Assessing Consumer Acceptability And Antioxidant Potentiality Of Foxtail Millet Meal Replacement Bar

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Abstract : Foxtail millet is an excellent functional food lacking commercial success due to difficulty in processing and consumption. The primary focus of this study is to formulate antioxidant rich meal replacement bar and analyze its nutrient composition. Foxtail millet is the principal ingredient in the meal replacement bars formulated. The consumer acceptability of these bars was assessed through organoleptic evaluation. In-vitro antioxidant assays such as DPPH, FRAP, Phosphomolybdenum, and Superoxide Anion Radical Scavenging Activity of the nutritionally finest variation was evaluated and compared against standard ascorbic acid. The foxtail millet meal replacement bars (FMRB) contains 249 to 283 Kcal energy, 49.23 to 55.90g carbohydrate, 7.14 to 9.05 g protein, and 2.34 to 2.63g fat. The FMRB were well accepted by the consumers. The in-vitro antioxidant activity of the ethanolic extract of FMRB and ascorbic acid was determined in a concentration-dependent manner (20µg-120µg). The sample extract concentration required for the half-maximal inhibition (IC_{50}) of DPPH and superoxide anion radical was calculated. The FMRB extract has good reducing power of ferric ion (0.8857 FRAP absorbance value) and phosphate-molybdenum IV (0.9180 absorbance value). Whereas, it exhibited a lower scavenging effect against superoxide radical (17.90%) but stronger scavenging activity of DPPH (86.69%). The present study indicates that the nutritional composition of the meal replacement bars was adequate to be a healthy snack and ethanolic extract of the FMRB possesses potent antioxidant activity which supports that bar is a potential ready-to-eat functional food.

Keywords: Antioxidants; DPPH; Foxtail millet; FRAP; Meal replacement bar; Superoxide radical.

1 INTRODUCTION

Antioxidants and substrate that is capable of being oxidized are present together in foods. The concentration of these antioxidants is less than the oxidizable substrates [1]. The antioxidants have a vital role in preventing undesirable changes in flavor and nutritional quality of food [2]. Furthermore, antioxidants are also an integral part of the body's defense system in fighting against the reactive oxygen species (ROS) and other oxidants synthesized in the body [3]. Polyphenols present in cereals, fruits, legumes, vegetables, are natural antioxidants that counteract the damaging effect of free radicals [4]. Foxtail Millets are abundant sources of these polyphenolic compounds. Research proves that consumption of foxtail millet reduces blood sugar level, lowers blood lipid and lipoprotein level, and hinders the growth of cancer cells. The nutraceutical properties of this millet are a result of the antioxidant capacity of specific bioactive components present in the grains [5]. Foxtail millet is identified as a vital crop considering its nutrient composition [6]. Since ancient times cultivation and consumption of foxtail millet (Setaria italica) are practiced.

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This major millet is the second most grown species of millets, and the sixth-highest yielding grain [7]. It has a high tolerance for drought conditions and environmental stress. The grains mature in 65-70 days, farmers in the grey regions prefer this over crops with more prolonged maturity periods. Morphologically, it has a similar grain structure as paddy rice [8]. The husk contains relatively high levels of fiber in addition to negligible amounts of anti-nutrients, and the remaining 79% digestible portion has higher amounts of proteins and minerals [9]. The anti-nutrients and fiber in the bran limits the access to the essential nutrients. The processing techniques enhance the physico-chemical accessibility and bioavailability of nutrients, reduce anti-nutrients, and improve the nutraceutical properties [10]. Traditional processing techniques enhance the nutritional, sensory, and palatable properties of millets [7]. This nutritionally superior crop is used for the preparation of porridge, nourishing soups or gruels, pancakes, noodles, and alcoholic beverages [11]. The non-glutinous Foxtail millet grains are easily digestible and do not aggravate acid production. In the culinary world, this trait makes it a potential functional food ingredient [12]. Meal replacement products (shakes, powders, and bars) are designed to provide the calorie and nutrients a typical meal supplies. Nutrition bar market is gaining popularity among young consumers, whose lifestyle and food trends play a key role in their consumption behavior [13]. The current study demonstrates the formulation of foxtail millet meal replacement bars, its nutritional content, consumer acceptability, and its antioxidant efficacy.

2 MATERIALS AND METHODS

2.1 Procurement of Raw Material

Dehulled foxtail millet grains, puffed rice, corn flakes, raisins, skimmed milk powder, honey, and sugar powder purchased from the local markets of Vellore, Tamilnadu.

