

Short Communication

The Effect of Fermentation on Thiamin, Phytic Acid and Tannic Acid Content of *Vicia Faba* (L) by *Rhizopus oligosporus*

A.B.M. Moslehuddin¹, Md. Motiur Rahman¹, Md. Abdus Shakoor², Abduz Zahar¹ and Moniruzzaman³.

¹Institute of Nutrition and Food Science, University of Dhaka, Bangladesh.

²Institute of post graduate Medicine and Research, Dhaka, Bangladesh.

³Sh. Suhrawardy Hospital, Dhaka, Bangladesh.

Introduction

Solid state fermentation refers to the cultivation of microorganisms on solid materials in the absence of free liquid and have been used for centuries in the Orient for the preparation of various fermented food products (1,2). The main advantages of solid state fermentation over submerged one include (a) the yields are much higher than those in liquid media (b) the space taken up by the fermentation vessel required is small relative to yield of product because less water is used and substrate is concentrated and (c) the operation cost is much lower (1.) Fermented foods make important contribution to the diet as source of protein, calories and of some vitamins, some workers found that the level of riboflavin, vitamin B6, nicotinic acid and pantothenic acid in the tempeh

(fermented soybeans) were much higher than those in unfermented soybeans (3).

Vicia faba is cultivated by farmers of north eastern part of Bangladesh. It is locally known as kalimotor. This study was performed to study the beneficial effect of fermentation on the *Vicia faba* (kalimotor).

Material and methods

Collection & preparation of Samples : *Vicia faba* (kalimotor) seeds were purchased from the local market in dehulled condition (split pulse). Collected seeds were manually cleaned and then stored at room temperature. Control (Unfermented) and tempeh (inoculated with *Rhizopus oligosporus*) samples were prepared.

Thiamin: The samples were hydrolyzed with 0.14N hydrochloric

acid and autoclaved at 15 psi for 30 minutes. After extraction, the samples were cooled to room temperature and the pH was adjusted at 4.5 using 2.5M sodium acetate. The samples were incubated over night with diastase enzyme suspension. Then the samples were filtered and the thiamin was determined according to the method of Kennerley and Peters (1932) (4).

Phytic Acid : Phytic acid was determined according to the method of AOAC (1975). 1g of dry sample was shaken with 10% Na_2SO_4 for 2 hours. Then they were centrifuged and heated with H_2SO_4 . In this way sample solutions were prepared. Then the absorbance readings were taken at 515nm using hydroxylamine-HCl, buffer acetate and dipyrilidil (5).

Tannic Acid : Tannic acid was determined according to the method of AOAC, 1975 (6). 0.5g of sample was taken in a beaker. 100 ml of distilled water was added and then refluxed for 2 hours. Cool and filtered through Whatman paper. In this way, unfermented and fermented samples of 24, 36 and 48 hours were prepared. Then the readings were taken in a spectrophotometer at 760nm.

Results and discussion

Thiamin : Thiamin content of unfermented (control) and fermented

vicia faba seeds of 24, 36 and 48 hours were found to be 0.29, 0.3, 0.2 and 0.42mg/100g respectively (Table-1).

Phytic acid : The Phytic acid content of unfermented (control) and 24, 36 and 48 hours fermented Vicia faba seeds of 24, 36 and 48 hours were found to be 0.29, 0.3, 0.2 and 0.42mg/100g respectively (Table-1).

Tannic acid : Tannic acid content of unfermented (control) and 24, 36 and 48 hours fermented Vicia faba seeds were found to be 1090, 715, 508 and 239 mg/100g respectively (Table-1)

Thiamin : In the study it was found that thiamin content increased only by 3.45% during 24 hours of fermentation but during 36 hours of fermentation thiamin content decreased 31.03%. The thiamin content again increased appreciably to 44.83% during 48 hours of fermentation (Table-1). From the results obtained it can be assumed that in the first phase of fermentation of 24 hours the mold *Rhizopus oligosporus* needed thiamin for sporulation and as a result thiamin content decreased between 24 hours and 36 hours of fermentation. After 36 hours, the thiamin content showed a significant increase. Here the vitamin was a secondary metabolite. Similar increase of thiamin was

Table 1. Effect of solid state fermentation on Thiamin, phytic acid and Tannic acid content of vicia faba (a)

Processing Method	Thiamin content (mg/100g)	%of increase or decrease (+), (-)	Phytic Acid content (mg/100g)	%decreased	Tannic Acid content (mg/100g)	%decreased
Soaked & steamed (content)	0.29±0.09		956±29		1090±36	
Soaked & Steamed & fermented for 24 hours	0.3±0.0ns	3.45 (+)	527±43*	44.87	715±39*	34.40
Soaked, steamed & fermented for 36 hours	0.2±0.01*	31.03(-)	368±31	61.51	508±39*	53.39
Soaked, steamed & fermented for 48 hours	0.42±13&	44.83(+)	128±47*	86.51	239±44*	78.07

a values are expressed as mean + SD of three samples, each analyzed in duplicate (on dry weight basis).

ns. Insignificant at 5% level of significance.

