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Nutritional, functional and shelf life studies of RTC little millet *bisibelebath* mix

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Abstract
The demand for millet based ready-to-cook (RTC) mixes among urban consumers is increasing due to ease of preparation steps and nutritional benefits against life style disorders. Little millet rice was explored for the preparation of RTC *bisibelebath* mix to meet the requirement of modern consumers. The little millet rice RTC mix was stored in MPP (Metalized Polyester Polyethylene pouches) under three different temperatures (Accelerated temperature of 37℃-AT, Room temperature, 18-24℃-RT and Refrigerated temperature, 0-5 ℃-RF) for a period of six months. Variations in cooking, functional, nutritional and bioactive compounds in RTC little millet mix was observed as against little millet rice. Higher hydration (445%) and swelling capacity (161.5%) was noticed in RTC mix as compared to little millet rice. The RTC little millet *bisibelebath* mix found to contain low carbohydrate (54.40%), protein (19.25%), crude fiber (7.60%), calcium (16.50 mg %), iron (7.87 mg %) and zinc (6.50 mg %). The bioactive compounds *in-vitro* studies revealed that, RTC mix contained phenolics (180.62 m.eq. gallic acid), flavonoid (60.74 mg. eq. quercetin) and antioxidants (41.23%). Among different storage temperatures, no significant differences were noticed between RT and RF with respect to shelf life and were acceptable up to six months.

Keywords: Ready to cook, flavonoids, tannin, swelling capacity, antioxidants

1. Introduction

Millets are group of highly variable small seeded grasses widely grown around the world as cereal crops or grains for human food and fodder. Millets are small seeded grains with different varieties such as pearl millet, finger millet, kodo millet, proso millet, foxtail millet, little millet and banyard millet. Currently 26.7 million metric tons of millets are being produced in the world from an area of 33.6 million hectare. Africa is the largest producer of millet (20.6 million metric tons) followed by Asia (12.4 million metric tons) and currently India is producing 10.5 million metric tons of millets (Dayakar *et al*, 2017) [9].

In spite of its rich nutritional quality, little millet (*Panicum miliare*) is neglected crop grown in both plains and hilly regions. The nutritional quality of little millet indicated that it contains energy (346.31 Kcal), carbohydrate (65.55%), protein (8.92%), fat (2.55%), iron (1.26 mg) and calcium (16 mg) with significant quantity of fatty acids such as oleic (868 mg), linoleic (1230 mg), total saturated fatty acid (589 mg) and total monounsaturated fatty acids (868 mg), hence it is nutritionally quite superior to any other cereal grain. Little millet plays a significant role in providing nutraceutical components such as phenols, tannins, phytate along with macro and micro-nutrients. (Dayakar *et al*, 2017) [9]. Superior nutritional value and storage feasibility of little millet grain has led to consider this grain as an important staple food by ancient people. As per Sridevi *et al*, (2010) [32] the consumption of little millet and their application in the fabrication of therapeutic foods for the management of metabolic disorders is well established fact. In spite of superior nutritional quality, it has not been found a place in larger portion of consumer's plate due to many reasons. The major reason being smaller grain size (1.8-1.9 mm) tend to accidental addition of pebbles, or stones, lack of availability of processing machinery at local places and non-awareness of women folk regarding methods of cooking has made it difficult for production of various products using this millet. Apart from this, high cost of processing units, non availability of raw material, increased production and availability of preferred cereals such as rice and wheat at subsidized prices also reduced the usage of this grain for the production of staple and convenience foods. However, with the support of governments, the area under millets is increasing now a days, so also millet consumption has increased among urban consumers which are mainly due to increasing awareness regarding health benefits of millet consumption.

Today's woman finds it difficult to cook many of our traditional recipes due to lack of time and requirement of elaborate preparations. This problem can be overcome by the advent of instant mixes or ready-to-cook (RTC) mixes. The concept of processing and product development of regional and traditional millets will benefit the non-consumers of millets with their specific nutrients in the form of ready to cook (RTC) products. The ready to cook (RTC) mixes normally in dry form need to be mixed with water before consumption will require only few minutes of cooking in water or steaming or frying and requires very less time for their preparation (Shobha *et al*, 2011) [31]. These RTC foods from millets will not only help the producers in improving their socio-economic status but also help in improving the health of the consumers to fight against life style disorders such as diabetes mellitus, obesity and cardiovascular diseases. Therefore, attempts have been made by many researchers utilizing and converting little millet into diversified forms of novel products such as convenient mixes which are readily available for the consumers. Popular breakfast food items such as *Idli* and *Dosa* were developed by substituting different types of millet flours such as finger millet, kodo millet, foxtail millet, proso millet and little millet with rice and black gram dhal (Srinivasan *et al*, 2013) [33] and were highly acceptable and popular among urban mass. In this line, *Bisibelebath* popular breakfast item normally prepared with rice and red gram dhal along with addition of spices has occupied top place in the minds of consumers as a mouthwatering breakfast item. Looking into the acceptability of *Bisibelebath* by the consumers and its superior nutritional quality, the instant *Bisibelebath* mix using little millet and pulse combinations was planned with an objective of developing RTC *bisibelebath* mix and study it's functional, nutritional and storage quality.

