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RESEARCH ARTICLE

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Standarisation, acceptability and digestibility of tempeh with cowpea and greengram

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ABSTRACT

A variety of indigenous fermented foods exist today, however, tempeh has been one of the most widely accepted and researched mold-modified fermented products. Tempeh is a traditional fermented food made from soaked and cooked soybean inoculated with a mould, *Rhizopus oligosporus*. After fermentation, the soybean is bound together into a compact cake by dense cottony mycelium. In this study, tempeh was prepared using different types of legumes such as soybean, greengram and cowpea in different proportions. The acceptability study of the tempeh was done by preparing chips and roast and it was found that all the treatments were highly acceptable. *In vitro,* starch and protein digestibility scores of the different treatments were also found to be high. Tempeh made with 100 % greengram gave the best result with the highest acceptability and digestibility scores.

Key words : Tempeh, Fermentation, Pulses, *In vitro* Starch and protein digestibilities

INTRODUCTION

Pulses are important foodstuffs in the dietaries of populations in the tropics and subtropics. In the technically underdeveloped areas of the tropics and subtropics where more than half of the world's population is concentrated, the scarcity of animal foods makes the use of comparatively low cost protein rich legumes to balance their diet (Schneider, 2002). In legumes, presence of antinutritional factors is a main drawback limiting their use.

Wide range of processing techniques could improve the protein and starch digestibilities of legumes(Alonso *et al* ., 1998). However, it is known that certain treatments, such as heat processing, could produce, in some conditions, physico-chemical changes in proteins, starch and in the other components of legume seeds affecting their final nutritional properties (Della, 1994). Fermentation is one of the household food technologies reviewed extensively as means by which the nutritive value of plant foods could be improved (Obadina *et al*., 2008). According to Tabera *et al.* (1995), the fermentation is associated with many chemical changes that enhance organoleptic response, contents of free sugars and vitamins, as well as digestibility. Generally, a significant increase in the soluble fraction of a food is observed during the fermentation and is known to improve nutrition, palatability and digestibility (Lin, 2007). Fermentation also preserves foods in a wide variety of flavours, aroma and texture.

Indigenous fermented foods were known before the recorded history but only recently, the world has taken a closer look to it as these are not only low cost and nutritious, but survived for centuries and time tested to be safe and wholesome. Tempeh or *tempe* in Indonesia, is made by a natural culturing and controlled fermentation process that binds soybean into a cake form (Hachmeister and Fung, 1993). It forms an important part of the diet of many poor people and supplies much of their protein (Djien and Hesseltine, 1990). Tempeh is one of the fastest growing categories in the Western food industry even as dairy to meat alternatives (Golbitz, 2000). Tempeh is very nutritious and serves as one of the best plant protein source containing over 40% protein. In Indian situation where a variety of pulses are used, tempeh could be prepared with pulses other than soybean thereby increasing the acceptability

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and digestibility of the common pulses. Hence, the present study is aimed to standardise tempeh with the pulses like soybean, greengram and cowpea in different combinations and proportions, to find out their *in vitro* starch and protein digestibilities and also to asses the acceptability of the tempeh.

MATERIALS AND METHODS

In the present study, tempeh was prepared using soybean, greengram and cowpea in different combinations. The selected legumes were purchased from the open market. The following treatments were tried.

T_1 - Soybean (100%) -control
T_2 - Greengram (100%)
T_3 - Cowpea (100%)
T_4 - Greengram (50%) + Soybean (50%)
T_5 - Cowpea (50%) +Soybean (50%).

Pure culture of *Rhizopus oligosporus* was obtained from Institute of Microbial Technology (IMTECH), Chandigrah. This was subcultured on 3% Malt agar medium. Starter culture was prepared by the modified procedure of Jurus and Sundberg (1976). To the sub cultured Malt agar medium, added 10ml of distilled water and vortexed for 3 minutes. The spore suspension thus obtained was further used for inoculation of the soybeans. The soybean (1 kg) was cleaned, washed, and boiled for 30 minutes, just making it soft. This was again washed and soaked overnight (12 hours). The legumes were dehulled by floatation in water and were surface dried. This was mixed with vinegar to adjust the pH to 4.5 (100 ml of vinegar for 1 kg of soybean). To this pH adjusted soybean added the spore suspension (7 ml of spore suspension for 100 g of raw soybean) and mixed well. The inoculated soybean was packed in perforated polyethylene pouches by pressing them flat to a thickness of 3 cm and were sealed. Packed pouches were incubated at 32 ^{0}C for 48 hours. After 48 hours, thick firm cake of soybean tempeh was obtained. This soybean tempeh was used as the starter culture. Two gram of soybean tempeh was used for inoculating one kilogram of the substrate for tempeh preparation.

