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Nutraceutical characterization and shelf life analysis of millet incorporated nutrition bars

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ABSTRACT

Recently, the nutraceutical sector of the food trade is unfolding, and designer foods such as nutrition bars have found their place in this competitive industry. The inclusion of underutilized food sources in the development of new value-added products is ingenious. Millets, the indigenous crop, are a good source of nutrients. The Nutri-cereal is still lacking commercial success and deserves recognition in the food-processing sector. In the current study, foxtail and pearl millet are used to develop foxtail millet meal replacement bar (FMRB) and pearl millet protein bar (PPB), respectively. Three variants of each type (25%, 27.5%, and 30% incorporation of millets) were developed to derive the nutritionally preferred variants. Estimation of macronutrients, essential amino acids, and vitamin content was done. The storage stability of the selected variants was evaluated for 42 days under accelerated conditions. The peroxide value, moisture content, water activity, total plate count, and yeast & mold count was assessed. The result revealed, among the variants, 30% FMRB (V-3) and 25% PPB (V-4) are the nutritionally finest bars. The shelf-life testing pointed out that the protein bar deteriorates rapidly than the meal replacement bar. The correlation between the nutrient composition and shelf-life assessment factors indicated the shelf-life parameters negatively correlate with carbohydrates present in the bars. However, fat and protein have a positive correlation with shelf-life parameters ($r = 1.00$, $p<0.01$). Favorable storage conditions and appropriate packing material that is conducive to retain the stability of the product can extend the shelf-life. Millet nutrition bars would revolutionize the agriculture and food industry. Thus, increasing the consumption of millets.

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INTRODUCTION

Millets are heterogeneous, small-seeded cereals that can grow in adverse climate-related areas. The crops' genetic makeup assures quality nutrient content, which can strengthen the pillars of food security of a nation (Mal *et al.*, 2010). The grains' rich nutrient elements establish their nutraceutical properties that help in managing diseases (Veena, 2002). This miracle crop is the staple food source in Africa and parts of Asia (Chandrasekara and Shahidi, 2011).

In India, it is extensively cultivated due to its tolerance to extreme climatic stress. Traditional food

recipes such as dosa, upma, khichdi, idli, desserts are made using millets. However, the consumption rate has dropped drastically over the years (FAO-STAT, 2016). There is a lack in the commercialization of millet products but the practice of cooking theses nutritionally superior crops at the household level is still followed (Subramanian and Viswanathan, 2003). This creates a void in the cultivation of millets, hence international and national research bodies have taken the initiative to promote the production and utilization of millets on a large scale.

The focus is on improving processing techniques to generate an easily consumable form of millets. Since millet grains have tough structural build, it requires efficient storage conditions and preparation techniques. The structural complexity also acts as a barrier in accessing the nutrients; processing techniques are known to break this barricade and increase the bioavailability of indispensable nutrients. Research conducted on millets has established that processes like milling, fermentation, germination, popping, puffing, extrusion, roller drying, enhances protein quality, in vitro digestibility and availability of macro and micronutrients (Cissé *et al.*, 1998; Arora *et al.*, 2011).

An understanding of suitable processing, salubrious alternatives, product development, lifestyle trend, and consumer buying behavior are key factors in determining the future scope of large scale production of millet-based ready-to-cook/eat (RTC/RTE) products (Nehir and Simsek, 2011; Bchir *et al.*, 2017). The food industry considers these inevitable factors and has made changes in the product range of RTC/RTE foods.

The nutrition bar industry is rapidly growing and conquering the functional food market, as it meets the need and wants of demanding customers. A myriad of nutrition bars are now available; exploration of ingredients to create a new line of designer foods is the latest trend in the food industry (Tech-Sci Research, 2020). The global food trend gives an opportunity to use underutilized ingredients such as millets to formulate nutraceutical foods designed to provide health benefits. In this study, foxtail millet and pearl millet are used to develop meal replacement bars and protein bars, respectively. Their nutraceutical properties and shelf-life are analyzed.

