

# The Nutritive Quality of Bengal Gram Tempe

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## Abstract

A study was undertaken on Bengal gram tempe prepared from Bengal gram by fermentation with *Rhizopus oligosporous* to observe the changes of nutritive values. Fermentation process changes the nutritional value of the finished product (tempe) by increasing the protein (8.4%) and fat (8.5%) and decreasing the fiber (32.2%). It decreased the phytate and tannin contents by 52 and 60 percent respectively. Zinc and copper contents of Bengal gram tempe increased by 43 and 19 percent respectively. Tempe contains an ample amount of unsaturated fatty acid and low in saturated fatty acid. The P/S ratio is higher in tempe than in the raw beans. The C<sub>18:2</sub> fatty acid increased by 22.87% while C<sub>18</sub> decreased by 83.66%. After fermentation the amino acid composition remained fairly constant. The Bengal gram tempe could be used for supplementary feeding.

**Keywords:** Nutritive Values, Tempe, Fermentation, *Rhizopus oligosporous*, Bengal Gram

## Introduction

Tempe is an Indonesian food generally made from soybean fermented by *Rhizopus oligosporous*. Fermentation is an indigenous processing technique, which transforms beans, grains and other substrates into highly digestible and palatable foods. Studies have confirmed that fermentation process enhances the nutritional value of the finished products by increasing the digestibility of the proteins, reducing the anti nutritive factors and enriching it with vitamins and minerals. Tempe possesses antimicrobial activity and has a hypocholesterolemic effect. Its supplementation in the diet of malnourished children has been found to be beneficial. It is also recommended as a nourishing food for pregnant and lactating mother<sup>1</sup>.

In developing countries plant proteins are cheaper and more accessible than animal proteins<sup>2</sup> and could be used to lower the protein energy malnutrition (PEM) rate and other dietetic problems, and improve the low nutritive quality of cereals and starch staple foods<sup>3</sup>. Grain legumes are rich and cheap source of dietary proteins in

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developing countries. Their utilization, however, is undermined by the presence of enzyme inhibitor, flatulence causing by oligosaccharides<sup>4</sup>, phytate causing reduced bioavailability of mineral<sup>5</sup>, toxic tannins, haemagglutins<sup>6</sup> and prolonged cooking time. Rhizopus fermentation of cereal legume mixture was reported to produce tempe with enhanced nutritive quality<sup>7</sup>.

In Indonesia tempe has been successfully used in community-based therapy for the management of diarrhoea and for PEM case<sup>8</sup> in synthesis of vitamin B<sub>12</sub> and might help in anemia management. Tempe can be incorporated in many foods causes no malabsorption syndrome and can be used by all age groups.

Despite a substantial increase in protein intake per capita per day in Bangladesh, protein energy malnutrition (PEM) and micronutrient deficiency are still major problem. The average mortality rate of 67 per thousand live births was reported to be associated with PEM and diseases, the level of severe PEM being 7 percent and moderate PEM 54 percent<sup>9</sup>.

The aim of the study was to prepare bengal gram tempe, to alleviate the nutritive quality of the bengal gram, which could be use to lower the nutrient deficiency of the vulnerable groups.

## **Materials and Methods**

### ***Raw materials and microorganisms***

Splitting Bengal gram (Chick pea, *Cicer arietinum*) was purchased from the local market in Dhaka, Bangladesh. Laru the rice grown mixed culture of *Rhizopus oligosporous* and *Rhizopus oryzae* (1:1) was obtained from the production unit of Research and Development Center for Applied Chemistry, Institute of Sciences, Bandung, Indonesia. Laru prepared in the laboratory of Institute of Nutrition and Food Science, Dhaka, Bangladesh was also used for tempe preparation .

### ***Preparation of Bengal gram tempe***

Tempe was prepared by the method developed by Slamet *et al.* (Personal communication).

The clean beans were washed, boiled and soaked in water for 2 hours. The pH was adjusted at 5.0-5.5 by adding 4% acetic acid. The beans were then washed and surface dried at room temperature. The beans were inoculated with 0.2% inoculum by dry weight of raw materials to obtain an acceptable tempe with respect to better

mold growth. Incubation was done at room temperature ( $23 \pm 2^{\circ}$  C) in 500 g polythene bags for 28-30 hours. Tempe was steamed, blanched for 5 minutes, dried in air oven at  $50-55^{\circ}$  C for 21 hours and pulverize through a 100 mesh laboratory mill. Samples of cooked uninoculated beans were treated simultaneously and referred as zero hours or control through out the experiment.

