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STUDIES ON THE PREPARATION OF TEMPEH AND TEMPEH KABABS

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Tempeh has been prepared from soybean cultivated in Pakistan by fermentation with *Rhizopus oligosporus* NRRL-2710 in shallow stainless steel trays. Excellent tempeh has been prepared after 20 hr incubation at 31°. The percentage of trypsin inhibitor has been determined at various stages involved during the tempeh formation. It was found to be maximum in the raw soybean and remained unchanged after soaking overnight. Boiling soybean for half an hour resulted in complete destruction of the trypsin inhibitor. After fermentation the other type of trypsin inhibitor was released which was completely destroyed by boiling the fermented material for 20 min. in water or deep fat frying. A procedure has been described for the preparation of tempeh and food preparations according to local conditions.

INTRODUCTION

Soybean is a relatively low cost rich source of highest quality vegetable protein. It contains 40-45 % protein and 18-22 % oil. Due to its high protein content soybean is known to be excellent as food supplement. Soybean contains nearly all essential amino acids and is largely free of cholestrol. For building the acceptance of soy products it is necessary to apply it in different varieties of foods to enhance their nutritional qualities.

One such product is tempeh. Tempeh is a product made in the East Indies by fermenting, soaked, partially cooked and dehulled soybean. In this method the work of tenderizing the bean has been given over to a process of natural fermentation. Moreover during this fermentation the mould digestion of soybean enhances the digestibility of the soybean, destroys the undesirable odours of raw substrate, improves taste and texture and produces a tasty product.

Food legumes constitute a major source of protein in developing countries. However the prolonged cooking time required to make them palatable and to destroy their antinutrients is a major constraint to their utilization.

The objective of the work was to investigate a method of processing soybeans by fermentation in order to prepare a ready-to-eat, quick cooking, and acceptable bean product of a bland and palatable nature, achieving significant reduction in cooking time and developing an economical process which requires a minimum of time, energy and cost. For this purpose tempeh preparation is the simplest way of making soybeans eatable.

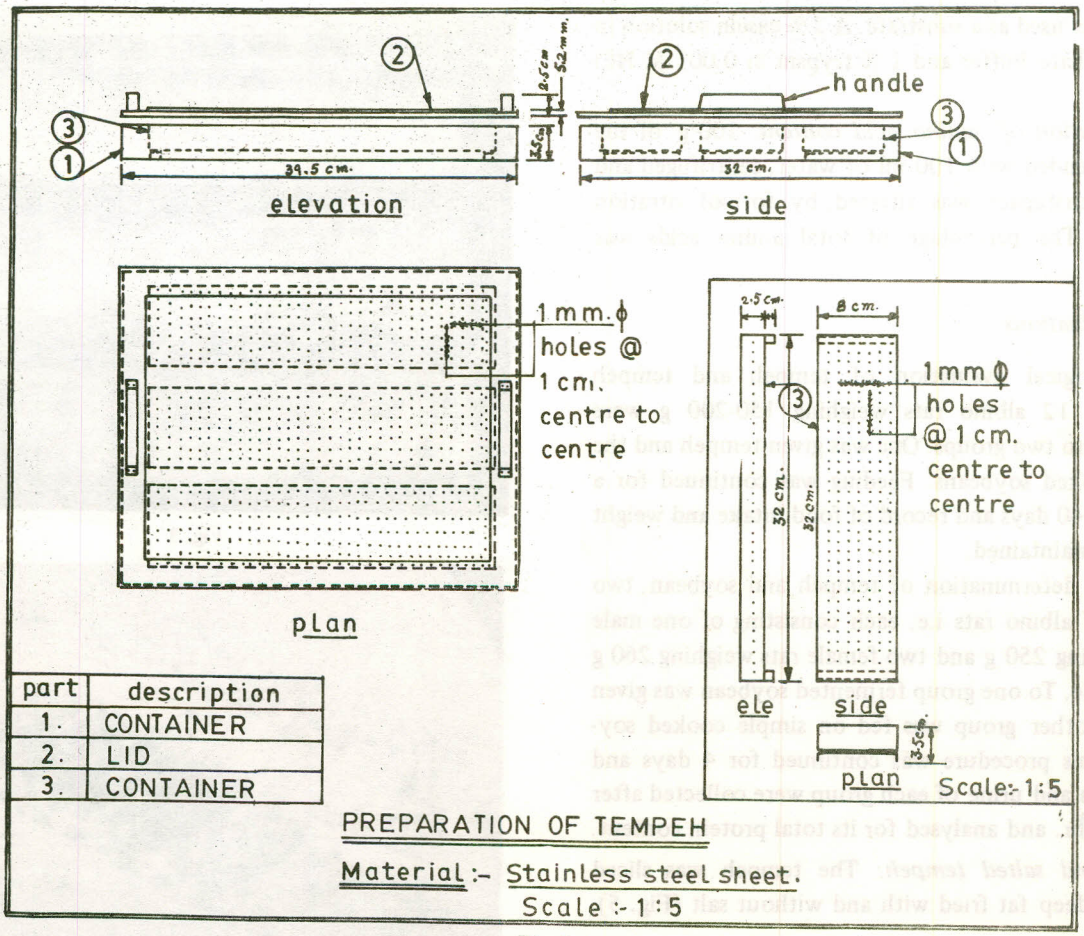
Since the food is bland, it can be modified to suit local taste by adding spices etc. A process has been standardized for the preparation of tempeh and food preparations according to the local conditions.

