J Food Sci Technol (November 2023) 60(11):2782–2791
https://doi.org/10.1007/s13197-023-05796-y

ORIGINAL ARTICLE

Quality evaluation of nutri-premix prepared by using millets and seeds of fruits and vegetables

Meenakshi Garg[1] · Manjoor Ali[1] · Vandana Batra[1] · Susmita D. Sadhu[1] · Sadhana Sharma[2] · Suradeep Basak[3] · Vandana Sablania[4]

Revised: 20 May 2023 / Accepted: 1 June 2023 / Published online: 4 August 2023

Abstract The objective of the present research was intended to formulate multigrain premix powder which could be utilized for the development of nutritional rich products. The multigrain premix was prepared by blending the seeds of pumpkin, jackfruit, and mango with barley, pearl millet, finger millet, sorghum, and other ingredients such as cardamom, and sugar. Before optimizing the composition of premix flour, around 8 combinations of each flour and seed powders were made to obtain the preeminent quality premix with high nutritional value. The formulation of flour was optimized on the basis of sensory analysis done by using 9-hedonic scale. The formulated multigrain premix was analysed for its nutritional and sensorial characteristics. Multigrain premix resulted in protein content of 5.35 g, carbohydrate 80.25 g, fat 6.88 g, ash 3.87 g, dietary fibres 8.67 g, calcium 73.25 mg, and iron 2.94 mg per 100 g of the mixture and many more minerals were also estimated in the given premix. Total energy was noted as 404.32 kcal. The GC–MS analysis was also performed to identify the composition of fat in terms of their saturation. Moreover, the shelf life study of multigrain premix was carried out for a period of 45 days at a temperature and relative humidity of 25 °C and 91% respectively. The overall quality of the multigrain premix was accepted in term of overall acceptability. The optimized premix was also taken for its microbiological analysis, and sensorial quality attributes to understand the shelf life study of the product when stored for longer period of time.

Keywords Multigrain premix · Microbiological analysis · Free fatty acids · Water activity · Shelf life

Abbreviations

AOAC	Association of Official Agricultural Chemists
FFA	Free fatty acids
FSSAI	Food safety and standards authority of India
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids

Introduction

In recent time, the demand of fast food, read to eat, ready to serve food has been increased tremendously due to globalization and modernization. People have started preferring light and quick meal in order to save cooking time and ease of consumption anywhere while travelling. Due to which people usually consume imbalance and unhealthy diet. Thus result in various diseases and health issues. In order to combat with such deficiency or unhealthy diet, the demand of protein and dietary rich ready to eat or ready to cook products have been increased exponentially. Moreover, malnutrition is a major disorder in India which is described by excess or deficiency of certain nutrients which creates adverse health effects and reduced life expectancy. In the market, different types of snack foods like Patty, burger, potato chips, nachos, samosa, extruded products, chocolates bars, toffees etc. are easily available but these snacks do not meet the requirement of a healthy individual (Njike

