Effect of Fermentation on the Hemagglutinating and Antitrypsin Factors in Legume seeds

A.B.M. Moslehuddin; Professor R.I. Tannous
Institute of Nutrition and Food Science University of Dhaka and Department of Food Technology and Nutrition American University of Beirut, Lebanon.

Introduction

The gap between protein supply of the world and the growth of the global population continues to be widen, and proper ways for narrowing this gap have become an urgent matter. Animal protein sources are costly and it is difficult for the developing countries to increase their animal protein sources due to increasing costs. On the other hand, fifteen to thirty per cent 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 [1]. But these legumes are deficient in sulphur containing amino acids, like methionine and cystine, and rich in lysine. The protein value of lentils, chickpaas and fava beans varies between 20 to 27%.

Inspite of high content of protein in legumes, there are some natural chemical substances which give undesirable reactions [2]. For example, fava bean contain toxic substances which cause the homelytic disease favism in individuals with an heriditary deficiency of glucos-6-phosphate dehydrogenase in red blood cells [3]. It has been also reported that fava beans contain hemagglutinating factors [4]. The other legumes, lentils and chickpeas also reported to have hemagglutinating and antinutritional factors [2].

There was extensive study of the fermented products from soybeans in South East Asia. One of these popular fermented products of soybeans by Rhizopus oligosporus is Tempeh, whose nutritional quality is better than the unfermented soybeans in respect of vitamins like riboflavin, nicotinic acid, vitamin B 12, pantethenic acid and protein [5,6]. Moreover this fermented product is safer than raw beans in respect of food poisoning bacteria and antinutritional toxic factors. The shelf life of the products are also long and they are pallatable to eat. An investigation of the nutritional quality of the Tempeh had led to suggest in being used as a possible source of low-cost protein food for child feeding in developing countries.

Therefore, this present study was designed to investigate the effect of fermentation by the same mold Rhizopus oligosporus on the toxic substance in lentils, chickpea and fava bean to improve their nutritional quality.

Materials and Methods

Dry fava beans, lentils and Kabuli chickpeas were selected for the process of fermentation by mold. These legume seeds were purchased from the local market, cleaned and undesirable seeds were removed.

The mold selected was the Tempeh producing Rhizopuas oligosporus (NRRL) 2710). This culture was brought from Dr. Hesseltine of U.S.A. and the freeze dried culture was revived on peptone dextrose agar (P.D.A.).

Chemicals used for the analysis of antitrypsin factors on the legumes were casein, purchaded from Nutritional Biochemical corporation, U.S.A. Trypsin EDTA was used from GIBCO (Grand Island Biological Co. California) U.S.A. in concentration of 5 mg/ml instead of Trypsin (E. Merck) mentioned in the method. All other chemicals used were of analytical grade.

Fermentation of Fava beans

The methods of Murata et al. (1967)[5] were followed for the fermentation of legumes with some modifications. Fava beans were soaked overnight in water in the proportion of 1:3. Then they were dehulled by hand and skins were separated to allow for growth of mold [8] Then the beans were kept in the incubuter at 37°C, on a wire basket. As soon as the temperature of the beans reached 37°C, they were taken out of the incubater and the process of inoculation with the inoculum of Rhizopus oligosporus was followed.

Preparation of mold

The mold took 4-5 days for revival from the freeze dried culture and several peptone dextrose agar plates were inoculated for the preparation of sufficient inoculum for the next step.

Preparation of inoculum

Preparation of inoculum for fermentation was done from the 48 hours growth of the fungal spores streaked on P.D.A. These spores from the P.D.A. plate were mixed with 2 gms of whet flour (sterile) by spreading with a sterile glass sprea 'er. Then the mixed spores from P.D.A. plate were used as the inoculum for 100 gms of dehulled legumes. In a clean beaker, these spores and 100 gms of fava beans were mixed and transferred to a polyethylene bag (16x8 cm). This polyethylene bag with inoculated beans was sealed, perforated every 5 cm apart and incubated at 37-40°C for fermentation.

Optimum condition for fermentation

The mold Rhizopurs oligosporus requires three factors for better production of fermented products. These are oxygen, moisture and heat. The various factors are closely interrelated and somewhat independent (Hermana, 1962)[8]. Sufficient oxygen is required for rapid growth of the mold. If air flow is too high, mold metabolizes too rapidly which produces enough heat to injure the growth. In insufficient oxygen supply there will be poor growth of the fungal spores on the legumes. So, a slow uniform diffussion of air was needed on the beans. This was done by perforating the polyethylene bag containing inoculated fava beans. Moisture for the growth of the mold is also required. There must be sufficient moisture for the better growth of the mold on the legumes. When the legumes became dry, sterile water was introduced into the polyethylene bag using a sterile Pasteur pipette to have optimum moisture content. Introduction of water was done in such a way that no excess moisture remained inside the polyethylene bag because it prevents optimum oxygen diffusion into the fermented legumes and ultimetely inhibits the mold growth. On the other hand, excess moisture favours food spolling bacterial growth[9]. Fermentation was done for 48 hours.

