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Rice and green gram based 'Tempeh'- Development and *in vitro* mineral bioavailability

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Abstract
Fermentation is one of the household food technologies by which the nutritive value of foods could be improved. 'Tempeh' is one of the most widely accepted and researched mould modified fermented product. Tempeh is traditionally made with soybeans only. In Indian situation where a variety of cereals and pulses are used, tempeh could be prepared with these. Hence, in the present study, tempeh types were standardised using different combinations of rice and green gram and their mineral content and *in vitro* mineral bioavailability was assessed. Tempeh developed with 100 per cent green gram had the best texture and firmness and was comparable with the control (100 % soybean). The tempeh types showed a reduction in the total calcium, iron, phosphorus, potassium and zinc when compared to the mineral content in the raw grains used for tempeh preparation. Mineral availability studies revealed that the availability of minerals from T_1 (soybean 100%) tempeh was the lowest and the highest availability of minerals was from T_4 (green gram 50% + rice 50%) tempeh. The mineral bioavailability ranged from 49 to 88.99 per cent among the treatments. The availability of zinc was found to be the highest among the minerals studied.

Key words: Tempeh, Fermentation, Rice, Green gram, Soybean, Calcium, Iron, Phosphorus, Potassium, Zinc, *In vitro* mineral bioavailability.

Introduction

Fermented foods, whether from plant or animal origin, are an intricate part of the diet of people in all parts of the world. Indigenous fermented foods were known before recorded history, but only recently the world has taken a closer look at them as these are not only low cost and nutritious, but survived for centuries and were time tested to be safe and wholesome. Fermented foods play an important socioeconomic role in developing countries as well as in making a major contribution to the protein requirements of the population. During last few years, much interest has been generated in the fermented foods of the Orient, where such foods are still being manufactured at cottage industry scale by means of natural micro flora from the staples and surrounds (Devi, 2004). Tempeh is a solid fermented soybean product that is consumed widely in Indonesia. Tempeh is the name ordinarily used for soybean fermented product. In recent years, there has been considerable interest in the West in popularising tempeh as an alternative protein source.

Cereals and legumes are one of the richest and least expensive sources of carbohydrates and proteins in the human diet and contribute substantially to the diets of a large part of the Indian population. The supplementation of cereals with high protein legumes is considered to be one of the best solutions to protein calorie malnutrition, particularly in the developing countries. However, the presence of anti-nutrients such as protease inhibitors and phytates reduces the digestibility or bioavailability of proteins and minerals in legumes

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(Gibson et al., 2006), and the presence of flatulence producing compounds, particularly in beans, may cause adverse effects in the gastrointestinal tract. In Indian situation where a variety of pulses, cereals and millets are used, tempeh could be prepared with pulses and cereals other than soybeans thereby increasing the bioavailability of nutrients of the common pulses and cereals. Tempeh preparation does not require any special skills. Because of its high nutritional value and acceptability, tempeh and its second generation products will have an impact on the consumers. Hence in the present study, tempeh was developed using rice and green gram and mineral content and the *in vitro* availability of minerals in the fresh tempeh was assessed.

Materials and Methods

Collection of raw materials

Tempeh was prepared with rice, green gram and soybean. The cereals and legumes were purchased in one lot from the local market. Tempeh is an Indonesian fermented food consisting of soybeans partially digested and bound together by mycelium of *Rhizopus*. *Rhizopus oligosporus* is the most frequently isolated organism from natural tempeh samples (Jurus and Sundberg, 1976). Hence, a pure culture of *Rhizopus oligosporus* was used for tempeh fermentation. Pure culture of *Rhizopus oligosporus* – MTCC 556, was obtained from Institute of Microbial Technology (IMTECH), Chandigarh.

Standardisation of tempeh with rice and green gram

Tempeh fermentation was carried out with pure cultures of *Rhizopus oligosporus*. The standard procedure adopted by Jurus and Sundberg (1976) was followed for preparing tempeh with soybeans as control, with green gram and also with the green gram in combination with different proportions of rice.

Preparation of starter culture

Pure cultures of *Rhizopus oligosporus* – MTCC 556 obtained from IMTECH was sub cultured on three per cent malt agar medium. Starter culture was prepared by the modified procedure of Jurus and Sundberg (1976). To the sub cultured malt agar medium, added 10 ml of distilled water and vortexed for 3 minutes. The spore suspension thus obtained was further used for inoculation of the soybeans. The soybeans (1 kg) were cleaned, washed, and boiled for 30 minutes, until they were just 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 1Kg of soybean). To this pH adjusted soybeans, added the spore suspension (7 ml of spore suspension for 100 g of soybean) and mixed well. The inoculated soybeans were packed in perforated polyethylene pouches by pressing them flat to a thickness of three centimetres and were sealed. Packed pouches were incubated at 32^{o} 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 grams of fresh soybean tempeh was used for inoculating one kilogram of the substrate for tempeh preparation.

