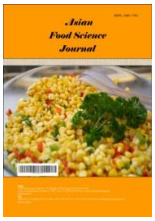

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Studies on an Alternative Method for Preparation and Fermentation of *idlis* Made from Proso Millet (*Panicum miliaceum* L.), Horsegram (*Macrotyloma uniflorum (Lam) Verdc.*) and Fenugreek (*Trigonella foenum-graecum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author AS performed the experiments and developed the manuscript in consultation with other authors. Author SJ verified the analytical methods and supervised the work of author AS. Authors KP and RRM contributed to the design and implementation of the research. All authors read and approved the final manuscript.

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ABSTRACT

The present study was undertaken to develop an alternative *idli* from proso millet with horse gram and fenugreek seeds. Two types of wet batters (Batter A & Batter B) were prepared by soaking the ingredients proso millet, horse gram and fenugreek separately (in the ratio by weight 7:2:1) in excess water for 8 hours at room temperature (25±2ºC). In method A, the ingredients were soaked in excess water and whereas in method B, fenugreek was soaked in buttermilk. The soaked ingredients were ground separately with adequate amount of water using a grinder and mixed with the addition of 1% salt. The resulting batter was fermented for 10 hours at room temperature. The batter was analyzed for its microbial load (Total Plate Count, Lactobacillus and Yeast and Mold)

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and physico-chemical properties (height rise, pH and titratable acidity) by drawing aliquots at fixed intervals. Microbial analysis revealed maximum load at 6th hour of fermentation for TPC, LAB and Yeast growth. Suppressing activity of the organisms was seen after this stage and there was a sharp decline in microbial growth (LAB and Yeast). The pH and total titratable acidity ranged between 4.88-6.20 and 0.19–0.51% respectively. The nutritional analysis and sensorial acceptance of the *idli* formulated with both the batters were also studied. Both the *idlis* were found to have a two-fold increase in protein content and a 50% reduction in carbohydrate content when compared with the traditional rice *idli*. The sensory analysis was conducted with 100 untrained panelists and the results revealed that *idli* prepared from Batter A was preferred. A dry ready to cook *idli* mix was also formulated and was studied for the above said properties. The study highlights that proso millet, horse gram and fenugreek can be used as an effective alternative for preparation of *idli*.

Keywords: Proso millets; horse gram; fenugreek; fermented food; traditional idli.

1. INTRODUCTION

Idli, a popular traditional cereal / legume-based naturally fermented steamed product, has a soft and spongy texture which is widely consumed as a breakfast food in India [1]. *Idli* makes an important contribution to the vegetarian diet as a source of protein, calories and vitamins, especially B-complex vitamins, compared to the raw unfermented ingredients [2]. Millets, the world's earliest food plants used by human, and are often grown in difficult conditions [3]. This nutrient house; also termed as poor man's food; originated in South East Asia and is found in India, America, Australia, and South Africa. In contrast, Millets are a major source of energy and protein [4]. They are unique among the cereals because of their richness in calcium, dietary fiber, and protein [5]. Hence, Millets can be used as a substitute for the staple grains rice or wheat. They can also be used to substitute the rice or wheat content of fermented foods like *dosa* or *idli* [6].

Proso millet *(Panicum miliaceum* L.*)* is a warm season crop, best identified by the ligule having a tuft of dense hair, and with a growing season of 60–100 days [7]. It is a highly nutritious cereal grain used for human consumption, bird seed, and/or ethanol production. Unique characteristics, such as drought and heat tolerance, make proso millet a promising alternative cash crop [8].

Horse gram (*Macrotyloma uniflorum (Lam) Verdc.*), a pulse crop grown under a wide range of adverse climatic conditions, is a rich source of protein (23.89-25.91%), carbohydrates (59.3%), essential amino acids, energy, iron, molybdenum, minerals and vitamins, but with a low content of lipid (0.53-0.63%) [9]. The pulse demonstrates hypoglycemic and hypolipidemic activity. The use of dry seeds as human food in large populations is limited due to its poor cooking quality [10].

Fenugreek is an aromatic plant which belongs to the family *Fabaceae* [11]. It is used both as an herb (leaves) and as a spice (seeds) [10]. It is cultivated worldwide as a semi-arid crop. Fenugreek seeds are rich in Folic acid, Vitamin A, Vitamin K, Vitamin C and are a storehouse of minerals such as iron, potassium, and calcium. It also contains high protein and nicotinic acid content. It is frequently used in curry and also as a main ingredient in the *idli* preparation as it improves the texture by adding sponginess to the steamed product [12].

