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Hastening of idli batter fermentation using Pearl millet resistant starch

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Abstract: **Idli is a traditional fermented rice and black gram based food. Idli batter was prepared by soaking of parboiled rice and decorticated black gram for 6 to 8 h at 30±10C in water. The present study is focused on hastening of fermentation process in idli batter by incorporation of pearl millet resistant starch (PMRS). Pearl millet resistant starch (5% and 10%) was incorporated into idli batter and its quality was evaluated. Control and resistant starch incorporated idli batter were fermented at room temperature, physico- chemical and microbiological properties were analyzed. Nutrients, texture and sensory evaluation were carried out for control idli as well as RS incorporated idli . The pH and total titratable acidity of control batter at 12 h was 5.70 and 0.20% respectively, however RS 5% and 10% incorporated batter were found to be 5.65, 0.31% and 5.61, 0.35% respectively. Lactic Acid Bacteria (LAB) count was increased in RS (Resistant starch) incorporated batter. RS incorporated idli had higher protein and fiber content compared to control idli. Incorporation of RS at 5% and 10% in idli batter improved the texture and appearance of the idli and it also had better nutritional quality compared to control idli.**

Keywords: **Idli batter; incorporation of pearl millet resistant starch; fermentation; nutrition; sensory evaluation.**

1. INTRODUCTION

Cereals are used world –wide as staple food, they are considered to be the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fiber for people all over the India. Most commonly cereals are utilize in combination with legumes to improve the overall protein quality of the fermented products. This combination can be replaced by the small seeded grains that are known as millet in breakfast food, convenience foods, and snack foods (FAO 2012). Resistant starch has been introduced in recent years as a food ingredient important to human nutrition, because consumers are demanding functional foods, i.e. foods with nutraceutical properties (Fuentes-Zaragoza et.al 2011). Idli is rice based steamed product made from a fermentedbatter comprising of rice and black gram in the ratio of 3:1. It has acharacteristic sour taste and a soft, spongy texture. It is very popular insouth India and is usually consumed for breakfast or as an evening snack (Nishaet.al., 2005).The biochemical changes occur during natural fermentation include increase in non-protein nitrogen, total acids, soluble solids, methionine, cystine and a decrease in reducingsugars, pH and soluble nitrogen (Desikachar et al. 1960 and Steinkraus et al. 1967).During fermentation of idli batter overnight, the naturally occurring microorganisms viz. Leuconostoc mesenteroides and Streptoccous thermophilus in grains/legumes/utensils grow rapidly, outnumbering the initial contaminants and dominating the fermentation. These microorganisms produce lactic acid (≥1.0%) and carbondioxide that make the batter anaerobic and leaven the product. Several aspects such as effect of raw materials, effect of fermentation or processing temperature and microorganisms involved in biochemical and nutritive changes have been investigated. Two significant changes occurring in idli batter fermentation are leavening and acidification. These two parameters have been used as the criteria for judging the progress of fermentation (Renuet.al 2000). Production of *idli* batter is a time consuming process sometimes requiring 24 h to get the desired quality of *idli* and hence hastening of fermentation process is an important parameter. Hence, the prime objective of this study was to hasten the fermentation process of idli batter using pearl millet resistant starch.

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2. MATERIALS AND METHODS

2.1 Procurement of raw materials

Pearl Millet grains (CO10), rice (T4M13) and black gram dhal (VRB8) were procured from Tamil Nadu Agricultural University, Coimbatore. Chemicals like sodium chloride (NaCl) sodium hydroxide (NaOH) and phenolphthalein (Analytical Grade) were purchased from Nice chemicals Kochi, India. De Man Rogosa and Sharpe (MRS) Agar, Sarbouraud Dextrose Agar (SDA)was purchased from Sigma- Aldrich.

2.2 Isolation of Pearl millet starch

Millet grains were steeped in distilled water (1:2) containing 0.01% sodium azide to inhibit microbial growth at 4°C for 24 h. The excess water was decanted, steeped and the washed grains were ground in a waring blender with sufficient water. The slurry was sieved on 85 mesh nylon bolting cloth. The left-over (hulls, germ and endosperm) was re-slurried with water to float off the germ and hulls. The grinding, sieving and regrinding of the left over endosperm particles had been repeated until the left-over was essentially free of starch. The starch-protein slurry was centrifuged at 2000 rpm for 20 min. The supernatant had been discarded and the protein layer on top of the starch was removed with spatula. The starch was washed repeatedly by re-dispersing in distilled water and centrifuging until it appeared clean. The cleaned starch was air-dried on a glass plate for 12 h, re-dispersed in water and wet-sieved through 100-mesh screen

(Bhupenderet al., 2013).