2.2 Preparation of Foxtail millet Meal Replacement Bar

Three variations of the foxtail millet meal replacement bar were prepared based on the proportion of ingredients: FMRB-1, FMRB-2, and FMRB-3 with 25%, 27.5% and 30% incorporation of foxtail millet respectively. All the ingredients were mixed and poured into rectangle silicone molds (length- 6.5cm , breadth- 3.5cm, height -2.5cm). The bars were then baked at 180°C for 30 minutes. Each bar weighed approximately 50g. It was packed individually in aluminum foil and stored in the refrigerator until further use.

2.3 Nutritional analysis

Nutritional analysis and sensory evaluation were carried out to select the nutritionally superior and acceptable variation for further analysis. The foxtail millet meal replacement bars were subjected to nutrient analysis. Proximate composition, total phenolics and total flavonoids content were estimated using standard laboratory protocol. Anthrone method of carbohydrate estimation was adopted [14]. The Kjeldahl method as described in AACC (1986) [15] was used to determine the protein content. Fat content was estimated by hydrolyzing sample with diluted acid and extracted with petroleum ether using Soxhlet apparatus according to AOAC method (2005) [16]. Atwater factor method was used to determine the energy value of each variation [17]. The total fiber was estimated by digesting the samples (fat-free) in sulfuric acid (1.25%) followed by sodium hydroxide (1.25%) solution [15]. Iron in each sample was estimated using the standard procedure (Sadasivam et al., 2005) [14]. Calcium was estimated using the titrimetric method of AOAC (1990) [18]. The Folin-Ciocalteu method or the Gallic acid equivalence method (Singleton et al., 1965) [19] was adopted to estimate the total phenolic content. The aluminum chloride colorimetric method for estimation of the total flavonoid content in a sample was followed [20] .

2.4 Organoleptic evaluation

The organoleptic properties of the foxtail millet meal replacement bars were evaluated using the 9-point hedonic rating scale. The numerical scale denotes the degree of likeness, where 9 signifies like extremely, 5 stands for neither like nor dislike (neutral choice), and 1 indicates dislike extremely. The panelist (20 judges) evaluated the bars in a clean, inodorous, naturally lighted room.

2.5 Ethanolic extraction by maceration technique

The solvent ethanol was used for extraction. For the maceration technique, 50 gram of the sample was added to 100 ml of 70% ethanol (prepared by adding 30% water to pure ethanol thereby increasing its polarity) [21].

2.6 In- vitro Antioxidant Activity Assays

The in-vitro antioxidant assays: DDPH, Phosphomolybdenum Antioxidant, FRAP, and superoxide radical scavenging activity, were done to assess the antioxidant capacity of the selected variation at different concentrations. Ascorbic acid was used as the standard for comparison of antioxidant activity. The percentage of 2, 2-diphenyl-1-picrylhydrazyl radical scavenged by the test sample and ascorbic acid was determined by slightly modifying the procedure by Tung et al., (2009) [22]. The FRAP Assay was done for the sample and ascorbic acid by the method described by Yen et al., (1995) [23] to estimate the antioxidant power. The reducing power of the sample and standard ascorbic acid was evaluated by the Phosphomolybdenum reduction assay elaborated by Prieto et al., (1999) [24]. The procedure followed by Winterbourn et al., (1975) was used to determine the scavenging activity of Superoxide (O_2^-) radical by the sample and standard [25].

2.7 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 23 Software package. The experiments were done in triplicates and results were statistically analyzed to choose the best variation among the three. The data were subjected to analysis of variance (One-way ANOVA) with Duncan's Post Hoc test ($p<0.05$). The IC 50 value for DPPH and Superoxide Anion Radical Scavenging Activity of the sample was calculated by the Linear Regression Test using GraphPad Prism version 5.00 for Windows.

3.0 RESULTS AND DISCUSSION

3.1 Nutritional composition

The comparative analysis of nutrient content of the three variations of foxtail millet meal replacement bars showed that 30% foxtail millet incorporated bar was nutritionally superior to 25% and 27.5% of foxtail millet added bars. The energy content of the three bars ranged from 249.33to 282.67 kcal/100g. The macronutrients estimation of the bars showed low amounts of fat (2.34 to 2.63 g/100g) and adequate measures of carbohydrate (49.23 to 55.90 g/100g) and protein (7.14 to 9.05 g/100g). The fiber estimated ranged from 11.43 to 15.96 g/100g. The bars have a high content of iron 37.90 to 59.16 mg/100g and sufficient quantity of calcium 149.00 to 215.67 mg/100g. The foxtail millet meal replacement bars have high total phenolic and flavonoid content.