2. Material and Methods

The little millet rice was procured from Shakthi Farms, Metagalli, Mysuru and other raw materials such as red gram dhal, salt and spices were procured from the local market, Mysuru in a single lot and refrigerated till further use.

2.1 Development of RTC little millet *Bisibelebath* mix

The optimization of RTC little millet *Bisibelebath* mix was achieved using statistical software central composite rotatable design expert® version 7.1.5 from stats ease Inc, USA. The procedure for the preparation of RTC mix was adopted from Brundha *et al*, (2019) [7], wherein the little millet, red gram dhal and spice mixture were taken as independent variables and sensory attributes were taken as responses. Among the 15 formulations tested, combination containing 50g each of little millet rice, red gram dhal and 15.36g of spice mixture was the optimized solution with best fit desirability of 0.9.

2.2 Cooking and Functional properties of ready to cook little millet *Bisibelebath* mix:

The functional property or functionality is defined as any property of a food or food ingredients besides its nutritional value which affects its utilization. The various cooking and functional qualities studied includes cooked weight, cooking time, elongation ratio, bulk density hydration capacity, swelling capacity, water and oil absorption capacity (Sareepuang *et al*, 2008) [30]

2.3 Proximate analysis

The micro kjeldhal method was employed to determine the total nitrogen and crude protein content (N x 6.25) of the samples as per the standard method (AOAC, 2000). Crude fat (Socs plus - SCS 06AS) was estimated by extraction with petroleum ether (60–80°C) while, crude fiber (Fibraplus-FES04) and ash contents were determined as per AOAC, (2000). Carbohydrate (by difference method) and energy was computed by taking protein (4 kcal/g), carbohydrate (4kcal/g) and fat (9 kcal/g) values using the formula as per AOAC,(2000).The energy content of sample was calculated using the formula given below.

Energy (kcal) = [Protein (g) x 4] + [Carbohydrate (g) x 4] + [Fat (g) x 9]

Minerals such as calcium, iron, zinc, manganese and copper were estimated by Atomic Absorption Spectrophotometer (Agilent Technologies 200 series AA spectra 240 AA) as per AOAC, (2000) protocol.

2.4 Analysis of major anti nutrients

The anti-nutrients analyzed in this study includes phytic acid and tannins which were known to be the major anti nutrient compounds in little millet grain which hinder the absorption of minerals.

2.4.1 Estimation of phytic acid

The phytic acid phosphorus of the samples was estimated by wade reagent method with slight modifications. The Phytic acid phosphorus content of the samples was calculated through regression equation (Absorbance =0.0338* phytic acid phosphorus (µg) +0.552, R^2 =0.9986) plotted with the sodium salt of phytic acid (1.12 to 11.2 µg/L) as standard. Results were expressed as percentage of dry weight (mg/100g). Phytate-phosphorus was converted to phytate by multiplying with factor 3.55 (Onivogui *et al*, 2014) [21].

2.4.2 Estimation of tannins

Tannins in the RTC mix as well as little millet grain were estimated calorimetrically based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid in alkali solution. The absorbance was measured at 760 nm and the tannin content of the samples was calculated as tannic acid equivalents from the standard graph (Onivogui *et al*, 2014) [21].

$$\text{Tannins (\%)} = \frac{\text{mg of tannin acid X Dilution X100}}{\text{ml of sample taken for colour development X Weight of sample}} \text{ X 100}$$

2.5 Estimation of bioactive compounds using *in vivo* method

"Bioactive compounds" are extra nutritional constituents that typically occur in small quantities in foods. They are being intensively studied to evaluate their effects on health; these compounds vary widely in chemical structure and function and are grouped accordingly. The major bio active compounds of nutritional significance include phenolic compounds including their subcategory flavonoids present in all plants and have been studied extensively in cereals, legumes, nuts, olive oil, vegetables, fruits, tea and red wine (Penny *et al.*, 2002) [23]. The different bioactive compounds analyzed in this study are total phenols, total flavonoids and antioxidant activity.

2.5.1 Determination of total phenolic content: The total phenolic content in the methonolic extract of RTC mix was determined spectrophotometrically using folin-ciocalteu method as described by Onivogui *et al*, (2014) [21] with slight modification. The amount of total phenol was expressed as gallic acid equivalent (GAE) in milligram per gram of dried extract.

2.5.2 Determination of Antioxidant activity: Antioxidant activity was determined in terms of free radical scavenging capacity using the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) as described by Sandra *et al*, (2009). Absorbance was measured at 517nm and the percent inhibition activity was calculated as per the formula

$$\text{Inhibition activity (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample X 100}}{\text{Absorbance of blank}}$$

2.5.3 Determination of Total flavonoids: The total flavonoid content (TFC) of the extract was determined according to Onivogui *et al*, (2014) [21]. The total flavonoid content was expressed as mg quercetin equivalents per 100 ml of ready to cook little millet *Bisibelebath* mix.