*Significant at 5% level of significance.

observed with Tempeh gembus by Ganjar *et al.* (1986) using the similar results during fermentation of *Lathyrus sativus* by *Rhizopus oligosporus* (8).

Phytic acid : From the present study it was found that the phytic acid content of *vicia faba* decreased throughout the fermentation period. The phytic acid content of *vicia faba* reduced to 44.87% after 24 hours of fermentation, 61.51% after 36 hours of fermentation and 86.61% after 48 hours of fermentation. The fact the *Rhizopus oligosporus* for its growth thiamin might be used in the preparation of *vicia faba*. Tempeh has strong phytic acid activity was also supported by sudarmadji and Markakis in research work (9). This phytase, an

enzyme capable of hydrolyzing phytases, is responsible for the reduction of phytic acid content in the *vicia faba* tempeh prepared by *Rhizopus oligosporus*. Brune *et al.* (1992) showed that prolonged fermentation for a couple of days can almost completely degrade the phytate (10). *Rhizopus oligosporus* strain can produce both extra and intracellular phytases. Intracellular phytase activity was reported higher than that of extracellular enzyme so, mold, *Rhizopus oligosporus*, with its phytase activity reduces the phytic acid content, which in turn increases the bioavailability of certain minerals.

Tannic acid : In the present study it was found that the tannic acid

content of *Vicia faba* seeds decreased with the fermentation period (24, 36 and 48 hours). Compared to control the tannic acid content of *vicia faba* seed reduced 34.40%, 53.39% and 48.07% during 24,36 and 48 hours of fermentation respectively (Table-1). This *vicia faba* prepared by *Rhizopus oligosporus* containing lesser amount of tannic acid have better nutritive value in terms of improved protein digestibility.

Summary

The effect of fermentation of dehulled legume *Vicia faba* (kalimotor) by *Rhizopus oligosporus* on vitamin like thiamin and antinutritional factors like phytic acid and tannic acid were studied. The fermentation method used was similar to the traditional fermentation procedures used in the preparation of Tempeh from soybean in the orient. The B-vitamin thiamin content first increased by 3.45% in 24 hours fermentation, then decreased to 31.03% fermentation and again increased 44.83% in 48 hours of fermentation. The antinutritional component phytic acid decreased by 44.87%, 61.51% and 86.61% after 24,36 and 48 hours of fermentation respectively. Tannic acid content decreased by 34.40%, 53.39% and 78.07% in 24,36 and 48 hours of fermentation respectively.

Reference

1. Hesseltine, C.W. Solid state fermentation. *Biotechnology Bioeng.* 1972; 14 : 5
2. Hang, Y.D. Luh, B.S. and Woodmas, E.E. Microbial production of citric acid by solid state fermentation of Kiwifruit peel. *J. Food science.* 1987; 52 : 226.
3. Muratak, Ikehata H, Miyamoto. Studies on the nutrition value of Tempeh, Osaka City University, Osaka, Japan. *J. Food Science.* 1967; 37 : 580-585.
4. Kennersly and Peters Spectrophotometric Analysis of Thiamin. *H of Biochem.* New York. 1934; 28 : 667.
5. Hernnana Introduction of Tempeh-an Indonesian. Fermented Soybean Food in National Academy of sci, National Research Council publication 1972; 843 : 275-79.
6. AOAC. Official Methods of Analysis 8th ed. Washington D.C. 1975; 878 : 880
7. I Ganjar, D.S. Stamet J. Satikoan Nur Endah. Wahyunigish. The thiamin 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, Yogyakarta-Indonesia, July, 1985.
8. Moslehuddin, A.B.M and Tannous, R.I. Effect of fermentation on the hemagglutinating and antitrypsin factors in legume seeds. *Bangladesh J Jtr* 1987; 1(1) : 42-49.
9. Studarmadji s. And Markakis. The phytic and phtase of soybean tempeh. *J. Sci. Food and Agr.* 1977; 28 : 381.
10. Brune M. Rossander-Hulten L, Hallberg, E landsson M. Sandberg A-S Iron absorption from bread. Inhibiting effect of cereal fiber, fiber, phytate and inositol with different number of phosphate groups. *Journal of Nutrition.* 1992; 122 : 442-449.