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Nutritional and microbial composition of *Sithu*, a traditional fermented sesame seed of Kuki-Chin-Mizo tribes of Manipur, India

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ABSTRACT

Sithu is a traditional fermented sesame seed product produce and consume by ethnic Kuki-Chin tribes of Manipur, India. The method for traditional preparation and mode of consumption and associated indigenous knowledge were documented. The preliminary nutritional and microbial profiling of the product was carried out. Mesophilic bacteria and lactic acid bacteria were recorded as 4.35 x $10^8$and 3.76 x 10^7cfu/g respectively whereas no fungi or yeast were detected. Carbohydrate content was reduced (14.86 ±3.42 to 9.45 ± 0.67g/100g) while crude protein content increased (22.38 ± 2.36 from 19.54 ± 1.67 g/100g) and crude oil contentslightly increased (45.06 ± 1.78 from 44.67 ± 2.07g/100g)when the seed was fermented to *sithu*. 5.02 ± 1.88 g fibre and 3.82 ± 1.06 g ash was recorded for sesame seed against 4.95±1.68g and 4.95±0.89g respectively for *sithu* from 100g samples.pH, total titratable acid, total phenolic content and antioxidant capacity were recorded as 8.2 ± 0.05, 0.034%, 73.08 ± 7.08% and 580 ± 88.9 mg/100g respectively.The nutritional and physiological quality makes this food an excellent food supplement for the improvised tribal population.

1. Introduction

Food fermentation is perhaps the most economic and simple method of food processing that has been employed from time immemorial and popular throughout the world. Its contribution to the diet of millions and associated intimately with the culture and tradition of many indigenous people in various parts of the world. The fermented food products supplemented protein, minerals and other nutrients, add variety, flavours to the otherwise bland diets. It also removes anti-nutritional factors present in the substrates and enhances functionality (16). Fermented foods contribute roughly about one third of all food categories globally with many different types of fermented foods of varied substrate origin with different microorganisms that yielded unique products with characteristics flavours and taste (6). Fermented foods and alcoholic beverages were produced from substrates of plant or animal origins mostly by natural fermentation or in the case of a few products by black-slopping or the addition of a traditionally prepared starter culture(s) containing functional microorganisms altering the substrates biochemically and organoleptically into food products that are socially acceptable (19). Fermented foods could be classified as acidic, neutral or alkaline but more commonly categorised based on the substrates used viz- fermented beverages, fermented meat, fermented vegetables etc (8).

Sesame (*Sesamumindicum* L.) is cultivated in several countries of the world such as India, China, Ethiopia, Uganda and Nigeria (1).Edible seeds comprised an alternative diet for nutrients and energy among the poor and under privileged populations where protein- energy malnutrition (PEM) crisis plagued the growth and development (5). In Japanese tradition the sesame is revered for being very good for health (12). As such sesame seeds are highly valued for their high content of oil which is known for its resistant to

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rancidity. However, the seed is used in preparations of puffed rice ball locally called *kabok*for its nutty flavours or it is fermented to prepare condiment in Manipur, India where oil extraction of sesame seed is confined to household activities with crude wooden equipment. *Sithu* is a fermented sesame seed product, alkaline in nature with characteristic odour and taste. People of Manipur, India traditionally savour several types of fermented food (8) and *sithu* is produced and relished particularly by Kuki-Chin-Mizo ethnic people throughout the year.

In recent time the traditional foods have been given tremendous importance and studied with renewed vigour for health and cultural reason. Despite the fact that traditional fermented food tend to be nutritious and healthy; consumed for many generations, many traditional fermented foods of smaller ethnic communities were overlooked by researchers. *Sithu* is one such popular traditional fermented food which has not been documented properly and not studied scientifically for its nutrition, associated microorganisms and safety concerns. In this article we are reporting preliminary studies on its production, ITK, proximate composition and microbiology.

2. Research Materials

Sithu and the raw sesame seed were collected from different parts of Churachandpur district of Manipur, India. Village elders and select families who were producing quality *sithu* for generations were interviewed for ITK and documentation of preparation of *sithu.*

2, 2-Diphenyl-1-picrylhydrazyl DPPH (D9132) was procured from Sigma, India. All other chemicals used in the analysis were of AR grade or higher from HiMedia, India.

