



INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ASSESSMENT OF P^H, CALCIUM CONTENT AND MOISTURE LEVELS IN FERMENTED TEMPEH

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ABSTRACT

Fermentation is the process where the energy yielding anaerobic metabolism of nutrients like sugars without oxidation are converted to organic acids like lactic acid, acetic acid, etc. and ethanol. The process involves the microbial growth in food and change its characteristics by their enzymes. There are many fermented foods available with high nutritive value to overcome the challenges of nutritional deficiency. One of the fermented foods widely accepted globally is tempeh produced from soybeans which is highly nutritive food and also a valuable plant-based protein source. The present study focuses on production of tempeh from soybeans and determination of Ph, moisture content and calcium ions presence by strip method, oven drying method and flame test respectively to assess the nutritive quality of soybeans after fermentation.

KEYWORDS:

Fermented foods, Tempeh, Starter culture, strip method, flame test

INTRODUCTION :

Tempeh, fermented food originated from Indonesia (1). The fermentation takes place from soybeans and is nutritious and plant based protein source (2). Through fermentation the nutritional values of soybean is enhanced and preserved. The popularity of ethnic food is gradually increasing over the last few years (3). The consumption of ethnic food provides the required taste and also provides the cultural habitat and the origin of the food to the consumer . Fermented soy food , tempeh is the staple food for the people of Eastern Asia and is taken as daily intake. as it improves bio availability of nutrients . Tempeh is produced by solid state fermentation of soybean with Mold starter culture, *Rhizopus* spp (4). As the soybeans are fermented to tempeh the nutritional profile of the food product is enhanced, and anti- nutritional content is decreased (5). By this process the desirable texture of the food is also improved. During fermentation of tempeh, enzyme beta- glucosidase secreted by molds breakdown the bond forming conjugated isoflavones that are stronger antioxidants and more easily absorbed in higher amount in human intestine compared to conjugated isoflavones (6) The present study focuses on determination of pH changes, moisture content and calcium content in the fermented soybeans which indicate the quality of tempeh.

MATERIALS AND METHODS :

1.1 COLLECTION OF SOYBEAN SAMPLE :

Soybeans were purchased from local market store , Hyderabad .

1.2 PREPARATION OF SOYBEANS FOR FERMENTATION :

250 g of soybeans were soaked in sterile water overnight at room temperature. Then the hulls were removed, and soybeans were boiled for 40 min . After boiling the soybeans were air dried for 30 min. The air dried soybeans were used as raw material for fermentation.

1.3 STARTER CULTURE:

50 g of *Rhizopus oligosporus spp* and rice powder were added to the air dried the soybeans to initiate the fermentation process .

1.4 FERMENTATION PROCESS:

The air dried soybeans with the starter culture were incubated for 48 hrs at room temperature.

1.5 IDENTIFICATION OF FUNGAL ISOLATE :

The fungal isolate in the fermented sample was identified by lacto phenol cotton blue staining method as indicated in Microbiology manual by S.M Reddy and S.Ram Reddy, 2023 (7).

1.6 DETERMINATION OF PH IN TEMPEH:

The fermented tempeh was tested for the change in PH before and after fermentation, by strip method. (8) The pH test strip was dipped in the sample for 10 seconds and observed for the change in the strip colour.

1.7 DETERMINATION OF MOISTURE CONTENT IN TEMPEH:

Moisture content in the tempeh affects the texture and shelf life of the product. to determine the moisture content in tempeh 2.5 g of the samples (before and after fermentation) were taken in petri plates and incubated for 4-8 hrs at 160°C in a hot air oven. After the incubation period the mass of the samples was measured and analysed for the total moisture content. (9). The moisture content was calculated by,

$$\text{MOISTURE (\%)} = \frac{W1-W2}{W1} \times 100 \quad (10)$$

1.8 DETERMINATION OF CALCIUM BY FLAME TEST: Tempeh the fermented soy product is the most popular replacement of meat and good source of dairy free calcium according to nutrition value.org (11). The presence of calcium ions in the sample was determined by flame test as tempeh is considered as rich source calcium.

RESULTS AND DISCUSSION:

Tempeh the fermented food soybeans is gaining importance as a rich source of nutrients which is plant based in origin. The soybeans were soaked in water overnight, air dried for the fermentation to tempeh. The air-dried soybeans were incubated with the starter culture, *Rhizopus oligosporus* and rice flour at 37° for 48 hrs (fig1).



Fig.1 Addition of starter culture to soybeans.

The mycelial growth was observed on the surface of the soyabeans indicating the growth of the fungus (fig 2). During fermentation the pH plays an important role in favouring the mold growth. The acidity of product was determined by strip method where the colour of the strip changed from pH 7 to pH 4 (fig 3) which may be due to production of organic acid by lactic acid bacteria which are natural habitants on soybeans during the initial period of fermentation and is in accordance with the earlier studies (12). *Rhizopus* species eliminates the unwanted beany flavour and softenss the pains by producing proteases which increase its digestibility (13).



Fig.2 Growth of fungus on soaked soybeans



Fig.3 pH of the tempeh before fermentation (A) and after fermentation (B)

The characteristic flavour and texture in tempeh is dependent on specific moisture level. Moisture is important for the growth of mold during fermentation process. The moisture content in the tempeh was determined by oven drying method fig(4a, b) and it was observed that after 48 h , the percentage of moisture was found to be 60 indicating relatively high-water content in the product. Earlier studies indicated 60.14% of moisture in tempeh produced from soy protein (14). A study by Wikandari *et al.* , 2020 demonstrated higher moisture content in tempeh prepared from yellow soybeans. According to Astawan *et al.*, 2013 the soybean varieties influence the water content of tempeh because of the differences in water penetration into the soybean matrix and fungal growth activity during fermentation. Fermentation of soybeans was by the starter culture, *Rhizopus oligosporus* which was identified by lacto phenol staining after the fermentation process (fig5).



A

B

Fig.4 Oven dried soybeans before (A) and after (B)

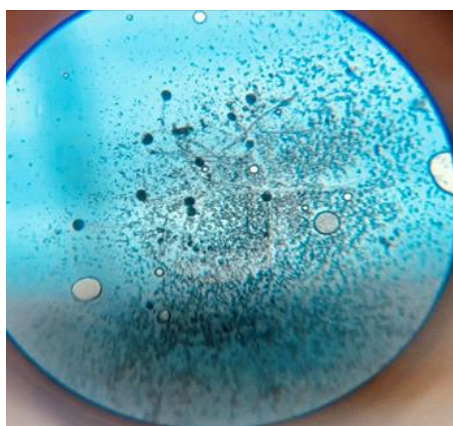


Fig.5 Microscopic view of *Rhizopus oligosporus*

Tempeh is considered as a good source of calcium compared to milk (15). The presence of calcium ions in the fermented tempeh was determined by flame test. It is an inorganic qualitative analysis method used in laboratory to spot the specific metal ions in compound (16). The sample when exposed to flame on bunsen burner, orange-red flame was observed indicating the presence of calcium ions (fig 6). According to Hasnah *et al.*, calcium in tempeh is excellent option for increasing own density and prevent bone loss (17).



Fig.6 Flame test for calcium ion determination in tempeh.

CONCLUSION:

Tempeh provides a sustainable nutritious food choice with numerous health benefits. The study concludes that the fermented tempeh has enhanced nutritive value when compared to unfermented soybeans confirming its role as an effective plant based nutritive supplement to the consumers.

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