MATERIALS AND METHODS

Raw Materials

Dehulled foxtail millet and pearl millet grains, corn flakes, honey, peanuts, puffed rice, raisins, skimmed milk powder, soy nuts, and sugar needed for the preparation of foxtail millet meal replacement bar (FMRB) and pearl millet protein bar (PPB) were purchased from the local markets of Vellore, Tamilnadu. Whey protein was purchased online from the manufacturer.

Preparation of Millet Nutrition Bars

The ingredients required for the preparation of FMRB are steamed and oven-dried dehulled foxtail millet grains, corn flakes, puffed rice, skimmed milk powder, raisins, honey, and sugar (finely powdered). Similarly, for the preparation of PPB, steamed and oven-dried dehulled pearl millet grains, roasted peanuts, roasted soy nuts, whey protein, raisins, honey, and sugar powder are required. Three variants of each FMRB and PPB were prepared based on the proportion of ingredients: V-1, V-2, and V-3 with 25%, 27.5%, and 30% addition of foxtail millet respectively and V-4, V-5, and V-6 with 25%, 27.5%, and 30% incorporation of pearl millet respectively. 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 and refrigerated until further use.

Nutritional analysis

Nutritional analyses were carried out to select the nutritionally superior and acceptable variation for further analysis. Proximate composition: Energy (*Atwater factor method*) (Chima and Igyor, 2007), carbohydrate estimation (*Anthrone method*) (Sadasivam and Manickam, 2005), protein estimation (*Kjeldahl method-AACC 46-10.01*) (Yang *et al.*, 2015), and fat estimation (*Soxhlet method*) (AOAC, 2005). The essential amino acid composition was determined according to the method of Schuster (1988). Vitamins A, C, and D content were assessed using standard protocols (Ugbogu and Ogodo, 2015; Achi *et al.*, 2017)

Shelf-life Analysis

The nutritionally best variation from each type of nutrition bar was further analyzed to understand its keeping quality. The nutrition bars were packed individually in an aluminum foil and stored in a sterile room at $40\pm2^{\circ}$C temperature and $80\pm5\%$ relative humidity for a period of 42 days and every seventh day the samples' shelf-life quality was assessed. The accelerated shelf-life testing focused on chemical spoilage and microbial spoilage. The chemical tests carried out to evaluate product stability were moisture content (*AOAC 934.01*) (Ileleji *et al.*, 2010), peroxide value (*AOCS Cd 8-53*) (Crowe and

White, 2001), and water activity (AOAC, 2016). The total plate count (IS 5402, 2012), yeast and mold count (IS 5403, 1999) was tested to check microbial growth.

Statistical Analysis

All the tests related to nutritional and shelf-life were done in triplicates and analyzed using descriptive statistics such as mean and standard deviation. The inferential statistical test, single-factor ANOVA with Duncan post hoc was employed to compare the nutritional components of the bars and to determine the best variation of FMRB and PPB, also to evaluate the changes in intrinsic factors during storage of the bars. Bivariate correlation, another inferential statistics was done to assess the interrelationship between nutrient content and shelf-life parameters using Karl Pearson's correlation. The IBM SPSS 23.0 was used for the statistical analysis.

RESULTS AND DISCUSSION

Nutritional composition

The comparative analysis of nutrient content of the three variants of foxtail millet meal replacement bars (FMRB) and three variants of pearl millet protein bars (PPB) showed that the proximate composition of the protein bars was higher than the meal replacement bars. The meal replacement bars provide 249 to 283 kcal, a moderate amount of carbohydrate (49 to 56 g) and protein (7 to 9 g), and low fractions of fat (2 to 3 g). The protein bars impart more than sufficient energy (332 to 379 kcal), high protein (16 to 18 g), moderate carbohydrate (54 to 56 g), and fat (5 to 10 g). Among the variants, 30% FMRB (V-3) and 25% PPB (V-4) were the nutritionally finest bars.

