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Development of Punugulu from foxtail millet (*Setaria italica*)

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Abstract
Punugulu is the delightful deep-fried dish from South Indian cuisine, traditionally made by black gram dhal and rice. Foxtail millet (*Setaria italica*) is an ancient crop that contain substantial amount of protein, fiber, minerals and phytochemicals. Fermentation has been found to enhance the bioavailability of nutrients by reducing anti-nutritional factors and improving its functional properties. This study was aim to develop Punugulu by partially replacing rice with foxtail millet. Six formulations (FMP1 to FMP6) were made, with varying proportions of foxtail millet flour (0% to 75%). The research also involved determining optimal fermentation time and analyzing the proximate composition. Fermentation time was fine-tuned and identified through a range of 6-24 hours, followed by sensory evaluations. Notably, Punugulu made using 45% foxtail millet and fermented for 12 hours received the highest sensory scores. As evident from the data of proximate composition, they are low-glycemic in nature when compared to traditional rice-based Punugulu due to their reduced carbohydrate (11.42%) and elevated fiber content (96.6%) and also rich in protein (27%), Iron (64.04%), Calcium (20.28%) and Phosphorus (27.05%).

Keywords: Foxtail millet (FM), *Setaria italica*, Fermentation, Punugulu, low glycemic index

1. Introduction
Millets are the 'Nutri-cereals' that contain comparatively high amount of fibre, vitamins and minerals than rice. *Setaria italica* which is commonly known as Foxtail millet (FM) is the world's second most cultivated millet; it possesses health-promoting properties owing to its unique protein composition containing high content of essential amino acids. The mature grains mainly consist of proline-rich, alcohol-soluble proteins (Prolamin) called Setarins, comprising about 60% of the total. It is rich in Vitamin B12 which is essential for maintaining a healthy heart and smooth functioning of the nervous system. A diet including FM may improve glycemic control and reduce insulin, cholesterol and fasting glucose in Type-2 diabetes patients [1, 2].
Punugulu, also familiar as Punukulu is a deep-fried delicacy which is a blend of key ingredients and selected spices that infuses mild aromatic flavours and it is prepared by using rice, black gram dhal (2:1) and spices [3]. As rice has high glycemic index, we have replaced with FM to bring down the carbohydrte level. Thus, it makes the developed product is low Glycemic than the standard Punugulu.
Fermentation is a biochemical process that utilizes microorganisms to partially oxidize sugars, producing energy, along with acids and alcohols as intermediate by-products. This process enhances the sensory and nutritional value of foods while extending their shelf life. Key microorganisms like lactobacillus species, *Streptococcus thermophilus*, *Lactococcus lactis* and *Bifidobacterium* are responsible for this process. Lactic acid bacteria not only facilitate fermentation but also prevent pathogen growth, degrade mycotoxins and offer probiotic benefits [4, 5]. This study was an attempt to develop low-GI naturally by traditional fermented product Punugulu by partially replacing rice with FM.

2. Objectives
- To develop Punugulu by partially replacing rice with FM and its organoleptic evaluation
- To determine the optimum fermentation time for FM Punugulu
- To analyze its Proximate composition

3. Materials and Methods

3.1. Raw materials

The research was carried out in the Department of Food Science and Nutrition, Yuvaraja's college, (Autonomous) University of Mysore, Mysuru. Raw materials viz., Rice, foxtail millet, black gram dhal and cumin seeds were procured from local market of Mysuru.

3.2. Method of preparation

Punugulu was prepared by soaking FM and black gram dhal for 4 hours. After rinsing and draining it was ground into a thick batter. The batter was allowed to ferment for 12 hours and then mixed thoroughly by adding cumin seeds and salt. Oil was heated in a kadai/shallow pot; batter was made into round balls, and deep-friedon medium flame until it turned golden brown and crisp.

3.3. Optimisation of fermentation time

The selected variations of foxtail millet and black gram dhal were soaked for 4 hours. Following the soaking process, they were ground into a paste and left to ferment at room temperature for different time: 6-24 hours. This fermentation period allows for the development of flavours and textures in the mixture, enhancing the overall quality of the final product.

