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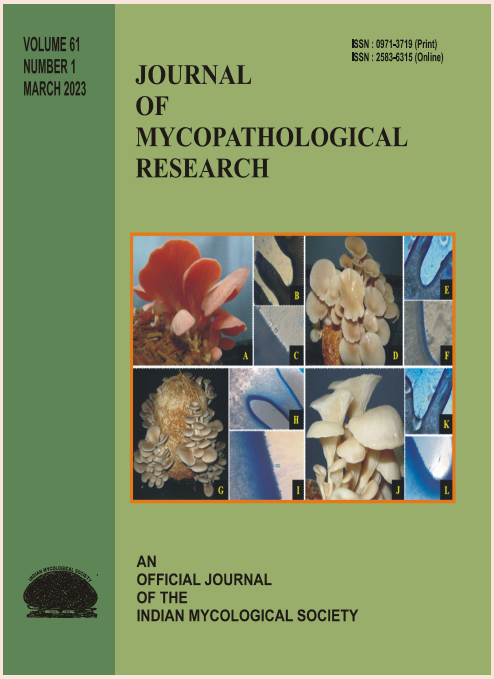

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Proximate analysis and nutritional values of some fermented food products of Nagaland, India

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The proximate biochemical and nutritional composition of fermented foods of Nagaland namely fermented pork fats, *Ashikumna*, fermented bamboo shoot, *Kese* and *Zusem*, dried fermented bamboo shoot, dry *Bastenga*, fresh bamboo shoot, rice beer *Katsing* and fermented crab, *Jangpangngatsu* were analyzed using various standard biochemical parameters. Our results indicate that most of the fermented food products are acidic in nature except JAP which was found to be neutral in nature. The food products also showed high antioxidant activity with standard and all the samples exhibiting a dose dependent activity on the DPPH radicals. The radical scavenging activity of the extracts were effective in the order KES > ZUPC > DBA > ZU > FBS > JAP > FPF > KAPC > KAT > ASK. Details on other biochemical parameters such as carbohydrate, reducing sugar, protein, crude fiber, total phenol and total flavonoid contents have also been highlighted. Scientific knowledge about fermented food diet to establish healthy and safe food consumptions among the indigenous people of Nagaland have been discussed.

Key words: Fermented foods, nutrition, antioxidants, ethnic, Nagaland

INTRODUCTION

Fermented foods constitute about one third of all food consumed by humans (Katz, 2012) and myriad studies support the beneficial roles of these foods in promoting human health. Microorganisms convert the chemical composition of raw materials during fermentation, which enrich the nutritional value, and improves metabolism. Microbial community comprising the acetic acid bacteria, lactic acid bacteria, non- lactic acid bacteria, gram-negative bacteria, filamentous mold, and alcohol producing yeasts dominates the Indian fermented foods and alcoholic beverages. Study on the natural microbial flora of bamboo shoot showed the presence of *Lactobacillus plantarum, L. brevis, L. casei, L. fermentum, L. curvatus, Leuconostoc mesenteroides, L. fallax* and *Tetragenococcus halophilus* as predominant microorganisms with various lactic acid bacteria playing a dominant role in imparting flavor, taste and aroma (Nongdam 2015).Other fermented foods of Nagaland such as *Tsutuocie* (fermented cucumber) and *Bastenga* (fermented bamboo shoot) were found to be dominated by *Bacillus subtilis* whereas *Bacillus licheniformis* formed the predominant micro-organism in *Anishi* (fermented taro leaves) and *Hungrii* (fermented mustard leaves) (Deb and Jamir, 2020). Limited studies are available on the microbial profile of fermented food products of Nagaland and hence the authors aim to promote the nutritional value of the food products which may further aid in promoting studies on microbes.

