence of moldy or damaged seeds. For the preparation of the sub-strate, the seeds were soaked in water overnight (seeds and water ratio being 1:3). After soaking, water was drained off, washed and cleaned . Preparation of Tempeh was followed by the modified method of william Shurtleft and Akiko Aoyagi, (195)11.

The seeds were then steamed for 10 minutes to remove some of the microflora. Then the seeds were dried at oven for removal of excess water for one hour. After removal of excess water the seeds were inocluted with 0.5% inoculum of **Rhizopus oligosporus** obtained from The Nutrition Research and development centre at Bogor, Indonesia.

The inoculated seeds were packed in petridishes and incubated at 30c for 24, 36 and 48 hours. The control seeds were also inoculated by the same way and was kept in the refrigerator. The fermented and unfermented samples were freeze dried for vitamin analysis and powdered was kept in the refrigerator.

THIAMIN: The samples were hydrolysed with Takadiastase. Thiamin was extracted from the filtrate by using Xylene and was determined spectrophotometrically at 520 nm according the method fo Lyman CM et al, (1952)[12].

NIACIN: The samples were hydrolysed with 1N H_2so_4 at 120° c for 30 minutes, adjusted to pH 4.5 and filtered. The filtrate was used for the determination of niacin spectrophotometrically at 44 nm using cyanogen bromide according to the method of official methods of naalysis (1984)[13].

RIBOFLAVIN: The sample was diluted by adding 0.1N HCI nixed and hydrolysed by autoclaving for 25 minutes. The sample was then cooled, adjusted to pH 6-6.6 with 0.1N NaoH and diluted to volume to give concentration of 1 µg/ml. The mixture was then filtered. In the experiment, 10 ml. Of the sample Was added to 2 efluorescence cuvettes and 1 ml working standard to one tube and 1 ml glacial acetic acid was added to each tube, mixed and added 5ml 4% $KMnO_4$ to each tube, mixed, kept for 2 minutes, then added 0.5 ml. 3% H_2So_4 to each tube, mixed thoroughly and shaked vigorously.

Fluorescence of each unknown sample was read against its standard blank by adding 20 mg Na_2SO_4 to each unknown, inverted and read (AOAC 1984)[14]. Standard vitamin samples were purchased from sigma chemical company. St. Louis, Mo 6317 USA.

Results and Discussion

Thiamin content in the Lathyrus sativus seeds and fermented Tempeh from the seeds are given in the table-1 & fig. Thiaming content of unfermeuted Lathyrus sativus seeds was found to be 0.44 mg% (Table-1). Thiamin first increased in 24 hours fermentation, then decreased in 36 hours. After 48hours, this vitamin increased again.

The decrease of thiamin was observed by Kiku Murata et al. (1967) in Tempeh, but thiamin content increased significantly in the Lathyrus sativus Tempeh. Similar increase of thiamin was observed in Tempeh gembus by gandjer et al. (198)[15] using the similar mold inoculum. From the result it can

Table 1 : Change in Vitamin content of unfermented and solid state Fermented Lathyrus sativus seed.

Vitamins	Unfermented Lathyrus Sativus seed.	Fermented Lathyrus Sativus seed in hours.		
		24 hr.	36 hr.	48 hr.
Thiamin(mg./100g)	0.44	0.49	0.47	0.79
Increase(xtimes)	0	1.1	1.0	1.8
Niacin(mg/100g)	1.76	6.4	9.6	9.8
Increase(xtimes)	0	3.16	5.4	5.6
Riboflavin(mg/100g)	0	0.120	0.33	
Increase(xtimes)			3	

Each figure is the average of Triplicate samples.

be assumed that in the first phase of fermentation of 24 hours, the fungi needed no thiamin for its own growth, but after 24 hours during sporulation, inoculum needed no thiamin and at the Same time increased thiamin content in the Lathyrus sativus Tempeh. During 48 hours fermentation, thiamin content showed a significant increase. Here the vitamin was mere a secondary metabolite.

