

## Nutritional Composition of Shithu: A Traditional Fermented Food of Churachandpur District, Manipur.

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**ABSTRACT:** Shithu is an indigenous traditional fermented foods prepared from seeds of *Sesamum indicum*(L). This fermented food has been consumed in different recipes over a long period of time by the people of Churachandpur. It is either consumed directly or used as a flavouring agent in vegetable items and its addition makes the vegetable items more soft and tasty. This fermented food is mainly prepared by women and the knowledge is passes down from generation to generation in words of mouth and no written record is found so far. Despite the fact that traditional fermented foods are widely consumed with no cultural inhibition and tend to be nutritious the nutritional values of *Shithu* has not yet been determined before. Thus, the nutrition value such as carbohydrate, soluble protein, total free amino acids, reducing sugar ,total phenol, flavonoid etc. and some trace elements like calcium, magnesium, iron, zinc and manganese were access following standard protocols for biochemical analysis. It was found that *Shithu* has showed potential content of total free amino acids 22.33mg/g, soluble protein 8.62mg/g and carbohydrate 3.30mg/g. The total phenol and flavonoid were found to be 8.26mg/g and 2.79mg/g respectively. In the analysis no ascorbic acid could be detected in the fermented Shithu. Among the trace elements calcium was found to be the highest with 56mg/g followed by iron 22mg/g. The present study aimed at documentation of traditional knowledge of this fermented foods preparation and a brief highlight on the mode of consumption and to generate information about the favorable nutritive values of this lesser known fermented foods locally called *Shithu* in churachandpur district, Manipur India

Keywords: Shithu, *Sesamum indicum* (L) seeds, nutritional value, traditional fermented foods. Churachandpur.

### INTRODUCTION

Fermented foods form important nutritional requirements of large number of people of the world. It is one of the oldest and most economical methods of producing and preserving traditional foods. In addition to preservation, fermented foods provide the bio nutrient minerals and fortified with bio active compounds enhancing the flavor and aroma and exert health promoting beneficial (Darby, 1979, Cambell-Platt, 1994, Steinkraus, 1998.) Traditional fermented foods and beverages have been reported from Darjeeling and Sikkim Hills (Tamang et al.,1988, Tamang 1996), Manipur (Jeyaram et al.,2009) Himachal Pradesh (Navdeep and Bhalla 2004) and Naga tribes of North-eastern India (Ashiho and Odyuo 2009) Churachandpur district which lies in the southern part of Manipur, India is inhabited by various tribes of the Chin-Kuki- Mizo groups of community sharing common culture and food habits '*Shithu*' is an indigenous traditional fermented product of *Sesamum indicum*(L) seeds with charismatic smell and flavor. It has been consumed as a regular food items in every household. It is either consumed directly or used as a flavouring agent in vegetable items throughout the year and its addition makes

the vegetable items more soft and tasty. Despite the fact that traditional fermented foods are widely consumed with no cultural inhibition and tend to be nutritious. So far no research has been carried out and no literature report is available pertaining to traditional fermented foods and its nutritional components of *Shithu*. It is fact that the quality of food depends upon the presence of relative concentrations of various nutrients such as proteins, fats, carbohydrates, vitamins and minerals. Thus, the work is a brief highlight or to generate information about the favorable nutritive values and to document the indigenous knowledge of the fermented food preparation of this lesser known fermented food locally known as *Shithu*

### MATERIALS AND METHODS

*Shithu* was collected from the fermenting sites and was dried in the oven at 20<sup>0</sup>C and grinded into powder and passed through a sieve and kept safe in an air tight container for the biochemical analysis.

#### Traditional fermentation process of *Shithu*.

A seed of *Sesamum indicum* (black seed varieties) is fried without oil and stilled till it produces charismatic smell. It is then pounded into powder

by using traditional wooden crusher. The paste is then mixed with water to make a sticky colloid and then put inside an air tight container and place near the fire for five days to undergo fermentation. This fermented *Sesamum indicum* seed is called *Shithu* by the people of Churachandpur.