The protein quality of food is the totality of its amino acid composition (Sloan, 1999). The essential and conditional amino acids in food are a good determinant of its nutraceutical property. The amino acid content of the protein bars is more than that of meal replacement bars. It is observed that the essential amino acid tryptophan is the limiting amino acid in all the variants, and leucine content is high (Table 1 and Table 2). Past studies also have reported a low concentration of tryptophan and a high concentration of leucine in both foxtail and pearl millet (Kamara *et al.*, 2009; Amadou and Le, 2013). The protein bars have a high content of leucine, whereas valine content of the meal replacement bars is significant. Cysteine and tyrosine are conditional amino acids that were measured with methionine and phenylalanine, respectively are found to be in reasonable concentration. Vitamin C content of the PPBs is relatively higher than FMRBs. Vitamin A and D were below the level of quantification and hence couldn't be detected. The nutraceutical characteristic of vitamin C makes it an inevitable nutrition marker of health. The anti-oxidative property of ascorbic acid hinders the oxidation of nutrients, thereby improves stability, delays rancidity, and inhibits microbial growth.

The foxtail millet and pearl millet are known for their nutritional components. In this study, foxtail millet incorporated nutrition bars meet the nutritional criteria for meal replacement bars or balance bars. These bars are convenient to consume and provide the needed calories for day-to-day functions. Comparing the results yielded with past studies on protein bars reveal that pearl millet-based protein bar has excellent protein quality (Pramitasari *et al.*, 2018; Kumar *et al.*, 2018). The other ingredients complement the nutrient quality of the bars.

Shelf-life of the selected variants

The shelf-life testing of both the bars carried out for a period of forty-two days points out that the protein bar deteriorates rapidly than the meal replacement bar (Table 3). The accelerated storage condition hastens the process of food spoilage. The shelf-life testing demonstrates the substantial influence extrinsic factors have on the intrinsic parameters. As the relative humidity and temperature of the storage room were high, the moisture content of the bars has increased drastically ($p<0.01$).

Peroxide value, a reliable measure of rancidity, was not detected even on the fourteenth day. Indeed till the 21^{st} day, the peroxide value of meal replacement bars was below the level of quantification. The moisture and water activity in protein bars are more pronounced than meal replacement bars. It is also noted that microbial spoilage of the protein bar is drastic.

It is observed that there is a notable relationship between the nutrients present in the bars and shelf-life parameters (Table 4). The energy content of the bar has a positive correlation with all the parameters ($r= 0.99$, $p<0.01$). The shelf-life parameters negatively correlate with carbohydrates present in the bars. However, fat and protein have a positive correlation with shelf-life parameters ($r= 1.00$, $p<0.01$).

Peroxide value is the degree of oxidation of extracted fat. The internal and external factors such as light, heat, moisture, pro-oxidizable substrate, and certain microbes catalyze the oxidation of fat (Patterson, 2011). Acknowledging these factors, the correlation between total plate count, moisture, water

Table 1: Essential amino acid and vitamin composition of FMRB

Essential amino acid and vitamin content	Variant Code		
	V-1	V-2	V-3
Isoleucine (mg/g)	$55.54\pm0.88^{b**}$	$41.92\pm0.52^{a**}$	$60.39\pm0.20^{c**}$
Leucine(mg/g)	$95.49\pm0.33^{b**}$	$90.44\pm0.19^{a**}$	$98.93\pm0.21^{c**}$
Lysine(mg/g)	$73.53\pm0.68^{b**}$	$63.05\pm0.39^{a**}$	$79.95\pm1.55^{c**}$
Histidine(mg/g)	$46.53\pm0.51^{b**}$	$38.51\pm0.31^{a**}$	$51.52\pm1.19^{c**}$
Valine (mg/g)	$113.50\pm0.10^{d**}$	$110.97\pm0.51^{c**}$	$118.83\pm0.01^{e**}$
Threonine (mg/g)	$73.59\pm0.01^{b**}$	$71.48\pm0.11^{a**}$	$74.75\pm0.05^{c**}$
Tryptophan (mg/g)	$34.25\pm0.32^{b**}$	$29.37\pm0.19^{a**}$	$37.58\pm0.18^{c**}$
Methionine+Cysteine(mg/g)	$57.74\pm0.11^{b**}$	$54.82\pm0.41^{a**}$	$58.53\pm0.26^{c**}$
Phenylalanine+Tyrosine(mg/g)	$100.47\pm0.40^{b**}$	$99.33\pm0.21^{a**}$	$100.70\pm0.17^{b**}$
Vitamin A (mg/kg)	ND	ND	ND
Vitamin C (mg/kg)	$316.33\pm3.21^{a**}$	$356.00\pm1.00^{b**}$	$428.33\pm1.53^{c**}$
Vitamin D (mg/kg)	ND	ND	ND