The present study investigates the alternative method for producing *idli* batter utilizing proso millet, horse gram and fenugreek. The objective was to study the behavior of *idli* batter, prepared by excluding rice and black gram dhal. The millets were combined with horse gram in order to improve the nutrient balance. Fenugreek seeds are used to enhance the fermentation process [13]. The study also analyses the physical, chemical, and microbial characteristics of the formulated batter and ready to cook mix.

2. MATERIALS AND METHODS

The main ingredients, proso millet, horse gram and fenugreek were procured from a local market, cleaned, and stored at ambient conditions until further use.

2.1 Preparation of *Idli* Batter

The wet batter was prepared by two different methods A & B. In method A, proso millet, horse gram and fenugreek (in the ratio 7:2:1) were

soaked in excess water for 8 hours at room temperature, whereas in method B, Fenugreek alone was separately soaked in excess buttermilk. The soaked ingredients were ground separately using a grinder with adequate amount of water and then mixed with the addition of 1% common salt [13]. The resulting batter was allowed to ferment and was studied for a period of 10 hours. In order to remove the bitterness, two variations were adopted - 3% of palm sugar was added to the fermented Batter A and in Batter B, fenugreek was soaked in excess buttermilk before grinding.

For the formulation of ready to cook dry mix, the ingredients were soaked for 8 hours in excess water and sun dried for 3 days. The dried grains were powdered and mixed along with 1.5 % of baking powder and 3% salt [14].

2.2 Physical and Microbial Properties of Developed Batters before and after Fermentation

Various physical properties such as pH, titratable acidity and height increase were studied for both the batters at fixed intervals during fermentation (once in every 2 hrs) under controlled conditions [15,16]. Microbial parameters such as determining the colony counts of aerobic mesophilic bacteria, lactic acid bacteria (LAB) and yeasts by pour plate technique on plate count agar, lactic agar and yeast glucose chloramphenicol agar respectively were determined at a regular time interval of 0, 4^{th}, 6^{th}, 8^{th} and 10^{th} hour [17]. For the dry mix; properties such as bulk density, water absorption capacity, oil absorption capacity, swelling power and solubility and alcoholic acidity were studied. Microbial parameters similar to the wet batter were studied at 0, 30 and 60 minutes.

2.3 Nutritive Value of the Developed *Idlis*

Nutrient analysis (moisture, ash, fat, protein, crude fiber, carbohydrate, and energy value) was carried out for the wet batters A & B, and the dry mix, (proso millet, fenugreek, and horse gram) using standard procedures as per FSSAI methods of analysis [18]. The formulated batter was also compared with traditional *idli* batter containing rice and black gram dhal. Protein was determined by the macro Kjeldhal method using a conversion factor of 6.25; fat using the Soxhlet apparatus and petroleum ether BP 60 to 70 degrees centigrade; and fiber by the AOAC method 991.43.

2.4 Organoleptic Evaluation

The sensorial acceptability of the formulated *idlis* was studied with 100 untrained panelists. The panelists evaluated the *idlis* for the factors - appearance, colour, flavor, taste, texture, and overall acceptability on a 5-point hedonic scale. 5 to 1 representing – like extremely and dislike extremely, respectively. The quality parameters were quantified, and the mean scores were evaluated.

3. RESULTS AND DISCUSSION

3.1 Height Increase, pH and Titratable Acidity of the Batter and Physical Properties of Dry Mix

The main parameter for dough rising was determined by measurement using a vernier caliper using standard measurement techniques [19]. From the study, it is recorded that considerable increase in batter height was observed during the fermentation period of 4 to 6 hours in Batter A and from 4 to 7 hours in Batter B as given in (Table 1). After this period, there was a steady decline in the height of both batters by the end of 10 hours of study. The reason for increase in batter volume can be attributed to the microbial growth and secretion of enzymes, which catalyze the hydrolysis of carbohydrates, lipids, proteins, anti-nutritional, and toxic factors [20].

The pH value of batter determined using a pH meter, at different fermentation time was found to be between 6.20 and 4.71. There is an increasing trend of acidity level, i.e., decrease in pH value with fermentation time, irrespective of batter. Acidification and leavening are the two most important changes that occur during fermentation [21].

The percent total acidity of *idli* batter estimated by titration using standard sodium hydroxide solution, at different fermentation time ranged between 0.19 and 0.51%.

