Selected Nutrient Contents of Mixed Solid State Fermented Millet *(Seteria italica)* and Bengal Gram by *Rhizopus oligosporus*

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Abstract

Mixed solid state fermentation by *Rhizopus oligosporus* on nutritional and antinutritional factors of dehulled cereal *Seteria italica* (millet or kaon) and legume *Cicer arietinum* (Bengal gram or chola or chick pea) were studied. The fermentation method used was similar to the traditional fermentation procedures used in the preparation of Tempeh from Soybean in the orient. The duration of fermentation was 24 hours at 37°C under controlled laboratory conditions. In this work protein content were increased by 5.02%, 5.97% and 9.25% after 24 hours of fermentation of (50:50), (60:40) and (75:25) ratios of mixed *Seteria italica* (millet or kaon) and *Cicer arietinum* (Bangal gram or chola or chick pea) respectively. Crude fat content decreased by 11.9%, 17.10% and 27.16% after 24 hours of fermentation. Ash content decreased by 3.73%, 4.57% and 4.60% respectively after 24 hours of fermentation. Crude fibre content decreased to 9.15%, 7.00% and 6.28% respectively after 24 hours of fermentation. Carbohydrate & Calorie were decreased 0.16%, 0.29%, 0.50% & 0.72%, 0.85%, 1.43% respectively after 24 hours of fermentation.

After 24 hours fermentation, thiamine content increased by 20.58%, 19.44% & 21.05% when compared with the thiamine content of raw state and 64.0%, 79.16% & 53.33% when compared with the thiamine content of soaked and steamed state respectively. Niacin content increased by 30.43%, 27.19% & 33.47% when compared with the Niacin content of raw state and 42.11%, 48.72% & 45.00% when compared with the Niacin content of soaked and steamed state respectively. Vitamin B_6 content increased by 37.68%, 35.84% & 23.80% when compared with the vitamin B_6 content of raw state and 69.64%, 63.64% & 67.72% when compared with the vitamin B_6 content of soaked and steamed state respectively.

The nutritional factors like protein and B vitamins (Thiamine, Niacin and Vitamin B_6) were increased. While crude fat, ash and fibre decreased. So temph can be prepared with millet or Bengal gram for meeting nutritional needs of young children and old people.

Key wards: Solid state fermentation, Nutrient content, *Rhizopus oligosporus,* Bengal gram.

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Introduction

Bangladesh is a developing country where malnutrition is a serious problem. The diet of Bangladesh is based on cereals and legumes. Legumes (pulses) are the cheapest source of protein and considered to be the poor's meat. Availability of protein from animal source is already scarce because fish production has gone down than requirement due to indiscrimate use of fertilizer and pesticide. So to meet the requirement of protein from plant source it is a need fenrich Bangladeshi diet[1,2].

By traditional solid state fermentation process of cereal & legume 'tempeh' is prepared. 'Tempeh' is a popular, delicious and nutritious food in Indonesia. 'Tempeh' could also be useful for our population as protein source as well as calorie. 'Tempeh' produced pleasanat aroma with alcoholic smell which increases its acceptability and is becoming popular food in USA, Japan, China, Taiwan and India. Due to fermentation, protein availability and digestibility improves, so also B vitamins along with reduction of antinutrient factors present in legumes[3,4]. There are many legumes & cereals, among them Bengal gram & millet are available. *Seteria italica* (millet or kaon) and *Cicer arietinum* (bengal gram or chola or chick pea) are the important sources of carbohydrate, protein, B vitamins and minerals[1,2]. The purpose of this study is to increase the availability of protein and of B-vitamins in these seeds by solid state fermentation. Through this process even weaning food (with or without milk or sugar) for the growing children and old people and other vulnerable group may also be fed.