The strain needed some thiamin for sporulation and as aresult thiamin content decreased. After 36 hours the inoculum needed no thiamin and at the same time increased thiamin content in the Lathyrus sativus Tempeh. During 48 hours fermentation, thiamin content showed a significant increase . Here the vitamin was mere a secondary metabolite.

Niacin content of the fermented and unfermented seeds of Lathyrus sativus is given in the Table -1 & Fig-2. Niacin contents of unfermented Lathyrus sativus seeds was found to be 1·76 (Table-1) This increased of niacin was 3.16 times in 24 hours fermentation, 5.4 times and 5.8 times in 36 and 48 hours fermentation. Niacin content increased first 3.16 times in 24 hours which was significant changes in

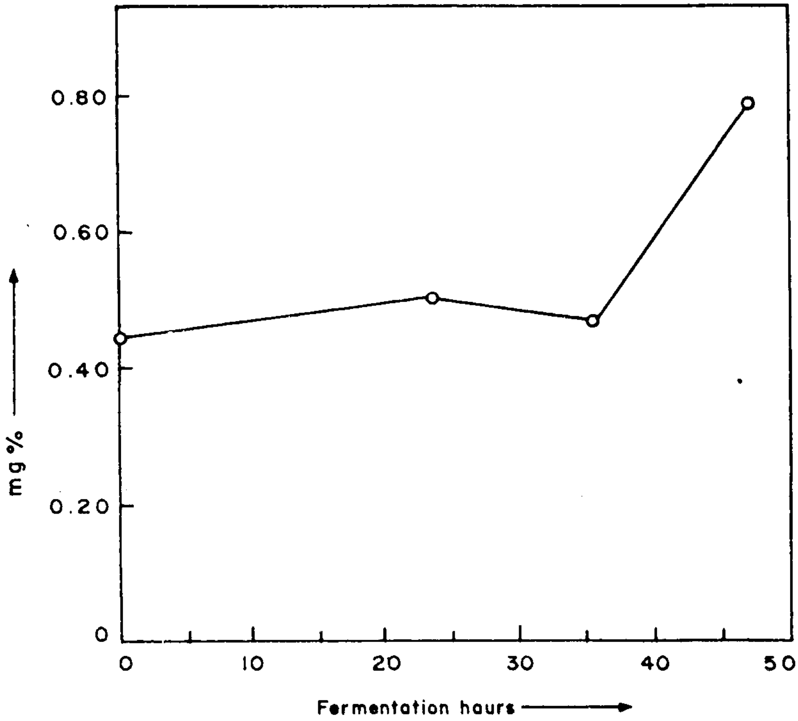

Fig.1. Thiamin content of solid state fermented Lathyrus sativus seeds

niacin content during optimum period of growth. The data strongly suggest that **Rhizopus oligosporus** can produce niacin under the conditions of fermentation. Sorenson and Hesseltine (1966)[16] reported that **Rhizopus oligosporus** can grow on a defined media free of vitamin. The present data support that the organism might be able to synthesis niacin for its own purpose and its activity of synthesis contributes upto certain time. In soybean Tempeh, niacin content increased during 36, 48 and 72 hours fermentation. In our experiment the similar observation was observed with **Lathyrus sativus** seeds fermented by the same mold inoculum.

Riboflavin content of the fermented and unfermented **Lathyrus sativus** seeds is given in the table-1 & Fig.3. Riboflavin content of unfermented **Lathyrus sativus** seeds was found to be nil (Table-1) .The vitamin could not be detected in the raw seeds, this may be due to small amount of the vitamin present in the seeds. After fermentation for 24 & 36 hours, the seeds found to contain 0.12 mg% and 0.35 mg% which will contribute to the

