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RESEARCH ARTICLE

Production, Quality and Acceptance of Tempeh prepared using *Dolichos lablab (Indian bean)* Legume

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ABSTRACT

Tempeh has been one of the most widely accepted fermented products and usually produced by fermenting soybeans inoculated with *Rhizopus oligosporus / Rhizopus oryzae.* Tempeh could be prepared with legumes other than soybean thereby increasing the acceptability and digestibility of the common legume. *Dolichos lablab* is a herbaceous plant with high nutritional benefits and still under-used in various countries because of anti-nutrient factors, such as enzyme (trypsin, chymotrypsin, amylase) inhibitors, phytic acid, flatulence factors, *etc*. A processing technique that removes anti nutritional factors to safe level will improve the nutritional quality of this legume. The purpose of this study is to prepare and standardize tempeh using fresh *Dolichos lablab* and *Arachis* hypogea in 75:25 ratio and to evaluate the physio-chemical, nutritional and organoleptic quality of tempeh and its value added products *viz.,* tempeh fry and tempeh gravy The quality analysis of *this* tempeh had 50.03 per cent of moisture, 20.13 g of carbohydrate, 19.37g of protein, 4.48 g of fat, 3.00 g of dietary fiber. On comparing the fresh tempeh, protein content of dried tempeh has increased considerably by 41.24 per cent and in the value added product *viz.,* tempeh gravy and tempeh fry, protein content has increased by 16.67per cent and 5.09 per cent respectively. There was 100 per cent removal of anti nutritional factors *viz.,* tannin and phytic acid due to various processing techniques. The overall acceptability of tempeh products was highly acceptable with score point of 8.0 ± 0.5 /9.0 and 7.5± 0.5 /9.0 for tempeh fry and gravy respectively. The unit cost production of 200 g of tempeh was Rs.50.00 only.

Keywords: *Tempeh; Dolichoslablab; Nutritional; sensory properties; Meat alternative.*

INTRODUCTION

Legumes are an important source of protein in many developing countries. Higher meat price in recent years and the need for protein rich foods have led people in most developing countries to shift their consumption to legumes. Tempeh is a traditional Indonesian solid-substrate fermentation in which soybean are hydrated and acidified, dehulled, cooked and then fermented with *Rhizopus oligosporus* as it is the dominant tempeh fungus which tastes like meat. With the growing health concerns of people across the globe, the importance of such health promoting foods is increasing steadily that had created awareness among the consumers to find meat alternatives. (Kumar *et al.*2017). The plant-based eating is recognized globally for its nutritional significance, a low cost food and also not only adds variety to the diet and reduce the risk of health issues. Developing new meat analogue that are attractive to the consumers is a challenge (Joshi et al.2015). Recently, the consumption of Tempeh has been increasing rapidly, in many countries. Tempeh could be prepared with pulses other than soybean thereby increasing the acceptability and digestibility of the common pulses (Erkan *et al.* 2019)

Dolichos lablab commonly called as Indian bean is the second most economically important species of Phaseolus family and one of the 12 primary grain legumes. In seasons due to its high availability it was sold at disrupted rate Very few farmers preserve and sold this legume as dried pulse.

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Moreover, they are under-used in various countries due to its anti-nutritional factors such as trypsin, chymotrypsin, amylase inhibitors, phytic acid, flatulence factors (Ramakrishna *et al.* 2008)

For enhancing the income of the farmers, value addition of fresh *Dolichos lablab* is a better choice. The objective of this work is to develop and standardize tempeh using fresh *Dolichos lablab* and for improving the nutrient content of the tempeh groundnut was incorporated to the legume in the ratio of 75: 25. In this study, the physicochemical composition, sensory evaluation of tempeh and tempeh based cooked products namely tempeh gravy and tempeh fry was evaluated. Buying intention studies and cost analysis was also done for promoting this value addition technique to the food processing industry due to its better nutrition and health advantage.