2.3 Preparation of Resistant starch

Pearl millet starch (10 g) was mixed with 40 mL of distilled water (starch:water 1:4) and the mixture was then autoclaved at 145^0C for 30 min. The autoclaved starch was allowed to cool to room temperature and then stored at 4^0C for various periods of time (24, 48 and 72 h).The autoclaving-cooling cycle was repeated three times, and then oven dried at 45^0C and grounded, sifted through 212 µm sieve (Zhao and Lin 2009).

2.4 Preparation of 'idli' batter

Rice and split dehulled black gram, in ratio of 3:1 (the ratio was selected based on prior of standardization studies), were soaked separately for six to eight hours. Idli batter was prepared by grinding wet rice to a coarse consistency and wet dehulled black gram to a smooth consistency, separately in a kitchen blender. Both were then mixed together with common salt (2% w/w). The batter was then transferred to a stainless steel vessel, covered with a lid and fermented ambient temperature for 12 h (Jama and Varadaraj 1999).

2.5 Incorporation of RS in'idli' batter

1. Rice 75g + Black gram 25 g (Control)

2. Rice 75 g + Black gram 25 g+ 5%RS

3. Rice 75 g+ Black gram 25 g+ 10% RS.

2.6 Analyses of idli batter

2.6.1 Determination of idli batter properties

The pH of the samples was measured using a pH meter. Titratable acidity was determined by the following method. Ten g of control and fermented batter was taken in a 100 ml conical flask to which 20 ml of distilled water was added. After adding 3–4 drops of phenolphthalein, the contents were mixed well and titrated against 0.1 N NaOH, an end point of pale pink color was appeared and expressed as % lactic acid produced (AOAC 2011).Bulk density was calculated as the ratio of mass to volume of the idli batter. Flow behavior index was determined using a viscometer (Brookfield, DV-E).

2.7 Microbiological analysis

The viable count of Lactic acid bacteria (LAB), mesophilic bacteria and yeast and mold of the naturally fermented 'idli' batter (control and RS) was estimated by the following methods. 10 g of sample was homogenized with 90 ml of sterile diluents (0.85% NaCl) for 2 min in a shaker at normal speed. Ten fold serial dilutions were prepared and pour plated on MRS agar for the enumeration of LAB. Spread plate technique was employed to determine the counts of total mesophilic bacteria and yeast and molds using Nutrient agar (NA) and Potato dextrose agar (PDA) respectively (John et al., 1960).

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2.8 Preparation of idli

The 12 h fermented idli batter of both control and RS incorporated idli batter were cooked by steaming for 15 min in idli moulds and the idlis were studied for the following characteristics.

2.8.1 Texture analysis of idli

The parameters like hardness, stickiness, springiness, cohesiveness, stringiness, chewiness, adhesiveness and gumminess of the idli were measured using aTAXT2 Texture Analyzer (Stable Micro system, USA) equipped with the AACC 26 mm cylindrical probe (P/26R). Firmness is defined as the force (in grams, kilograms or Newtons) required for penetrating the product.

2.8.2 Nutrient composition of Idli

Moisture and ash were estimated by AOAC method, protein by Micro-kjeldhal method, fat by Soxhlet extraction method using petroleum ether, crude fibre by AOAC method, carbohydrate by the method of Southgate (1991).Apparent amylose and total starch were estimated by the method of Williams (1970).

2.8.3 Sensory evaluation of Idli

Quality is the ultimate criterion for the desirability of any food product. Organoleptic properties of idli were evaluated by a panel of 25 semi-trained members. A nine point score card was developed on the basis of numerical rating scale and the samples were tested for their organoleptic properties namely appearance, colour, texture, flavor and taste.

