Reduction of Phytic acid and Hemagglutinating Factors in Solid State Fermented Legumes by *Rhizopus Oligosporus*

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Introduction

Legumes have been considered as poor men's meat since they are the cheapest source of protein. Legumes have not only twice the protein content but also contain more protein on weight basis than egg, fish and flesh food while the seeds contain large amount of protein and other nutrients.

Phytic acid is widely distributed in nature particularly in cereal and legumes and is the principal storage form of phosphorus.[1 2 3] Phytates represent more than 50% of the total phosphorus in cereal (2). Concentration of phytic acid in food depends on its stage of maturity at harvest (Sutard *et al* 1985).[4] At high levels phytates are responsible for the reduction of mineral bioavailability such as Ca, Zn, Fe and other trace elements.[5] protein is also able to form complexes with phytic acid. When bound to protein, it causes decreased solubility and functionability of the protein (Chen *et al* 1985)[6]. Phytate may also cause protein to be more resistant to proteolytic digestion

(Raholra *et al* 1974)[7]. The hydrolysis of phytate is catalyzed by the enzyme phytase to inositol and free orthophosphate (Lilian *etal* 1985)[8]. Many cereal and legumes contain phytase. Mucosal phytase was described in rat, in the chick and in human (Karouani *et al* 1985)[2].

Hemagglutinating lectins and other antinutrients are present in plant. Lectins are defined as carbohydrate binding protein (glycoprotein) on non-immune origin that agglutinate cells or precipitate polysaccharides or glycoconjugates (Kimura and Funsts 1986, Miguchi *et. al* 1968).[9. 10]

Lectins have the ability to agglutinate the red blood cells of various species of animal tissue (Higuchi *et. al* 1983).[11] legume seeds are specially rich in these protein. The hemagglutining may reduce the

Bangladesh Journal of Nutrition, Vol. 11, Nos. 1 & 2, June 1998. Printed in Bangladesh, Institute of Nutrition and Food Science, University of Dhaka, Bangladesh.

intestinal absorption of essential nutrients through their combination with the absorptive cell lining of the intestinal wall (Ikeda *et. al* 1986)[12].

Solid state fermentation refers to the cultivation of microorganisms on solid material in the absence of free liquid and have been used for centuries in the orient for the preparation of various fermented food products (Hesseltine 1972).[13] One of the traditional solid state fermented food is tempeh, popular Indonesian food, consisting of tender cooked soybean bound together by dense cottony mycelium of Rhizopus mold into compact 3/4 inch thick white cakes (Shurtleff *et. al* 1977).

The aims of this experiment were to investigate the reduction in phytic acid levels and hemagglutinating factors during solid state fermentation of legumes for enhenced nutritional quality as the cheapest source of protein.

Materials and Methods

The dehulled legumes, Lentil (*Lens culinaris*), Chick pea (*Cicer aritinum*), Mungbean (*Vigna radiata*). Black gram (*Vigna mungo*), pea (*Pisum sativus*), Pigeon pea (*Cajanus cajan*), Lathyrus, (*Lathyrus sativum*) were purchased from the local market of Dhaka.

Preparation of tempeh: Tempeh was prepared from several types of legumes, Tempeh inoculum was obtained from the Nutrition Research and Development Centre at Bogor, Indonesia. Preparation of Tempeh was same for all the legumes and was done by modifying the method of Shurtleff and Aoyagi.[8]

The seeds were soaked in water and steamed for 10 minutes and inoculated with 0.5% tempeh (Inoculated with *Rhizopus oligosporus*) inoculum. Then the seeds were kept in incubator at 37°C on a glass petridish for 24 hours and used as a sample for the experiment.

Extraction and determination of phytic acid : One gram of powdered sample was diluted with 10% sodium sulfate in 1.5% hydrochloric acid and was shaken for 2 hours. From it 10 ml filtrate was mixed with 10 ml of water and 10 ml of 2% ferric chloride in 1.2% hydrochloric acid, and was boiled in water bath for 30 minutes, then cooled to room temperature. This was centrifuged for 20 minutes, the pellets were then washed with 2.5% sodium sulfate in 0.6% hydrochloric acid, and was dissolved with 5 ml of concentrated nitric acid. Four drops of concentrated sulfuric acid were added and heated. Then 10 ml of 3 N hydrochloric acid was added and the mixture was then heated for 10 minutes and made 100

ml with deionized water and filtered. Five ml of filtrate was taken in a tube and 0.5 ml hydroxylamine-HCl, 2.5 ml bulfer acetate and 1 ml alphalpha dypyridil were added and mixed thoroughly and after 30 minutes, absorbance for each sample was measured at 515 nm and compared with Iron standard curve.[9]

Determination of hemagglutinating factors : The methods were followed according to Liener and Hill.[10].