Selected legumes were cleaned, washed and boiled for 30 minutes, just making it soft. This was again washed and soaked overnight. The legumes were dehulled by floatation in water and were surface dried. This was then mixed with vinegar to adjust the pH to 4.5(100 ml of vinegar for 1 kg of substrate). This substrate (1 kg) was inoculated with 2 grams of tempeh starter. This was then packed in perforated polythene pouches by pressing them flat to a thickness of 3 cm and was sealed. Packed pouches were incubated at 32^{0}C-36^{0}C for 48 hours, during which the tempeh fermentation took place. After fermentation , the pulses were bound together into a compact cake by dense cottony mycelium.

Acceptability of fresh tempeh were assessed by preparing tempeh chips and tempeh roast. Fresh tempeh was cut to thin uniform slices and fried in coconut oil with the addition of salt. Method for the preparation of meat roast was followed for the preparation of tempeh roast using fresh tempeh by replacing meat. Sensory evaluation of the tempeh chips and roast were carried out using score cards based on a nine point hedonic scale by a panel of 10 selected judges. The quality attributes namely, appearance, colour, flavour, texture, taste, and overall acceptability were evaluated.

In vitro starch digestibility (IVSD) was estimated as suggested by Satterlee *et al.* (1979). One gram of the sample in 100 ml water was boiled for one hour and filtered. One ml of the gelatinised solution was taken and one ml of the enzyme solution (saliva diluted with equal quantity of water) was added. The mixture was incubated at 37^0 C for 1- 2 hours. The reaction was stopped by adding 1ml of sodium hydroxide. Later, glucose was estimated by the method of Somoygi (1952).The method proposed by Sadasivam and Manikam (1992) was used to determine *in vitro* protein digestibility (IVPD). A multi-enzyme system, consisting of a mixture of porcine pancreatic trypsin type IX, bovine pancreatic chymotrypsin type II and porcine intestinal peptidase grade III, was used. Tempeh and distilled water were used to prepare 50 ml of an aqueous protein suspension (6.25 g protein/l) with pH adjusted to 8.0, while stirring in a water bath at 37^{0}C. The multi-enzyme solution was maintained in an ice bath. Five ml aliquots of the multi-enzyme solution were added with stirring to the protein suspension at 37^{0}C. The rapid pH drop was recorded automatically over a 10 minutes period using a pH meter. IVPD of the tempeh was calculated from the equation IVPD = 210.46 - 18.10X, where X = pH after 10 minute.

The scores obtained for the organoleptic evaluation were analysed statistically using Kendall's coefficient of concordance so as to assess the degree of agreement among the judges. The results of IVSD and IVPD statistically analysed using one way ANOVA.

RESULTS AND DISCUSSION

The fresh tempeh made with soybean, greengram and cowpea in different combinations fermented in perforated polyethylene bags were filled completely with white mycelium of the fungus, *Rhizopus oligisporus* and the entire contents could be lifted out as a whole piece. Thus, the fresh tempeh was a firm white cake with a very

good nutty flavour. According to Hachmeister and Fung (1993), in good tempeh, the beans are knitted together by a mat of white mycelia. Babu *et al*. (2009) also reported that fermentation of soybean, resulted in a firm textured product with a somewhat nutty flavour and a texture similar to a chewy mushroom.

Fresh tempeh obtained was cut into thin slices and cubes and were used in making different preparations like tempeh chips and tempeh roast, respectively. The results of the acceptability trials with fresh tempeh chips are given in Table 1. It is evident from the table that the tempeh chips made from all the treatments were highly acceptable in all sensory characteristics. Among the treatments, mean scores for appearance, colour, flavour , texture and taste were found to be significantly high in T_2 (100% greengram) which was reflected in its high overall acceptability score (8.9). This was followed by T_5 (8.5) where the tempeh was prepared with 50% cowpea and 50% soybean. The mean scores for the sensory evaluation of tempeh roast are given in Table 2. From the table we can see that the tempeh roasts were highly acceptable. Significant difference was observed among the treatments with respect to various sensory qualities and highest overall acceptability was for T_2 (100% greengram) with a score of 8.7. The organoleptic evaluation results are in accordance with the finding of Vaidehi *et al*. (1985) who had also reported that the tempeh products like chips and curries showed a high percentage (90%) of acceptability.