Development of rice and green gram tempeh

Tempeh was prepared with rice and green gram in different combinations. The treatments T_1 (soybean 100% - control), T_2 (green gram 100%), T_3 (green gram 75% + rice 25%) and T_4 (green gram 50% + rice 50%) were assessed.

Green gram was cleaned, washed, and boiled for 30 minutes, until it was just soft. This was again washed and soaked overnight (12 hours). It was then dehulled by floatation in water and was surface dried. In treatments with rice, raw rice was washed and boiled for 5 minutes, drained, surface dried and mixed with the treated surface dried legumes. The substrates were 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 two grams of tempeh starter (prepared as detailed above) and mixed well. This was then packed in perforated polythene pouches by pressing them flat to a thickness of three centimetres and was sealed. Packed pouches were incubated at 32^0C-36^0C for 48 hours, during which the tempeh fermentation took place, after which the mycelium appeared to be more or less uniformly distributed throughout to form a firm cake with a good flavour.

Analysis of mineral content in fresh tempeh

Minerals such as calcium, iron, phosphorus, potassium and zinc were assessed in fresh tempeh types. The calcium and iron content were estimated by atomic absorption spectrophotometric method using the diacid extract prepared from the sample (Perkin-Elmer, 1982). The diacid extract was made up to 100 ml and 10 ml of the made up solution was again diluted to 50 ml. This solution was read directly in atomic absorption spectrophotometer and calcium and iron content was expressed in mg per 100g. The phosphorus content was analyzed colorimetrically as suggested by Jackson (1973). To five ml of pre-digested aliquot, five ml of nitric acid vanadate molybdate reagent was added and made up to 50 ml with distilled water. After 10 minutes, the OD was read at 420 nm. A standard graph was prepared using serial dilution of standard phosphorus solution. The phosphorus content of the sample was estimated from the standard graph and expressed in mg per 100 g. The method suggested by Jackson (1973) was followed for the estimation of potassium using a flame photometer. One gram of the digested solution was made up to 25 ml and read directly in a flame photometer. The potassium content was expressed in mg 100 g^{-1} of the sample. The zinc content was estimated using atomic absorption spectrophotometer as suggested by Perkin-Elmer (1982). The diacid extract was made up to 100 ml and was read directly in an atomic absorption spectrophotometer and zinc content was expressed in mg per 100 g.

In vitro availability of minerals from selected fresh tempeh types

HCl extractability of minerals in foods is an index of their bioavailability from foods (Chompreeda and Fields, 1984). Thus the solubility of minerals in foods, subject to *in vitro* gastric intestinal digestion is a useful indicator of mineral bioavailability.

In vitro availability of calcium, iron, phosphorus, potassium, and zinc of the selected tempeh types were assessed. For this the HCl extractability of the minerals were analysed. The samples were extracted with 0.03 N Hydrochloric acid by shaking the contents at 37^0 C for three hours. The clear extract obtained after filtration with Whatman No. 42 filter paper was oven dried at 100^0 C and wet acid digested. The amount of the HCl extractable calcium, phosphorus, iron, potassium and zinc in the digested samples were determined by the methods as described above for the estimation of total minerals. HCl extractability of each mineral was derived by using the following formula (Duhan et al., 2001).

Mineral extractability, % =

$$\frac{\text{Mineral extractability in 0.03N HCl}}{\text{Total minerals}} \times 100$$

Results and discussion

Development of tempeh with rice and green gram

The tempeh prepared with 100 per cent soybeans (T_1) was found to be a thick firm cake with the white cottony mycelium distributed uniformly. The treatments with 100% green gram had a comparable firmness with the control T_1 and all the other treatments had less dense mycelium than the control. In treatments with rice (T_3 and T_4), the firmness was less when compared to control and T_2, but the tempeh could be easily sliced without any crumbling. Similar studies conducted by Srapinkornburee et al. (2009) by using red kidney bean as substrate for tempeh production revealed that, tempeh after 48 h fermentation looked much like soybean tempeh and could be easily sliced because of the firm texture.