10% saline extract (0.90% Sodium chloride) of powdered sample were prepared and refrigerated overnight. Then it was centrifuged (3000 ×g) for 10 minutes, 4% suspension of chicken red blood cell (RBC) was prepared. Different dilution of the extracts were made in three test tubes by adding 2 ml of 4% RBC suspension and refrigerated for overnight. Control samples were also prepared. Hemagglutination was detected by tappering the tube and clumping of the red blood cells. Cells which were not agglutinated dispensed readily to form uniform suspensions. The degree of hemagglutination was then measured according to method of Liener and Hill.[10]

Results and Discussions

The changes in phytic acid content during tempeh production are shown in Table - 1. From this table it can be seen that phytic acid among legumes ranged between

Table -1: Phytic acid content of raw, control and fermented legumes (mg/100)

Lagumes	Raw[a]	Control[b]	Fermented
Lentil	584	396	143
Chick pea	833	718	48
Mung bean	497	358	172
Black gram	823	555	124
Pea	431	344	86
Pigeon pea	746	670	198
Kheshari	631	560	90

a. Values are average of three determinations express on dry weight basis.
b. Soaked overnight audsteamed for 10 minutes.

431-833 mg/100g. Chick pea had the highest amount, followed by *Black gram*, *Pigeon pea*, *Lathyrus pea*, *Lentil* and *Mung bean*. The table shows that a significant reduction of phytic acid occurred after 24 hours of fermentation of the seeds with *Rhizopus oligosporus*.

Attempts to reduce phytate in legumes by cooking, autoclaving or germination have been partially successful.[11] It was shown that 25% reduction was possible in soybean by 4 hours autoclving. The present study shows that phytic acid content of chick pea decreased 82%, Black gram 84%, pea 80.4%, Pigeon pea 77.47% and *Lathyrus sativus* 56% from raw seeds after 24 hours of fermentation. Ferdiaz and Markakis[12] found that the phytic acid content of the fermented peanut press cake (Onchom) inoculated with *Rhizopus oligosporus* decreased to 0.05% phytic acid after 72 hours of fermentation. It is well known that the most promising procedure to decrease phytate in seeds involving activation of the phytase enzyme resulting in the catalytic cleavage of phytate.[13,14]

Germinaion has been shown to reduce the phytic acid content of mature seed due to increased phytase activity.[15] *Rhizopus oligosporus* strain commonly used in tempeh production produced both extra and intracellular phytases. Intracellular phytase activity was higher than that of extra cellular enzyme. The steaming step prior to inoculation should have totally or partially inactivated the phytase which might be present in the legumes. Therefore, any phytase found in tempeh must hav originated from the molds[1]. Sudarmadji and Markakis.[16] demonstrated that the *Rhizopus oligosporus* used in the tempeh fermentation has strong phytase activity. The enzyme a present in mold hydrolyzes phytates and make the minerals available, Fermentation thus offers the dual advantage of saving energy cost by shortening the cooking time as well as rendering the grains nutritionally superior by removing certain antinutritional factor like phytic acid.

The effects of fermentation by the mold *Rhizopus oligosporus* on the hemaglutinating factors in seven legumes as hemagglutinin units (HU) are shown in table-2.

High hemagglutinating factors found in Lentil (70HU/g), Pea (71HU/g) and Khesari (73HU/g). Chick Pea and pigeon pea contain less hemagglutinating factor than Lens or *Lathyrus*. In this study fermented sample showed significant reduction

Table 2. Hemagglutinating factor in raw control and fermented legumes, (Hamagglutinating unit/gm[a]).

Lagumes	Raw[a]	Control[b]	Fermented
Lentil	70	59	8
Chick pea	35	29	0
Mung bean	56	42	12
Black gram	56	50	0
Pea	71	58	7
Pigeon Pea	42	40	0
Kheshari	73	56	25

a. Values are average of three r. eplicats expressed on dry weight basis.

b. Soaked overnight and steaming for 10 minutes.

of hemagglutinating activity present in raw and control seeds. The fermentation procedure can yield a potentially useful biochemical and a residual legumes with higher nutritive value.

Summary

Seven legumes are solid state fermented with *Rhizopus oligosporus* to study the effect on hemagglutinatins and phytic acid (Inositol 1,2,3,4,5,6, hexaphosphates) which are known as antinutrients. They are widely distributed in plants particularlly in cereal and legumes. During fermentation the phytic acid content of all legumes decreased. The average content of phytic acid in legumes is about 649 mg/100g and the percentage decreased was 74.42 after fermentation. The fermen-tation totally reduced hemag-glutinating activity in three legumes and the average reduction in rest of the legumes are about 75.07%.

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