Materials and Methods

Millet and Bengal gram seeds procured from the local market were used for this study. Dehusked millet and "dal" cleaned and kept at room temperature untill tests were done. In a 100 ml. beaker 50g of seeds were soaked in water (1:3 seeds to water ratio) overnight (12h), washed and steamed for 10 min and dried at room tempereture to remove excess water. Steamed seeds were inoculated with 0.5% inoculum of *Rhizopus oligosporus* a mold collected from the Nutrition Research Center, Bogor, Indonesia. For the preparation of inoculum by growing tempeh mold on rice or steamed cassava flour. The mold culture was then dried in the sun to when the spores had fully matured. It was then ground to powder. Inoculum made by growing temph mold on rice has shelf life of six months at 25^0c when stored in a closed container

(0.5% means desire amount of inculum). One portion of the soaked and steamed seeds was kept in the refrigerator (kept at ^{0}c) as control. Inoculated seeds were packed in glass petrideshes and fermented in an incubator at 37^{0} c for 24h. After farmentation they were kept in a refrigerator as fermented sample. For the raw sample it also control dried and cleaned seeds were ground in a mechenical grinder and strored at room temperature. So we obtained three kinds of sample -

1. Inoculated seeds
2. Soaked and steamed seeds (control)
3. Raw sample-(dried and cleaned)

For the study purpose we mixed these three types of seeds each in three ratios like (millet 50: Bengal gram 50), (millet 60: Bengal gram 40), (millet 75: Bengal gram 25). Moisture, proximate principles of food (protein, fat, carbohydrate), ash, crude fibre and B- vitamins both for unfermented and fermented seeds are determined by standard methods[5,6,7,8].

Crude protein, ash, fibre, were determined by the ICMR methods[5]. Fat was determined according to the method of Folch[6]. Thiamine was determined according to Lyman et al[7]. Niacin was analyzed colorimetrically according to the method of AOAC[8]. Vitamin B_6 was estimated microbiologically using Saccharomyces Carlsbergensis (ATCC 9080) as assay organism according to Hyderabad manual method[5].

Statistical analysis

After coding and editing, the collected data were analyzed by using SPSS software pakage. The results were presented using mean ± SD and T-test.

Results

The effect of fermentation on chemical composition of *Seteria italica and Cicer arietinum* mixed seed is shown in Table 1. Before fermentation mixed seeds of soaked and steamed, the protein content of *Seteria italica* (millet) and *Cicer arietinum* (bengal gram) (millet 50: bengal gram 50), (millet 60: Bengal gram 40) and (millet 75 : bengal gram 25) were found to be 21.52 ± 0.10, 20.76 ± 0.07, 20.00 ± 0.1g/100g respectively. After fermentation, protein content was found to be 22.60 ±

0.13, 22.00 ± 0.16, 21.85 ± 0.14g /100g respectively which was increased to 5.02%, 5.97% and 9.25% respectively. After fermentation the fat content were decreased by 11.90%, 17.10% and 27.16% respectively when compared with the fat content of control (before fermentation soaked and steamed). Ash & Fibre contents were decreased by 3.73%, 4.57%, 4.60% & 9.15%, 7.00%, 6.28% respectively when compared with the ash & fibre contents of control. Carbohydrate & calorie were decreased 0.16%, 0.29%, 0.50% & 0.72%, 0.85%, respectively 1.43% when compared with the carbohydrate & calorie contents of control.

The effect of fermentation on vitamins content of *Seteria italica* and *Cicer arietinum* mixed seeds are shown in Table 2. After fermentation, thiamine content by *Seteria italica: Cicer arietinum* were increased 20.58%, 19.44% and 21.05% when compared with the thiamine content of raw state respectively. Thiamine content were also increased 64.0%, 79.16% and 53.33% respectively when compared with the thiamine content of soaked and steamed state. Niacin content were increased 30.43%, 27.19% and 33.47% respectively when compared with the Niacin content of raw state. Niacin content were also increased 42.11%, 48.72% and 45.00% respectively when compared with the Niacin content of soaked and steamed state. Vitamin B_6 content were increased 37.68%, 35.84% and 23.80% respectively when compared with the vitamin B_6 content of raw state. Vitamin B_6 content were also increased 69.64%, 63.64% and 67.72% respectively when compared with the vitamin B_6 content of soaked and steamed state.