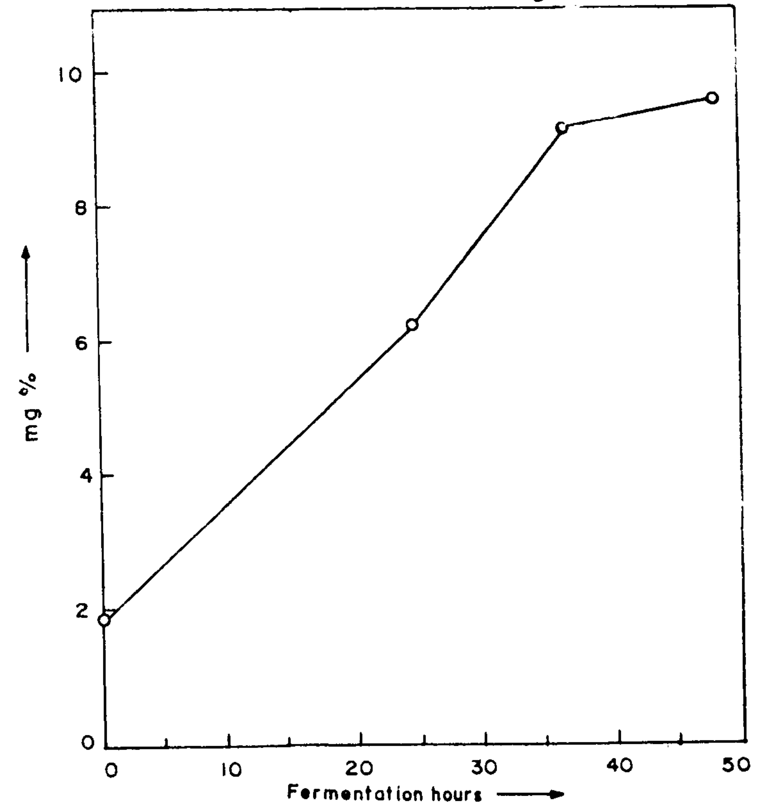

Fig.2. Niacin content of solid state fermented lathyrus sativus seeds.

people who consume these seeds. Similar observation was observed in soybean and wheat Tempeh. The importance of this vitamin in anemia and other field of nutrition are well recognised by (Briggset al,[17].

Though there are toxins in the seeds of Lathyrus sativus, people use it or different food preparations. During the preparations of different foods and fermentative products, the toxin are reduced and nutrients are increased. The fermentative products is safer in use and during experiments, it was shown that the effect of toxicity was negligible to zero in the fermentative products[2]. Moreover, durung soaking & washing for fermentation, most of the toxins are removed[7]. To meet the requirements of nutrients, the basic need is a balanced diet in that case those who use these seeds sometime in a day, may be benefitted by these Vitamins in the seed. In other words, along with other food items intake of fermented Lathyrus sativus seed may later-meet one's vitamin (niacin, thiamin and riboflavin) requirements.

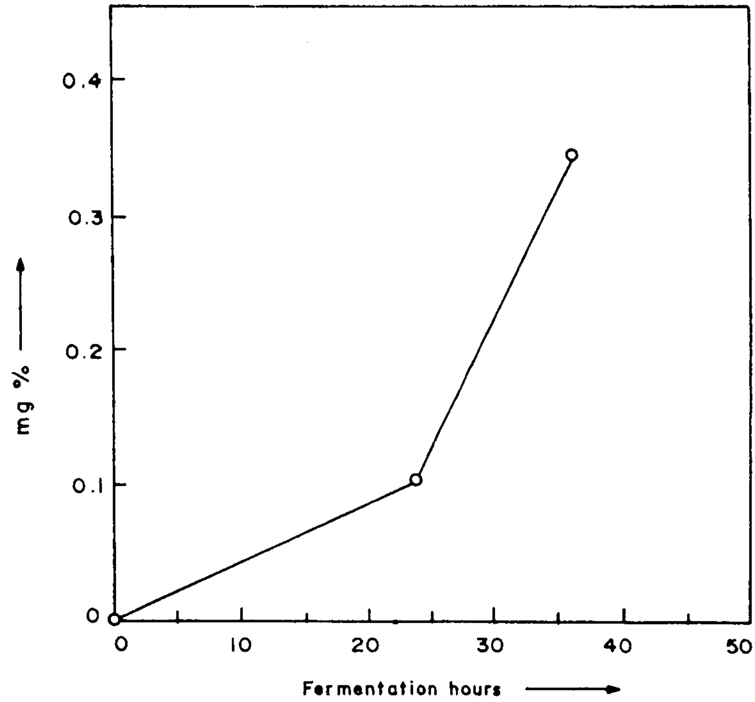

Fig.3. Riboflavin content of solid state fermented lathyrus sativus seeds.