2.9 Statistical analysis

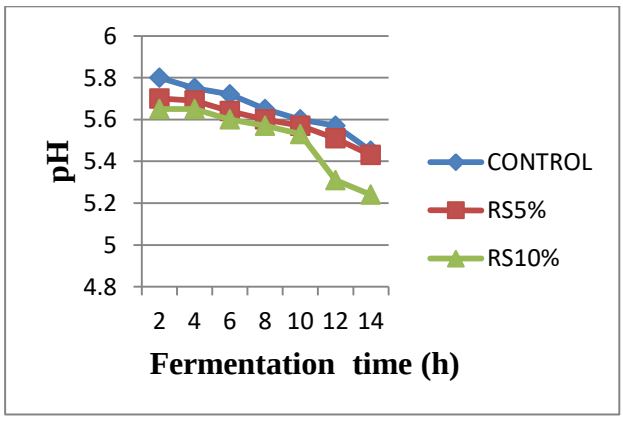
All the experiments were conducted in three replicates and statistical analysis was performed using SPSS version 18.0 software. One way analysis of variance (ANOVA) was used to determine the significant differences between means, with the significance level at5% ($P<0.05$).

3. RESULTS AND DISCUSSION

Idli batter properties

pH and Titratable acidity of control and RS incorporated idli batter are representing in Fig.1 (a) (b).The pH value of control idli batter was decreased (5.89 to 5.49) as the fermentation time increased from 0 to 12 h. This result was similar with Ghosh and Chattopadhyay (2010). Similarly significant reduction of pH was also observed in RS incorporated idli batter at 5 (5.80 to 4.66) and 10% (5.61 to 4.60). An increasing trend of acidity level was related with decrease in pH. This is mainly associated with the development of *S.faecalis* producing lactic acid, which lowers the pH value (Balasubramanian and Viswanathan 2006).

Total acidity of control idli batter at different fermentation time was in the range of 0.15 to 0.44% and it also increased at 5 (0.19 to 0.53) and 10 %(0.10 to 0.59) incorporation of RS. Rekha and Vijayalakshmi (2011) reported that in okara fortified batter, acidity was higher (0.18 to 0.64) than in unfortified batter (0.15–0.43). The increase in acidity may be due to the high amount of soluble protein, amino acid and free fatty acid present in okra fortified batter. pH and titratable acidity of Idli batter from polished parboiled rice and decorticated black gram blend in a ratio of 2:1, 3:1 and 4:1 (v/v) were in the range of 5.9–4.1and 0.443–0.910 %, respectively (Balasubramanian and Viswanathan,2007).



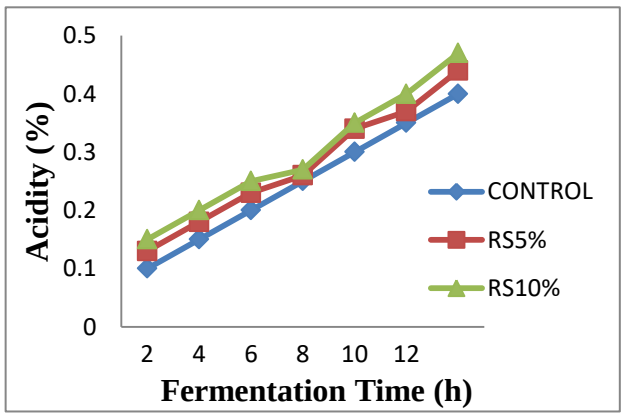


Fig.1(a):pH Values of control and RS incorporated idli batter Fig.1(b):Titratable acidity of control and RS incorporated idli batter

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The volume of control batter was increased two-fold after 12 h fermentation time while RS incorporated batter volume was increased at 8 h. This might be due to the CO_2 production by the yeast during natural fermentation and it is a measure of metabolic activity. This is also because of combined contribution of both hetero fermentative lactobacilli and non LAB (Thyagaraja et al. 1992). Since both leavening and acid development are required for 'idli', determination of the end point of the 'idli' fermentation becomes rather arbitrary. However, the use of different ingredients in different proportions resulted in raise in volume besides reduction of fermentation period. (Susheelamma and Rao 1978)

Idli batter viscosity was measured in centipoises (cps) it's an important parameter determining the end product quality. The viscosity of idli batter was significantly ($p<0.05$) reduced due to the incorporation of resistant starch. Control and RS 5 and 10% incorporated idli batter was found to be 7,912 cps, 9,405 cps and 9,507 cps respectively.