2.1 Proximate Composition

Moisture, crude protein, oil, ash and total carbohydrate were determined according to AOAC (2000) method. Briefly, *sithu*samples were dried at 105°C overnight and moisture content in percentage was calculated by subtracting the dried weight from the original weight of the samples and divided by 100. Total nitrogen in the sample was determined by micro kjeldahl and protein was calculated as N×6.25. Ash content was determined by incinerating the samples at 600°C in a muffle furnace to constant weight. Total crude oil was determined by carefully extracting 2g of sample with petroleum ether in a soxhlet apparatus. Total carbohydrate was determined by anthrone method. Crude fibre content was determined using acid hydrolysis method. Each sample was analysed in triplicate.

2.2 pH Measurement

1g sithu sample was added to 25 ml of double distil water and vortex vigorously before measuring the pH with pH meter (Mettler Toledo,FP20) after calibrating against the standard buffers.

2.3 Total Phenolic Content (TPC)

The total phenolic compound contents were determined by Folin-Ciocalteu reagent. 0.5g of dried powder of *sithu* or sesame seeds was weighed into a 250 ml flask. To this, 25 ml of 80% methanol was added and homogenize to form a uniform suspension and filtered through a Whatman No 42 Filter paper. To 3 ml of the above filtrate 1 ml of Folin-Ciocalteu reagent mixture was added and mixed thoroughly before adding 3 ml of 2% $Na_2CO_3$to the mixture which was vortex briskly again and allowed to stand still for a few minutes. The developed colour intensities were read using a single beam spectrophotometer (BioEra, India) at a wavelength of 760 nm. Similarly, absorbance of the working standard was also read to prepare standard curve. The amount of total phenol was calculated as a gallic acid equivalent (GAE) in mg per g of dry mass.

2.4 Determination of Antioxidant Capacity

1 mL of aqueous extract of the samples was mixed with 1 mL 0.4 mM 1,1-di phenyl-2-picrylhydrazyl (DPPH) methanolic solution, the mixture was left in the dark for 30minutes and the absorbance of sample at 517 nm was recorded with a UV-spectrophotometer against methanol as blank. The percentage concentration of DPPH in the reaction medium was calculated using the following formula:

$$\% \text{ DPPH} = \frac{(\text{Absorbance of the control} - \text{Absorbance of the test sample})}{\text{Absorbance of the control}} \times 100$$

2.5 Total Titratable Acidity (TTA)

TTA was determined by the method as described by Pearson (15). Briefly, 3 g of *sithu* was added in 30 ml distilled water and vortex vigorously before filtering through whatman No. 1 filter paper. 20 ml of the filtrate was titrated against 0.1 M NaOH with two drops of phenolphthalein indicator. Total titratable acid was calculated using the titre value as percentage lactic acid.

2.6 Microbial Enumeration

The aseptically collected samples in sample containers were transported to the laboratory using cooler boxes and stored at 4^{o}C. The samples were immediately processed for further studies. 10 g of sithu was transferred to a sterile stomatcher bag containing 90 ml of physiological saline (0.1% w/v bacteriological peptone, 0.85% w/v NaCl) and homogenized (Stomatcher 400-Circulator, Seward, UK) at 250 rpm for 30 min and used for microbial enumerations. Homogenates was serially diluted up to the level of 10^{-}

^{8}decimals using physiological saline. The decimal dilutions each for i) Plate count Agar (PCA) ii) de man Rogosa and Sharpe (MRS) agar supplemented with 1% $CaCO_3$ iii) Yeast extract Peptone Dextrose agar (YEPD) and iv) Potato Dextrose Agar (PDA) supplemented with chloramphenicol 100mg/L were spread plated in triplicates and incubated at 30 °C. PCA, PDA and YEPD plates were incubated for a period of 24-48 h. The MRS plates were incubated in anaerobic Jars (HiMedia) with anaerobic gas packs (anaerocult C, HiMedia) for maintaining a micro aerobic environment for 48-120 h. Microbial load was calculated by counting the colonies on the agar plate manually. These isolates on agar media were stored at 4°C for further studies.