MATERIALS AND METHODS

Collection and preparation of samples

Various samples of fermented bamboo shoots *Zusem* (ZU), fresh bamboo shoot (FBS), *Kese* (KES), dry *Bastenga* (DBA), fermented rice beer *Katsing* (KAT) , fermented pork fats , *Ashikumna* (ASK), fermented crab, *Jangpangngatsu* (JAP)

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were either bought from the market or collected from households in Nagaland and a control set of selected samples ZUPC (*Zusem* positive control), KAPC (*Katsing* positive control), FPF (fresh pork fat negative control) were prepared in the laboratory following the traditional method under sterile conditions. Samples were refrigerated at 4°C until further analysis.

Carbohydrates and reducing sugar analysis

Glucose was taken as the standard for both the analysis. The anthrone method (Hedge and Hofreiter, 1962) was used to evaluate the carbohydrates content. 100 mg FW of the sample was boiled in 2.5N HCL for 3 hrs. To 1 ml of the sample, 4ml of anthrone reagent was added, heated for 8 minutes in a boiling water bath, cooled rapidly and the absorbance taken at 630 nm.

Reducing sugar was estimated using 3, 5-dinitrosalicylic acid (DNSA) reagent (Miller, 1959) and ethanol was used for extraction procedure. 2 ml of extract and 2 ml of DNSA reagent was homogenized and the mixture boiled for 9 mins followed by cooling to room temperature. 0.5 ml of potassium sodium tartrate was added and the absorbance measured at 540nm.

Protein analysis

Protein estimation was measured following Bradford method (Bradford, 1976). Phosphate buffer was used for extraction. To 1m of sample, 5 ml of dye was added, homogenized by shaking and allowed to stand for 5 mins. The absorbance was taken at 595 nm and Bovine Serum Albumin (BSA) was used as standard.

Crude fiber

Crude fiber was determined following AOAC 978.10 (AOAC, 2005). Dried samples measuring 1 gram was subjected to defatting with petroleum ether. 200 ml of 0.25N Sulphuric acid was added to the defatted sample and boiled for 30 mins. This was then filtered using No. 1 Whatman filter paper and the filtrate boiled with 200 ml of 0.313N NaOH solution for 30 mins followed by filtration and subsequent washing with hot distilled water till the colour became translucent. A pre-weighed aching dish (W1) was taken for the residue and dried at 130±2ºC for 2 h and allowed to cool down. The aching dish was weighed (W2) and again ignited for 4 hours at 600°C followed by cooling in a desiccator and reweighed again (W3). The following formula was used to calculate the crude fibre content :

$$\text{Crude fiber (g/100g)} = \frac{\text{Loss in weight on ignition } (W2\text{-}W1) - (W3\text{-}W1)}{\text{Original weight of sample}} \times 100$$

Ash content

Total ash content was determined following AOAC 942.05 with slight modification (AOAC, 2005). 1 gram of the powdered sample (W3) was transferred to a pre- weighed crucible (W1) and combusted at 600°C for 4 hours (W2) and allowed to cool in a desiccator. The total ash content was determined using the formula

$$\text{Ash \%} = W2\text{-}W1/W3 \times 100$$

Moisture content

It was estimated following AOAC 930.15 with modification (AOAC, 2005). A pre weighed aching dish along with 30 g of sample was placed at 70±1°C in the oven for ~16 h till a constant weight was achieved and the value was determined using the formula :

$$\text{Moisture content (\%)} = \frac{\text{Loss of weight}}{\text{Weight of the sample}} \times 100$$

Determination of pH value: 5 g of the sample was homogenized with 10 ml of DW and its pH was calculated using a digital pH meter.

Determination of alcoholic content using hydrometer

The percentage of alcohol by volume and weight was measured using Pro Series Triple Scale Hydrometer.

