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STUDIES ON SOY BEAN TEMPEH

Part 1. Optimization of Factors Effecting Fermentation in Commercial Production of Tempeh with Respect to Pilot Plant Studies

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Trays with a holding capacity of 25 kg and housed in a chamber, were designed and fabricated for the commercial production of soybean tempeh. *Rhizopus oligosporous* was used for fermentation process and factors effecting pilot plant fermentation were optimized.

INTRODUCTION

Protein is one of the most essential and expensive nutrients among all other nutritive elements like fats, minerals and vitamins consumed by man. Tens of thousands of children every year in underdeveloped countries die due to diseases caused by protein deficiencies. It has been predicated that by the year 2000, protein deficiency will rise to a level of 20 million tons/years. One of the more effective means of combating the anticipated deficiency is to utilize protein products derived from low cost plant materials. Soybeans having 39–40% protein has been consumed by the Orientals for 4000 years. It was introduced into the United States in 1904 which now has about 62% of total world soybean cultivation. Soybean protein is not only low cost, but also plays a significant role from the nutritional point of view. Its amino acid content is very close to that required for human health. When properly processed this vegetable protein can meet the cultural profiles, acceptability, palatability eating habits, texture, shelf-life, storage as well as nutritional qualities. In Indonesia fermentation techniques have been employed by Djien and Hesseltine [1] to improve the legume's digestability, and to mask the undesirable flavour. This fermentated product known as tempeh forms an important source of protein in the Javanese diet. It is differentiated from "soysauce" and "miso" which are primarily used as a condiments supplying flavour and aroma to dishes, whereas tempeh is not only a high protein, low caloric meal but one of the first vegetarean food to contain nutritionally important amounts of vitamin B_{12}, essential for propagation of erythrocytes and prevention of anaemia, according to Van Veen and Steinkraus [2].

Various grain legumes (except soybean) have been used as staple food by Pakistanis in various regions. A high ratio of malnutrition exists in the country due to the shortage and the rising cost of animal protein. The present studies were, therefore, undertaken to develop organoleptically acceptable, high protein low cost food items from soybean tempeh. Keeping in view the cultural profiles and eating habits of people, tempeh was cooked and mixed up with spices to form minced meat and *kababs* which have been greatly accepted by local people and their increasing demand has led to the pilot production of Tempeh. Laboratory studies were, therefore, adopted for development of modern commercial methods.

EXPERIMENTAL RESULTS

Soybean variety *Bragg* was obtained from Tandojam (Sind), which contained 38–39% protein, 5.68% moisture and 8.75% ash. *Rhizopus oligosporous* culture NRRL 2710 was obtained from Indonesia and maintained on a malt extract agar medium. Experiments were carried out in a humidity and temperature controlled room. Protein nitrogen was estimated by the micro-kjeldahl N(6.25) method, while moisture and ash were determined according to AOAC method [3].

Processing of soybeans. Soybeans were cleanssed to remove stones, twigs, grits etc. and soaked with three times its weight of water containing an appropriate amount of lactic acid for 8–12 hr. It was then boiled for 90 min. after readjusting its water level to 1:3. The cooked beans were strained and washed in water to separate the cotyledons; the hulls drained out through washing and the pH checked. Finally the beans were cooled, and inoculated asceptically with *Rhizopus oligosporous.*

Conditions of Fermentation. For carrying out pilot plant studies, various factors effecting the fermentation

process i.e. pH; temperature, humidity and aeration were studied.

(a) pH. It was observed that beans soaked in water in the abscence of lactic acid at room temperature got spoiled due to bacterial growth, fungal inhibition and undesirable odours. In the present studies for the commercial production of tempeh, bacterial contamination and spoilage were prevented by the addition of only 0.6% lactic acid instead of 1% [4]. This amount was quite sufficient not only for controlling bacterial growth but also facilitated rapid mold growth, thus resulting in good quality tempeh in 18 hr.

(b) Temperature. During the initial stage of fermentation, the temperature was recorded to be 31° but as fermentation progressed, it reached 45°–49° (because of active growth and evolution of heat), leading to dehydration and inhibition of the mold. Therefore, a temperature control panel was installed in the room and the temperature was maintained at 32–34° during the entire fermentation process.

(c) Aeration. Surface area and thickness of the fermentation material plays an important role in tempeh production which indicates that sufficient oxygen should be available to the fermentation mass. Studies were, therefore, carried out using large perforated plastic trays filling upto three different levels.