MATERIALS AND METHODS

1. The soybeans used were of 'Lee' variety, cultivated at Swat during (1976). The moisture content of raw soybeans varied from 5.27 to 6.58% and contains 28.5% oil, 44% protein.
2. The inoculum was prepared by growing a pure culture of *Rhizopus oligosporus* NRRL-2710 on Malt extract agar slants for 2-3 days at 31°.
3. *Large outer trays:* Dimensions 39.5 x 32 x 3.5 cm with perforations in the cover of 1 mm on 1 cm center. The bottom of the trays contain no perforations (Fig. 1).
4. *Smaller inner trays:* Each large tray contains three smaller trays of 35 x 8x2.5 cm with perforations at the bottom and sides of 1 mm. on 1 cm centres. 1 cm high supports were fixed at the lower corner of the trays to attain uniform mould growth by providing space under the smaller trays for the supply of air to the lower surface of the substrate (Fig. 1).
5. Petri dishes of 9 cm and 14 cm diameters were used.
6. Large outer trays without perforations in the top cover.
7. Large outer trays with stainless steel sieve of 200 mesh in the top cover.

Procedure for preparing soybeans for the tempeh fermentation

a. Soybeans were soaked overnight in tap water at room temperature and then dehulled by rubbing them with



part	description
1.	CONTAINER
2.	LID
3.	CONTAINER

Fig. 1

hands. A minimum of 6 litres of water was used for soaking every kilogram of the dry soybeans.

b. The soaking water was discarded and the soybeans were autoclaved at 15 psi for 3 different periods i.e. 15, 20 and 30 min. or boiled in water for 1 hr.

c. Soybeans were placed on sterilized muslin cloth to drain off the excess water and to cool the beans. The beans became swollen and soft after this treatment.

d. The beans were inoculated with the spores of the tempeh mould, (*Rhizopus oligosporus* NRRL-2710) and 10 ml of sterilized water was added to a slant of the sporulated culture, and this spore suspension was utilized for 1 kg of dry soybeans. Excess incoculum was used to ensure rapid and uniform fermentation.

e. After inoculation and thorough mixing, the beans were loosely packed into containers (Fig. 2b).

f. The inoculated beans were incubated at 31°. A tray of water was placed at the bottom of the incubator in order to increase its humidity.