## ESTIMATION OF NUTRITIONAL COMPONENTS

### Estimation of carbohydrate

The carbohydrate content was measured by spectrophotometrically following Anthrone's method (Hedge and Hofreiter 1969) the samples 0.1g was extracted in 80% alcohol and centrifuge. The supernatant was used for analysis. To 1ml of the supernatant, 4ml of anthrone reagent was added and kept in a boiling water bath for 8 min. Then the test tubes were ice cooled rapidly. The absorbance of the test samples which is green to dark green colour was measured at 630nm. The amount of carbohydrate content was calculated using a standard curve prepared from glucose.

### Estimation of phosphate buffer soluble protein

The protein content in the fermented samples was determined by the Lowry's method (Lowry et al. 1951) Extraction was carried out with phosphate buffer (pH 7.5, 0.1M). The 0.5 g of sample was grinded well in 5–10 mL of cold phosphate buffer (pH 7.5, 0.1M) using a chilled mortar and pestle. The homogenate was then filtered through four layers of cheese cloth. The filtrate was centrifuged at 5000 rpm for 15 minutes in a centrifuge and the supernatant was collected into a graduated test tube and its volume was raised to a certain level (10 ml) with the same buffer and kept for estimation. Then, 1 ml of this supernatant was taken in another centrifuge tube and 1 ml of trichloro acetic acid (TCA) solution (10%, w/v in distilled water) was added to it. The precipitate (whitish in colour) was observed immediately. Precipitation was completed in 5 min. The precipitate was centrifuged and after centrifugation the supernatant was discarded. A little amount (5 ml) of ethyl alcohol (95%) was added into the protein precipitate so as to remove the excess, TCA from the surface of the protein. The washed precipitation was then mixed with 5 mL of alkaline copper solution and allowed to stand for 10 min. The 0.5 mL of Folin-Ciocalteu reagent was then added in the tubes that were incubated at room temperature in dark for 30 min. A bottle green to navy blue coloured appeared. Then its absorbance was read at 660 nm by using a spectrophotometer. Calculations were done from the standard curve prepared by using BSA (Bovine Serum Albumin) as the standard solution.

### Estimation of reducing sugar

Reducing sugar was done by using Somogyi's method (1944). 500mg of the samples was extracted with hot 80% alcohol twice (5ml each time). The supernatant was collected and

evaporated on a water bath. 10 ml of distilled water was added to dissolve the sugar. To 0.2 ml of alcohol free extract the volume is made up to 2 ml with distilled water. 1 ml of alkaline copper tartrate was added. The test tube was then placed in boiling water for 10 min and then cooled to room temperature. To it 1 ml of arsenomolybdate reagent was added. The volume of the test tube was made up to 10 ml with distilled water. After standing for 10 minute, the absorbance of the blue colour was read at 620 nm. The amount of reducing sugar present in the sample was calculated from the standard curve prepared by using glucose as the standard.

### Estimation of total free amino acid

The estimation of total free amino acid was done by the method of Yemm and Cocking (1955). In this; 50 mg of the samples were homogenized in 10 ml of 50% ethanol with a pinch of activated charcoal. The contents were centrifuged at 1000 rpm for 10 minute and the free amino acids were collected in the form of clear supernatant. To 1 ml of the supernatant, 25 ml of ninhydrin (2% w/v in isopropyl alcohol) and 2.5 ml of 0.1 M acetate buffer (pH 5.5) were added. The mixture was then heated on boiling water bath for 30 minutes and after cooling, aqueous isopropyl alcohol (1:1) was added to make up volume to 10 ml. The colour intensity of the violet complex was measured at 570 nm absorbance. The amount of total free amino acids were calculated with the help of a calibration curve prepared from glycine and is expressed as mg amino acids powdered per mg/ g of the sample.