Microbiological analysis

The microbial analysis of control and RS incorporated batter presented in Fig. 2 and it revealed that both bacteria and yeasts played an important role throughout the process of natural fermentation. The microbial population of LAB was increased from 6 to 12 h for the control batter, however the microbial population was comparable from6to 8 h in RS incorporated batters. RS incorporated idli batters showed higher microbial counts compared to the control idli batter. Ghosh and Chattopadhyay (2011) reported that in okra fortified idli batter there was a gradual increase in mesophilic bacterial and LAB count with fermentation time as well as yeast and mould count. Thus the bacterial counts were higher in okra fortified batter than control batter. It has been previously reported that ingredients in foods such as black gram affect the microbial population as they provide a source of nutrients for the growth of microorganisms during fermentation. RS5% and 10% incorporated idli batter had rapid growth of bacteria, yeast and thereby reducing the fermentation time from 12 to 6 h leading to hastening of idli batter fermentation. Iyer and Ananthanarayan (2008) reported that reduction (14–8 h) in fermentation time of idli batter by addition of an exogenous source of alpha -amylase enzyme.

Rekha and Vijayalakshmi (2011) reported higher bacterial counts along with gradual increase in yeast and mould counts in okra fortified idli batter. Ghosh and Chattopadhyay (2011) suggested that black gram, as a source of protein in foods enhances themicrobial population.

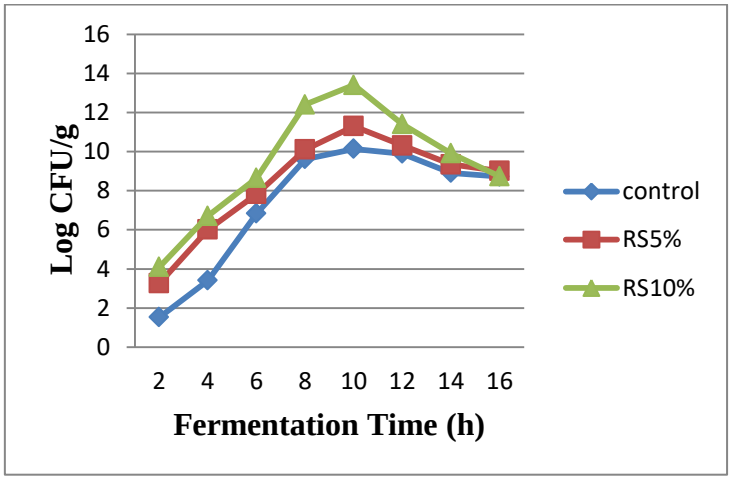

Fig.2: Microbial changes in control and RS incorporated idli batter.

Nutrients analyses of idli

Nutrient composition of control and RS incorporated idli is given Table 1. Protein and fibre content were higher in RS incorporated idli. High moisture and low carbohydrate content was observed in RS incorporated idli. Protein content of control idli was 11.56% and it was found to be high in RS 5 and 10% incorporated idli (12.01 and 14.5%).

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Table 1: Nutrients Composition of control and RS incorporated idlis

Parameters (%)	Control idli (%)	RS 5% idli (%)	RS 10% idli(%)
Protein	11.56±0.82[a]	12.01±0.20[b]	15.5±0.02[c]
Fat	0.11±0.09[a]	0.02 ±0.01[b]	0.02±0.01[b]
Fiber	0.28± 0.05[a]	0.42±0.03[b]	0.46±0.02[b]
Apparent Amylose	31.0 ±0.57[a]	35.54 ± 0.21[b]	37.43± 0.21[c]
Amylopectin	69.56±0.35[a]	64.45±0.30[b]	62.57±0.21[c]
Moisture	28.66 ±4.37[a]	29.8 ±0.05[b]	27.3±0.05[c]
Total CHO	60.66 ±0.33[a]	57.66±0.50[b]	56.2±0.06[c]

RS –Resistant Starch, % - Percentage, CHO- carbohydrate.

Mean values followed by the different letters within the row are significantly different ($p<0.05$).

The fat and fibre content of control idli were 0.11% and 0.28% respectively whereas fat content was decreased and fibre content was increased due to the addition of RS. Moreover the carbohydrate content was less in RS incorporated idli. Presence of amylose and amylopectin considerably influenced forrice starch digestion in the gastrointestinal tract, influencing faecal excretion and constitution, post- prandial blood glucose response and total cholesterol. Amylose content is normally used to evaluate some properties of product consumption such as cohesion and softness and also aid the control of biologically relevant parameters such as blood glucose and triglyceride concentration (Denardin*et al*, 2007).