Summary

Thiamin, riboflavin, niacin of the fermented and unfermented Lathyrus sativus seeds are analysed in this experiment. In unfermented Lathyrus sativus seed contents of thiamin and niacin were found to be 0.44mg% and 1.7 mg% respectively while riboflavin content was found to be nil. All of these vitamins after fermentation. Thiamin increased by 1.1 times after 24 hours, 1 time after 36 hours. and 1.8 times after 48 hours. Niacin increased by 3.16 times after 24 hours., 5.4 times after 36 hours. and 5.6 times after 48 hours and riboflavin increased by 3 times after 36 hours. Niacin & riboflavin significantly increased and thiamin increased only after 48 hours. The fermented product become suitable for consumption usually after 24 hours. to 36 hours. So, such increase in vitamins is significant in nutritional context of Bangladesh.

References

1. Biochemistry & Technology of chick pea seeds; Critical reviews in Food Science & Nutrition. CRC press INC . Bocaraton, Florida, USA. Editor thomas E, Furia. Vol 25, Issue 25(2),107,1986.
2. Moslehuddin, A.B.M. Solid State Fermentation of **Lathyrus sativus** seeds by **Rhizopus oligosporus**, Ph.D. Thesis, Dhaka University, Page No.1 & 78 ,1990.
3. Tannous, R.I., and Ullah, M. Effects of autoclaving on Nutritional factors in legumes seeds. Tropical Agriculture 48: 123,1969.
4. Shastri, B.N. Health of India vol. VI. pp. 36-47. (Council Sci. Res. Publ. New Delhi), 1962.
5. Ramchandran M phansalkor, S.V. Essential amino acid composition of certain vegetable food stuffs, Indian J. Med. Res. 44: 501-509, 1956.
6. Ganapathy, K.T. and Dwivedi, M.P. Studies on clinical epidemology of lathyrism, Indian council of Med. Res. Rewa, India, 1961.
7. Mohan, V.S. Nagarjan, V. and Gopalan C. Simple preactical procedures for the removal of toxic factors in Lathyrus sativus (Khesari dhal) Indian. J. Med. Res. 54:411, 1966.
8. Hermana, Tempeh: In Indonesian fermented soybean foods. presented at the world study on Waste Recovery by Microorganisms. University of Malaysia UNESCO/ ICRO (W.R. Stanten, editor) Published by the Ministry of Education: Malaysia, Koalalumpur for the Malayasian National Commission of UNESCO. P 221, 1972.
9. Wang, H,L. and C.W. Hesseltine, Wheat Tempch cereal chemistry: 43: 563-570, 1966.
10. Auret, M. and van veen A.G. Possible sources of protein for child feeding in under developed countries American J. Clin Nutri. 3, 234,1955.
11. William shurtleft and Akiko Aoyagi, The Book of Tempeh, Harper Colophon Books. New York, P. 104,1985.
12. Lyman, C.M. ,R. Ory, M. Trant and G. Richii. Determination of thiamin in rice and rice products Anal. Chem. 24(6), 1020-24,1952.
13. AOAC. Official methods of Analysis 10th ed. Washington. D.C.P. 827,1984.
14. AOAC. " Official Methods of Analysis 10th ed. Washington D.C. P. 826,1984.
15. I. Ganjar D.S. Slamet J. Satikoan Nur. Endah Wahyunigsih, The thiamine content of Tempeh gembus enriched with Rice. Polish, presented in the Association of Southeast Asian Institutions of Higher learning seminar on Food Technology and Nutrition, 8-10, Yogyakasta-Indonesia, July, 1985.
16. Sorensen, W.G. and C.W. Hesseltine, Carbon and nitrogen utilisation by **Rhizopus oligosporus** Mycologia, 58: 681-89,1966.
17. George M. Briggs and D.H. Calloway Begerts. Nutrition and Physical fitness, 10th edition. Sunders college publishing, West Washington square, philadelphia, PA, 1979.