Aflatoxin analysis was conducted for the dry soybeans, soybean after soaking overnight, also after autoclaving (before inoculation with tempeh mould) as well as in the tempeh by T.P.I. standard procedure and C.B. Method [5].

The trypsin inhibitor assay was performed at the various stages involved during tempeh formation, using the formol titration method [4].

Five g. of the sample was taken in 125 ml of water. The mixture was stirred and the pH was adjusted to 7.6 with N/10 NaOH solution. Again shaken for one hour and then centrifuged for 15 min. at 5000 rpm. The percentage of trypsin inhibited was determined in the clear centri fugate.

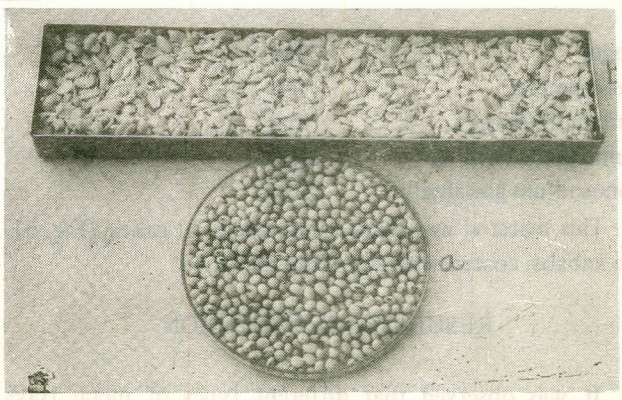


Fig. 2 (a, b)

Casein was used as a substrate. A 2 % casein solution in pH 7.6 phosphate buffer and 1 % trypsin in 0.001 M HCl was used.

Determination of amino acid content. 100 g. of the sample was blended with 100 ml of water, centrifuged and the clear centrifugate was titrated by formol titration method [6]. The percentage of total amino acids was calculated.

Biological evaluations

1. For biological evaluation of tempeh and tempeh products, 12 albino rats weighing 150-200 g were divided into two groups. One was given tempeh and the other cooked soybeans. Feeding was continued for a period of 40 days and record of food intake and weight gain was maintained.
2. For NPU determination of tempeh and soybean, two groups of albino rats i.e. each consisting of one male rat weighing 250 g and two female rats weighing 260 g were made. To one group fermented soybean was given and the other group was fed on simple cooked soybeans. This procedure was continued for 4 days and the faeces and urine of each group were collected after every 24 hr. and analysed for its total protein content.

Simple and salted tempeh: The tempeh was sliced (Fig. 4) and deep fat fried with and without salt (Fig. 5).

Tempeh kababs. The following spices were added to 1 kg of tempeh.

Onion	250 g
Garlic	30 g
Tomatoes	90 g
Pepper (red)	20 g
Salt	15 g
Corriander (dried)	15 g
Water	1.5 litre

The mixture was heated until a thick paste was formed, which was ground, and to the paste were added:

Ginger	20 g
Green pepper	4 g
Cardamom (large)	6 g
Cumin seeds (white)	15 g
Monosodium glutamate	2.5 g

This material was shaped into small flat cakes, (Fig. 6). The kababs, coated with egg white and fried.

RESULTS AND DISCUSSION

It was observed that different types of metal trays produced variable results. Metal trays without perforations