Texture analysis of control and RS incorporated idlis

Table 2 shows the result of textural parameters of 'idli's prepared using control and RS incorporated batter. Hardness is a measure as the peak force during compression in the first cycle. Hardness of traditional 'idli' was 8.64 Newton and 'idli' prepared with RS 5 and 10% incorporated batter fermented for 12 h 'idli' were 4.28 and 3.29 Newton, respectively. These values indicated that the control 'idli' offered more resistance to compression than that of RS incorporated 'idli'. Thus the RS incorporated samples were softer and easy to bite compared to control samples. On the other hand the values for stickiness of RS 'idli' were relatively low. The stickiness of control 'idli' was 0.12 Newton moreover 0.10 Newton was noticed in each of RS 5 and 10% incorporated idli. Chewiness of control idli was 3.64 Newton however it was decreased due to RS addition. None of the difference was observed in adhesiveness of control and RS incorporated 'idli'. The texture changes may also be due to the ionic changes in the protein network induced by the decrease in pH during fermentation. The disruption of protein would reduce the firmness and springiness of the idli. According to reported literature, natural fermentation time of the batter varies from 14 to 24 h with overnight natural fermentation being the most frequently practiced. Incorporation of RS in idli batter has a beneficial effect in terms of higher amount of microbial production and leavening during natural fermentation. Reduction in the fermentation time of the 'idli' batter is of great commercial significance for large scale 'idli' production (Bharti and Laxmi 2008) and this can be potentially achieved by incorporation of pearl millet resistant starch.

Table 2: Texture analysis of Control and Resistant Starch incorporated idlis

Parameters(N)	Control Idli	RS 5% Idli	RS10% Idli
Hardness	8.64 ± 0.80	4.282±0.21	3.29±0.13
Stickiness	-0.12±0.02	-0.10±0.10	-0.10±0.15
Springiness	0.925±0.02	0.941±0.05	0.95±0.05
Cohesiveness	0.455±0.07	0.475±0.20	0.49±0.04
Stringiness	0.520±0.31	0.147±0.05	0.22±0.89
Chewiness	3.647±0.43	1.915±0.16	1.55±0.15
Adhesiveness	0.003±0.05	0.003±0.01	0.03±0.19
Gumminess	3.935±0.40	2.033±0.10	1.62±0.07

N- Newtons, RS –Resistant Starch, %- percentage

Values reported as Mean± S.D of triplicates

OrganolepticProperties of Idli

The scores of sensory evaluation of control and RS incorporated 'idli'are presented in Table 3. The effect of incorporating PMRS 5 % and 10% levels on sensory attributes of idli like appearance, texture, flavor, taste, color and overall acceptability of was studied. Appearance attributes of control idli got the highest score (7.26) followed by 5% incorporated idli and 10% incorporated idli. Regarding the texture attribute, high score was obtained for 5% and 10% incorporated idlis. However control idli acquired the highest score for taste attributes. Darken color was observed in 10% incorporated idli compared to control and 5% incorporated idli. Soni and Arora (2000) reported that yeast involved in the fermentation not only contribute towards gas production, which results in good texture but also towards the sensory qualities of the 'idli'. ANOVA test reveals that there was significant difference between control and RS incorporated idlis for all the attributes such as appearance, colour, flavor, texture, taste and overall acceptability

Table 3: Organoleptic Properties of control and RS incorporated idli

Parameters	Control Idli	RS 5%	RS 10%
Appearance	7.26 ± 0.15^{a}	7.24 ± 0.14^{b}	7.13 ± 0.08^{c}
Texture	7.54 ± 0.14^{a}	7.10 ± 0.05^{b}	7.10 ± 0.30^{c}
Flavor	6.80 ± 0.18^{a}	6.80 ± 0.25^{a}	6.26 ± 0.21^{b}
Taste	6.95 ± 0.83^{a}	6.35 ± 0.94^{b}	6.30 ± 0.81^{c}
Colour	7.20 ± 0.58^{a}	7.30 ± 0.59^{b}	7.50 ± 0.67^{c}

Values are Mean ± SD of 9-point Hedonic scale. Mean values followed by the different letters within row are significantly different ($p<0.05$).

4. CONCLUSION

The present study concluded that incorporation of pearl millet resistant starch increased the acidity and decreased the pH level of idli batter. Fermentation time was reduced by the incorporation of resistant starch as a prebiotic as well as a symbiotic. It also enhanced the composition of nutrients in idli particularly fiber, and protein. Reduction in the fermentation time of the idli batter is of great commercial significance for large-scale idli production and has been potentially achieved by addition RS. Incorporation of pearl millet resistant starch up to 10 % hasten the fermentation process and it could be used for making a novel product.

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