2.6 Estimation of bioactive compounds of RTC little millet *Bisibelebath* mix using *in-vitro* digestion method
The *in vitro* digestion was done using sequential enzymatic steps based on slightly modified method reported by Salawu *et al*., (2018) [35]. It involved three distinct stages (oral, gastric, and total gastro-intestinal digestion) and each stage was terminated by inactivating the enzyme.

2.7 Evaluation of storage stability of RTC little millet *bisibelebath* mix
The developed RTC little millet *bisibelebath* mix packed in metalized polyester polyethylene pouches (420 gauge) was subjected to storage study under three different temperatures *viz*., refrigerated temperature (0-4 ^{0}C- RF), room temperature (18-24 ^{0}C- RT) and accelerated temperature (37 ^{0}C-AT) for a period of six months. The quality of stored RTC mix was assessed in terms of biochemical parameters such as peroxide value, free fatty acids (AACC, 2000) [1], non-enzymatic browning in terms of browning index (BI) as per Afoakwa *et al*, (2014). For browning index calculation, five gram of sample was extracted with 100 ml ethanol (67%) for one hr. The extract was filtered through what man No.1 filter paper and browning index in terms of absorbance at 460 nm of the filtrate was measured by taking 67% ethanol as blank.

2.8 Analysis of Sensory, microbial and consumer acceptability of RTC mix during storage: The sensory evaluation of RTC mix was carried out by 21 semi trained judges using 9-point hedonic scale and microbial quality and consumer acceptability were also conducted at monthly intervals till the end of the storage period as per Shobha *et al* (2011) [31].

2.9 Statistical analysis
Data was analyzed statistically in terms of mean and standard deviation for cooking, functional and nutritional properties whereas analysis of variance (ANOVA) was used to test the level of significance at $P< 0.05$ for storage studies.

3. Results and Discussion
3.1 Cooking and functional properties of RTC little millet *Bisibelebath* mix
Cooking and functional properties of little millet grain and little millet RTC mixes is depicted in Table 1. The cooking time, cooked weight, swelling capacity and water elongation ratio were found to be more in RTC mix compared too little millet rice which may be due to the presence of red gram dhal and spice mixture in the formulation. Results of Itagi *et al*, (2012) [12] indicated high bulk density in multigrain composite mixes. Water absorption capacity is important functional character in the development of ready to eat foods and high water absorption capacity indicates product cohesiveness (Shobha *et al*, 2011) [31]. In the present investigation, water absorption capacity was found to be more in RTC mix (500 ml /100g) than in little millet rice (200 ml /100g). In the present investigation, higher oil absorption capacity was observed in RTC mix (Table 1) which may be due to variation in protein concentration, degree of interaction with water, oil and conformational characteristics of developed formulations.

Table 1: Cooking and functional properties of RTC little millet *Bisibelebath* mix

Properties	little millet rice	RTC little millet *Bisibelebath* mix
Cooking time (min)	10±0.40	15±0.30
Cooked weight (g/10g)	6.47±0.01	8.65±0.01
Bulk density (g/ml)	0.81±0.01	0.83±0.01
Elongation ratio	1:1.12	1:1.66
Hydration capacity (%)	225±0.35	445±0.30
Swelling capacity (%)	100±0.40	161.5±0.1
Water absorption capacity(ml/100g)	200±0.1	500±0.1
Oil absorption capacity (ml/100g)	160±0.1	620±0.1

Note: Values are mean of three replications ± SD

3.2 Nutritional Composition of RTC little millet *bisibelebath* mix
The perusal of Table 2 depicts the nutritional composition of little millet rice and RTC little millet *Bisibelebath* mix. It was noticed that RTC mix exhibited significantly more protein (19.25g/100g) compared too little millet rice which could be due to addition of red gram dhal and spice mixture in the mix. Even the fat and crude fiber content were significantly more in developed mix (6.5% and 7.6%) compared to little millet rice (4% and 4.01%) which is due to addition of other ingredients in the development of RTC mix. Similar line of work on finger millet rice *Bisibelebath* mix was reported by Begum *et al*, (2017). The important minerals of nutritional significance such as zinc (6.50 mg/100 g) and iron (7.87 mg/100 g) were also significantly more in little millet RTC mix. The data on nutritional composition of little millet in the present study is in line with the reports of previous investigators such as Nazni and Devi, (2016).

Table 2: Nutritional and Anti-nutritional components of RTC little millet *Bisibelebath* mix

Parameters	Little millet rice	RTC little millet *Bisibelebath* mix
Energy (kcal)	359±0.01[a]	353± 0.01[b]
Carbohydrates (%)	68.81±0.01[a]	54.40± 0.01[b]
Moisture (%)	10.0±0.25[a]	8.40±0.14[b]
Protein (%)	12.0±0.1[b]	19.25± 0.01[a]
Fat (%)	4.0±0.5[b]	6.50 ± 0.70[a]
Crude fibre (%)	4.0±0.1[b]	7.60 ± 0.57[a]
Ash (%)	1.60±0.1[b]	3.75 ± 0.02[a]
Calcium (mg/100g)	14.5±0.1[b]	16.50± 0.1[a]
Iron (mg/100g)	7.50±0.1[b]	7.87 ±0.45[a]
Zinc (mg/100g)	6.05±0.1[b]	6.50 ±0.28[a]
Magnesium (mg/100g)	0.70±0.01[b]	1.90± 0.98[a]
Copper (mg/100g)	0.50±0.1[b]	1.25± 0.007[a]
Phytic acid (mg/100g)	0.66±0.01[b]	92±0.1[a]
Tannins (%)	0.74±0.01[b]	4.4±0.01[a]