In this way, another sample of fava beans boiled for 1 hour was fermented. Unfermented samples were kept in the deep freeze.

Fermentation of lentils

Preparation of lentils before inoculation was different from fava beans. Lentils were soaked for two hours and dried to remove excess moisture, then inoculated by the mold spores according to the previous methods of fava beans. Then the polpethylene bags containing lentils were incubated at the required temperature. Unfermented samples were kept in the deep freeze.

Fermentation of chickpeas

Chickpeas were soaked overnight and then dehulled like fava beans and fermented by the same procedure of fava beans and lentils. Unfermented samples were kept in the deep freeze.

Checking of fermentatron

After 24 hours incubation of legume samples, white mycelia of fungal growth were observed on the products surface. Moisture and temperature of the products were checked occasionally by naked eye and thermometer. After 48 hours incubation, samples were taken out of the incubator and kept into deep freeze for stopping the fermentation.

All the unfermented and fermented samples were prepared in duplicates. The following samples were prepared.

Fava beans

1) Fava beans (raw, soaked for 1 hour)
2) Favn beans soaked for overnight (unfermented control)
3) Fava beans soaked for overnight and fermented
4) Fava beans boiled for 1 hour (unfermented)
5) Fava beans boiled and fermented

Lentils

1) Lentils (Raw)
2) Lentils (soaked for 2 hrs, unfermented control)
3) Lentiis (soaked and fermented)

Chickpeas

1) Chickpeas (Raw, soaked for 1 hour)
2) Chickpeas (soaked for overnight, unfermented control)
3) Chickpeas (soaked and fermented)

All these samples were freeze dried, powdered into 20 mesh and kept in the deep freeze for further experiments.

Testing of Hemagglutinating and Antitrypsin factors in legumes: Hemagglutinating and Toxic Substances of Fava bean.

The hemagglutinating activity of the fava bean's extracts and other legumes were done according to Liener and Hill (1953)[10] and Tannous and Ullah (1969)[1].

Antitrypsin factors in lentiis and chickpeas

To determine the antitrypsin factors in lentils and chickpeas, the method of Roy and Rao et al.[11] for extraction was followed. For enzymatic activity measurement, the method of Kakade et al.[12] was followed with some modifications. Extracts of 5% powdered legumes in water were made by mixing for 3 hours using a microid shaker. These extracts were then centrifuged at 15°C and 700 r.p.m. for 20 minutes and the supernatant was separated for experiment.

Results

The effects of fermentation by the mold Rhizopus oligosporus on the hemagglutinating factors in fava beans are shown in Table I. From the data it can be observed that after fermentation for 48 hours by the mold, there was a decrease of 75% of the total hemagglutinating factors in the soaked and fermented beans.

In the Table II, the effect of fermentation on the hemagglutinating factors of lentils was 100% and in the Table III also no hemagglutinating factors could be detected in the raw and fermented samples.

Table IV showed that no antitrypsin factors were present in the lentils and Table V showed 54% decrease of antitrypsin factors in the fermented chickpeas.

Discussion

The results in general indicated that there were some effects on the hemagglutinating and antitrypsin factors of the legume after fermentation by the Rhizopus oligosporus (NRRL 2710). The decrease in the Hemagglutinating factors in fava beans after fermentation was 75% and there were total destruction of hemagglutinating factors in lentils after fermentation, No hemagglutinating factor in chickpeas was observed during our experiments and this was also confirmed by Tannous and Ullah, (1969)[1]. There was 54.0% decrease of antitrypsin factors in chickpeas after fermentation and no antitrypsin factor was present in lentils (Liener, 69)[2].

The hemagglutinating and antitrypsin factors which are found in the legumes are almost completly destroyed upon autoclaving and boiling, but main purpose of prepering Tempeh products from the legumes were to study the effect of fermentation on these two toxic factors. Moreover, it was shown that during the processing of the legumes by fermentation with the Rhizopus oligosporus, the products become digestible[13]. There are reports that during the process of fermentation, no aflatoxins[11] are produced in the products, on the otherhand, the Rhizopus oligosporus reduced aflatoxin B_1 content by 50%. This fermentation system not only reduces some of the toxic factors in legumes, but also enhance the nutritive value of the substracts.[5] There was no suitable methods to test the reduction of toxicity in fava beans by fermentation by the Rhizopus oligosporus. Therefore, antitrypsin and hemagglutinating factors of the fermented and unefrmented fava beans, lentils and chickpeas were studied.