### **Analytical procedure.**

Raw, cooked unfermented and Bengal gram tempe (fermented) were mechanically grounded in a grinder and kept in plastic bags for analysis.

### ***Proximate analysis***

Moisture, ash, fiber and protein contents were determined accordingly to the method of AOAC<sup>10</sup>.

Fat was extracted accordingly to the modified method of Folch *et.al.*<sup>11</sup> The crude fat was determined gravimetrically AOAC<sup>10</sup>.

Carbohydrate was determined by difference [100- (moisture + protein + fat + ash + fiber).

Caloric value was calculated by multiplying the carbohydrate, protein and fat content with factor 4, 4, 9 respectively<sup>12</sup>. All analysis were done in triplicate.

### ***Determination of minerals***

About 5 g of sample was wet ashed using a mixture of 18 M sulfuric acid, 12 M perchloric acid and 16 M nitric acid (0.5 :1.0 :0.5 by vol).<sup>13</sup>. After proper dilution, the concentration of Fe, Zn, Cu, Ca and Mg were determined by atomic absorption while Na and K were determined by atomic emission.<sup>14</sup>. An appropriate dilution was done with 0.4% lanthanum (w/w) to overcome ionic interference during the estimation of Ca and Mg. Estimation of phosphorus was done colorimetrically using the method of Fiske and Subbarow<sup>15</sup>.

### ***Determination of phytate phosphorus***

The extraction and precipitation of phytic acid were accomplished according to the method of AOAC<sup>10</sup>. The amount of phytate phosphorus was calculated from the iron content. Iron content was estimated by atomic absorption spectrophotometer.

### ***Determination of tannin***

Tannin was estimated by the method of Burns<sup>16</sup> using Folin Denis reagent. The extraction was done by refluxing the sample for 5 hours with distilled water and filtered.

### ***Determination of fatty acid***

Total lipid was extracted from the samples according to the modified method of Folch *et al.*<sup>11</sup> The free fatty acids were extracted from total lipid and were methylated according to the method of Morrison and Smith<sup>17</sup> Finally a portion of it was injected into a gas liquid chromatogram (Pye Unicum GC 304, glass column, 1500 nm X 4mm); a 10% DEGS on 100-120 mesh diatonitc CAW was used. Nitrogen was used as carrier gas at flows of 32 ml/min. The standards were run parallely.

### ***Determination of amino acid composition***

The amino acid composition was determined by fully automated liquid chromatography (JIL- 300 TEOL LTD TOKYO) by following the modified method of Moore *et al.*<sup>18</sup>

## **Results and Discussion**

The proximate composition of raw Bengal gram and Bengal gram tempe is shown in table 1. Mold fermentation increased the protein and fat content of tempe slightly while it decreased the fiber content. Wang *et al.*<sup>7</sup> attributed the increase in protein content with the decrease of other constituent utilized by mold for growth. Fermentation by *R. oligosporus* increased the nitrogen content of corn gluten<sup>19</sup> while Matsuo<sup>20</sup> reported a decrease in fiber content of okara tempe. A decrease in the mineral content was also observed in the present study. This could be due to dehulling<sup>21</sup>, leaching on soaking and cooking. Akinrele *et al*<sup>22</sup> reported the decrease of iron in maize by lactic acid fermentation, and Grandjar<sup>23</sup> reported the decrease in iron content of tempe from winged beans. Zinc and copper contents increased slightly after fermentation of Bengal gram. The level of anti-nutritive factors in Bengal gram tempe is also summarized in Table 1. Fermentation of Bengal gram decreased the phytate by 52 %. Pre fermentation procedure reduced phytic acid and a further by mold fermentation. Khokhar and Chauhan<sup>24</sup>, reported the decrease in phytate on soaking and cooking. The activity on phytase enzyme during mold fermentation has been reported<sup>5</sup>. Similar findings showed that cow pea tempe had a 60 percent

reduction of original phytate content.<sup>25</sup> The reduction of tannin content from raw beans to tempe was found to be 60 %. Reddy *et al.*<sup>26</sup> reported the reduction in tannin content by 37.5% to 77.0% by discarding the cooking water of the bean. Dehulling<sup>20</sup> and soaking reduce the tannin content<sup>27</sup>. This study shows that the processing of food was effective to remove the tannin from the legume.