The decrease in the height of the batters from the 7^{th} hour of fermentation reveals the suppressing activity of the microbial load. This trend is also evident from (Table 4) where there is a sharp decline in LAB and yeast count. Similar results were seen in dry mix sample as described in (Table 2). This increase in acidity in a shorter period was due to the addition of

Table 1. Results of pH, titratable acidity and volume increase of wet batter samples

Time	pH		Titratable Acidity (%)		Height increase (cm)	
	Batter A	Batter B	Batter A	Batter B	Batter A	Batter B
0th hour	6.20	5.80	0.21	0.23	0 cm	0 cm
1st hour	6.15	5.80	0.23	0.24	0.2 cm	0 cm
2nd hour	6.08	5.75	0.26	0.27	0.5 cm	0.1 cm
3rd hour	5.98	5.70	0.33	0.32	0.8 cm	0.1 cm
4th hour	5.63	5.54	0.37	0.37	1.1 cm	0.7 cm
5th hour	5.34	5.16	0.39	0.42	2.5 cm	1.5 cm
6th hour	4.99	4.88	0.44	0.48	4.6 cm	2.8 cm
7th hour	4.99	4.71	0.43	0.51	4.6 cm	3.2 cm
8th hour	5.33	4.91	0.32	0.42	3 cm	2.6 cm
9th hour	5.48	5.17	0.26	0.32	Suppressing activity	Suppressing activity
10th hour	5.63	5.45	0.19	0.28	Suppressing activity	Suppressing activity

Table 2. Results of pH, titratable acidity, and volume increase of reconstituted dry mix batter

Time	pH	Titratable acidity (%)	Height increase (cm)
0th hour	5.98	0.21	0 cm
½ hour	5.76	0.23	1.4 cm
1st hour	5.77	0.23	1.4 cm

bicarbonates, which supported faster fermentation process [22]. The dry mix was seen to have a solubility of 28% and acidity swelling power of 3.08 g/g as seen from (Table 3).

Table 3. Physical properties of dry mix

Physical parameters of dry mix	Results
Bulk Density	0.80 g/ml
Water Absorption Capacity	0.65 g/g
Oil Absorption Capacity	0.28 g/g
Swelling Power	3.08 (g/g)
Solubility	28 %

3.2 Microbial Analysis of Wet Batter Samples

The microbial colony counts of aerobic bacteria, lactobacilli, and yeast count of the two formulated wet batter were in the same trend as seen from (Table 4). The microbial growth of the two formulations increased from the start at 0th hour to the 4th hour marginally and more significantly from the 4th hour to 6th hour depicting the increase in fermentation [23]. During the 8th hour of colony count, the microbial count decreased which implied the initial stage of suppressing activity of fermentation process of the two formulated wet batter samples. Similarly, there is a decrease in LAB and yeast growth as seen from (Table 4). The 10th hour of colony count also showed suppressing activity of wet batter samples and no growth of LAB and yeast. This trend is applicable for both the formulated wet batter samples. Therefore, it is concluded that the optimal fermentation time is between four to six hours under ambient conditions for all the variants. Steaming for *idlis* before or after optimal times may not give the desired texture of the product.

3.3 Microbial Analysis of Dry Batter Samples

The microbial analysis of the reconstituted dry mix batter revealed the presence of bacteria and yeast and is listed in Table 5. The total aerobic bacterial count of the batter marginally increased from 0 to 30 mins and the count was stabilized in the 60 minutes of observation. There was no lactic acid bacterial count 0 to 60 minutes of observation. The yeast count of the batter increased from 0 to 30 minutes and the count decreased slightly during the 60th minute of observation. Reconstitution of the dry mix and insufficient time and availability of substrate, without proper aeration and time for fermentation and steaming, does not yield the desired quality of *idlis*. Moreover, there could be a change in the substrate composition, which could have altered the availability of nutrients for the growth of bacteria [24]. Batter fermentation requires the presence of oligosaccharides with galactose residues for growth of bacteria, which may have undergone changes due to prolonged drying [25 and 26]. This could be a probable reason why

Table 4. Results of microbial analysis of wet batter samples A & B

Time (hour)	Total aerobic bacterial count (log cfu/g batter)		Lactic bacteria count (log cfu/g batter)		Yeast count (log cfu/g batter)	
	Batter A	Batter B	Batter A	Batter B	Batter A	Batter B
0	8.51	8.59	No colonies		8.71	7.60
4	8.85	8.85	8.84	7.48	8.84	7.91
6	9.14	9.47	9.09	8.32	9.05	8.63
8	8.83	9.22	8.14	7.78	No growth	8.43
10	8.56	8.81	No colonies		No growth	

Table 5. Microbial analysis of reconstituted dry mix batter

Time (minutes)	TPC (log cfu/g batter)	LAB (log cfu/g batter	YC (log cfu/g batter)
0	8.65		8.68
30	9.54	No growth	8.89
60	9.32		8.84