### Estimation of ascorbic acid.

The ascorbic acid content were determined volumetrically (Raghu et al.,2007) by titrating with 2, 6 dichloro-indophenol dye. 250 mg of the fresh and fermented sample of bamboo shoots were weight and crushed with pestle and mortal in 5ml of 0.4% oxalic acid and centrifuged at 5000rpm for 20 minute. The supernatant was then transferred to a measuring cylinder and the volume was made up to 10ml. 5ml of the supernatant were pipette out and kept in a white porcelain disc and titrate against the standardized 2, 6 dichlorophenol indophenols reagent till the sample solution became pink, which persist for few seconds. The amount of ascorbic acid content in the sample was calculated, using the following formula.

$$\text{Ascorbic acid (mg/g of tissues)} = \frac{I \times S \times D}{A \times W} \times 100$$

Where,

I=ml of indophenols reagent used in the titration;  
S=mg of ascorbic acid reacting with I of the reagent; D=volume of the extract in ml; A=the

aliquot titrated in ml and  $W$ =the weight of the sample.

#### Estimation of total phenol content

Total phenol contents were determined by folin-ciocalteu method with sodium carbonate solutions following Mc Donald *et al.* (2001).500 mg of the sample were crushed with 10ml of 80% ethanol using pestle and mortar. The slurry was then centrifuged at 5000 rpm for 20 minutes. The supernatants were collected in a test tube. The residue is again re-extracted with 5 ml of 80% ethanol and centrifuge and pool the supernatant. Then the supernatants were then evaporated in a petri plate to dryness. Then the dried residue is dissolved in 5 ml of distilled water and the volume made up to 10 ml with distilled water. 0.1 ml of the dissolved residue was taken and its volume was made up to 3 ml with distilled water. In the test tubes containing test samples, 0.5 ml of folin-Ciocalteu reagent was added. Then after 3 minute, 2ml of 20% of  $\text{Na}_2\text{CO}_3$  were added and mixed thoroughly. The mixtures were then kept in water bath for exactly 1 minute and cooled to room temperature and the absorbance was measured at 765nm. The total phenol content was calculated and expressed in mg/g using chlorogenic acid as the standard.

#### Estimation of flavonoid content

Aluminium Chloride spectrophotometric method was used for flavonoids determination (Chang *et al.*2002) with slight modification. For extraction of sample, 500 mg of the powdered dried plant sample were crushed with 10 ml of methanol by intermittent maceration up to 48 hours. The solvent was evaporated and reduced up to 5 ml at room temperature. After evaporation, samples were centrifuged at 10,000 rpm for 15 minute at room temperature. The supernatant were collected and volume was made up to 5 ml with methanol. 0.1 of the supernatant was taken and it was added with 0.1 ml of aluminium chloride (10%), 0.1 ml of potassium acetate (1M) and 2.7 ml of distilled water to made volume up to 3 ml. The reaction mixture was kept at room temperature for 30 minute. The absorbance was measured at 415 nm using spectrophotometer. The calibration curve was prepared using different concentrations of quercetin.

#### Estimation of minerals:

Mineral contents were determined by atomic absorption spectrometry.

Wet Diacid Digestion: Wet diacid digestion method (Caper *et al.*1978) was adopted for different mineral analysis.500mg of the powder sample was weighed and added in 100ml volumetric flask., then 20ml of conc. Nitric acid was added and keep them in a sand bath for around 2-3 hrs until brown fumes ceased . After the sand bath, the sample was cooled at room temperature.

After cooling, 10ml of 70% perchloric acid was added in each volumetric flask and heat again for 1 hr. Then the digested sample was kept overnight. The volumes of the digest was made up to 50ml with distil water and the extracts was filtered in whatman no 42.This digest solution was used for different mineral analysis The macro-elements Ca, Mg and three micro-elements Mn, Fe, and Zn were analyzed using Parkin Elmer atomic absorption spectrophotometer, Analyst AA-200.For this analysis, respective standards are prepared using 1000ppm stock for each element. For the macro element, 2ppm, 4ppm and 6ppm standards were used. Micro- elements required lower concentration for making the standard, so 1ppm, 2ppm and 3ppm were prepared. For estimation of the elements, the instrument was set by using their respective standards curve for calibration of samples. Then the digest were analyzed using suitable lamps of each element.