The values are presented as mean ± standard deviation (n=3). ** Significantat 0.01 level. The superscripts indicate the difference in means in each row,where $^{a<b<c<d<e<f}$. ND- Not detected.

Table 2: Essential amino acid and vitamin composition of PPB

Essential amino acid and vitamin content	Variant Code		
	V-4	V-5	V-6
Isoleucine (mg/g)	$92.24\pm0.12^{f**}$	$79.14\pm0.05^{d**}$	$85.96\pm0.74^{e**}$
Leucine(mg/g)	$154.18\pm0.03^{f**}$	$133.59\pm0.01^{d**}$	$143.69\pm0.04^{e**}$
Lysine(mg/g)	$92.33\pm0.02^{f**}$	$89.01\pm0.02^{d**}$	$90.06\pm0.02^{e**}$
Histidine(mg/g)	$71.06\pm0.01^{d**}$	$70.91\pm0.01^{d**}$	$70.94\pm0.01^{d**}$
Valine (mg/g)	$94.26\pm0.03^{b**}$	$91.96\pm0.01^{a**}$	$92.01\pm0.02^{a**}$
Threonine (mg/g)	$86.80\pm0.01^{f**}$	$78.65\pm0.02^{d**}$	$81.86\pm0.02^{e**}$
Tryptophan (mg/g)	$60.01\pm0.06^{f**}$	$51.17\pm0.05^{d**}$	$52.09\pm0.02^{e**}$
Methionine+Cysteine(mg/g)	$106.67\pm0.12^{f**}$	$86.45\pm0.05^{d**}$	$99.42\pm0.15^{e**}$
Phenylalanine+Tyrosine(mg/g)	$136.36\pm0.02^{c**}$	$136.45\pm0.02^{c**}$	$140.26\pm0.03^{d**}$
Vitamin A (mg/kg)	ND	ND	ND
Vitamin C (mg/kg)	$553.67\pm2.08^{e**}$	$541.00\pm6.08^{d**}$	$572.00\pm5.29^{f**}$
Vitamin D (mg/kg)	ND	ND	ND

The values are presented as mean ± standard deviation (n=3). ** Significantat 0.01 level. The superscripts indicate the difference in means in each row,where $^{a<b<c<d<e<f}$. ND- Not detected.

activity, peroxide value, and fat is justifiable. The water holding capacity of food is the ability to retain water by components such as protein or starch in food. The interaction of the protein with water affects the shelf-life, texture, quality, and palatability of food (Zayas, 1997). This explains the reason for the faster deterioration of protein bars than meal replacement bars. The results prove that nutritional parameters and shelf-life parameters are strongly interrelated.

A perfect uphill correlation between the variables is noted (r=1.00, p<0.01). It indicates if the value of one parameter increases, then the value of the other variable increases simultaneously (Table 5). Water in foods is a key factor in deciding the quality of the food product. When the moisture content is increasing, there's a surge in the microbial load. The water activity expresses the free water available for microbial growth, enzymatic reactions, chemical, and biological changes in food. Therefore there is a positive relationship between water activity, peroxide value, and total plate count, and the results yielded conform with available data (Padmashree *et al.*, 2013).