Comparison of the three soybean levels after fermentation demonstrated the dehydration of mass and very mild growth in 3" level experiment. A cross section of the three masses revealed mycelial penetration and softening of beans in 1" and 2" levels, whereas the 3" level showed not only absence of mycelial growth, but also the beans remained hard. This showed that the thickness of soybean cake above 2" level hindered the passage of oxygen through bean mass, preventing mycelial growth penetration, and lack of enzymatic action in the deeper areas (Table I).

(d) Humidity. After cooking and straining the beans, the hot steaming water was transferred to the fermentation room to obtain 60% humidity at the start of the fermentation process and maintained by covering the front portion of the fermentation units with dripping wet cloth hanging from the trough like roof of the chamber which is filled with water.

During the normal fermentation process, it was observed that humidity rose to 65–70% after 6 hr. and reached a maximum of 85–90% in 16 to 18 hr, the stage at which fermentation was completed. A decline in humidity was recorded thereafter in 22–24 hr which steeped to 75–80% falling to 50% in 30–40 hr (Table 2).

Table 1. Effect of quantity of beans for mycelial growth during fermentation

Weight of beans in Kg.	Level of beans in inches	Time of fermentation in hrs.	Type of growth
1.5	1	18	Profuse
3	2	18-20	Profuse
6	3	24	Growth mild and confined to surface areas, with signs of dehydration.

Pilot Plant Studies. Laboratory scale experiments were conducted on 1 kg capacity stainless steel trays as described by Steinkrans (1959). Preliminary studies on pilot plant scale were conducted on plastic trays. After studying various fermentation factors (as described above) stainless steel trays as shown in Fig. 1. were designed and fabricated.

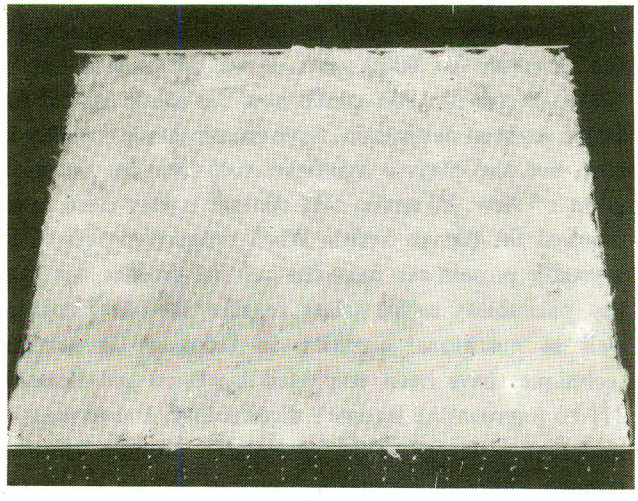

Fig. 1. Tempeh showing pure white growth of the fungus after 16–18 hrs. of incubation at 32°–34°C.

Stainless steel fermentation chamber and trays. A stainless steel chamber for holding trays was designed. Depending on the amount of tempeh desired, a series of such chambers with 25 kg capacity may be fabricated and can be placed one on top of the other and/or on either side of each other.

Table 2. Effect of fermentation time on humidity and growth

Fermentation time in hrs.	Humidity %	pH	Weight (in kg) Processed bean	Tempeh	Smell of ammonia	Growth	Acceptability of the product
	65 – 70	5.5	3.3	—	—	Slight	Not tested
18	85 – 90	6.0	3.3	2.75	—	Pure white mycelial growth with droplets	Highly acceptable
24	75 – 80	7.0	3.3	2.5	Slight	Dirty white growth, with indication of spore formation	Partially acceptable
48	50 – 55	8.0	3.3	1.8	too much	Heavy sporulation showing signs of dehydration.	Not acceptable,

After the completion of each run and removal of tempeh (Fig. 1) the fermentation chamber and the trays were sterilized by dipping in a large vessel of boiling water. The room was frequently sterilized by washing with hot water and disinfecting with a suitable disinfectant.

Inoculation. The processed soybean was inoculated with spore suspension and the processed-inoculated beans were lossely packed in stainless steel trays having 2" beans level and incubated for 18–20 hr. at 60–65 humidity and 31–32° temperature.

The fermented material turned into a white interwoven mycelial compact cake called tempeh in 18–20 hr. with droplets of water on its surface due to mold respiration. This product is of good quality and has a pleasant odour, having a pH of 6.0, and an acceptable taste. Fermentation when continued further, resulted in an unpleasant ammonia smell which was due to the breakdown of protein. Tempeh and its product at this stage were not organoleptically acceptable.

It was observed that from 15 kg soybean, 25–26 kg tempeh of good quality was obtained with 16% protein, 66% moisture and 14% ash. The tempeh is now ready for preparing minced meat and kabab by adding spices according to the taste.

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