✉ Vandana Sablania
212vandysablania@gmail.com

1 Bhaskaracharya College of Applied Sciences, University of Delhi, New Delhi, India

2 Asian Medical Institute, Kant, Kyrgyzstan

3 Department of Biotechnology, UIBT, Chandigarh University, Mohali, Punjab, India

4 Institute of Home Economics, University of Delhi, New Delhi, India

et al. 2016). Continuous consumption of fast food, alcohol and sedentary lifestyle are majorly responsible for increase in the rate of overweight, obesity, diabetes, coronary heart disease and various health threats. Due to lack of time for working women, there is an increased demand for nutritious, healthy, low priced, and easy to carry food products. Hence, consumption of nutrient rich diet can overcome such emerging problems by formulating the products either by fortification or enrichment with dietary fibres, proteins, minerals etc. This demand has provoked food manufacturers to develop healthy premix which can be eaten as such or can be modified in any shape like round ball, bar, flat bread, extruded products, soup powder etc. Food demanded by consumers should be rich in vitamins, minerals and major nutrients like carbohydrates, proteins and fats so that it can help to overcome the problem associated with malnutrition. Hence, the food industry is intensively working on the production of low-calorie, low-fat, and high-fibre food since many years in response to public interest. Multigrain products are good source of micronutrients (Sahu et al. 2015) and dietary fibres along with therapeutic health benefits like gut microflora retention, bowel movement, and reduction in cholesterol levels due to their better dietary fiber content (Angioloni and Collar 2011). Inclusion of whole grains in diet improves the nutritive value of the products as they are rich source of dietary fibres, minerals, antioxidants, and phenolic compounds which play a vital role in protecting against obesity, cancer, cardiovascular diseases, and diabetes (Slavin 2003). Some of the cereals and coarse cereals can be used for the development of multigrain premix along with some seeds such as pumpkin seeds and mango kernel flour which are rich in minerals like calcium, magnesium, manganese, copper, protein and zinc. It also contains phytosterols and free-radical which can boost the health (Rakcejeva et al. 2011; Kittiphoom 2012; Pradeep et al. 2014). The incorporation of required nutrients from coarse cereals (barley, sorghum, pearl millet, finger millet etc.) are rich in dietary fibres, minerals and antioxidants (Saleh et al. 2013). Pearl millet contains about 92.5% carbohydrates, 2.1% ash, 7.8% crude fat, 13.6% crude protein and 2.8% crude fibre which can significantly add value to new products (Saleh et al. 2013). There are various scientists who has developed cookies rich in fiber (21%) and iron (12.8%) by using whole multigrain flour and fructo-oligosaccharides (Handa et al. 2010). Subsequently, Mango (*Mangifera indica* L.) kernel powder and pumpkin seed powder observed as a good source of biological active components (such as stigmasterol, tocopherols, and phenolic compounds) due to which they can be used for the development of nutrient rich product (Gohari et al. 2011; Aslam et al. 2014; Syed et al. 2019). Currently, various types of fruit bars are lined up at super markets mostly in urban areas and the market price of these bars ranged between 85–130 rupees (1.04–1.59 USD) per 35–45 g bar. Due to the high cost of these healthy bars, low and middle income group families are unable to consume them on regularly basis. Therefore, it was decided to explore underutilized crops for the production of low cost healthy food or multigrain premix with improved nutritive value. This study had been proposed to optimize and formulate a functional multigrain premix from an indigenous ingredients such as pearl millet, barley, finger millet, sorghum, and seeds and peels released by the industry such as pumpkin seeds, mango kernel flour etc., in addition to reduce the calories with high nutritive value, and high fibre content.

Materials and methods

Materials

Various ingredients such as sorghum (Jowar), pearl millet (Bajra), finger millet (Ragi), Barley (Jau), mango kernel powder (*Mangifera indica* Linn.), pumpkin seeds powder, and jack fruit seed powder (*Artocarpus heterophyllus* Lam.) were purchased from the local market of Dwarka, New Delhi, India for the formulation of multigrain mix. Brown sugar, glucose powder, and cardamom were purchased from "SPAR" located at Pacific Mall, Subhash Nagar, New Delhi, India. The Chemicals used in analysis were of analytical grade and ordered from Sigma Aldrich (Seelze, Germany).

Processing of raw ingredients

Seeds and kernels

Whole pumpkin and mango fruit was purchased from the market and they were washed thoroughly before cutting. Pumpkin seeds were separated from the fruit and then dried in tray drier at 50 °C for 3–4 days until it was dried completely. Dried pumpkin seeds were roasted directly in a pan at a temperature of 120 °C to reduce the anti-nutritional factors from the seeds and ground into coarse powder using Philips food processor (model No. HL7707/750W). Mango kernel was separated from the pulp, washed under running tap water and boiled in water for 10–15 min to remove its bitter taste. After boiling the kernel was dried in tray drier at 50 °C for 3–4 days and the ground into fine powder. Cardamom powder was also added to impart the flavor during roasting. Care should be taken while roasting to avoid over-roasting of the ingredients.

Cereals and millets flour

Sorghum, pearl millet, barley, and finger millet were cleaned properly and sieved by using 100 ASTM sieve size to separate the dust particles from them. These millets were allowed to puff at a temperature ranged between 210 to 240 °C using 2.5% salt (w/w) in a deep bottom pan and agitated continuously until it get puffed up maximum (Mishra et al. 2014).

Flavors

Brown sugar with particle size 150–1000 microns (Mawana brand) and glucose powder was added as an adjunct into the optimized multigrain premix to have better taste in it.

Formulation of multigrain premix

A number of trials were made to optimize the proportion of multigrain and seeds for making premix. A list of 8 different proportions with different flour and seed powder was designed to obtain best mixture of multigrain premix (Table 1). On the basis of preliminary trials and 9- hedonic scale sensory analysis (like taste, flavor, colour), the best combination of the premix was selected. The sensory analysis was performed by 25 number of semi-trained panelist. On the basis of overall acceptability, the optimized multigrain premix was accepted by the panelist and was further taken for various analyses.