Table 1: Effect of Fermentation on Chemical Composition of *Seteria italica and Cicer arietinum* Mixed Seeds[a]

Nutrients	Samples Pattern	Before fermentation soaked and steamed (control).	After fermentation (24 h after)	% of effect	P-value
	Seteria italica : Cicer arietinum				
Protein (g/100g)	(50 : 50)	21.52 ± .10	22.60 ± .13	5.02(+ve)*	P<0.05
	(60 : 40)	20.76 ± .07	22.00 ± .16	5.97(+ve)*	P<0.05
	(75 : 25)	20.00 ± .11	21.85 ± .14	9.25(+ve)*	P<0.05
	Seteria italica : Cicer arietinum				
Fat (g/100g	(50 : 50)	6.47 ± .022	5.70 ± .041	11.90(-ve)*	P<0.05
	(60 : 40)	4.97 ± .016	4.12 ± .022	17.10(-ve)*	P<0.05
	(75 : 25)	4.86 ± .024	3.54 ± .029	27.16(-ve)*	P<0.05
	Seteria italica : Cicer arietinum				
Ash (g /100g)	(50 : 50)	1.61 ± .022	1.55 ± .024	3.73(-ve)*	P<0.05
	(60 : 40)	1.53 ± .039	1.46 ± .016	4.57(-ve)*	P<0.05
	(75 : 25)	1.52 ± .033	1.45 ± .029	4.60(-ve)*	P<0.05
	Seteria italica : Cicer arietinum				
Fibre (g/100g)	(50 : 50)	1.53 ± .029	1.39 ± .022	9.15(-ve)*	P<0.05
	(60 : 40)	1.57 ± .045	1.46 ± .039	7.00(-ve)*	P<0.05
	(75 : 25)	1.59 ± .054	1.49 ± .029	6.28(-ve)*	P<0.05
	Seteria italica : Cicer arietinum				
Carbohy-drate (g/100g)	(50 : 50)	68.87 ± .029	68.76 ± .036	0.16(-ve)*	P<0.05
	(60 : 40)	71.17 ± .039	70.96 ± .045	0.29(-ve)*	P<0.05
	(75 : 25)	72.03 ± .11	71.67 ± .036	0.50(-ve)*	P<0.05
	Seteria italica : Cicer arietinum				
Calorie (100g)	(50 : 50)	419.35	416.74	0.72(-ve)*	P<0.05
	(60 : 40)	412.45	408.92	0.85(-ve)*	P<0.05
	(75 : 25)	411.86	405.94	1.43(-ve)*	P<0.05

(a) Values are expressed as mean ± SD of three samples, each analyzed in duplicate (on dry weight basis).

* (+ ve) = increased   * (– ve) = decreased

Table 2: Effect of Fermentation on Vitamins content of *Seteria italica and Cicer arietinum* Mixed Seeds[a]

Vitamins	*Seteria italica : Cicer arietinum*	Vitamins content (mg/100g)			% of Effect	P-value
		Before fermentation		After fermentation (24 h) fermented		
		Raw	Soaked and steamed (control)			
Thiamine	50:50	0.34± .029	0.25 ± .016	0.41 ± .022	20.58 (+ve)* 64.0 (+Ve)*	Significant p≤0.05
	60:40	0.36 ± .036	0.24 ± .029	0.43 ± .022	19.44 (+ve)* 79.16 (+Ve)*	Significant p≤0.05
	75:25	0.38 ± .022	0.30 ± .016	0.46 ± .029	21.05 (+ve)* 53.33 (+Ve)*	Significant p≤0.05
Niacin	50:50	2.07± .029	1.90 ± .022	2.70 ± .037	30.43 (+ve)* 42.11 (+Ve)*	Significant p≤0.05
	60:40	2.28 ± .036	1.95 ± .041	2.90 ± .041	27.19 (+ve)* 48.72 (+Ve)*	Significant p≤0.05
	75:25	2.48 ± .033	2.00 ± .044	3.31 ± .051	33.47 (+ve)* 45.00 (+Ve)*	Significant p≤0.05
Pyridoxine	50:50	0.69± .057	0.56 ± .036	0.95 ± .029	37.68 (+ve)* 69.64 (+Ve)*	Significant p≤0.05
	60:40	0.53 ± .036	0.44 ± .045	0.72 ± .050	35.84 (+ve)* 63.64 (+Ve)*	Significant p≤0.05
	75:25	0.42 ± .029	0.31 ± .036	0.52 ± .044	23.80 (+ve)* 67.72 (+Ve)*	Significant p≤0.05

a Values are expressed as mean ±SD of three samples, each analyzed in triplicate (on dry weight basis)

