The Effect of Solid State Fermentation on the Niacin Content of Cajanus cajan (L.) by Rhizopus Oligosporus

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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 (Hesseltine, C. W. 1972 & Hang, Y. D. 1987). The main advantages of solid sate fermentation in relation to another kind of fermentation named submerged include (a) the yields are much higher than those in liquied 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 operating costs are much lower than those for liquied phase fermentation (Hesseltine, C. W. ; 1972). Fermented foods are essential components of diets in many parts of the world especially South East Asia, the Near East and parts of Africa (Shurtleff, W. & Aoyagi, A. 1985). Fermented foods make important contributions to the diet as a source of protein, calories and of some vitamins. Murata et al, (1967) found that the level of riboflavin, vitamin B_6. nicotinic acid, pantothenic acid in the tempeh (fermented soybeans) were much higher than those in the unfermented soybeans (changes in riboflavin, micotinic acid, production of vitamin B_{12} were also found by Steinkraus et al. (1961).

Cajanus cajan (Arhar) is highly nutritious, like any othr legume (Deshio Khaddyadrabber Pushtiman, 1992). but there is no known research done to study the changes of its niacin countent due to fermentation. Niacin is the generic name for nicotinic acid and nicotinamide, either of which may act as a source of the vitamin in the diet, Nicotinamide is a component of the respiratory co-enzymes NAD^+ and $NADP^+$, concrned with tissue oxidation. Lack of niacin causes the deficiency syndrom "Pellagra". The purpose of this study was to find out whether solid state fermentation increases or reduces the content of niacin in Cajanus cajan.

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Materials and Methods

Cajanus cajan (Arhar) seeds were purchased from the local market of Dhaka, Bangladesh, in the form of 'dal' (split pulses). Collected seeds were cleaned manually and then stored at room temperature until all tests could be made. To prepare control and sample, the seeds were soaked in water overnight (seed to water ratio being 1 : 3). Soaking, water was drained off, seeds were washed twice. Tempch was prepared from the seeds by modifying the method of william Shurtleff and Akiko Aoyagi (1985). The method is as follows.

The soaked and washed seeds were steamed for 10 minutes to destroy some of th microflora. Then the seeds were dried at room temprature to remove excess water. Then half of this preparation was kept as control and stored at)°C. The other half of the seeds were inoculated with 0.5% tempeh inoculum (Rhizopus oligosporus), obtained from Nutrition Research and Development Centre at Bogor, Indonesia. Inoculated seeds were packed in glass petridishes and fermented in an incubator at 37°C for 24 hours.

Then, after observing the appearance of Cajanus cajan tempeh (The fermented product) it was kept in the freeze at 0°C to stop any further fermentation.

Niacin was analysed colorimetrically according to the method of AOAC (Sidney Williams, 1984). 5g of fresh sample was taken into a 250 ml erlenmeyer flask. 33.27ml 1N sulphuric acid was mixed and heated at 15 1b preessure for 15 minutes in an autoclave. After autoclaving, th sample was cooled and adjusteed to PH 4.5 with 10N sodium hydroxidee, Then it was diluted to 44.1 ml and filtered. In a 25 ml volumetric flask 8.5g of ammonium sulphate was taken, in whcih 20 ml sample solution was added and diluted to volume with distilled water. Then the solution was shaked vigorously and filtered. This was used as the sample solution.

50 mg Niacinamide (Sigma Chemicals, USA) dissolved in 25% alcohol to make 500 ml. An aliquot of 2 ml from this solution was taken and diluted to 50 ml with distilld water. Concentration of the solution (niacin stock) was 4ug/m/. 20ml of stock solution was added with 8.5g of ammonium sulphate. The volume was made 25 ml. 0.5, 1.0, 2.0, and 2.5 ml of this solution was taken in five, 15 ml dilute ammonium hydroxide, 5 ml cyanogen bromide solution, 2 ml/10% sulfonilic acid solution, and water was added accordingly. Standard blank was prepared by taking 6ml water, 0.5 ml of ammonium hydroxide, 2 ml of sulfonilic acid, and 0.5 ml of dilute hydrochloric acid in a 15 ml tube. 30 seconds after adding sulfanilic acid, reading was taken in a spectrometer at a wave length of 450 nm.

Sample solution of unfeermented and fermnted sample was preepared by adding 1 ml of each sample with 0 : 5 ml of dilute ammonium hydroxide, 5 ml of cyanogen bromide, 2 ml of 10% sulfanilic acid and 0.5 ml water. Then absorbance reading was taken at 450 nm.