Physicochemical analysis of multigrain premix

Nutritional analysis

The optimized multigrain premix was analyzed for its nutrient composition as per AOAC standard methods in which the crude protein was estimated by micro-kjeldahl method, crude fat was estimated by soxhlet extraction apparatus, ash content was determined by hot air oven method and carbohydrate content was calculated by method of difference {100- (moisture% + crude protein % + crude fat % + ash %)} (AOAC 2005). The detailed method for each parameter is explained further.

Protein The protein content of the given sample was determined by using micro-kjeldahl method. In this method, crude protein content was determined from the organic Nitrogen present in the sample. This method includes three different steps (Digestion, distillation and titration) for the determination of protein. During digestion, 2 g of the sample was heated for 2 h at higher temperature (350 °C) after adding 3 g of the digestion mixture (including potassium sulphate, and copper sulphate) and 25 mL of concentrated sulphuric acid which leads the conversion of nitrogenous compounds into ammonium sulphate. The produced ammonium sulphate is decomposed in the presence of 4% boric acid and 30 mL of 40% sodium hydroxide for distillation. This result in the liberation of ammonia is the presence of standard solution of excess acid and then back titrated with standard solution of 0.1 N Hydrochloric acid (HCl). Nitrogen content and protein content was calculated by using the formula mentioned below where 6.25 is a protein factor (FSSAI 2011).

$$\text{Nitrogen content} = \frac{V2 - V1 \times N \times \text{Dilution factor} \times M}{W \times 10} \tag{1}$$

where V1—Titre volume of blank, V2—Titre volume of sample, N—Normality of HCl, M—Molecular weight of nitrogen (14.007), W—Weight of the sample taken initially

$$\text{Protein content} = \text{Nitrogen content} \times 6.25$$

Fat Crude fat content was determined by using Soxhlet apparatus. In this method 5 g of the sample was taken followed by the addition 120–150 mL of petroleum ether (80–120 °C) and heated continuously for 5–6 h. After evaporation of the solvent, the fat content was determined gravimetrically (Ranganna 2005). Fat content was determined by using the formula mentioned below.

Table 1 Various proportions of multigrain and seeds used in preparation of 100 g of multigrain premix

	Jackfruit seed powder		Mango kernel seed powder	Pumpkin seed powder
Optimization of seeds from fruits and vegetables	1		1	5
	1		1	7
	1		1	9
	1		1	11
	Pearl millet	Sorghum	Barley	Finger millet
Optimization of grains and millets	4	1	1	1
	3	1	1	1
	2	1	1	1
	1	1	1	1

$$\text{Fat content}\,(\%) = \frac{\text{Weight of the sample remained after evaporation}}{\text{Weight of the sample taken initially}} \times 100 \quad (2)$$

Fat obtained from the multigrain premix was used for the preparation of Fatty acid methyl esters according to a laboratory method explained in earlier studies. Principally, 1 mg of oil was allowed to react with 0.1 M NaOH-MeOH for 5 min followed by reacting with 4% HCl-MeOH for 5 min, at ambient temperature. Once the reaction was over, fatty acid methyl esters were extracted with iso-octane. GC analysis was conducted with a Shimadzu GC-2010 equipped with a FID. A capillary column having length and breadth of 30 mm × 0.25 mm was used with helium as the carrier gas at a flow rate of 1 mL/min. Injection volume was 1 µL with initial temperature of 142 °C, it was increased to 184 °C, held for 3 min, and then increased to 244 °C. Fatty acid methyl esters were identified by comparing their retention times with those of fatty acid methyl esters standards. Area under each fatty acid peak relative to the total area of all fatty acid peaks was used to quantify the fatty acids present. Results are observed as g fatty acid/100 g of total fatty acids (Lutterodt et al. 2011).

Crude dietary fibre It was determined by using fibraplus fibre extraction instrument (Pelican equipment, Tamilnadu, India).