Different letters in a row indicate significant differences ($p < 0.05$) from Duncan's multiple range tests

3.3 Anti-nutritional components

The term anti-nutrients refer to defense metabolites having specific biological effects depending upon the structure of specific compounds which range from high molecular weight proteins to simple amino acids and oligosaccharides (Bora, 2014) [6]. The anti-nutritional contents of RTC mix as well as little millet rice are shown in Table 2. Addition of red gram dhal and spice mixture to the RTC mix may tend to enhance the phytic acid and tannin contents in RTC mix (92% and 4.4%) than in little millet grain (0.66%and 0.74%) respectively. Similar findings were reported in case of foxtail millet *Bisibelebath* mix developed by Sridevi *et al*, (2010) [32]. Even the study of Thilagavathi *et al*, (2015) [36] indicated phytate and tannin contents of little millet flour were found to be 24.42% and 18.62% respectively. Even our results are in agreement with Pradeep *et al*, (2011) [24] for anti-nutrient content of little millet grains.

3.4 Bioactive compounds in RTC little millet *Bisibelebath* mix using *in vitro* enzymatic digestion method

The impact of gastrointestinal digestion of bioactive compounds such as total phenols, flavonoids and antioxidant activity in terms of DPPH is depicted in Figure 1.

The conditions of the gastric digestion process (low pH and enzyme action) may favor the release of gallic acid bound to other structures present in the food matrix such as carbohydrates (eg: gallotannins, manogalloyl-glucoside, digalloyl-glucoside and tetragalloyl-glucoside) thus increasing the content of this compound (Lingua *et al*., 2018) [18]. The present study, the phenolic contents present in the RTC little millet *bisibelebath* decreased after reaching intestinal phase (180.62 mgeq gallic acid /100g) when compared with oral (192.18 m geq gallic acid/100g), gastric (218.30 mgeq gallic acid/100g) phases and undigested (186 mgeq gallic acid/100g) samples. The increased phenolic compounds in gastric digestion phase at acidic medium (Figure 1) were due to the release of phenolic compounds from the matrix or the increased reactivity of phenolic compounds towards Folin-Ciocalteu reagent. Similar findings were observed by Goulas *et al*., (2019) [11].

The RTC little millet *bisibelebath* had higher amount (102.32%) of DPPH radical scavenging activity (DRSA) in oral phase followed by gastric (96.63%), intestinal (41.23%) phase and undigested (68.85%) samples (Figure 1). It may be due to the presence of bioactive compounds that can inhibit alpha-amylase and less starch was converted to maltose in little millet grains (Oyedemi *et al*., 2017) [22]. Similar study was conducted by Chandrashekhara *et al*., (2012) by subjecting the kodo, finger, prose, foxtail and pearl millet grain samples into simulated *in vitro* digestion and microbial fermentation. The millet samples treated with the fermentation process and gastrointestinal method had higher DRSA when compared to pH treated and aqueous method.

Flavonoids are hydroxylated phenolic compounds having a benzo-γ-pyrone structure mostly present in plants (Rohn *et al*., 2002) [26]. In the present study, flavonoid contents (Figure 1) were significantly low in oral phase (48.82 mg eq quercetin/100g) which further reduced in the gastric phase (32.12 mg eq quercetin/100g) but significantly increased in the intestinal (60.74 mg eq quercetin/100g) phase. It may be due to the sensitivity of the enzymes to pH conditions, proteolytic enzymes used and also weak glycosidic bond which was linked to food matrix by flavonols (Inada *et al*., 2015) [13]. Contradictory study was observed by Quatrin *et al*., (2020) [25] where in authors reported that jaboticaba fruit peel had highest recovery index of flavonoid and small decrease was found in the initial phases of digestion (salivary and gastric phase).

3.5 Effect of storage on bioactive compounds of RTC little millet *Bisibelebath* mix

The total phenols, antioxidants and total flavonoids together considered to be bioactive compounds. Since red gram dhal and little millet grains contain antioxidant activity, it is essential to find the bioavailability of the same in the food prepared out of the mix. As depicted in Figure 2, storage duration and temperature affected the total phenolic contents decreased significantly during storage under the experimental conditions applied at accelerated temperature (AT) followed by room temperature (RT) and refrigerated temperature (RF). During second month of storage at AT and RT, the phenolic contents decreased significantly at ($p < 0.01$) under AT followed by RT and RF. It is noteworthy to mention that between RF and RT there was no significant reduction was noticed. Similar findings were also observed in carrot and mango beverages by Saci *et al*, (2015) [28]. Even the results of Maity *et al*, (2016) [19] also support our results, wherein, significant reduction of phenolic compounds was observed between AT, RT and least under RF conditions.