**Statistical analysis:** All assays were carried out in triplicate and values were obtained by calculating the average of three experiments using Excel program (Microsoft Excel .2007) and data are presented as Mean $\pm$ SEM.

#### RESULT:

**Table1. Nutritional content of traditional fermented *Sesamum indicum*(L) seeds *Shithu*.**

Parameters	Concentration mg/g
Carbohydrate	3.30 $\pm$ 0.65*
Soluble protein	8.62 $\pm$ 0.89
Reducing sugar	0.02 $\pm$ 1.03
Total free amino acid	22.33 $\pm$ 1.52
Ascorbic acid	Not detected
Total phenol	8.26 $\pm$ 1.00
Flavonoid	2.79 $\pm$ 0.37

\* Data presented as mean of three replicates  $\pm$  SD.

**Table2. Minerals content of traditional fermented *Sesamum indicum*(L) seeds *Shithu*.**

Parameters	Concentration mg/g
Calcium	56.30 $\pm$ 0.65*
Magnesium	1.14 $\pm$ 0.89
Manganese	0.13 $\pm$ 1.03
Iron	22.33 $\pm$ 1.52
Zinc	5.20 $\pm$ 0.91

\* Data presented as mean of three replicates  $\pm$  SD.

#### DISCUSSION

The nutritional value of *Shithu* is depicted in **table 1**. The finding shows that *Shithu* has showed potential content of total free amino acids 11.92mg/g and soluble protein 8.62mg/g amino acids are the building block of protein. Protein is an

indispensable requirement for the growth and maintenance of all biological organisms. Every cell in our body needs protein to carry out the metabolic activities that sustain us. The amount of protein required for normal health is variable depending on many factors mainly body weight, age physical activity, health condition (Nirmala et al., 2011). The amount of carbohydrate detected is 3.30mg/g which shows that it can provide some source of energy as carbohydrate is an energy giving food. Less amount of reducing sugar with 0.02mg/g was found and no ascorbic acid was detected in the fermented *Shithu*. Traditional fermented protein-rich foods offer excellent opportunities for improving the diets of people. The total phenol and flavonoid were found to be 8.26mg/g and 2.79mg/g respectively. Phenolics compounds and flavonoid are found to be associated with antioxidant activity in biological system (Hasan et al., 2006). These compounds can act as antioxidant by radicals scavenging in which they break the free radicals chain reaction through hydrogen atom donation (Kosem et al., 2007). The present finding on phenol and flavonoid shows that the fermented *Shithu* is having a good potential of antioxidant property.

**Table 2** shows the minerals content of *Shithu*. Among the minerals calcium was found to be the highest 56mg/g followed by iron which is 22mg/g. The amount of magnesium, manganese, and zinc is 1.14mg/g, 0.13mg/g and 5.2mg/g respectively. Calcium constitutes a large proportion of the bone, human blood and extracellular fluid (Indrayan et al., 2005). Iron is a component of hemoglobin and helps in oxygen transportation. Iron together with hemoglobin and ferredoxin plays an important role in body metabolism and manganese is an antioxidants nutrient important in the nourishment of the nerves and the brain (Okwu and Morah 2004) The present finding of zinc may be useful as

zinc is essential for the production of insulin, a hormone and carbonic anhydrase, an enzyme in the body (Okwu and Morah 2004) Magnesium is very important in humans, especially in the formation of bones and teeth like the function of calcium (Llelaboye and Pikuda, 2009)

## CONCLUSION

Based on the experimental finding presented in this study, it appears that *Shithu* is highly nutritious and can be concluded that this traditional food can be promoted in addressing the protein, amino acids, phenols, flavonoid and minerals such as calcium Iron, magnesium and Zinc deficits prevalent in the diets particularly among the low income families as *Shithu*, is prepared and consumed throughout the year. Despite the advancement of science and technology the production of this fermented food still remains rudimentary. This traditional fermented food preparation is of low cost as it needs less labour-input and the raw material is easily and locally available. If proper scientific and technical support is extended to the existing indigenous practices of this home based fermentation of *Shithu* there is a strong potential of increasing food production, improving the nutritional and economic status of the rural population.

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