Ash content Ash content was determined by referring FSSAI method (FSSAI 2011). In this 5 g of the sample was ignited over the burner for the complete removal of organic compounds and transfer to a muffle furnace maintained at 550–550 °C and continue ignition till grey ash was obtained. Sample was cooled in a dessicator and weigh. The process was repeated until a grey ash is obtained.

$$\text{Ash content}\,(\%) = \frac{\text{Weight of the ash}}{\text{Weight of the sample taken}} \times 100 \quad (3)$$

Mineral content The mineral estimation (calcium, iron) was done by using Atomic Absorption Spectrophotometry method No. 922.06#960.0 (AOAC 2000) and sample preparation was done by referring Abdulrahman and Omoniyi method (Abdulrahman and Omoniyi 2016) with slight modification in sample preparation. A sample weighed 0.5 g was digested by using 50 mL of 0.1 N Nitric acid on a heating system, heated until the samples turns into colorless solution. Once the digestion was complete, the solution was transferred into an amber colored bottle until used for the estimation. The concentration of calcium, iron, phosphorus, zinc, manganese, and copper were determined by flame Atomic Absorption Spectrophotometer. Around 0.2–5 µL/mL of the sample was injected into the sampler in the presence of air-acetylene mixture. The samples were passed through an array of spectrophotometer at a specific wavelength. The wavelength used for the estimation of calcium, iron, phosphorus, zinc, manganese, and copper were set as 440, 325, 480, 220, 280, and 340 nm respectively (Rybicka and Gliszczyńska-Świgło 2017). For estimation of each mineral, the standard curve was calibrated by using appropriate concentration of an individual standard sample.

Total energy Total energy estimation was done by multiplying the carbohydrate, crude protein and crude fat values by 4, 4, and 9 respectively.

Microbiological analysis

The microbiological study of multigrain premix was done by IS-5402 and IS 5887 (Aneja 2007) which included the analysis of total plate count, coliform counts, E.*coli*, salmonella, S. *aureus*, yeast and mould counts.

Shelf-life analysis

The multigrain premix was packed in aluminium laminates having thickness of 25 micron and then stored in an Environmental Chamber (Ocean life science Corp. model no.-OLSC-116E) at 25 °C and 91% RH storage conditions. The sample was withdrawn periodically at an interval of 15 days up to 45 days. The stored sample was analyzed for water activity, free fatty acid value, peroxide value and sensory attributes.

Water activity

Free fatty acid The free fatty acid (FFA) value can be expressed as oleic acid, linolinic acid, linoleic acid, lauric acid, palmitic acid, and ricinoleic acid using various equations. FFA relatively measures the intensity of rancidity due to the degradation of triglycerides with change in temperature, moisture content and lipolytic enzyme's activity. In this study 5 g sample was taken into a 250 mL Erlenmeyer conical flask. To neutralize the sample, 50 mL of ethanol was added (95% ethyl alcohol solution) followed by the addition of few drops of phenolphthalein indicator. Boil the sample for 5 min and titrate while hot by using 0.1 N NaOH until a faint permanent pink color appears. In this case FFA (%) was estimated in terms of oleic acid (FSSAI 2011).

$$\text{FFA}(\text{mg KOH}/100\,\text{g}) = \frac{28.2 \times \text{Titre value} \times N\ \text{of KOH}}{\text{Weight of sample(g)}} \quad (4)$$

Peroxide value

Peroxide value of the multigrain mix was done by modifying the method given by Ogungbenle (2003). In this method, 2 g of the multigrain mix was taken and added to 20 mL of solvent (3 parts of glacial acetic acid: 2 parts of chloroform) in a 250 mL conical flask and heated for 30 s in a water bath. Followed by the addition of 20 mL of 50% aqueous potassium iodide solution and 25 mL of distilled water. The mixture was titrated against 0.001 N sodium thiosulpahte solution. Total disappearance of the milky white precipitate indicated the end point.

$$\text{Peroxide value}\left(\frac{\text{milliequivalent}}{\text{Kg}}\right) = \frac{\text{Titre value(Blank-sample)} \times N \text{ of sodium thiosulphate}}{\text{Weight of sample(g)}} \quad (5)$$

Sensory analysis

Consumer acceptance of the multigrain premix was judged by a group of 25 members. 5 g of premix was added into 25 mL of milk and given to panel members for the sensory analysis. The sensory test was done at a temperature of 25 ± 5 °C in Food Technology Laboratory 1 of Bhaskaracharya College of applied Science, Dwarka, New Delhi. The panelists were asked to evaluate the product with different descriptive attributes like colour, appearance, texture and flavor. Sensory scores were recorded and average scores were calculated. 9- point hedonic scale was used to assess the degree of like/dislike ranging from 'Like very much' to 'Dislike very much' with 'Neither like nor Dislike' as the average. The data was analysed and computed the number of responses as percentage.

Statistical analysis

The statistical analysis for the various parameters was performed in SPSS 20.0 (IBM Corporation, Armonk, New York). The results were expressed as mean ± standard deviation of duplicate readings and the mean values were separated using Duncan's multiple range test ($p \leq 0.05$).