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 flavor and a texture similar to a chewy mushroom. The growth of mycelium was highest in tempeh prepared with 100 per cent soybean (control) and binding of cotyledons with the fungal hyphae was found to be less all other treatments. This may be due to the fact that the process of dehulling during the preparation of tempeh was very effective in soybeans whereas in green gram some hulls still remained after the process. The fungal mycelium was found to adhere firmly to the dehulled cotyledons. All the treatments had good nutty flavor which is a characteristic property of a good tempeh. Hachmeister and Fung (1993) developed several tempeh like products with wheat, triticale, yellow sorghum and red sorghum and revealed that the type of grain as well as the strain of *Rhizopus oligosporus* used influenced the product's appearance and flavour. In their study, they found that red sorghum yielded a product with good texture, aroma and appearance, yellow sorghum and triticale were found to be unacceptable substrates for tempeh production, and wheat though it produced a product with a desirable aroma and flavour, crumbled when sliced because of the less firm texture, as observed in the present study also in tempeh types with wheat.

Analysis of mineral content in fresh tempeh

In the present study, the calcium content (Table 1) of the selected fresh tempeh types ranged from 40.37 to 149.10 mg $100g^{-1}$ with the lowest calcium content in T_4 (green gram 50% + rice 50%) tempeh and highest in T_1 (100% soybean) tempeh. Calcium content was significantly high in T_1 when compared to other tempeh types. Vaidehi (1993) also reported a calcium content of 142 mg $100g^{-1}$ in fresh soybean tempeh. According to Tee et al. (1997) tempeh contains about 129 mg calcium 100 g^{-1} and in Malaysian diet, tempeh is also one of the calcium rich foods besides milk and dairy products. Studies conducted by Haron et al. (2008) showed calcium content of 56.8 + 1.6 mg $100g^{-1}$ in tempeh. Babu et al. (2009) also stated that tempeh was as an excellent source of calcium. The significantly high calcium content observed in 100 per cent soybean tempeh may be due to the high calcium content observed in raw soybean when compared to other pulses.

The iron content (Table 1) was significantly higher in T_1 (soybean 100%) tempeh (3.6 mg 100 g^{-1}) and there was no significant variation in the iron content of other tempeh types which ranged from 1.53 to 1.9 mg $100g^{-1}$. The iron content in T_1 in the present study was found to be higher than iron content of 1.5 mg $100g^{-1}$ in soybean tempeh reported by Vaidehi (1993).

The phosphorus content (Table 1) was also significantly higher in 100 per cent soybean tempeh (270.61 mg $100g^{-1}$) and significantly lower in T_4 (green gram 50% + rice 50%) tempeh (158.52 mg $100g^{-1}$). The results are in accordance with the findings of Shurtleff and Aoyagi (2001) who reported a phosphorus content of 240 mg $100g^{-1}$ in fresh soybean tempeh.

Table1. Calcium, iron, phosphorus, potassium and zinc in selected fresh tempeh types (100 g^{-1}).

Treatments	Calcium (mg)	Iron (mg)	Phosphorus (mg)	Potassium (mg)	Zinc (mg)
T_1	149.10^{a}	3.6^{a}	270.61^{a}	296.62^{d}	2.17^{a}
T_2	61.03^{b}	1.9^{b}	188.46^{b}	525.11^{a}	1.79^{b}
T_3	51.05^{bc}	1.75^{b}	185.25^{b}	419.99^{b}	1.60^{c}
T_4	40.37^{c}	1.53^{b}	158.52^{c}	330.12^{c}	1.34^{d}

DMRT column wise comparison
Values with same super script do not have significant difference
Values are mean of three independent determinations

The potassium content (Table 1) of the selected fresh tempeh types varied from 296.62 to 525.11 mg 100g^{-1} with the highest potassium content in T_2 (green gram 100%) and lowest in T_1 (soybean 100%). The lowest zinc content was found in T_4 (green gram 50% + rice 50%) tempeh (1.34mg 100g^{-1}) and the highest in T_1 (soybean 100%) tempeh (2.17 mg 100g^{-1}).

According to Kozlowska (1996), fermentation does not usually increase the level of minerals present in foods unless unusual circumstances are present (as in fermenting food in a metal or earthen container), but it decreases the activity of phytic acid naturally present in grains. In this study also, all the tempeh types showed a reduction in the total calcium, iron, phosphorus, potassium and zinc when compared to the mineral content in the raw grains used for tempeh preparation. This loss in mineral content can be attributed to soaking and dehulling of legumes in the process of tempeh preparation. Studies conducted by Appukuttan (2010) also revealed that soaking of green gram, bengal gram and horse gram for six hours and dehulling before cooking showed a considerable reduction in calcium, iron, phosphorus, potassium and zinc content.