Total Phenolic assay

The Folin- Ciocalteu assay (Singleton and Rossi, 1965) with modification was used to estimate the total phenolic content. The samples were extracted using ethanol and gallic acid was used as the standard. The mixture consisted of 3 ml of sample solution, 0.5 ml of Folin- Ciocalteu reagent and

Table 1: Proximate nutritional composition of fermented bamboo shoot products of Nagaland,

Proximate analysis	ZU	ZUPC	FBS	KES	DBA
Moisture content (%)	95.29	95.76	92.66	92.68	91.98
Crude fibre (mg/100mg)	13.89	14.70	9.6	15.95	15.1
Ash (%)	11.2	17.7	13	12.2	7.5
pH	5.1	5.4	6.9	4.4	4.1
Carbohydrates (mg/100mg)	2.72± 0.002	2.87± 0.611	3.47±0.096	0.10± 0.01	2.35± 0.585
Reducing sugar (mg/100mg)	1.08± 0.001	1.12± 0.001	2.20±0.003	0.03±0.001	0.94± 0.004
Proteins (mg/100mg)	0.06± 0.005	0.05± 0.004	0.08±0.004	0.02±0.005	0.10± 0.011
Phenol (mg/100mg)	0.16± 0.008	0.15± 0.02	1.00± 0.7	1.95± 0.9	1.02± 0.1
Flavonoid (mg/100mg)	5.52± 2.72	4.15± 1.044	6.73±0.356	8.03±1.175	2.58± 0.911

ZU (*Zusem*), ZUPC (*Zusem* positive control), FBS (fresh bamboo shoot), KES (*Kese*), DBA (dry *Bastenga*)

Table 2: Proximate nutritional composition of fermented rice beer (KAT) against its positive control KAPC

Proximate analysis	KAT	KAPC
pH	2.9	3.9
Carbohydrates (mg/100ml)	631.85± 0.975	512.7± 0.579
Reducing sugar (mg/100ml)	382.5± 0.018	396± 0.006
Alcoholic content	7 %	5 %
Proteins (mg/100ml)	32.28± 0.494	8.928± 1.037
Phenol(mg/100ml)	1.427± 0.105	1.08± 0.335
Flavonoid (mg/100ml)	377.5± 4.376	353.95± 4.765

KAT (*Katsing*), KAPC (*Katsing* positive control)

Table 3: Proximate nutritional composition of fermented pork fats (ASK), its negative control (FPF) and fermented crab (JAP) of Nagaland

Proximate analysis	ASK	FPF	JAP
pH	4.2	5.3	7.1
Carbohydrates (mg/100mg)	0.75± 0.001	0.68± 0.044	2.62± 0.43
Reducing sugar (mg/100mg)	0.43± 0.003	0.48± 0.008	0.86±0.004
Proteins (mg/100mg)	0.02± 0.008	0.01± 0.001	0.14± 0.09
Phenol (mg/100mg)	0.01± 0.005	0.01± 0.005	0.35±0.006
Flavonoid (mg/100mg)	7.62± 1.53	17.5± 0.285	14.4±5.09

ASK (*Ashikumna*), FPF (fresh pork fats), JAP (*Jangpangngngatsu*)

1.5 ml of 20% Sodium Carbonate. The absorbance was taken after an hour at 510 nm.

Total flavonoid content (TFC)assay

Theassay was performed following the technique of Sahreen and Khan (Sahreen *et al*. 2010) with slight modification. To 0.3 ml of methanolic dried sample extracts, 3.4 ml of 30% methanol, 0.15 ml of 0.5M sodium nitrite and 0.15 ml of 0.3M aluminum chloride were added. After 5 mins, 1 ml of 1M NaOH was added and the absorbance measured at 510 nm. The standard curve was prepared using Quercetin and expressed as mg Quercetin equivalents (QE) / 100 mg of extract.