MATERIAL AND METHODS

Acquistion of the material

Fresh *Dolichos lablab* used in this study were purchased from the market in the harvest season *thaipattam* and sorted manually and only the whole and healthy ones were selected, placed in polyethylene bags and stored in a cold room until use. *Rhizopus oligosporus* MTCC 556 culture was obtained from Microbial Type Culture Collection and Gene Bank (MTCC) of CSIR-Institute of Microbial Technology Chandigarh.

Starter culture for tempeh and inoculums preparation

Rhizopus oligosporus strains were sub cultured and preserved in Potato Dextrose Agar (PDA) medium. Tempeh inoculum was prepared by inoculating *Rhizopus oligosporus* spores in 100 g of sterile rice flour and incubated at 30ºC for 3 to 5 days. After mycelium formation, rice flour was powdered and stored in pouches and used as inoculums. Tempeh production was prepared following the methodology of Starzynska-Janiszewska *et al. (*2014)

Inoculum Preparation

For the production of the flour inoculum, 100 g of rice was grounded and sieved using 200 mesh sieve. The flour was placed in glass containers with metal lid, sterilized and cooled at room temperature. Twenty milliliters of the inoculum were inoculated into each vessel and incubated in an oven at 30°C for 5 days. After this period, the containers were refrigerated and used up to 30 days.

Tempeh Production

Soyabean tempeh was prepared (T_0) by taking two hundred grams of soyabeans. It was cleaned in running water, submerged in one liter of sterilized water at room temperature for hydration/maceration with the addition of 20 ml of commercial vinegar containing acetic acid (5 per cent) for 20 hours. For the removal of surface water, beans were dried on paper towels at room temperature and heat treatment was performed by conditioning the beans in beakers capped with laminated paper and autoclaving them for 15 min at 121°C and then draining them and cooling at room temperature. The fresh Indian bean tempeh's was prepared by dividing into two treatments Tempeh with 100per cent Indian beans (T_1) and tempeh with Indian beans and ground nut in ratio of 75:25per cent (T_2.)(Fig .1). Groundnuts was soaked for six hours and autoclaving them for 15 min at 121°C and then draining them and cooling at room temperature. After these procedures, beans were placed in two packaging material *viz.* ,polyethylene bags, banana leaves and inoculated with 20 g of the inoculum previously produced with rice flour and strain of *Rhizopus oligosporus*. The bags were sealed and small holes were made with a fork to allow the contact of the fungus with oxygen. Finally, beans were incubated in an oven at 30°C and visually monitored to follow up on the development of the mycelium (about 30 h)

The treatment T_3 is the dried form of fresh tempeh (T_2) that is done by microwave drying and reduce the moisture content to 25 per cent thereby increasing the shelf life of the produce to two weeks. Using fresh T_1 tempeh, two recipe product were prepared *viz.,* tempeh gravy (T_4) and tempeh fry (T_5)

The flow chart for the preparation of functional tempeh is as follows

Sorted fresh Indian legume (75per cent) + Soak groundnut for 6 hrs (25 per cent)

↓

Wash thoroughly and rinse with water

↓

Autoclaving them for 15 min at 121 °C

↓

Beans were dried on paper towels at room temperature

↓

Mix the cooked pulses with 1 per cent tempeh inoculum

↓

Pack pulses in HDPE bags with holes of 1mm size,

↓

Seal, flattened and keep it on a tray

↓

Incubated in oven at 30°Cfor 30 hrs

↓

Tempe

Figure 1.Preparation of Tempeh

Proximate Nutrient composition:

Dolichos lablab tempeh samples were dehydrated for 48 hrs until the material was completely dehydrated. The nutritional characterization was performed by official methods according to the AOAC (2007) in raw beans, cooked bean, fresh, dried tempeh and products prepared using tempeh. The moisture content was determined by oven-drying at 105°C until constant weight, the lipid content was determined by continuous extraction in a Soxhlet apparatus, the protein content was obtained by the micro-kjeldahl method, the total crude fiber, dietary fiber, carbohydrate, phytic acid and Tannin were estimated as per Sadasivam and Manickam (2008)

Tempeh fry and Tempeh gravy Preparation

For the sensory analysis, recipes of tempeh fry and tempeh gravy were chosen, because these are popular among the surveyed public. The same ingredients and amounts were used in the formulation of *tempeh fry and compared with control sample soyabean tempeh fry*. Another product prepared was tempeh gravy and compared with mutton gravy. The same ingredients of mutton gravy and amounts were used in the formulation of tempeh gravy.