The antioxidant activity of the extracts as depicted in figure 2, was decreased significantly under AT compared to RF and RT. In the current investigation, the analysis of variance

(Table 3) showed that interaction effects of storage duration and temperature were decreased significantly ($p< 0.01$) on radical scavenging activity of developed RTC mix. The radical scavenging activities of the developed beetroot bar decreased to the extent of about 32, 52 and 70% under RF, RT and AT respectively as documented by Maity *et al*, (2016) [19]. Flavonoid content of the RTC little millet mix was also decreased in the similar pattern as that of antioxidants.

Table 3: Effect of storage on biochemical parameters of RTC little millet *Bisibelebath* mix

Storage duration (months) (M)	Peroxide value (Meq/kg of fat)			Mean	Free fatty acid (% of oleic acid)			Mean	Browning index (%)			Mean
	0-4 °C	18-24 °C	37 °C		0-4°C	18-24 °C	37 °C		0-4 °C	18-24 °C	37 °C	
0	4.8^{r}	4.8^{r}	4.8^{r}	4.80^{g}	0.22^{q}	0.22^{q}	0.22^{q}	0.22^{g}	1.30	1.30	1.30	1.30^{g}
1	5.0^{q}	5.2^{p}	6.4^{m}	5.53^{f}	0.23^{q}	0.24^{qp}	0.25^{p}	0.24^{f}	1.39	1.42	1.50	1.44^{f}
2	5.6^{o}	6.0^{n}	8.0^{i}	6.53^{e}	0.27^{o}	0.29^{n}	0.34^{l}	0.30^{e}	1.40	1.45	1.56	1.47^{e}
3	6.8^{l}	7.6^{j}	8.4^{h}	7.60^{d}	0.32^{m}	0.39^{j}	0.47^{h}	0.39^{d}	1.61	1.64	1.73	1.66^{d}
4	7.2^{k}	8.0^{i}	9.2^{f}	8.13^{c}	0.36^{k}	0.45^{i}	0.66^{e}	0.49^{c}	1.76	1.79	1.80	1.78^{c}
5	8.8^{g}	10.0^{d}	11.2^{b}	10^{b}	0.54^{g}	0.77^{d}	0.97^{b}	0.76^{b}	1.88	1.90	1.94	1.91^{b}
6	9.6^{e}	10.8^{c}	12.0^{a}	10.80^{a}	0.64^{f}	0.85^{c}	1.0^{a}	0.83^{a}	1.95	1.97	2.00	1.97^{a}
Mean	6.83^{c}	7.49^{b}	8.57^{a}		0.37^{c}	0.46^{b}	0.56^{a}		1.61^{c}	1.64^{b}	1.69^{a}	
Interactions	F-value	S.Em	CD		F-value	S.Em	CD		F-value	S.Em	CD	
Storage period	**	0.031	0.088		**	0.003	0.009		**	0.01	0.029	
Storage temperature	**	0.02	0.058		**	0.002	0.007		**	0.007	0.021	
Storage period X storage temperature	**	0.053	0.153		**	0.006	0.016		**	0.018	NS	

Different letters in a row indicate significant differences ($p< 0.05$) from Duncan's multiple rangetests.
Note: RF: 0-5°C, RT: 18-24°C and AT: 37°C

3.6 Effect of storage on biochemical changes of RTC little millet *Bisibelebath* mix

Storage of any product determines its wholesomeness during the definite period of time. The perusal of Table 3 indicated that as the storage duration and temperature increases, the biochemical parameters (peroxide value, free fatty acid and browning index) also increased significantly ($p< 0.05$). Similar findings were observed by Gahalwat and Sehgal, (1992) [10] that the peroxide value and fat acidity of weaning food developed from locally available food stuffs increased with increase in storage period. In this study, peroxide value increased significantly ($p< 0.01$) under AT followed by RT and RF from second month (Table 3) to till the end of the storage period. Our values were within the limits as per Kirik and Sawyer, (1991) [17] who reported that the limitations of noticeable rancidity in oil containing product, the peroxide value should be between 20-40 meq/kg fat. Free fatty acid (FFA) contents were also increased in similar patterns as peroxide value. The values started increasing significantly ($p< 0.01$) from second month under AT (0.34 to 1.00%) followed by RT (0.29% to 0.85%) and RF (0.27% to 0.64%) respectively. Hydrolysis of lipids during storage is normally brought about by the naturally occurring lipases. However, during cooking, lipase activity was destroyed and therefore the formation of free fatty acids in stored RTC little millet *bisibelebath* mix must have resulted from the decomposition of hydro peroxide. Similar trend in FFA was observed by Khan *et al*, (2008) [14] where in free fatty acid values were increased significantly in groundnut burfi. Significant differences were observed between AT, RT and RF, but between room and refrigeration storage, non-significant differences were observed (Table 3). Even the results of Khan *et al*. (2014) [15] reported that the instant porridge mix remained stable up to 52 weeks at 15–34 °C. In the present study, the RTC little millet *Bisibelebath* mix was stable up to 180 days at RT and RF. Browning index observed to be increased significantly ($p< 0.05$) between three storage temperatures (Table 3) and the increase was pronounced under AT. This may be due to the formation of intermediate browning compounds in MPP stored samples at higher temperatures which react with water during reconstitution leading to formation of melanoids results in brown colour Results reported by Maity *et al* (2016) [19] showed that browning index increases with increase in storage period and temperature.