Results

The niacin content of the unfermented control seeds and fermented tempeh of Cajanus cajan are given in the Tabl 1. The table shows that the niacin content has inereaseed almost 19% after 24 hours of fermentation.

Discussion

In the present work it was found that fermentation make Cajanus cajan seeds richer in niacin content. but there is no information available to support the ability of Rhizopus oligosperus to synthesize vitamins. In general niacin has been found to be associated with some filamentous fungi. Fun (1913) isolated niacin from yeast. The present experiment seems to support that the organism might be able to synthesize niacin. The amount of nicotinic acid, Riboflavin, vitamin B6, and pantothenic acid in soybeans, fermented by Rhizopus oligosporus at 37°C. for 36 hours was found to be much higher than those in unfermented soybeans, by Murata et al., (1967). It was also observed that these B vitamins increased further during 48 and 72 hours of fermentation (Murata, K. et al. 1967). However due to a bitter taste of spored sample, fermenting longer than 48 hours is not desirable. Increase in nicotinic acid, riboflavin, and vitamin B_{12} content were also found by Hermana (1972) and Steinkraus, K. H. et al. (1961). P. A. Roclofsen and Anneke Talens (1964) found considerable increase in niacin and riboflavin content of soybean after fermntation by Rhizopus oryzae. The increase in niacin content found by Murata et al. (1967) in first 24 hours, can be

Table 1. Effect of solid state fermentation on niacin content of Cajanus cajan seeds (dry basis).

Proceessing method	Niacin mg/100g	Average niacin mg/100g	% of increase
Soaked, steamed (control)	0.9344 1.0431 0.9763	0.9846	
Soaked, steeamed & feermented (Tempeh)	1.1860 1.1359	1.1704	18.87

compared with the finding of the present study. The differnce may b due to the conditions used for fermentation.

Summary

Niacin content of unfermentd and fermented Cajanus cajan were studied in this paper and found that fermentation make the seed richr in niacin. content. Moreover, by this solid state fermentation, the other nutritional contents are also increased (Moslehuddin, A. B. M. 1990) and at the sam time harmful antinutrients (Sudarmadji, S., and Merkais, p. 1977) and toxin (Meslehuddin, A. B. M. and Tannous, R. I. 1987) substances aro reduced. Therefore it is more beneficial to ferment the legume seeds in terms of nutrition.

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Letter to the Editor

The Editor
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Madam/Sir,

If one looks at the map of the developing world it would be immediately noticed that vitamin A deficiency and iodine deficiency go together. The picture of Bangladesh, Indonesia, India, Thailand and Nepal are among them. It would be noted further that more children of poor families have nightblindness/xerosis etc. even though the consumption of carotene bearing fruits and vegetables by them are not insignificant. In the etiology of vitamin A deficiency may not only dietary but also enviromental factors that cause infection must be playing a role. Dr. Carl Bauman of the University of Wisconsin[1] showed some fifty years ago that rats rendered hypothyroid with antithyroid compounds were not able to convert carotene into retinol. Could then iodine deficiency be a contributing factor to the manifestation of vitamin A deficiency.

We chose a village in Norsingdhi, Bangladesh where both clinical symptoms vitamin A deficiency (nightblindness, xerosis) and of iodine deficiency (goitre of different grades) were visible. We chose children of both sexes (12-16 years) and randomly distributed them between two groups, group I and II. Group I children were given 1 ml lipiodol injection iodine in oil and Group II children had an injection of vitamin B-Complex. None of them ever had iodised salt or any other iodine preparation prior to or during the period of the study. One ml of lipiodol had 0.48 gram of iodine in poppy seed oil.

All the children came from the same socio-economic status and had comparable intake of various nutrients. They had hardly any intake of food from animal source (source of retinol). Blood samples were taken for estimation of T_3, T_4, and TSH, as well as serum retinol, serum β carotene, serum RBP and prealbumin prior to the administration of lipiodol and vitamin B complex. Food intake survey was also done at the begining of the study and at the end.

While the blood analysis as contemplated has not been yet possible the clinical examination for eye symptoms and thyroid gland has been made.

We found that the children who received iodine (as lipiodol) no longer showed any symptoms of vitamin A deficiency but the size of thyroid gland did not change remarkably. Those who received placebo (vitamin B complex) showed no change with regard to nightblindness or xerosis, details will be published elsewhere.

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