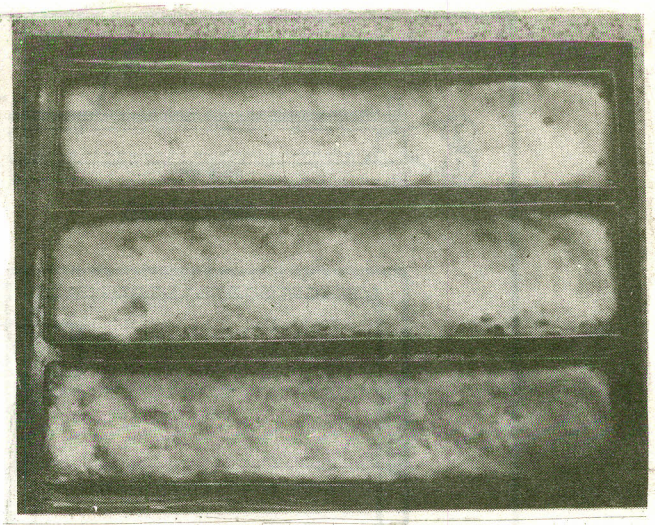


Fig. 3

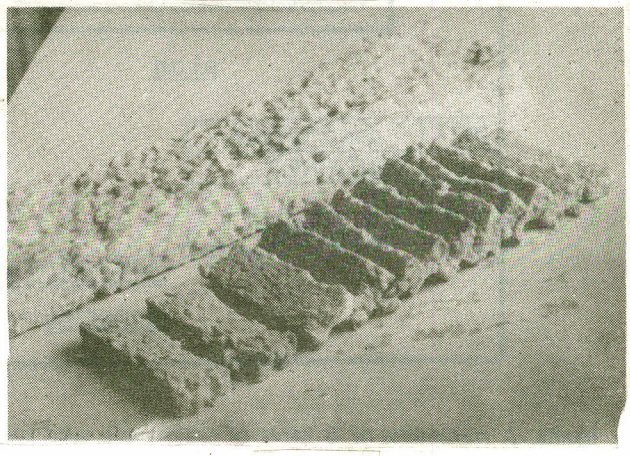


Fig. 4

Fig. 5

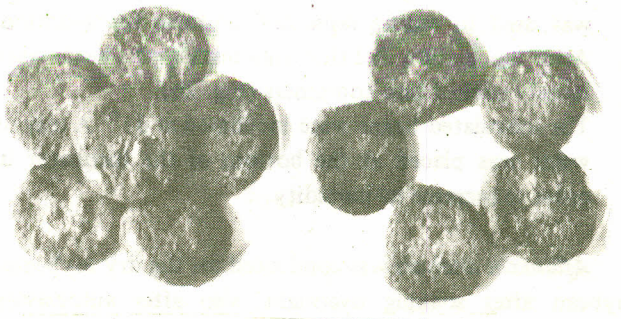


Fig. 6

did not produce good tempeh, as determined by the development of white mycelium, by odour and moulding throughout the soybeans in 20 hr, while the metal trays with stainless steel sieve evaporation of water caused dryness of the cake. However the metal trays with perforation of 1 mm on 1 cm centers in the top cover. The growth was excellent throughout the whole mass of the cake.

The amount of aeration is very critical for the growth of the mould. If insufficient air is supplied, it will not promote the growth of the mould, on the other hand if the amount of air is in excess the soybeans at the surface will dry out before the mould starts to develop and no tempeh will be produced.

Due to perforations at the bottom and walls of the smaller trays, excellent growth occurs. Mould growth also depends upon the number of soybean pieces. If the soybean grain is split into more than 8 pieces mould growth is poor, because the air spaces in between the particles are reduced causing the air supply in the interior to be inadequate resulting in inadequate mould growth, when the soybean grain was split into 2-3 pieces the growth was maximum.

During hot months of summer some times soaking of soybean results in unfavourable bacterial growth, which causes putrefaction and disagreeable odours. This can be controlled by soaking the beans in running water. During cooking of the soybeans 1% lactic acid or 0.5 percent acetic acid is added. The beans become slightly acidic (pH – 5.0) which is favourable to the growth of the mould but inhibits many bacteria that could cause spoilage of the tempeh.

EFFECT OF AUTO CLAVING

Soybeans were autoclaved at 15 psi for 15, 20 and 30 min. The beans which were cooked for 15 min. were not soft enough to be digested by the mould; Those autoclaved for 30 min. were too soft and mould growth was also poor whereas the growth was maximum in the beans autoclaved for 20 min. or boiled in water for one hr. The growth is visible after 18 hr incubation at 31° and reaches its maximum after 20 hr (Fig. 3).