3.7 Changes in sensory quality during storage of RTC little millet *Bisibelebath* mix

The perusal of Figure 3 depicts the changes in sensory attributes during storage of RTC little millet *Bisibelebath* mix. Increase in storage temperature had a detrimental effect on the color, appearance, texture, flavor, taste and overall acceptability scores. It can also be seen that the storage temperature significantly affected the sensory score of the product (Figure 3). It is evident that, significant reduction in sensory characteristics was observed between AT, RT and RF. The overall acceptability (OAA) scores during six months of storage decreased from 8.65 to 8.16, 7.58 to 7.25 and 7.28, 7.05 for the developed mix, stored at RF, RT and AT respectively. However, when stored at RF and RT condition, the RTC mix received significantly higher acceptable scores in terms of all sensory characteristics up to six months of storage. The most affected sensory parameters was colour which decreased significantly ($p< 0.01$) during storage period of six months at accelerated temperature which was due to increase in browning index at accelerated temperature. The decrease in sensory scores of the stored product at different temperatures was reported by Maity *et al*., (2016) [19].

3.8 Consumer acceptability of RTC little millet *Bisibelebath* mix

The consumer acceptance of food product largely depends on its sensory perception. The consumer acceptability of cooked little millet *bisibelebath* (data not shown) revealed that 84% of the consumers including rural and urban areas rated the little millet *bisibelebath* as "very good". Similar line of work was conducted by Shaviklo *et al*., (2013); Sudharani *et al*., (2013) [27, 34]. Microbiological profile of RTC mixes (data not shown) indicated that there were no bacteria, fungi and moulds throughout the six month of storage period under RF and RT. But under AT, the total bacteria and fungi counts were 2.0 and 5.0 CFU /g. There was no E. coli reported in the entire sample stored at different temperatures till the end of storage period.