Table 3: Shelf life analysis of selected FMRB (V-3) and PPB (V-4)

Storage period (days)	Nutrition Bar	Moisture @ 105°C (g/100g)	Water activity @ 25°C (a_w)	Peroxide value (meq/kg)	Total Plate Count (cfu/g)	Yeast & Mould (cfu/g)
0	V-3	$15.03\pm0.06^{a**}$	$0.700\pm0.000^{a**}$	ND	ND	ND
	V-4	$16.60\pm0.10^{b**}$	$0.758\pm0.001^{b**}$	ND	ND	ND
7	V-3	$15.63\pm0.06^{a**}$	$0.700\pm0.000^{a**}$	ND	ND	ND
	V-4	$17.03\pm0.06^{b**}$	$0.769\pm0.001^{b**}$	ND	ND	ND
14	V-3	$15.93\pm0.06^{a**}$	$0.700\pm0.000^{a**}$	ND	$59\pm3^{a**}$	ND
	V-4	$19.87\pm0.06^{b**}$	$0.778\pm0.001^{b**}$	ND	$81\pm1^{b**}$	ND
21	V-3	$16.10\pm0.09^{a**}$	$0.701\pm0.001^{a**}$	ND	$104\pm5^{a**}$	ND
	V-4	$20.33\pm0.06^{b**}$	$0.781\pm0.001^{b**}$	$1.66\pm0.01^{a**}$	$213\pm15^{b**}$	ND
28	V-3	$17.50\pm0.10^{a**}$	$0.704\pm0.001^{a**}$	$1.10\pm0.01^{a**}$	$122\pm4^{a**}$	ND
	V-4	$20.70\pm0.10^{b**}$	$0.782\pm0.001^{b**}$	$2.49\pm0.01^{b**}$	$300\pm10^{b**}$	ND
35	V-3	$18.10\pm0.02^{a**}$	$0.709\pm0.001^{a**}$	$1.50\pm0.01^{a**}$	$156\pm3^{a**}$	ND
	V-4	$23.10\pm0.10^{b**}$	$0.787\pm0.001^{b**}$	$2.79\pm0.01^{b**}$	$435\pm5^{b**}$	ND
42	V-3	$18.70\pm0.02^{a**}$	$0.712\pm0.001^{a**}$	$1.72\pm0.01^{a**}$	$218\pm3^{a**}$	ND
	V-4	$23.73\pm0.06^{b**}$	$0.789\pm0.001^{b**}$	$2.96\pm0.01^{b**}$	$612\pm3^{b**}$	ND

The values are presented as mean ± standard deviation (n=3). ** Significantat 0.01 level. The superscripts indicate the difference in means between V-3and V-4 based on the storage period, where $^{a<b}$. ND- Not detected.

Table 4: The relationship between the nutrient content of the bars and their shelf life quality

Shelf-life Parameters	Nutrients			
	Energy	Carbohydrate	Protein	Fat
Moisture	0.99**	-0.98**	1.00**	1.00**
Water Activity	0.99**	-0.97**	1.00**	1.00**
Peroxide Value	0.99**	-0.98**	1.00**	1.00**
Total Plate Count	0.99**	-0.97**	1.00**	1.00**

**Significant at 0.01 level

Table 5: The relationship between chemical deterioration factors and biological deterioration factor

Variables	Moisture	Water Activity	Peroxide Value	Total Plate Count
Moisture	1	-	-	-
Water Activity	1.00**	1	-	-
Peroxide Value	1.00**	1.00**	1	-
Total Plate Count	1.00**	1.00**	1.00**	1

**Significantat 0.01 level

CONCLUSION

The foxtail millet meal replacement bars and pearl millet protein bars are designer foods that meet the current trend. The results pertaining to the study indicate the nutritional importance of these bars. Millet is the principal ingredient and it is well endowed with nutraceutical properties. The inclusion of other ingredients has complemented the nutrition quality. Since the bars developed are made without the addition of artificial sweeteners and preservatives, it is safe to eat. The storage stability of the bars under high temperature and humidity showed that the bars deteriorated quickly. The protein bar was the first to show microbial spoilage. The conclusive decision on the shelf-life is one month. Favorable storage conditions and appropriate packing material that is conducive to retain the stability of the product can extend the shelf-life.

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