**Table 1. Proximate composition , mineral composition and antinutritive factors in raw Bengal gram and Bengal gram tempe**

<i>Proximate composition</i>	Bengal gram (g/100g)	Bengal gram tempe (g/100g)
<i>Moisture</i>	7.8	7.97
<i>Ash</i>	3.03	1.45
<i>Fat</i>	5.62	6.10
<i>Protein</i>	22.31	24.18
<i>Fiber</i>	1.18	0.80
<i>Carbohydrate*</i>	60.01	59.50
<i>Calorie (Kcal/ 100g dry wt.)</i>	380	289
<i>Minerals</i>	(mg/100g)	(mg/100g)
<i>Iron</i>	9.23	8.11
<i>Zinc</i>	2.25	3.22
<i>Copper</i>	0.67	0.80
<i>Calcium</i>	60.00	40.00
<i>Magnesium</i>	86.00	55.00
<i>Sodium</i>	88.00	15.00
<i>Potassium</i>	655.0	278.00
<i>Phosphorus</i>	391.0	328.00
<i>Antinutritive factors</i>	(mg/100g)	(mg/100g)
<i>Phytate</i>	211.00	101.00
<i>Tannin</i>	38.00	15.0

\*Carbohydrate = 100 -(Protein + fat + ash + moisture + fiber)

Note: Results are expressed as per 100 g dry wt. basis.

**Table 2. Percent composition of fatty acid of raw Bengal gram and Bengal gram tempe**

Fatty acid	Fatty acid content (%)	
	Bengal gram	Bengal gram tempe
C <sub>14</sub>	0.41	Trace
C <sub>16</sub>	15.02	16.36
C <sub>16:1</sub>	Trace	Trace
C <sub>18</sub>	9.73	1.59
C <sub>18:1</sub>	24.91	22.14
C <sub>18:2</sub>	47.50	57.89
C <sub>18:3</sub>	2.43	2.01

**Table 3. Percent composition of saturated, monounsaturated and polyunsaturated fatty acids and P/S ratio of Bengal gram and Bengal gram tempe**

Fatty acid	Fatty acid content (%)	
	Bengal gram	Bengal gram tempe
Total Saturated	25.15	17.95
Total Monounsaturated	24.91	22.14
Total Polyunsaturated	49.93	59.9
P/S Ratio	1.98	3.33

**Table 4. Percent composition of amino acid of Bengal gram and Bengal gram tempe**

Amino Acid	Amino acid content (%)	
	Bengal gram	Bengal gram tempe
Aspartic Acid	0.09	0.09
Threonine	4.54	4.35
Serine	6.53	5.98
Glutamic Acid	16.13	13.83
Glycine	7.75	7.04
Alanine	7.05	7.66
Cysteine	0.74	0.53
Valine *	5.63	5.56
Methionine.*	1.03	0.86
Ileucine.*	4.90	4.79
Leucine*	8.54	8.09
Tyrosine*	2.43	2.53
Phenylalanine*	5.38	5.24
Histidine*	2.44	2.34
Lysine*	6.80	5.94
Arginine*	8.43	6.13
Hydroxyproline.	0.12	0.99
Proline	1.42	13.02

\*Essential amino acid.

In this method tryptophan was destroyed during digestion, aspartic acid contains asparagine and glutamic acid contains glutamine (peak shows together). Peak of cysteine did not come (reason is unknown).

Table 2 summarizes the percent composition of fatty acid of raw Bengal gram and the tempe. The amount of C<sub>14</sub> in raw bengal gram was 0.41 percent and tempe had trace amount. C<sub>16</sub> content in both raw and tempe was almost same. Trace amount of C<sub>16:1</sub> was found in both beans and tempe. The amount of C<sub>18</sub> was higher in raw beans than in tempe, C<sub>18:1</sub> and C<sub>18:3</sub> were almost same in raw and tempe while C<sub>18:2</sub> was found higher in tempe than in the raw beans. Total saturated, unsaturated fatty acid and P/S ratio of Bengal gram and its tempe are shown in Table 3. Tempe was low in saturated fatty acids, contains an ample amount of unsaturated fatty acid. The P/S ratio was also found to be higher in tempe than in the raw beans. The ability of the fungus to hydrolyze fat is well-documented<sup>28</sup>. It is also reported that the soy tempe is low in fat and has the ability to lower the blood cholesterol level<sup>29</sup>. Therefore, Bengal gram tempe containing highest amount of polyunsaturated fatty acid, could be included in the diet for the hyper cholesterolemic patients.

The amino acid content of raw Bengal gram and its tempe are shown in Table 4. There was no remarkable difference found in the essential amino acid content between the raw Bengal gram and its tempe. Hydroxyproline and proline contents of tempe were 0.99 and 13.02 mg/100g respectively, which were higher than raw Bengal gram (0.12 and 1.42 mg/100g). It was also reported that after fermentation the amino acid composition remained fairly constant<sup>30</sup>.

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