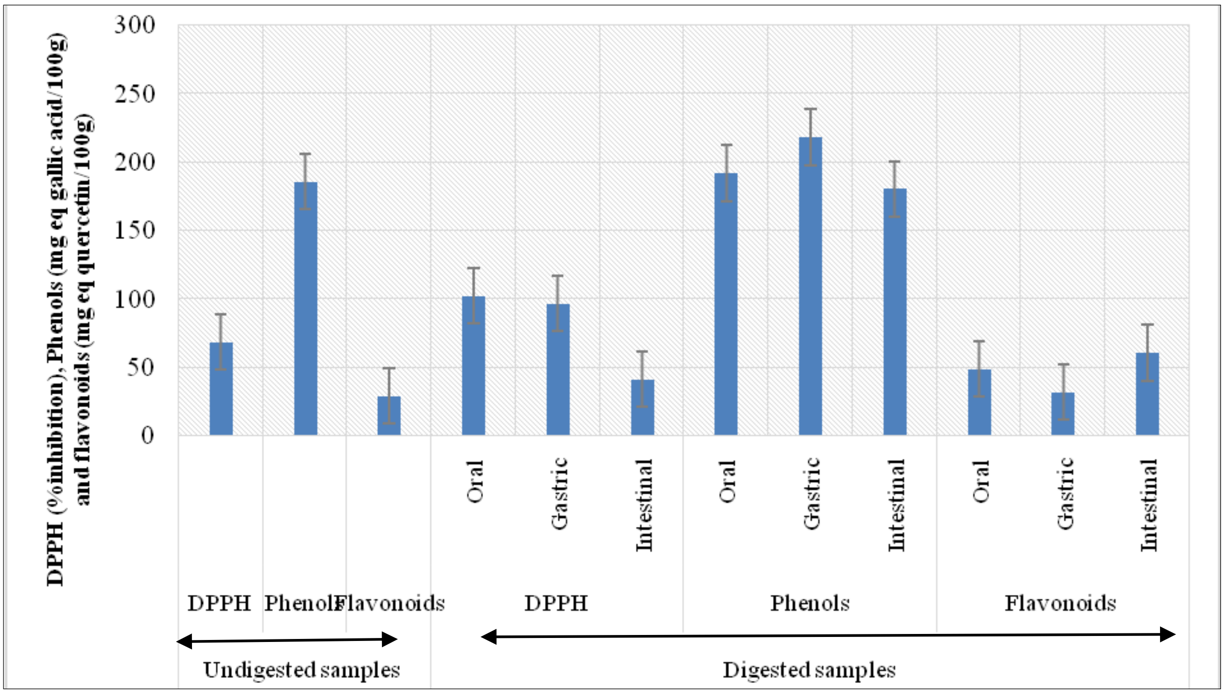
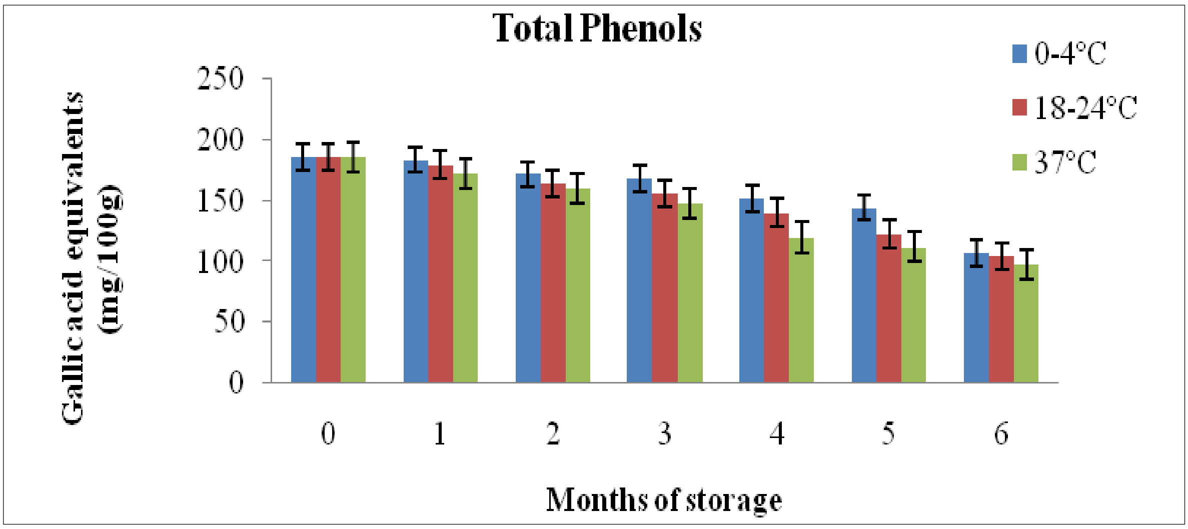
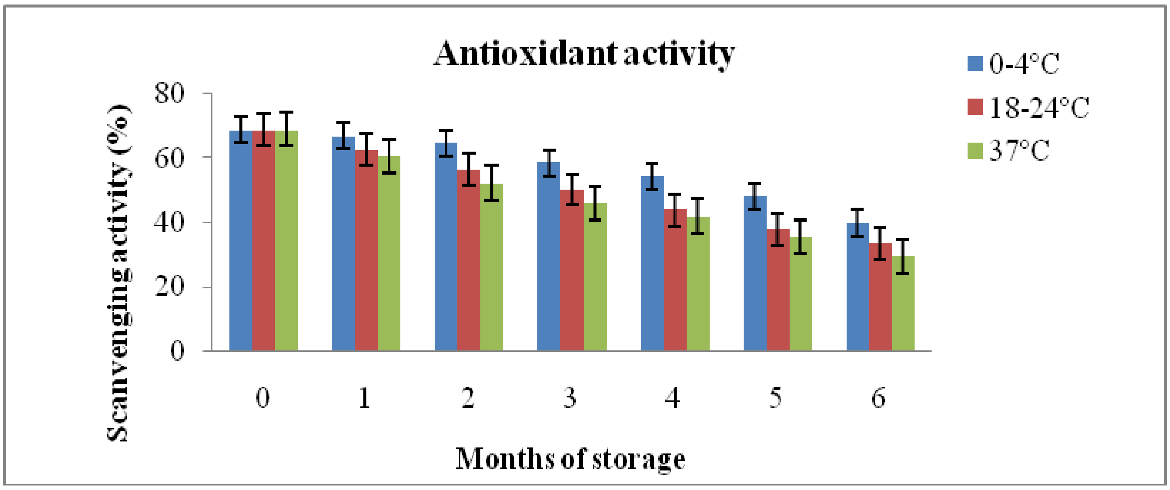


Fig 1: Bioavailability of bioactive compounds in RTC little millet *Bisibelebath* mix using *in-vitro* digestion method





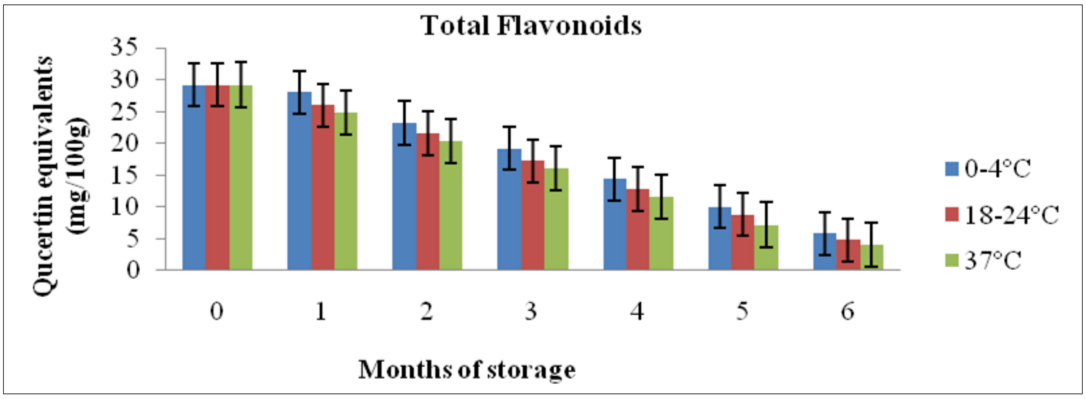


Fig 2: Effect of storage on bioactive compounds of RTC little millet *Bisibelebath* mix