3.4. Determination of batter volume and pH

The changes in volume and pH levels were assessed for each variation during the fermentation process at room temperature. pH values were assessed using pH meter, while changes in volume were determined by pouring the batter into a 250 ml measuring cylinder and recording the measurements after 6, 12, 18, and 24 hours.

3.5. Sensory Analysis of Punugulu

The sensory assessment of developed product was conducted to ascertain the level of acceptability concerning different attributes. The evaluation involved 30 Semi-trained panelists who rated the product using a 9-point hedonic scale, and the final score was derived from their collective feedback.

3.6. Nutritional analysis of Punugulu

The nutritional composition of the chosen variation (45%) of FM Punugulu and the control was determined in triplicates using the established A.O.A.C. (1980) methods [6]. To assess the constituents, the moisture content was estimated via hot air oven at 98 to 100 °C, protein content was determined using the Micro-Kjeldhal method for total nitrogen, ash percentage was derived through high-temperature incineration in a muffle furnace, and fat content was estimated using the Soxhlet apparatus [7, 8]. Crude fiber content was determined using a crude fiber analyzer. The carbohydrate content was calculated by subtracting the sum of moisture, protein, fat, and ash content from 100 for every 100 g of the sample. Additionally, minerals such as calcium, iron, and phosphorous were analyzed through Atomic absorption spectrometry (AAS), known for its precision and accuracy [9-12].

3.7. Statistical analysis

The gathered data was statistically [13], along with the application of Duncan's multiple range test to determine significance, with a level set at $p \leq 0.05$ [14].

4. Formulation of the product

Table 1: Formulation of product (ingredients g/100gm) for preparation of Foxtail Millet Punugulu

Ingredients	0%	15%	30%	45%	60%	75%
Foxtail millet (g)	-	15	30	45	60	75
Rice (g)	75	60	45	30	15	-
Black gram dhal (g)	25	25	25	25	25	25
Cumin seeds (g)	1	1	1	1	1	1
Salt (g)	2	2	2	2	2	2

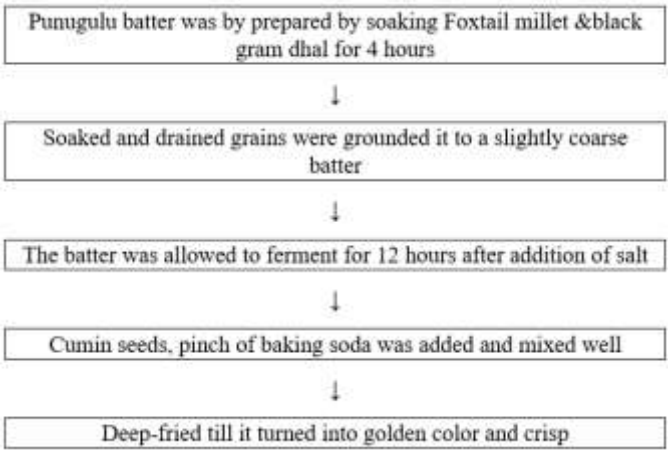

Fig 3: Flow chart for the preparation of Foxtail Millet Punugulu

Fig 4: Different variations of Punugulu developed from Foxtail millet in comparison of rice Punugulu

Fig 5: Foxtail millet Punugulu developed by varying the time of fermentation

5. Results and Discussion

The study was carried out to standardize the fermentation time and quality evaluation of Punugulu prepared by replacing rice with FM and its nutritional analysis.