DPPH radical scavenging assay

The assay was examined *in vitro* using DPPH radical as described by Aoshima, (Aoshima *et al*., 2004) with modification. Methanolic extracts (2-10 mg/ml) of various concentrations measuring 1.0 ml was mixed with 1.0 ml of 0.8 mM DPPH solution. The mixture was homogenized by shaking and kept in a dark place for 30 mins after which the absorbance was measured at 517 nm against a reagent blank (methanol). Ascorbic acid was used as standard and the percentage of inhibition was calculated using the formula

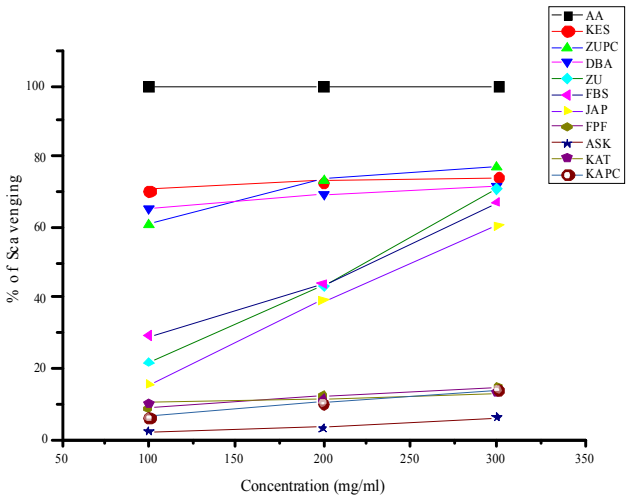

Fig. 1 : DPPH radical scavenging activity (%) of different extract concentration of the fermented food samples against Ascorbic acid on DPPH radicals.

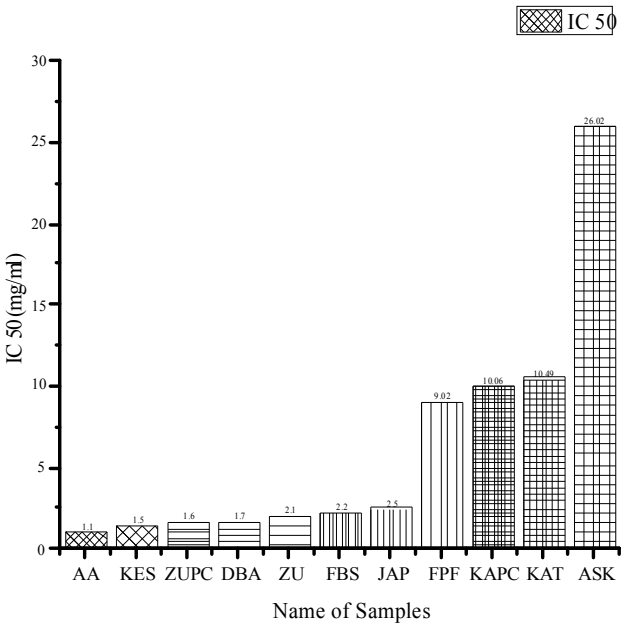

Fig. 2 : IC 50 values of the fermented foods against Ascorbic acid.

% Decolorization = (ABS control - ABS sample/ABS control) x 100

Where ABS control= absorbance of control at 517 nm

ABS sample= absorbance of sample at 517 nm

Calculation of IC.. value

IC.. values which shows 50% inhibition was calculated using regression analysis in MS excel.

Statistical analysis

Data are reported as mean ± standard deviation of three determinations.

RESULTS

General biochemical characteristics on fermented bamboo shoots of Nagaland

All the fermented bamboo shoots **(**Table 1) were found to be acidic in nature with pH ranging between 4.1- 5.4 except for the negative control (FBS) which was found to be neutral in nature (pH 6.9). Crude fiber was found to be highest in KES (15.95) while the carbohydrate and protein content was found to be the lowest in the particular sample (0.109 ± 0.01,0.028 ± 0.005). FBS (3.474 ± 0.096) showed the highest carbohydrate value while DBA (0.106 ± 0.011) showed the highest protein content among the samples studied. Reducing sugar exhibited the highest value in FBS (1.08 ± 0.001) and lowest in KES (0.038 ± 0.001) respectively. The total phenolic and flavonoid content was also found to be highest in KES (1.959 ± 0.9, 8.035 ± 1.175) which corresponds to its high antioxidant activity. It was also observed that in fermented bamboo shoots, differences in terms of physical and bio-chemical properties were observed in the sample against its negative control. The presence of ash and protein in minimal amount in the fermented bamboo shoot fits its use as a balanced nutritional food.