Table 1: *The nutritional content of the Foxtail Millet Meal Replacement Bars.*

Nutrients/ 100g	FMRB-1	FMFR-2	FMRB-3	p value
Energy (kcal)	257.67± 1.15 [b]	249.33± 1.15 [a]	282.67± 1.15 [c]	0.000
Carbohydrate(g)	50.81± 0.21[b]	49.23± 0.31[a]	55.90± 0.26[c]	0.000
Protein (g)	8.32± 0.08[b]	7.14± 0.05[a]	9.05± 0.18[c]	0.000
Fat (g)	2.34± 0.01[a]	2.63± 0.01[c]	2.57± 0.02[b]	0.000
Fiber (g)	11.43± 0.35[a]	15.96± 0.15[c]	13.53± 0.25[b]	0.000
Calcium (mg)	154.33± 2.08[b]	149.00± 1.00[a]	215.67± 3.21[c]	0.000
Iron (mg)	37.90± 0.10[a]	46.76± 0.21[b]	59.16± 0.25[c]	0.000
TPC (mg GAE/g)	120.39± 0.44[b]	118.94± 0.02[a]	123.10± 0.02[c]	0.000
TFC (mg QE/g)	13.63± 0.02[b]	12.07± 0.01[a]	15.69± 0.05[c]	0.000

FMRB- Foxtail millet Meal Replacement Bar. The outcome is expressed in Mean ± STDEV (n=20). Statistically significant at $p < 0.05$, where $^{a<b<c}$ in each row.

3.2 Organoleptic evaluation

The consolidated rating from the organoleptic evaluation shown in Table 1 suggests that bars were well received in

all aspects of a sensory profile and the acceptability of the three variations were not statistically significant.

Table 2: *Organoleptic Evaluation of the three variations of Foxtail millet Meal Replacement Bar.*

Parameters	FMRB-1	FMFR-2	FMRB-3	pvalue
Appearance	8.60± 0.59[a]	8.40± 0.68[a]	8.25± 0.55[a]	0.092
Flavour	8.20± 0.62[a]	8.25± 0.64[a]	7.95± 0.76[b]	0.190
Colour	8.15± 0.58[a]	8.05± 0.76[a]	8.30± 0.66[a]	0.273
Texture	8.20± 0.62[a]	7.95± 0.76[a]	7.70± 1.08[a]	0.080
Taste	7.85± 0.75[a]	8.15± 0.75[a]	8.20± 0.69[a]	0.157
Overall acceptability	8.15± 0.67[a]	8.15± 0.48[a]	8.35± 0.67[a]	0.339

The outcome is expressed in Mean ± STDEV (n=20). Statistically significant at $p < 0.05$, where $^{a<b}$ in each row.

3.3 In-vitro antioxidants Assays

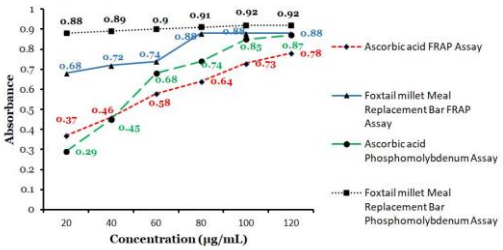
The nutritionally finest meal replacement bar (FMRB-3) was further assayed to assess in vitro antioxidant potential. The results from Table 3 indicate that the FMRB-3 foxtail millet meal replacement bar is effective against DPPH free radical. The IC50 value of the same is 74.59 µg/ml, though it is more than the ascorbic acid concentration (45.139 µg/ml) required for a 50% inhibitory effect against DPPH.

Table 3: *In-vitro Antioxidant Activity of selected Foxtail millet Meal Replacement Bar.*

Concentration of Sample (µg/ml)	DPPH %		FRAP absorbance value		Phosphomolybdenum absorbance value		O_2^-RSA %	
	AA	FMRB	AA	FMRB	AA	FMRB	AA	FMRB
20	34.57± 0.01[a]	17.05± 0.39[a]	0.3789± 0.0001[a]	0.6860± 0.0060[a]	0.2914± 0.0001[a]	0.8830± 0.0000[a]	25.83± 0.01[a]	6.27± 0.16[a]
40	45.76± 0.02[b]	21.96± 0.59[b]	0.4618± 0.0001[b]	0.7190± 0.0050[b]	0.4479± 0.0001[b]	0.8927± 0.0006[b]	33.17± 0.01[b]	12.86 ±0.16[b]
60	58.34± 0.03[c]	41.60± 0.45[c]	0.5854± 0.0001[c]	0.7370± 0.0040[c]	0.6792± 0.0000[c]	0.9030± 0.0000[c]	48.05± 0.01[c]	13.02 ±0.16[b]
80	73.24± 0.01[d]	52.97± 0.44[d]	0.6430± 0.0001[d]	0.8797± 0.0015[d]	0.7414± 0.0002[d]	0.9110± 0.0000[d]	54.63± 0.01[d]	14.63 ±0.16[c]
100	86.12± 0.02[e]	60.98± 0.45[e]	0.7336± 0.0001[e]	0.8837± 0.0015[d]	0.8504± 0.0002[e]	0.9170± 0.0000[e]	68.82± 0.01[e]	16.13 ±0.19[d]
120	89.93± 0.02[f]	86.69± 0.23[f]	0.7812± 0.0001[f]	0.8857± 0.0012[d]	0.8738± 0.0001[f]	0.9180± 0.0000[f]	72.67± 0.01[f]	17.90 ±0.09[e]