Table 2: pH of Foxtail Millet Punugulu batter values are mean ± SD (n=20) *p value < 0.05 (Holm Sidak method)

Fermentation time / Variations	0 hrs	6 hrs	12 hrs	18 hrs	24 hrs
Standard (SP1)	6.8±0.22	6.2±0.37	5.6±0.20	5.1±0.16	4.2±0.08*
SP2 (20%)	6.7±0.57	6.2±0.48	5.6±0.56	5.0±0.31	4.2±0.10*
SP3 (40%)	6.8±0.63	6.3±0.81	5.4±0.43*	5.1±0.12	4.4±0.05*
SP4 (60%)	6.8±0.72	6.2±0.52	5.4±0.28*	5.2±0.51	4.2±0.12*
SP5 (80%)	6.7±0.34	6.2±0.21	5.5±0.35	5.2±0.26	4.1±0.19*
SP6 (100%)	6.8±0.18	6.4±0.19	5.4±0.64*	5.1±0.17	4.3±0.15*

5.1 pH and volume of Sorghum Punugulu batter

The pH values of the Punugulu batter exhibited a range of 5.0 to 6.0 during different fermentation periods (6, 12, 18, 24 h). The initial pH was 6.8 – 6.7 and as fermentation progressed, the pH dropped to 5.2-5.0 till 18 h. At 24 hrs it dropped to pH 4.4-4.1 as detailed in Table 2. The experiment was conducted in triplicates and the obtained values were calculated statistically by standard deviation. This rise in acidity is primarily attributed to the activity of Lactic acid bacteria, which generate lactic acid, thereby decreasing the pH. The batter also experienced an incremental increase in volume, starting from an initial 40.5±.50 ml for all variations, as indicated in Table 3. This expansion is attributed to the integration of lactic acid bacteria into the batter during fermentation and the subsequent entrapment of air.

Table 3: Determination of volume of Foxtail Millet Punugulu batter values are mean ± SD (n=20) *p value < 0.05 (Holm Sidak method), h-hour

Variations of Punugulu with different fermentation time	Volume of batter (ml)				
	0 h	6 h	12 h	16 h	24 h
Standard (STP1)	40.50±0.12	50.60±0.74	56.00±0.48	58.15±0.83	60.35±0.26
FMP1 (20%)	40.81±0.42	52.00±0.56	55.60±0.76	56.20±0.41	58.00±0.18
FMP2 (40%)	41.00±0.69	55.40±0.81	65.30±0.63	75.60±0.39	78.25±0.45
FMP3 (60%)	40.00±0.83	53.10±0.62	57.00±0.25	59.25±0.72	62.50±0.27
FMP4 (80%)	40.00±0.29	48.20±0.38	50.30±0.91	55.00±0.60	57.30±0.46
FMP5 (100%)	40.60±0.47	42.40±0.70	42.80±0.45	45.55±0.72	47.20±0.58

5.2 Sensory evaluation of Foxtail Millet Punugulu prepared by varying proportions of rice

The data pertaining to the effect of incorporation of various levels of FM on sensory attributes of Punugulu are shown in Table 4. Standard punugulu obtained maximum score for its superior organoleptic properties. The scores obtained for all sensory attributes for FMP2, FMP3 and FMP4 were almost similar on par with control. FMP5 and FMP6 showed decreased scores and were less acceptable compared to other variations. The variations FMP2, FMP3 and FMP4 were crisp in texture it was due to deep frying in oil. Compare to all the variations FMP3 was more acceptable on par with control in terms of sensory attributes.

Table 4: Sensory scores of different variations of Punugulu developed from foxtail. Values are mean ± SD (n=20) *p value < 0.05 (Holm Sidak method)

Parameter	Standard Punugulu	FMP2 15%	FMP2 30%	FMP3 45%	FMP4 60%	FMP5 75%
Appearance	8.82±0.39	8.29±0.58	8.19±0.58	8.47±0.21*	7.32±0.93	6.88±0.80
Colour	8.82±0.35	8.17±0.72	8.17±0.72	8.41±0.79*	7.29±0.91*	6.54±0.87*
Texture	8.70±0.58	8.05±0.74	8.14±0.74	8.29±0.77*	7.17±0.95*	6.52±0.79
Flavour	8.58±0.61	7.5±0.79*	8.01±0.79	8.17±0.88*	7.31±0.86*	6.35±0.31*
Taste	8.70±0.58	7.8±0.69*	7.98±0.69	8.15±0.80*	7.29±0.98	6.47±0.71*
Overall acceptability	8.75±0.55	8.05±0.65	8.12±0.31	8.23±0.75*	7.29±0.92	6.43±0.23*