General biochemical characteristics on fermented rice beverage of Nagaland

Our result (Table 2) revealed that the average alcoholic content of KATwas 5 % which can be considered a safe level of consumption according to the guidelines given by World Health Organization (WHO). It was also observed that, even though the same method was adopted for

preparation of positive control, differences in terms of alcoholic content and bio- chemical properties appeared. The plausible reason for this variation might be attributed to differences in the variety of rice used in preparing the starters, their ratio and also the duration of the product stored.

General biochemical characteristics on other fermented foods of Nagaland, India

The details of fermented pork fats (ASK), its negative control (FPF) and fermented crab (JAP) are discussed in Table 3. The pH of JAP (7.1) revealed its neutral nature with low protein content (0.148 ± 0.09mg/100mg of FW) and high flavonoid content (14.461 ± 5.079mg/100mg of DW). ASK showed negligible difference against its negative control in all the biochemical parameters observed, with slightly higher values inclined towards fermented product except for flavonoids. It can be ascertained that fermentation of pork fats reduces its pH but undergoes moderate biochemical and physiochemical changes based on the parameters studied.

Antioxidant capacity and IC50 values of fermented food products of Nagaland, India

The radical scavenging activity of DPPH by various fermented foods analysed is shown in (**Fig.1)**. The extracts exhibited DPPH radical scavenging activity ranging between 100-300 mg/ml concentration and the IC50 values of KES, ZUPC, ZU, FBS, JAP, FPF, DBA ,KAPC, KAT and ASK was found to be 26.02, 1.7, 2.1, 2.5, 2.2, 9.02, 1.6 and 1.5 mg/ml respectively **(Fig.2).** KES exhibited the highest radical scavenging activity corresponding the highest phenolic content as well. The samples exhibitng the highest to the lowest activity were effective in the order KES> ZUPC> DBA> ZU> FBS> JAP>FPF>KAPC> KAT> ASK. Ascorbic acid was used as the standard and standard and all the extracts exhibited a dose dependent inhibition on the DPPH radical.

DISCUSSION

Our results revealed the acidic nature of the bamboo shoots which corresponds to the dominance of Lactic Acid bacteria reported from various studies (Badwaik *et al*. 2014). The presence of LAB also creates unique flavor profiles as a result of fermentation and enhance biochemical activities such as bacteriocins, antioxidants (flavones, phenols and steroids) etc that shows the highest antioxidant activity among the food samples reported from our study, (Sharma *et al*. 2020). In case of fermented rice beer, the difference between the positive control and the marketed product may be attributed to differences in the variety of rice used in preparing the starters, their ratio, the duration of the product stored that ultimately hosts diverse microbes. The fermentation of pork fats reduces its pH but undergoes moderate biochemical and physiochemical changes based on the parameters studied. This maybe due to the modest biochemical activities of the microbes present in ASK limited by the fat content of the substrate as microbes fail to accumulate lipids at high levels of fermentation.

CONCLUSION

Our study revealed that fermented foods of Nagaland can be further exploited for their health-giving attributes as they are highly valued for their promising prebiotic and probiotic prospects. However elevating the production of traditional fermented foods, emphasis should be placed on the role of the tribal communities associated with it and how they will benefit from the modifications. Furthermore, the palatability and sensory characteristics of progressive foods must take into consideration the local population preferences. At present, the fermented food products of Nagaland augments the culinary diversity, vitalizes the local economy and hearten the livelihood of the Nagas pre-eminent by their food culture.

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