IVSD and IVPD of the developed tempeh is given in Table 3. *In vitro* starch digestibilities of the different treatments were found to be significantly high compared to the control (T_1-100% soybean). There was also significant variation in IVSD of different treatments. The IVSD values ranged from 69.18% (T_1) to 80.32% (T_2).The IVSD of treatments T_2 and T_4 were included in the same group (a) and showed no significant difference. The high starch digestibility of fermented products may be related to enzymatic properties of microbes, which ferment the substrate. The fermenting microflora brings about the breakdown of starch to oligosaccharides. The enzymes produced by the fermenting microflora also bring about the cleavage of amylose and amylopectin to maltose and glucose (Sindhu and Khetarpaul, 2002).

The IVPD of different treatments were high and were found to be high ranging from 72.03 to 88.57 % . The highest IVPD was for the treatment T_2 (88.57%) and lowest for the control (72.03%). No significant variation in the IVPD was observed between the treatments T_3 and T_4. Similar results were obtained in a study conducted in tempeh flour by Bejarano *et al*.(2008) and according to them, the solid state fermentation process like tempeh fermentation improved the protein digestibilities

Table 1 : Mean scores for sensory qualities of tempeh chips

Treatments	Mean scores					
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
T_1 - Control	8.9 (3.90)	8.9 (3.95)	7.8 (2.45)	8.4 (2.95)	7.4 (1.75)	8.3 (3.05)
T_2	9.0 (4.10)	9.0 (4.15)	9.0 (4.60)	8.9 (4.15)	8.8 (4.35)	8.9 (5.00)
T_3	8.1 (2.30)	7.8 (1.65)	8.7 (4.10)	7.3 (1.30)	8.5 (3.90)	8.1 (2.20)
T_4	7.5 (1.30)	7.8 (1.75)	7.4 (1.75)	8.2 (2.65)	7.6 (2.15)	7.6 (1.15)
T_5	8.7 (3.40)	8.7 (3.50)	7.6 (2.10)	8.8 (3.95)	8.0 (2.85)	8.5 (3.60)
Kendall's (W) value	0.751**	0.766**	0.763**	0.655**	0.633**	0.860**

Figures in parenthesis are mean rank scores
** indicates significance of value at P=0.01

Table 2 : Mean scores for sensory qualities attributes of tempeh roast

Treatments	Mean scores					
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
T_1 - Control	9.0 (3.65)	9.0 (3.70)	7.1 (1.65)	8.6 (3.25)	7.5 (2.35)	8.3 (2.85)
T_2	9.0 (3.65)	8.8 (3.20)	8.7 (4.00)	8.7 (3.40)	8.3 (3.65)	8.7 (4.25)
T_3	8.9 (3.40)	8.8 (3.20)	9.0 (4.65)	7.3 (1.10)	8.8 (4.60)	8.2 (3.20)
T_4	8.3 (1.90)	8.6 (2.70)	7.7 (2.45)	9.0 (4.05)	7.6 (2.35)	8.3 (2.80)
T_5	8.5 (2.40)	8.4 (2.20)	7.6 (2.25)	8.6 (3.20)	7.4 (2.05)	8.1 (1.90)
Kendall's (W) value	0.515**	0.226*	0.811**	0.672**	0.599**	0.309*

Figures in parenthesis are mean rank scores
* and ** indicates significance of value at P=0.05 and 0.01

Table 3 : IVSD and IVPD (%) of different tempeh		
Treatments	IVSD (%)	IVPD (%)
T_1 - (Control)	69.18[d]	72.03[c]
T_2	80.32[a]	88.57[a]
T_3	72.43[c]	77.33[b]
T_4	80.78[a]	78.04[b]
T_5	78.32[b]	72.31[c]

Means with same super scripts form a homogenous group

and in their study, the protein digestibility ranged between 72.2% to 83.7%. The improvement in IVPD caused by fermentation could be attributed to the partial degradation of complex storage proteins to more simple and soluble products and it could also be attributed to the degradation of tannins, polyphenols and phytic acid by microbial enzymes (Jood and Khetarpual, 2005).

Conclusion:

It is concluded that the indigenous fermented food tempeh can be prepared with different legumes and in this study the tempeh made with 100 % greengram was the best treatment with regard to acceptability and digestibility of starch and protein. The digestibility of starch and protein were found to be high in different treatments and by using commonly available cereals and legumes in different combinations we can prepare different types of tempeh which can solve the problems of under nutrition and malnutrition.

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