Sensory evaluation of Tempeh fry and Tempeh gravy

Tempeh recipes were evaluated for sensory properties by 50 non trained participants for their appearance, aroma, flavor and overall impression using a nine-point hedonic scale. The tasters were also asked about their purchase intent. The cost analysis was also done.

Statistical analysis:

All the analytical measurements were carried out in three replicates and results were expressed in mean values of standard deviation. The collected analytical data was analyzed by using AGRES Software statistical tool.

RESULTS AND DISCUSSION

Proximate composition:

The proximate nutritional content was analyzed for the tempeh's prepared using T_0- Soya bean tempeh; T_1-Tempeh *Dolichos lablab* (100per cent) T_2: Tempeh (75 % *Doilchos and 25% Arachis hypogea*), T_3: Tempeh dry (T2 in dried form). T_4 Product – Tempeh gravy, T_5 Product tempeh fry and the results are presented in Table 1.

The moisture content of tempeh ranged from 37 to 50 per cent in different treatment and in the dehydrated tempeh, moisture was 24.5 per cent. On comparing soybean and Indian bean tempeh, tempeh prepared using *Dolichos lablab* (T_1) had higher moisture content (50.03 per cent), This could be due to use of Indian legume in fresh form and soybean in dried form. Erkan *et al* (2019) also observed similar results that the broad bean tempeh had maximum moisture content (63.10per cent) while minimum moisture content (44.88 per cent) was seen in chickpea tempeh.

The results revealed that there was wide difference in nutrient content of T_1 and T_2 samples *i.e.*,100 per cent *Dolichos lablab* tempeh and *Dolichos lablab* and groundnut (75:25) tempeh. There was an increase in the protein and fat content by 15.47 per cent and 453 per cent respectively. By just adding 25% groundnut the protein content of tempeh (T_1 and T_2) has increased from 16.38 to 19.38. Wheras the fresh bean had 16.0 per cent protein only. Similar results were also reported by Astuti *et al.* (2000) in soybean *tempeh* and soybean. The increase in protein content could be due to the action of the protease enzyme produced by the fungus during fermentation, the soluble protein content increases markedly. According to Catur 2021 the protein value of the white bean *tempeh* does not increase significantly after fermentation. Vital *et al. (*2018) also reported similar results.

Maximum protein content was obtained in dried form of tempeh when compared to fresh Indian legume. The protein content of tempeh has increased to 41.24 per cent in dried tempeh and in

the product using fresh tempeh the protein content has increased by 16.67 and 5.09 per cent in tempeh gravy and tempeh fry respectively. This increase might be due to the addition of protein rich ingredients *viz.,* cashew, coconut in the recipe preparation. There was an increase in the fat content by 28 per cent in tempeh gravy and 36 per cent in tempeh fry.

On comparing raw pulse and fresh tempeh there was an increase in the calcium and dietary fibre by 68 per cent and 19 per cent respectively. The calcium content increased to 44 per cent in dried tempeh. In tempeh fry and gravy because of various processing there was complete removal of phytic acid and tannin content as it undergoes the process of fermentation, boiling *etc.,* as quoted by Kamalasundari *et al*. (2019).