Result and discussion

Optimization of multigrain premix

For preparing 100 g of premix, different proportions of cereals and seeds were taken and mixed in the ratio of 70:30, 60:40, 50:50, and 40:60 out of which 60:40 proportion (Table 2) was selected as best combination on the basis on sensory score. After optimization, the selected proportion included 8.571 g of puffed sorghum, 34.285 g of puffed pearl millet, 8.571 g of puffed barley, 8.571 g of puffed finger millet, 31.111 g of roasted pumpkin seed powder, 4.444 g of mango kernel powder, and 4.444 g of jackfruit seed powder. The obtained premix was further taken for various qualitative analyses. To improve the taste of multigrain premix 10 g of glucose powder, 10 g of brown sugar, and 3.5 g of cardamom powder was added (Table 3).

Table 2 Final optimized proportion of grains and seeds for the preparation of 100 g of multigrain premix

Multigrain proportion	Fruit and vegetable seeds proportion
70	30
60	40
50	50
40	60

Table 3 List of ingredients used for the preparation of 100 g of multigrain premix

Name of the ingredients	Amount used
Pearl millet	34.285 g
Finger millet	8.571 g
Sorghum	8.571 g
Barley	8.571 g
Pumpkin seed powder	31.111 g
Mango kernel powder	4.444 g
Jackfruit seed powder	4.444 g
Brown sugar	10 g
Glucose syrup	10 g
Cardamom powder	3.5 g

Nutrient composition of multigrain mixture

The proximate analysis of multigrain premix was shown in Table 4. The product showed reduced moisture content (2.85%) when it was compared with the premix (3.6%) formulated by Chauhan et al. (2017) by using whole wheat flour, pearl millet, finger millet, sorghum and maize for the development of biscuits.

Carbohydrate content

The carbohydrate content of the multigrain premix was found to be 80.25% (Table 4). There was no significant difference was observed in the carbohydrate content of multigrain premix when compared with multigrain khakra

Table 4 Nutrient composition of multigrain premix

Nutrients	Amount
Energy (Kcal)	404.32 ± 0.55
Moisture content (%)	2.85 ± 0.54
Carbohydrate (%)	80.25 ± 0.14
Protein (%)	5.35 ± 2.86
Fat (%)	6.88 ± 0.51
Ash (%)	3.87 ± 0.56
Dietary fibres (%)	8.67 ± 0.55
Calcium (mg/100 g)	73.25 ± 0.13
Iron (mg/100 g)	2.94 ± 0.77
Phosphorus (mg/100 g)	28.58 ± 0.11
Zinc (mg/100 g)	0.59 ± 0.03
Manganese (mg/100 g)	9.62 ± 0.25
Copper (mg/100 g)	0.92 ± 0.0.17
Total fat (%)	
Saturated fat	2.40 ± 0.03
Unsaturated fat	4.48 ± 0.05
Monounsaturated fatty acids	2.94 ± 0.01
Polyunsaturated fatty acids	1.54 ± 0.01

All the values are mean ± standard deviation for the duplicate readings

(81.1%) prepared by Chauhan et al. (2017). Higher value of carbohydrates could be results of roasting. Obadina et al. (2016) confirmed in their study that the unroasted pearl millet showed carbohydrate content of 69.44% which was comparatively lower than roasted pearl millet (72.01%). Higher value of carbohydrate might also be due to the higher proportion of pearl millet which constitutes about 70% of the starch depending upon the variety of grain (Punia et al. 2021).

Protein content

Protein content of multigrain premix was reported to be 5.35% which might be due to high proportion of pearl millet. According to Taylor (2016) protein content of pearl millet was found to be 8.6 to 19.4 g/100 g on dry basis due to its large germ size whereas other grains have comparatively less protein content such as finger millet 7% (Singh and Raghuvanshi 2012), sorghum 8–12%, barley 8–13% (Abah et al. 2020).