Table 6. Proximate analysis

Proximate analysis	Proso millet	Horse gram	Fenugreek	Batter A	Batter B	Reconstituted Dry mix batter	Traditional *Idli* Batter
Moisture content (%)	11.9±0.01	11.8±0.01	13.7±0.01	66.18±0.02	68.56 ±0.02	69.92 ±0.03	67.15±0.03
Total protein (%)	12.5±0.01	22.0±0.01	26.2±0.01	10.33±0.01	9.65 ±0.02	9.82 ±0.03	5.03±0.01
Total fat (%)	1.1±0.01	0.5±0.01	5.8±0.02	1.00±0.01	0.50 ±0.02	1.50 ±0.02	0.05±0.00
Crude fibre (%)	2.2±0.05	5.3±0.01	7.2±0.01	1.48±0.02	1.60 ±0.01	1.43 ±0.02	0.95±0.02
Total ash (%)	1.9±0.01	3.2±0.01	3.0±0.01	8.20±0.02	4.80 ±0.01	7.70 ±0.02	1.13±0.01
Total carbohydrate (%)	70.4±0.06	57.2±0.04	44.1±0.04	12.81±0.08	14.89 ±0.06	9.63 ±0.03	25.69±0.03
Energy value (Kcal)	341±0.20	321±0.10	333±0.20	101.56±0.19	102.66±0.10	91.30 ±0.10	123.2±0.10

Table 7. Mean Organoleptic scores of formulated *idlis*

Formulated *Idli*	Appearance	Colour	Texture	Taste	Flavour	Mouth feel	Overall Acceptability
Batter A	3.79 ±0.03	3.37±0.01	3.81±0.02	3.31±0.03	3.18±0.02	3.21±0.01	3.46 ±0.03
Batter B	3.76 ±0.04	3.27±0.01	3.74±0.02	2.87±0.01	2.84±0.04	2.87±0.01	3.18 ±0.02
Reconstituted Dry Mix	3.72 ±0.02	3.17±0.01	3.65±0.03	3.12±0.01	3.16±0.03	3.17±0.01	3.24 ±0.02

instant *idli* mixes have been a failure in the market as the required texture is not achieved.

3.4 Nutritive Value of the Developed *Idlis*

The data pertaining to the nutritive value of the ingredients and formulated products are depicted in (Table 6). From the following proximate results, it is evident that all the raw materials in the millet formulations used had high protein content and the resulting batter also revealed a high protein and a low carbohydrate content [27]. As seen from the table, there is a two-fold increase in the protein content of the formulated batter when compared with the traditional *idli* batter. The carbohydrate content has reduced to nearly 50% due to the utilization of millets in the

formulated batters. With the objective of providing a more nutrient idli with enhanced protein and lower carbohydrates, the formulations were developed. The wet batters with proso millets provided the necessary substrate for fermentation by bacteria and could yield soft idlis. However, the reconstituted dry mix batter had a lower carbohydrate level indicating its unavailability qualitatively and quantitatively for fermentation by bacteria to produce soft *idlis*.

3.5 Organoleptic Evaluation

The mean acceptability scores obtained by the sensory evaluation of formulated *idlis* are tabulated in Table 7. The batter A *idli* showed a higher acceptability factor when compared to batter B *idli* due to the slight bitterness taste in the latter. The formulated *idlis* were well accepted by the untrained panelists. Batter A *idlis* had the optimal conditions for formulation, processing, and acceptability tests. Batter B had a slight bitterness due to the presence of differentially treated fenugreek. Batter A had palm sugar added, which may have reduced or neutralized the bitterness and also aided fermentation. The dry mix batter had fenugreek in its composition as described above, but on soaking and drying for long periods might have resulted in the loss of its pungency. Not much difference was observed in the texture (porosity) of idlis, but the same from batter A did give a better texture. When served hot, without any accessory servings, the overall acceptability was good, but on storage for about an hour the idlis from the instant mix revealed hardness in the texture, shrinkage in porosity, rendering it unacceptable. This is due to the shrinkage of the *idlis* observed and loss of moisture and the subsequent closure of the porosity holes.

4. CONCLUSION

An alternative method for preparation and fermentation of traditional *idli* batter was developed using proso millet, horse gram and fenugreek and studied for its properties. The batter developed had a two fold increase in protein and low carbohydrate content than the traditional *idli* batter. Though the results of sensory analysis were on similar grounds for the three variants, Batter A was most acceptable. Thus, the Batter A formulation proves to be an effective alternative for traditional *idli* with a good sensorial acceptance.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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