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STUDIES ON SOYBEAN TEMPEH*

Part II. Propagation and Preservation of *Rhizopus oligosporous* spores for Commercial Production of Tempeh from Soybean

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Experiments were carried out to propagate and preserve *Rhizopus oligosporous* spores for the commerical production of tempeh from soybean. It was observed that 18–20 hr. were needed to complete fermentation process and the formation of tempeh. There appears to be no appreciable difference with regard to the fermentation process if processed soybeans are inoculated with sporulation mass or tempeh under a given set of conditions. The sporulation mass stored for different lengths of time did not show any significant decrease in the viability of spores even after a period of 15 months.

INTRODUCTION

Tempeh, an Indonesian food and an important part of Javenese diet, is prepared by the fermentation of soybeans. Tempeh thus prepared contains 15–25% protein. Due to its high content of essential amino acid, it is regarded as a good substitute for meat. The product involves inoculation of socked, dehulled and cooked soybeans with a pure culture of *Rhizopus oligosporus,* which after 18 hr. of incubation at 31° results in a white mycelial cake known as "tempeh".

Mold *Rhizopus oligosporus* produces the enzymes protease and lipase [1] which break down the complex protein molecule into amino acids, and hydrolysis fats into fatty acids, thus enhancing the digestibility of soybeans. Besides destroying unpleasant odour, it imparts a pleasant flavour and gives additional texture to beans.

Van Veen and Scheafer [2] observed that compared to cooked beans, tempeh when consumed was well tolerated, even by persons suffering from gastro-intestinal upsets as noted in prisoner's of war camp during World War II. Antibiotic production by tempeh mold has been reported by Wang et. al. [3] who suggested its relation to disease resistance among tempeh consumers. Van Veen and Steinkraus [4] reported increased levels of niacin, riboflavin and vitamin B_{12} in the fermented tempeh.

Non-availability of animal protein due to livestock shortage and its rising cost has led to a high degree of malnutrition and protein deficiency diseases, especially among lower socio-economic groups in underdeveloped and developing countries. Utilization of vegetable protein like soybean tempeh, therefore, can play an important role in combating nutritional deficiencies.

Keeping this in view, a study on tempeh formation from soybeans was undertaken, initially on laboratory scale and subsequently on pilot plant level. The most essential factor for the commercial production of tempeh is the mass production of *Rhizopus oligosporous* spores, preservation of spore mass and prolonged storage without the loss of purity and viability of strain.

The use of culture media available in the market is economically not feasible for its commercial production. Steinkraus et. al [5] reported the preservation of freeze-dried fermented soybeans for use as inoculum. Rusmin and Ko [6] developed the inoculum by growing *Rhizopus oligosporous* spores on cooked rice. Hesseltine [7] reported the mass production of spores using rice, pearled wheat bran and cracked soybean and preserving the spores after freeze drying and grinding the fermented mass.

The present studies have been carried out in an effort to determine the storage life, viability and total spore count of *Rhizopus oligosporus* in relation to tempeh production under certain sets of conditions.

MATERIALS AND METHODS

Locally available soybean, variety Bragg, cultivated in Tandojam, Sind, was used in these studies. *Rhizopus*

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oligosporous (NRRL 2710) culture obtained from Indonesia was used for fermentation. The mold was cultured on malt extract agar (Difco) slants at 31° for 48 hr. and stored at −4°.

Preparation of Sporulation Mass: Rhizopus oligosporous was inoculated to freshly prepared malt extract agar slants and incubated at 31° for 72 hr. During this period spores developed profusely on the surface of the agar slants. 20 to 25 ml sterlized distilled water alongwith sterilized beads was added to the tubes and vigorously shaken to dislodge the spores and get them suspended in water. This spore suspension was then used for inoculating processed soybeans which were subsequently placed in a single layer in perforated stainless steel trays. The trays containing the inoculated soybean were incubated at 31° at a relative humidity to 60%. The mycelium thus produced from the germinating spores completely engulfed the beans in 10–12 hr. Fermenation was allowed to continue for 28–30 hr. till the development of spores was completed. This sporulated fermented mass of beans was dried at 50–55° packed in polyethylene bags in measured quantity and preserved at −4°.

Methods for the preparation of Inoculum: Two methods of inoculation were employed.