DPPH- 2, 2-diphenyl-1-picrylhydrazyl; FRAP- Ferric Reducing Antioxidant Power; O_2^-RSA- Superoxide Anion Radical Scavenging Activity %; AA- Ascorbic acid;. Each value in the table are represented as Mean ± STDEV (n=3). Statistically significant at $p < 0.05$, where $^{a<b<c<d<e<f}$ in each column.

Figure 1: *Absorbance value of extracted Foxtail millet Meal Replacement Bar and Ascorbic acid for FRAP and Phosphomolybdenum Assay.*



The reducing ability of the bar determined through FRAP and phosphomolybdenum assay, showed the highest absorbance values of 0.8857±0.0012 and 0.8738±0.0001 respectively at a concentration of 120 µg/ml of sample extract. Figure 1 illustrates that the sample has higher absorbance than standard ascorbic; indicating a higher reducing power compared to ascorbic acid. The foxtail millet meal replacement bar has relatively poor superoxide anion scavenging activity with an IC50 value of 436.61 µg/ml than ascorbic acid (IC50=68.933 µg/ml). The meal replacement bar has good antioxidant efficacy.

4.0 DISCUSSION

Millets, in general, are a remarkable source of protein, calcium, dietary fiber, and polyphenols [26]. Foxtail millet is the key ingredient of the meal replacement bar and its contribution to the nutritional value of the bar is irrefutable. Studies have investigated the nutritional content of foxtail and reported that it has 9.5-18.9 g/100g protein, total carbohydrate of 71.5-83.8 g/100g and the crude fat content was 4.4.-7.3 g/100g [27]. Mineral content is also sufficient, certain genotypes of foxtail has 1.99-22.69 mg/100g of calcium and 2.47-16.46 mg/100g of iron [28]. Kim et al., (2010) [29] reported that ethanolic extracts of foxtail used in their study have total phenol content (TPC) of 12.0-26.7 mg GAE/ g and total flavonoid content (TFC) of 4.0-8.1 mg QE/g. Cereal meal replacement bars typically have a protein content of 4.4 to 25 g/100g, 50.3 to 72.9 g/100g of carbohydrate, 3.1 to 17.6 g/100g of fat, 2.1 to 10 g/100g of fiber, 3.18-16.3 mg/100g of iron and 15-953 mg/100g of calcium. The wide range of nutrient content is attributed to the type and variety of ingredients used [30]. The meal replacement bars formulated do meet the nutritional standard. For the study, we have used 70% ethanol as the solvent. Studies have shown that ethanolic extracts have higher antioxidant activity as compared to other aqueous extracts. Lapornik et al., (2009) [31] have concluded that the presence of higher amounts of polyphenols in ethanolic extracts increases the antioxidant activity; the non-polar character of the solvent contributes to its efficiency in the cell wall and seed degradation, which causes polyphenols to be released from cells. The polyphenols in foxtail are known to inhibit oxidants [32-35] and contribute to the antioxidant property of the bar. Furthermore, natural antioxidants present in foxtail such as, protein hydrosylates [36], polysaccharides [37], fiber [38], also exhibit optimum radical scavenging activity and reducing power.

5.0 CONCLUSION

Antioxidants in food are necessary to reduce the oxidative stress on cells by free radicals generated in the body. Instead of consuming high dose antioxidant supplements,

that may be harmful; it is better to eat antioxidant-rich foods. Foxtail millet is certainly a potential functional food, and preparation of ready-to-eat foxtail millet-based foods definitely helps overcome the barrier of reduced consumption due to cooking difficulty. The study focused on preparing ready-to-eat antioxidant rich meal replacement bar using foxtail millet as the key ingredient. This foxtail millet-based meal replacement bar has sufficient nutrients as well as good antioxidant activity (86.69% scavenging activity of DPPH and 88.57% FRAP). The organoleptic scores suggests that the bars will be accepted by most consumers.An understanding of the health benefits of foxtail millet is essential for the utilization of this cereal as a dietary supplement. In conclusion, more foxtail millet incorporated ready-to-eat food has to be produced, as it can be a healthy snack for all age groups.

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7.0 APPENDICES

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