3. Results

3.1 Traditional preparation of *Sithu*

Though *Sithu* is prepared throughout the year based on availability of sesame seed and requirement it is usually prepared during the month of October and November after harvesting paddy, the staple food of people of Manipur. The detail procedure is described in fig.1. The sesame seed (Fig.1a) were cleaned washed with water and drained out the water. Then it was roasted (Fig.1b) until a characteristic odour was released which was ground (Fig. 1c) in a traditional wooden mortar and pestle after cooling. The fermentation was usually carried out in a dried specially made (Fig.1d) bottle gourd container with cap. Appropriate quantity of warm water was also added to the ground seed in the container, packed and capped nicely and kept in the sunlight or in the kitchen for appropriate temperature. Water could be added a little again after day or so if the whole water was soaked. The cap was closed tightly and kept in the sunlight or kitchen until the desired odour was released from the fermentation which normally takes 4-6 days. Thus prepared sithu (Fig. e) is ready for consumption but it could be stored for months and use routinely as side dishes or condiment in preparation of meat and vegetables.

Figure1. Traditional preparation of *sithu*; a. sesame seed, b. roasting of sesame seed, c. pounding of seeds, d. fermentation in gourd bottle container, e. *sithu*

3.2 Proximate Composition of Sesame Seed and *Sithu*

The proximate composition of both sesame seed and sithu is presented in the Table 1. The proximate content show some significant changes in some nutrients and little or no changes were observed during fermentation of *sithu* from sesame seeds. The moisture content of sesame seed was 5.78 ± 0.07 % whereas the moisture content of fermented product *sithu* was 67 ± 1.12 %. Carbohydrate content was reduced from 14.86 ±3.42 to 9.45 ± 0.67 g/100g when the seed was fermented to *sithu*. Crude protein content was estimated to be 19.54 ± 1.67 in the sesame seed but it was increased to 22.38 ± 2.36 percent (g/100g) in sithu of the test samples. Crude oil content of sithu was also slightly higher in *sithu*with 45.06 ± 1.78 from 44.67 ± 2.07per 100g sample in seed. 5.02 ± 1.88 g fibre and 3.82 ± 1.06 g ash was recorded for sesame seed in comparison to 4.95±1.68g and 4.95±0.89g respectively for *sithu* from 100g samples.

3.3 Physicochemical Properties of *Sithu*

The physicochemical properties such as pH, TTA, antioxidant capacity and TPC were measured using standard methods which were already published. pH was recorded as 8.2 ± 0.05. TTA being the measure of acidity of the food was estimated and found to be 0.034%. Antioxidant capacity as estimated by DPPH method was 73.08 ± 7.08% and TPC as assayed by Folin-Ciocalteu method was 580 ± 88.9 mg/100g.

3.4 Microbial enumeration of *Sithu*

Four commercially available media Potato dextrose agar (PDA), Plate count agar (PCA), Yeast extract peptone dextrose agar (YEPD) and deManRagosa Sharpe (MRS) were employed to enumerate fungi, mesophillic bacteria, yeast and Lactic acid bacteria (LAB). Both fungi and yeast were not detected upto the level of 10^{-4} dilution in the laboratory conditions. 4.35 x 10^{8}mesophillic bacteria and 3.76 x 10^{7} LAB was recorded by serial dilution and streaking method.

4. Discussion

The study was conducted to document the traditional method of *sithu* preparation for improvement in process of preparation and product in future. Primary microbial and nutritional profile of the fermented sesame -sithu was studied systematically to give a overview of its role in the diet of the natives. Womenfolk of the Kuki-Chin community are skilled in preparing *sithu*traditionally and this favourite condiment is readily available in kitchen of the household. The traditional knowledge associated with *sithu* has been passed down from the earlier generation orally as it has not been documented previously (9). Fermentation of sesame reduces anti-nutrients like phytate and oxalate that inhibits or reduces bio availabilities of minerals and other nutrients which are otherwise normally present in raw sesame seed (3). This