5.3 Sensory evaluation of Punugulu prepared by varying fermentation time

Specific fermentation time improved sensory qualities, with the highest scores for a 12h fermented Punugulu and hence, the best fermentation time for Punugulu was 12 h. Shorter fermentation time (6 h) lowered the sensory scores, while excessive fermentation negatively impacted the last variation as a result of hyper-fermentation.

Table 5: Sensory scores of Punugulu developed from Foxtail by varying fermentation time. Values are mean ± SD (n=20) *p value < 0.05 (Holm Sidak method)

Parameter	Standard Punugulu	FMP(6hr)	FMP (12hr)	FMP(18hr)	FMP(24hr)
Appearance	8.62±0.39	8.19±0.58	8.46±0.65*	8.06±0.75*	7.36±0.03*
Colour	8.82±0.35	8.17±0.72	8.71±0.29*	8.01±0.89*	7.72±0.01*
Texture	8.40±0.58	8.25±0.74	8.39±0.17*	7.92±0.56*	7.17±0.05*
Flavour	8.98±0.61	8.05±0.79	8.17±0.58*	7.86±0.51	7.69±0.56
Taste	8.60±0.58	8.28±0.69	8.35±0.30*	7.82±0.20*	7.57±0.88
Overall acceptability	8.75±0.55	8.15±0.65	8.23±0.65*	7.7±0.58*	7.61±0.42

5.4 Proximate Composition of Punugulu

A thorough analysis of proximate composition of selected Punugulu (FMP3) and control version was conducted and the results have been presented in Table 6. Notably, the moisture content across all variations of Punugulu remained consistent. Interestingly, FMP3 exhibited higher protein and fat content compared to the control, while showing a decrease in carbohydrate content. Furthermore, FM Punugulu had an elevation in key nutrients such as fibre, ash, iron and phosphorus content.

Table 6: Proximate composition of Punugulu (Control and STP3) developed from Foxtail. Values are mean ± SD (n=3) *p value < 0.05 (Holm Sidak method)

Nutrients	Standard Punugulu 0%	FMP3 (45%)
Energy (kcal)	393.70±3.11	366.36±1.81
Protein (g)	11.1±0.71	14.07±0.04*
Fat (g)	10.3±0.15	9.2 ± 0.83
Fibre (g)	0.25±0.13	2.44 ± 0.28*
Moisture (%)	13.00±2.12	11.87 ± 0.34
Ash (g)	1.25±0.11	2.47 ± 0.1
Carbohydrate (g)	64.15±1.10	56.82±1.62*
Phosphorus (mg)	216.25±1.19	274.75±2.01*
Iron (mg)	1.475±0.11	2.42±0.32*
Calcium	46.10±0.19	55.45±0.02*

6. Conclusion

The current research endeavours was mainly focused on enhancing the nutritional value of Punugulu and this led to the incorporation of foxtail millet in variable proportions. The results were encouraging, as this modified version of FM Punugulu (45%) fermented for 12 hours garnered approval for its sensory characteristics and nutritional benefits. Fermentation contributed for its unique flavour profile and resulted in rise of batter volume as well. When compared to standard Punugulu, the selected variation (45%) of FM Punugulu had enhanced level of Protein (27%), fibre (96.6%) Iron (64.04%), phosphorus (27.05%) and calcium (20.28%) content. FM Punugulu has remarkable attributes like high fibre content and notably low carbohydrate (11.42%) profile. These qualities contribute to lowered glycemic index, an important factor in supporting weight management and regulating blood glucose levels for type-2 diabetes patients. Thus, FM punugulu would be the best option for those who seek both flavour and well-being in their food choices.

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