In vitro availability of minerals from fresh tempeh types

Mineral availability of cereals and pulses can be improved by decreasing the antinutritional factors especially by hydrolysing the phytates. In the present study calcium availability of different tempeh types (Table 2) varied from 49.00 to 61.77 per cent, the highest being in T_4 (green gram 50% + rice 50%) tempeh and the lowest in T_1 (soybean 100%) tempeh. Even though there was a reduction in the total calcium content in tempeh when compared to the raw ingredients, the bioavailability of calcium was found to be very high in different tempeh types. Appukuttan (2010) had reported that in green gram soaked for 12 hours and dehulled, calcium availability was 24.32 per cent whereas in the present study, in T_2 (green gram 100%) tempeh, the calcium availability was found to be 56.63 per cent. The comparatively low calcium availability in T_1 (soybean 100%) tempeh (49 %) may be due to the very high phytate content in raw soybeans.

The availability of iron from different tempeh types was also found to be high, and ranged from 64.52 to 66.82 per cent without significant variation among themselves. Appukuttan (2010) had reported that in green gram soaked for 12 hours and dehulled, iron availability was 30.62 per cent whereas in T_2 (green gram 100%) tempeh, iron availability was 66.07 per cent, indicating the effect of tempeh fermentation in increasing the iron availability.

Phosphorus availability from different tempeh types varied from 49.68 to 65.72 per cent with T_1 (soybean 100%) tempeh having significantly lower phosphorus availability, compared to other treatments. Statistically there was no significant variation in the phosphorus availability of the treatments T_2, T_3 and T_4. The study by Appukuttan (2010) revealed that, in green gram soaked for 12 hours and dehulled, phosphorus availability was

Table 2. In vitro availability of calcium, iron, phosphorous, potassium and zinc from fresh tempeh types.

Treatments	Calcium (%)	Iron(%)	Phosphorus (%)	Potassium(%)	Zinc(%)
T_1	49.00^c	64.52^a	49.68^b	46.30^d	82.23^a
T_2	56.63^b	66.07^a	61.49^a	54.15^c	86.17^a
T_3	60.70^a	64.74^a	62.15^a	60.36^b	87.17^a
T_4	61.77^a	66.82^a	65.72^a	63.60^a	88.99^a

DMRT column wise comparison

Values with same super script do not have significant difference

Values are mean of three independent determinations

48.25 per cent whereas in 100 per cent green gram tempeh (T_2), the phosphorus availability was 61.49 per cent. The comparatively low phosphorus availability in T_1 (soybean 100%) tempeh may be due to the high phytate content in soybean.

Potassium availability was found to be 46.30 to 63.60 per cent in different tempeh types, the highest being in T_4 (green gram 50% + rice 50%) tempeh. Significant variation was observed among all the treatments with regard to their *in vitro* availability of potassium. Potassium availability in green gram soaked for 12 hours and dehulled was 45.69 per cent as reported by Appukuttan (2010), but after tempeh fermentation , the potassium availability was 54.15 per cent in T_2 (green gram 100%) tempeh.

The availability of zinc was found to be the highest among the minerals studied. Zinc availability varied from 82.23 to 88.99 per cent without significant variation with the tempeh types. Zinc availability reported by Appukuttan (2010) was 62.78 per cent in soaked and dehulled green gram, but in T_2 (100% green gram) tempeh, zinc availability was 86.17 per cent.

Mineral availability studies revealed that the availability of minerals from T_1 (soybean 100%) tempeh was the lowest and the highest availability of minerals was from T_4 (green gram 50% + rice 50%) tempeh. The high availability of minerals as revealed in the present study was supported by Sudermadji and Suparmo (1997) who also found a high bioavailability of elements such as zinc, iron, manganese, calcium and phosphorus in tempeh. Astuti et al. (2000) also revealed that fermentation process of tempeh decreased the phytic acid and enhanced the bioavailability of minerals such as calcium, zinc and iron. Babu et al. (2009) reported that fermentation neutralizes the phytate acid present in the soybeans; therefore tempeh does not restrict the body's absorption of minerals. Jood and Khetarpaul (2005) found that reduction in antinutrients due to fermentation may increase the bioavailability of various minerals, but there need not be an increase in the total mineral content in fermented foods. This is in line with the results of the present study which showed a reduction in the total mineral content in all the tempeh types mainly because of soaking and dehulling process in tempeh preparation.

Thus the results of the present investigation clearly brought out that, besides 100 per cent soybean tempeh (T_1) which was used as the control, T_2 (100% green gram), T_3 (green gram 75% + rice 25%), and T_4 (green gram 50% + rice 50%) tempeh types had proved to be efficient for the preparation of tempeh. All the treatments had good mineral content and mineral bioavailability.

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