But the favism causing factors of fava beans were not related with hemagglutinating factors as reported by Baba (1977)[4]. To study the actual effect of the fermented fava beans on the toxicity of the bean may be conducted in health centres with human volunteers under a physisian surveillance. As these beans are popular in the countries of Asia and Africa, these can be potential source of nutrients of these people of these countries if toxic components in them can be eliminated by fermentation processes.

Tabe I-Effect of Fermentation on the Hemagglutinating Factors in fava beans[a]

Treatment	Soaked for 1 hour	Soaked for 1 hour and Fermented	Boiled for 1 hour	Boiled for 1 hour and Fermented
Hemagglntinating factors	80	20	0	0
% Decrease		75	100	100

a. Each figure represents average of duplicating determinations.

Table II-Effect of Fermentation (48 hours) on the Hemagglutinating Factors in Lentils[a]

Treatment	Raw	Soaked for 2 hrs. and Fermented
Hemagglutinating Factors (Unit/gm)	640	0
% Decrease	—	0

a. Each figure represents average of duplicating determinations.

Table III Effect of Fermentation (48 hrs.) on the Hemagglutinating Factors in Chickpeas[a]

Treatment	Soaked for overnight	Soaked and Fermented
Hemagglutinating	0	—
Factors (Unit/gm)	—	0

a. Each figure represents average of duplicating determinations.

Table IV Effect of Fermentation (48 hrs.) on the Antittrypsin Factors in Lentils[a]

Treatment	Raw	Soaked for 2 hours	Soaked for 2 hours and Fermented
Antitrypsin Factors %	0	0	0
% Decrease	0	0	0

a. Each figure represents average of duplicating determinations.

Table V. Effect of fermentation on the Antitrypsin Factors in Chickpeas[a]

Treatment	Soaked for 1 hour	Soaked for overnight	Soaked and Fermented
Antitrypsin factors %	20.1	20.9	9.6
% Decrease	—	0	54.0

a, Each figure represents average of duplicating determinations.

References

1. Tannous, R.I., and Ullah, M.: Effects of Autoclaving on Nutritional Factors in Legumes Seeds. Tropical Agriculture, 48,123, 1969.
2. Liener, I.E.: Toxic Constituents of Plant Food Stuffs. Academic Press, New York. United States of America, p. 500, 1969.
3. Jamalian, J.: Favism inducing toxins in Broad beans (vicia fava). Determination of Vicine content and investigation of other nonprotein nitrogenous compounds in different Broad bean cultivar. J. Sci, Food Agric , 29, 136, 1978.
4. Baba. N.H.: Favism: The study of the toxic factors in fava beans, Ph.D. Thesis. Faculty of Medicine. American University of Beirut. Beirut, Lebanon. p. 106, 1977.
5. Murata, K.: Ikehata, H. and Miyamote, T.: Studies on the Nutritional Value of Tempeh. J. Food Sci., 32, 580, 1967.
6. Gyorgy, P.: The Nutritive value of Tempeh. Meeting the protein needs of infants and children. Publ 843. National Academy of Science, National Research Council, Washington, D.C. United States of American. pp. 281-289, 1961.
7. 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.
8. Hermana, Tempeh: An Indonesian fermented soybean foods. Presented at the World Study on Wests Recovery by Microorganisms. University of Malaya-UNESCO/ICRO (W.R. Slanten, editor). Published by the Ministry of Education: Malayasia, Kualalumpur for the Malayasian National Commission of UNESCO. p. 221, 1972.
9. Steinkraus; K.H., Hard, D.B. Van Buren, J.P and Hackler, L.R : Pilot Plant Studies on Tempeh. Proceeding Conference, Soybean Products for Protein in Human Food. Peuria, Illinois, United States of America. p. 83, 1961.
10. Liener, I.E. and Hill, E.G : The effect of heat treatment on the nutritive value and hemagglutinating activity of soybean meal. J. Nutr. 49, 609, 1953.
11. Roy, N. Dwijendra, and Rao, Y.P.: Evidence, isolation, Purification and some Properties, of Inhibitee in Lathyrus. J. Agric. Food Chem., 19, 257, 1971.
12. Kakade, M.L., Simon, N and Liener, I.E.: An evaluation of natural vs synthetic substracts for measuring the antitryptic activity of soybean samples. Cereal chem., 46.518, 1969.
13. Maga, J.A. Lorenz, K. and Onayemi, O. Digestive acceptability of Protein as measured by the initial rate of in Vitro Proteolysis. J. Food, Sci. 38, 173, 1973.
14. Swanke Dijen. Self protection of fermented foods against aflatoxin: Proc, IV Int. Congress Food Sci. and Technel. 111: 244-253, 1974.