Table 1. Proximate composition (dry weight basis) of sesame seed and *sithu*

Nutrients	Sesame seed	Sithu
	g/100g	g/100g
Moisture	5.78 ±0.07	67 ± 1.12
Carbohydrate	14.86 ±3.42	9.45 ± 0.67
Crude protein	19.54 ± 1.67	22.38 ± 2.36
Crude oil	44.67 ± 2.07	45.06 ± 1.78
Fibre	5.02 ± 1.88	4.95 ± 1.68
Ash	3.82 ± 1.06	4.95 ± 0.89

Table 2. Physiological properties of sithu

Sl. No.	Parameters	Properties
1.	pH	8.2 ± 0.05
2.	Antioxidant capacity %	73.08 ± 7.08
3.	Total phenolic content	580 ± 88.9 mg/100g
4.	TTA %	0.034 ± 0.006

Table 3. Microbial load enumeration of *Sithu*

Media	Cfu/g	Conditions	Remarks
PDA with antibiotic	Nd	30 ^{o}C, 72 h	Not detected upto 10^{4} level
YEPD with antibiotic	Nd	30 ^{o}C 72 h	Not detected upto 10^{4} level
PCA	4.35 X 10^{8}	35 ^{o}C 24 h	Mesophillic aerobic bacteria
MRS	3.76X 10^{7}	35 ^{o}C 48 h	Microaerophillic Lactic acid bacteria

product is very similar to fermented sesame seed products called ogiri, a flavouring condiment of some African countries (20, (13)).The flavour and taste of *sithu* produce an appetising effect and usually preferred as side dish in every meal. It is frequently added to various vegetable and meat items as taste enhancer or may consume directly. Addition of *sithu* in cooking made the vegetable and meat more tender and chewable along with its characteristic flavour. Some of the favourite items with *sithu* are *sithubai*, *sithumaltameh* and *Sithuai*. *Sithubai* is prepared with baby pumpkin, bean leaf and chilli; *Sithumaltamah* is chutney of *sithu* with red chilli and perhaps most cherished item is *Sithuai* which is cooked with crab and chilli. In the remote villages the food offered the task of supplementing protein, mineral and other nutrients along with potential probiotics where there is a limited food option and depends on mostly the staple foods.

The *sithu*was found to be significantly reduced in total carbohydrate and slender increase in crude protein was observed. Similar observation was reported in some alkaline fermented soybean foods (16). It may be due to utilisation of carbohydrate as energy source by the microorganisms for its growth and development. The protein of sesame seeds have lysine (2) and are rich in sulphur containing amino acids like methionine and cystine (4). Therefore sesame is an ideal supplement for the poor whose primary protein sources are soybean and groundnuts and other pulses that are generally deficient of such sulphur amino acids. The lignans, sesamin and sesamolin in the sesame seed synergistically improved the vitamin E activity ofα and γtocopherol(7) that produce cholesterol lowering effects in humans (11)to maintain healthy heart and skin. Additionally our results show both raw seed as well as *sithu* have good amount of minerals. Therefore regular consumption of *sithu* by the natives provides a healthy supplement to the regular diets. TTA of 0.034% indicate lesser organic acid formation by LAB or yeast and it will not be acidic in taste. The phenolic compounds in the *sithu* influenced the unique smoky taste and probably have a role in antioxidant activity.

Our results show healthy bacterial population of both mesophilic bacteria and Lactic acid bacteria but fungal growth was not detected. This would be due to anti fungal activity of sesame oil (10), (14)). The colony and cell morphology along with preliminary biochemical test (results not shown) reveal that the major bacterial population were *Bacillus* spp.Functional properties like fibrinolytic activities were usually associated with alkaline fermented seed products (17). Further studies need to carry out to ascertain functionality, microbial dynamics and therapeutic activities of the product as sesame products were reported with various health claims. Attempts are being made to produce starter culture and standardised method for production for hygienic and more presentable product.

5. Conclusion

Traditional foods are culturally very important and closely linked with the ethnic identity. *Sithu,* the traditional fermented sesame seed product of Kuki-Chin tribes of Manipur is an excellent food supplement to the improvised tribal population. The traditional production method and presentation should be improved for a more appealing and hygienic product to attract the larger consumer particularly youths by showing the potential and actual health benefits.

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