* (+ Ve) = Increased   * (– Ve) = Decreased

Discussion

In this study it was found that the protein content of *Seteria italica* and *Cicer arietinum* mixed seeds increased after fermentation (24 h). Soluble nitrogen increases from 0.5% to 2.5% on tempeh, while the total nitrogen remains relatively constant[9]. This increased soluble nitrogen might have improved protein efficiency ratio (PER) of tempeh as reported by Gyorgy[10]. It might be attributed to better availability of amino acids liberated from the soy protein during the fermentation and better digestibility of tempeh due to increase of water soluble solids and nitrogen[11,12]. Murata[13] showed that the quantity of free amino acids increased as fermentation progressed.

Fat decrese gradually after fermentation. The presence of lipase in the fermented seeds may be the cause of these decrease. The mold, *Rhizopus oligosporus*, possesses strong lipolytic activity. Wangenknect et al[14]. Also reported that in the course of the fermentation hydrolyzing about one third of the neutral fat. The antioxident that protect oxidation of fatty acids, produced by the tempeh mold had been extensively studied by Gyorgy[17]. Murata[13] attributed to the improved nutritive value of tempeh to stabilization of oil by antioxidents produced during fermentation. Ash content decreased by fermentation which is due to washing of sample (millet and Bengal gram) and decantation of water. Crude fibre content decreased to about one third to one fourth of the actual dietary fibre. Total dietary fibre content was not influenced by fermentation or baking, even though a slight decrease in soluble fibre and increase in insoluble fibre components observed[16,18].

Carbohydrate and energy remained same after fermentation but fermentation plays a positive role in increasing the nutritive value of carbohydrate as shown by Akinrele[19].

Thiamine, niacin and vitamin B_6 content increase during fermentation. Roelofsen[16] found higher levels of thiamine, riboflavin and nicotinic acid both in tempeh and soybeans than those observed by Murata[13] found that the level of riboflavin, vitamin B_6, niacin and pantathonic acid in 'temph' were much higher after fermentation. Similar increase of thiamine was observed in tempeh gembus by Gandjar using the similar mold inoculum[21]. Hermana[23] and steinkraus[9] found considerable increase in niacin content of soyabean after fermentation. Wang and Hesseltine[24] verified vitamin content of tempeh through fermentation of wheat with *Rhizopus oligosporus* and found marked increased in niacin and riboflavin. Mosleuddin and Mahmud[25], Murata[13], Hermana[23], Robinson and Kao[26], observed that tempeh mold *Rhizopus oligosporus* can synthesize large amount of vitamin B_6. B_6 found rich in meat (organ meat) which is costly. Whole grain cereals and peanut are also rich source[29]. *Seteria italica* and *Cicer arietinum* are grown in Bangladesh and content of vitamin B_6 is rich. Though the vitamin B_6 content of the fermented *Seteria italica* and *Cicer arietinum* mixed seeds are not sufficient, the product will also be enriched with other nutrients after this process.

The B vitamins act as a cofactor in enzymatic reactions. They work side by side with some minerals. In this way they could increase the utilization of those minerals. So, increased amount of B vitamins in tempeh is beneficial for the utilization micronutrient as well as for macronutrient.

Since solid state fermentation is a cheap, easy and less time consuming process, so by mixed cereal and legume through solid state fermentation we can make diet nutritionally sound for adults and through this process even weaning food for the growing children may be prepared. Due to fermentation protein availabihty improves, so all B-vitamins. We have long lasting tradition of consumption of fermented food stuff like pantabhat and dadhi, fermented foods are likely to remain and important part of the human food for many years to come. Tempeh can be used to improve nutritional status of poor people.

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