The trypsin inhibitor assay [4] revealed that the inhibitor exists in soybean seeds and overnight soaking in water had no effect on trypsin inhibitor activity. After boiling in water for 20 min. or autoclaving for 10 min. at 15 psi, the trypsin inhibitor is completely destroyed. After fermentation with *Rhizopus oligosporus* (NRRL-2710) the bound form of trypsin inhibitor is again released, and is destroyed by boiling the tempeh in water for 20 min. or deep fat frying (Table 1).

There are two types of trypsin inhibitors in soybean. One is heat labile and is proteinous in nature and the other exists in an inactive, bound, heat resistant form. These are free fatty acids in nature which are liberated from oil in the soybeans by fungal lipase [3]. Once released, it can be easily destroyed by boiling or fat frying. The bound trypsin inhibitors are known to get free during gastric digestion [7]. Thus the prior release of the bound trypsin inhibitors and their inactivation is naturitionally important.

Tempeh contains 52.5% moisture, 20-22% protein (Table 2), while beef contains 21% protein. Kababs prepared from tempeh possessed 20% protein. (The analysis were based on wet basis).

Amino acid content (hydrolysed protein) in the tempeh has been determined [6]. It has been found that

Table 1. Soybean trypsin inhibitor (STI) assay, during the process of tempeh formation.

S. No.	Treatment	Trypsin inhibited (per cent)
1.	Dry whole soybean	28.6 trypsin inhibited
2.	Soybean after soaking for an overnight in tap water	28.6 " "
3.	After boiling the soaked soybean for ½ an hour in tap water	0.0 " "
4.	Fresh tempeh	13.16 " "
5.	Tempeh boiled in water for 20 minutes	0.0 " "
6.	Tempeh, deep fat fried	0.0 " "

Calculated on dry basis

Table 2. Moisture content of soybeans at various stages involved during tempeh formation.

Treatment	Moisture content (per cent)	Protein (per cent)
Dry seed	5.27	44.0
After soaking for an overnight in water	51.40	21.8
After boiling: for ½ hr. in water	54.8	20.5
Fresh tempeh	52.5	21.2

Table 3. Organoleptic evaluation (mean score of 24 judges).

	Appearance (10)	Flavour (10)	Texture (10)	Taste (10)	Total Score	Percentage
Simple tempeh (fried)	6.35	6.15	6.82	5.90	25.22	63.05
Salted tempeh (fried)	6.50	6.40	6.44	6.20	25.54	63.85
Tempeh kabab	8.96	7.95	7.51	8.73	32.44	81.10

Table 4. Biological evaluation of tempeh and soybean.

Group	Total protein intake	Protein in faeces	Protein in urine	NPU (per cent)	Mean weight gain per rat per day
Group I Fermented soybean (tempeh)	42 g	5.5 g	2.0 g	82.1	1.5 g
Group II Unfermented soybean	40 g	6.4 g	2.3 g	78.25	1.25 g

nearly 44% protein of soybean is hydrolysed into simple amino acids by the proteolytic enzymes produced by the mould culture.

Aflatoxin analysis confirmed the absence of any aflatoxin in the tempeh due to mould growth.

Simple tempeh, salted tempeh and tempeh kababs were organoleptically evaluated by twenty-four judges. The mean score of these judges for appearance, flavour, texture and taste of each product is given in Table 3.

Both simple and salted tempeh were almost equally liked by the taste testing panel whereas the score for tempeh kababs was much higher than the other two products. It appears that the introduction of tempeh in the form of kababs, i.e. traditional Pakistani food is much more accepted than the other two products.

Feeding studies using rats show that the values for weight gain are slightly greater when rats were fed on tempeh as compared with those given simple cooked soybeans (Table 4). The Net Protein Utilization (NPU) was determined by total protein intake and the total protein excreted through faeces and urine [8]. The results indicate that NPU value for fermented soybean i.e. tempeh is higher than for the unfermented soybean.

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