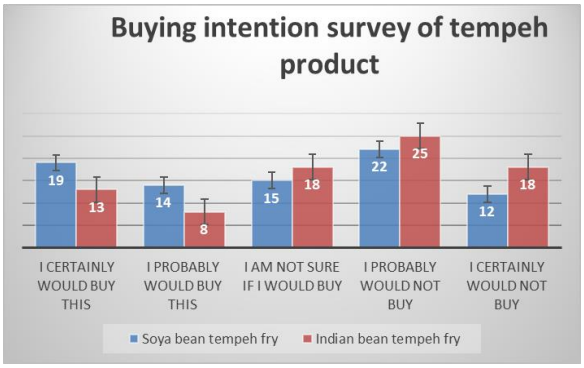

Figure 2 Buying intention of tempeh product

Tempeh prepared using soyabean

Dolichous lablab tempeh

Tempeh fry

Tempeh gravy compared with mutton gravy

Table: 1 Nutritional analysis in different tempeh and in tempeh preparation g/100g

S.No	Treatment	Moisture g per cent	Fat g per cent	Protein g per cent	Crude fibre g per cent	Dietary fibre g per cent	CHO g per cent	Calcium mg	Phytic acid mg	Tannin mg
1.	T_0	37.00	14.23	27.21	3.38	2.463	14.30	67.00	54.50	60.8
2.	T_1	50.03	0.81	16.37	4.01	2.533	24.47	58.13	4.50	3.8
3.	T_2	50.03	4.48	19.37	2.11	3.000	20.13	69.33	6.80	0.56
4.	T_3	24.47	6.22	32.97	3.02	3.560	27.67	125.00	-	-
5.	T_4	35.36	6.25	22.60	2.97	2.033	28.83	110.27	-	-
6.	T_5	37.02	7.00	20.41	4.26	4.410	24.43	103.27	-	-
	Sed	0.507	0.018	0.019	0.024	0.0267	0.375	0.372		
	CD (P=0.05)	0.890	0.032	0.034	0.042	0.0471	0.375	0.656		

T_0- Soya bean tempeh; T_1 Tempeh (100per cent) *Dolichos lablab* T_2: Tempeh fresh (*Doilchos lablab*-75 per cen and Arachis hypogea -25per cent), T_3: Tempeh dry (75:25), T_4Product – Tempeh gravy, T_5Product tempeh fry

Table 2: Organoleptic Evaluation of Tempeh products with control samples

Parameters	*Mutton gravy (Control)*	*Indian bean Tempeh gravy*	*Soyabean 65 Control*	*Tempeh 65 Indian bean*
Appearance and colour	8.5	8.0	9.0	9.0
Flavour	9.0	7.5	8.0	7.5
Texture	8.5	8.0	8.5	8.5
Taste	9.0	7.5	8.5	8.0
Overall acceptability	8.5	7.5	8.5	8.0

(9—I liked very much, 8—I liked it a lot, 7—I liked it moderately, 6—I liked it slightly, 5—I did not like it or disliked, 4—I disliked it slightly, 3—I Disliked it moderately, 2—I did not like it much and 1—I did not like it very much).

Conclusion

Tempeh prepared using Dolichos lablab is an innovative food; it has good nutritional value with a considerable amount of protein. Hence, it may be an alternative and eventually an option for non-vegetarian preparations. As compared to 100 per cent *Dolichos lablab* tempeh the combination of *Dolichos lablab and Arachis hypogea* tempeh in the ratio of 75:25 had better sensory scores and improved protein and fat content by 15.47per centand 453 per cent respectively. The calcium content increased by 44 per cent in dried tempeh and 36 per cent in the food product developed using tempeh. On comparision to fresh Indian bean there was an increase in the protein and fat by 44per cent and 28 per cent in dry tempeh. Further, there was a complete removal of phytic acid and tannin content in the tempeh recipes. The overall acceptability of *Dolichos lablab* tempeh fry was observed as highly acceptable with score point of 8.0 ± 0.5. Likewise the *Dolichos lablab* tempeh gravy has a score of 7.5± 0.5. The unit cost production of 200g of the functional *Dolichos lablab* tempeh packed with HDPE was Rs.50.00

Finally, it was concluded that value addition to Indian bean is possible with potential social acceptability and economic impacts. It gives nutritionists the opportunity to explore the versatility of common beans in gastronomy.

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Originality and plagiarism, Consent for publication,

All the authors agreed to publish the content

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There were no conflict of interest in the publication of this content

Data availability:

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail;.

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