(i) Tempeh Production by Mass Sporulation Technique. Sporulated mass preserved as above has a total viable count of 89×10^7/g material. For commercial production, the inoculum was initially prepared from the frozen spore mass by thawing at room temperature (25–28° approx.) for 30–40 min. This was then suspended in cooled sterilized water and used as an inoculum as described above. For each kg of processed beans 20 g sporulation mass/200 ml water is used.

(ii) Tempeh Production by Tempeh to Tempeh Inoculation. Traditionally in Indonesia, the inoculum is taken from a previously fermented cake or from the wrapper of the cake [8]. Keeping this in view, a study was undertaken to inoculate a batch of processed beans with portions of the previous day's tempeh by suspending the fermented mycelial bean cake in water and spraying it over the processed beans. The tempeh thus produced needed the same fermentation time and the taste and flavour were the same as that of spore fermented tempeh. This tempeh to tempeh cultivation method can safely be used for four to five consecutive days by retransferring the inoculum from subsequent days trays to the next day's tray and so on. Thereafter the mold growth slows down and hence after the 5th day, sporulation mass technique should be remployed for initiating tempeh fermentation.

Storage Viability: Storage viability was determined after 2½ months', 5 months', 10 months', 15 months', period by making total spore count and determining the germination percentage. The total spore count was made by making appropriate dilutions of 1 g sporing mass in distilled water and making a direct microscopic examination using Thoma haemocytometer counting chambers [9]. The Viability of the spores was determined by the plate count method. 0.01 ml of spore suspension was made by dilution method and a known number of spores were pipetted out into test tubes containing 10 ml. of melted malt extract agar. After thorough mixing this malt extract inoculated agar was poured in sterilized petri dishes. The petriplates were then allowed to solidify and incubated at 27°-28° for 24 hr. The experiment was triplicated. The viable spores germinated into isolated colonies were counted and compared with initial spore count for evaluating percent viability.

RESULTS AND DISCUSSION

Inoculation of processed beans by both sporulation mass technique as well as the tempeh to tempeh method resulted in equally good quality tempeh in 18–20 hr.

It was also observed that the tempeh to tempeh cultivation method can safely be used for four to five consecutive days by retransferring the inoculum from subsequent days' trays to the next days' trays and so on. Subsequent retransfers declines the viability of fungi which is a natural phenomenon as has been observed during this study by the slow growth rate of mold after 5th transfer. Therefore, sporulation mass technique has to be re-employed thereafter for initiating tempeh production.

Sporulation mass was stored at −4° for different periods. The initial viability was 95% which after 2½ months' storage was reduced to 89%. However, it further decreased to 84%, 81% and 75% after 5, 10 and 15 months respectively (Fig. 1). According to Rusmin and Ko [6], the initial viability was 72% for spores adhered to the glass beads and 69% for spores in the inoculum pieces. They mentioned that these numbers decreased rapidly during the early storage period. Later the germination percentage levelled off for some time and subsequently declined to very low points. However these authors did not observe any severe decline in the viability of spores even after 15 months of storage.

It appears that no significant difference occurs in tempeh formation on inoculation with sporulation mass or with pieces of tempeh to processed soybean. Moreover the

viability of spores was not reduced to any appreciable extent even on storage for more than a year.

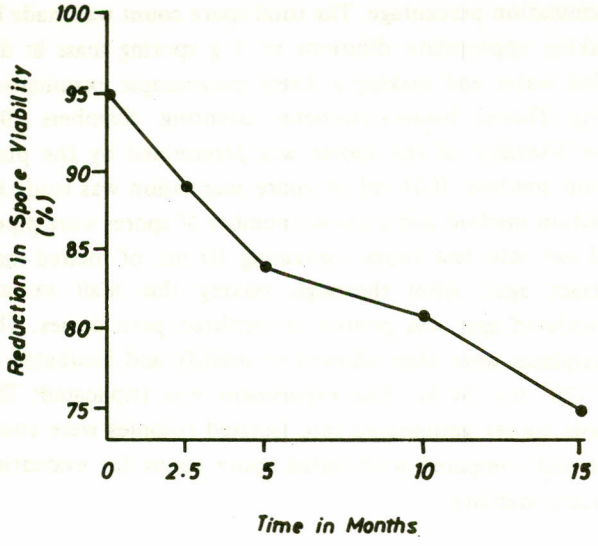

Fig. 1. Reduction in spore viability after 2½, 5, 10 and 15 months time.

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