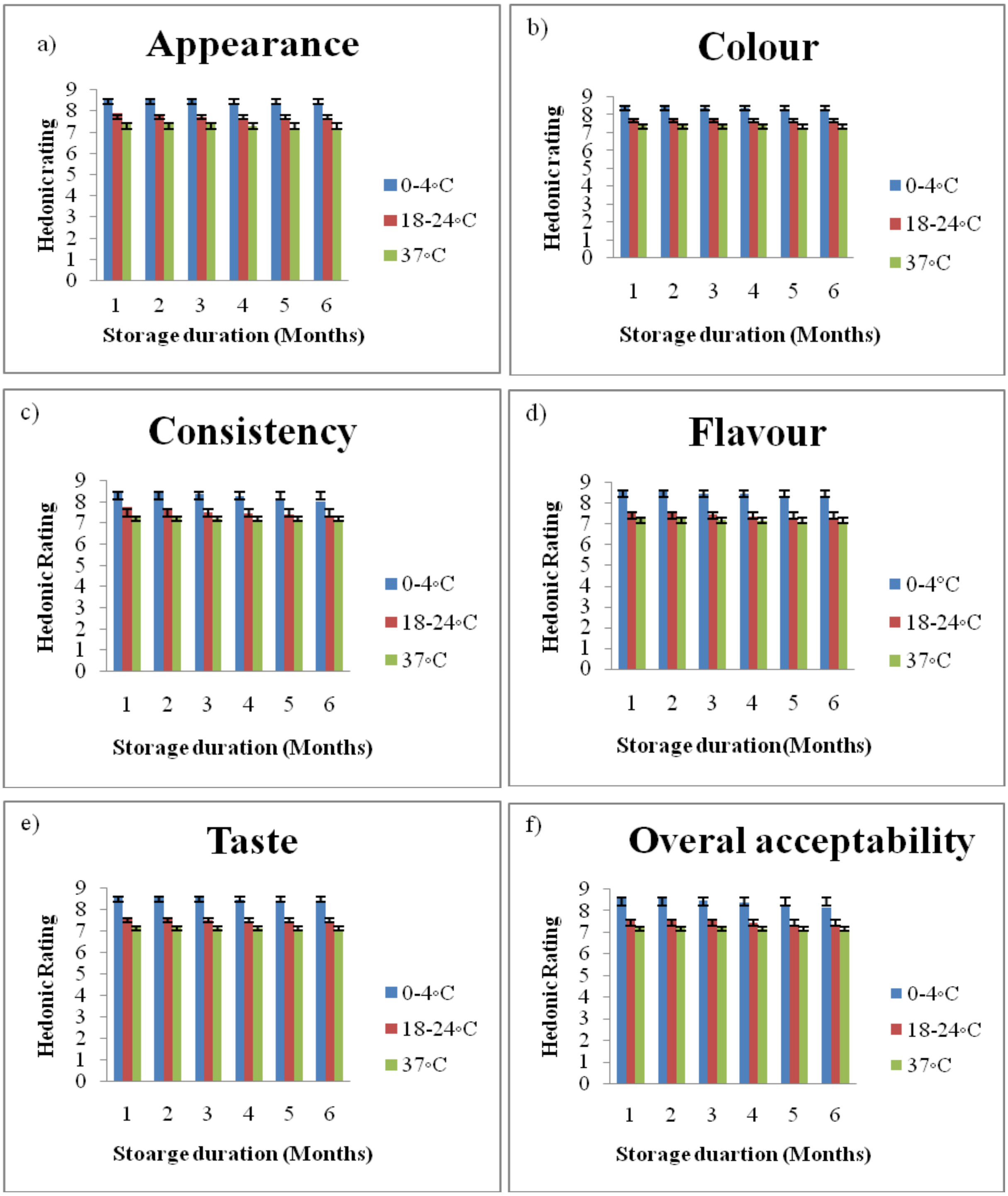


Fig 3: Effect of storage on sensory quality of RTC little millet *Bisibelebath* mix

4. Conclusion

An acceptable RTC little millet *bisibelebath* mix can be prepared by combining little millet rice and broken red gram dhal in 50:50 ratio with 15.36 g of spice mixture. Nutritionally, RTC mix was found to be superior in protein, fiber, low carbohydrate along with bio-active compounds such as phenols, flavonoids which contributed for enhanced antioxidant capacity. Stored mix in MPP pouches showed better retention of bioactive compounds, sensory and biochemical parameters under room and refrigerated conditions compared to accelerated temperature. Hence, the RTC mix can be stored up to six months under room temperature with lesser storage cost.

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