Fat content

It was found that the optimized product showed a good amount of fat content (Table 4). The fat content of the multigrain premix (6.88%) was found to be comparatively higher than the multigrain kharkra (1.6%) produced by Chauhan et al. (2017). This could be due to the incorporation of pumpkin seed flour which is known for a potential source of fatty acids (Veronezi and Jorge 2012). Multigrain premix observed to contain both saturated (2.40%) and unsaturated (4.48%) fatty acid. Moreover, it resulted in higher amount of monounsaturated FA as compared to polyunsaturated fatty acid (PUFA). The result showed 2.94% of the total MUFA whereas PUFA was reported to be 1.54% with a significant difference. The value of MUFA and PUFA in multigrain premix is an indication of presence of unsaturated fat which is effective in decreasing cardiovascular diseases, and helps in the formation of cellular membrane. PUFA is also useful in reducing the low density lipoprotein (Pradeep et al. 2014). Higher value of unsaturated fat could be due to the incorporation of roasted pumpkin seed powder. Aktaş et al. (2018) mentioned in their study that the dry salt roasted pumpkin seed revealed higher PUFA value as compared to unsalted roasted pumpkin seeds. However, having a higher value of oleic acid (MUFA) confirms better heat stability and nutrition composition rather than linoleic acid (PUFA) due less unsaturation. It was also reported that higher unsaturation (PUFA) is more prone to get oxidized at higher temperature due to their less stability.

Dietary fibre and ash content

The dietary fibre content of multigrain premix was reported as 8.67% due to inclusion of various millets and cereal grains which is responsible for higher fibre content in the multigrain premix (Table 4). Ashwath Kumar et al. (2019) observed increase in fibre content from 2.17 to 9.09% when the biscuits were prepared by using multigrain premix containing barley, sorghum, chickpea, soy flour, and pea. This is mainly due to the incorporation of whole grains for the preparation of multigrain premix.

Mineral content

Mineral content of the multigrain premix was reported as calcium (73.25 mg/100 g), iron (2.94 mg/100 g), phosphorus (28.58 mg/100 g), zinc (0.59 mg/100 g) and manganese (9.62 mg/100 g) and copper (0.92 mg/100 g). The amount of iron and zinc (Table 4) was found to be comparatively low when compared with the individual grains which could be due to the utilization of pearled grains. Majorly grains contain most of the minerals in their pericarp, germ, and aleurone layer which gets lost during the milling process (Taylor 2016). The higher content of calcium, and phosphorus could be due to the addition of pumpkin seeds. Devi et al. (2018) reported that the mineral content in pumpkin seed kernel were noted to be Zinc (907 mg), Phosphorus (848.6 mg), manganese (487 mg), potassium (404.9 mg), magnesium (335.6 mg), copper (124 mg), calcium (25.7 mg), iron (16.1 mg), sodium (2.2 mg) and cobalt (0.6 mg). Even, Ashoush and Gadallah (2011) mentioned that mango seed kernel has high content of polyphenolic compounds, and micro elements such as selenium, copper and zinc which could also be responsible for increase in mineral content of the formulated multigrain premix.

Shelf life analysis

In most of the food, lipid peroxidation and enzymatic hydrolysis leads to reduction in shelf-life. The most regulating factor in determining the shelf-life of dried premix is the presence of fats and oils which undergoes auto-oxidation during storage causing development of off flavor. Therefore, in order to consume quality product, the shelf life study of the formulated multigrain premix was done by analyzing the water activity, free fatty acid and peroxide value.

Water activity

Water activity is defined as the availability of free water molecules in any food commodity. Presence of free water determines the rate of change in any biochemical reactions and microbial activity. In this analysis, the optimized multigrain premix showed increase in water activity over a period of time (Fig. 1). Initially the water activity was observed to be 0.528 which was further found to be increased 0.53 after 15 days of storage. No significant difference was observed till 15 days. The value of water activity increased significantly from day 30 to 35 whilst causing in reducing the quality of the product. Reduced water activity values do not deliver a complete inactivation of microorganisms, but partially control their activities just to facilitate longer shelf-life of the product. Low water activity is rather favourable for stability of multigrain premix because it controls the microbial and oxidative activity (Sablania et al. 2018).

Free fatty acid

Free fatty acid value generally explains the hydrolysis of fats and oils in the presence of hydrogen ions. Multigrain premix showed FFA value ranged from 2.5 mg KOH/100 g to 3.04 mg KOH/100 g over a period of 45 days (Fig. 1). Increase in FFA value could be due to inappropriate storage condition or higher room temperature which will leads to deterioration fats and oils present in the sample and promotes increase in FFA value. Due to which it causes higher acidity, bitterness and rancid flavor to the end product. Nikolić et al. (2008) as observed increase in free fatty acid value of wheat flour during storage which showed a direct influence on quality of wheat flour. Earlier studies on biscuits obtained by using composite flour have also reported increase in FFA with increase in storage period (Sharma et al. 2013).

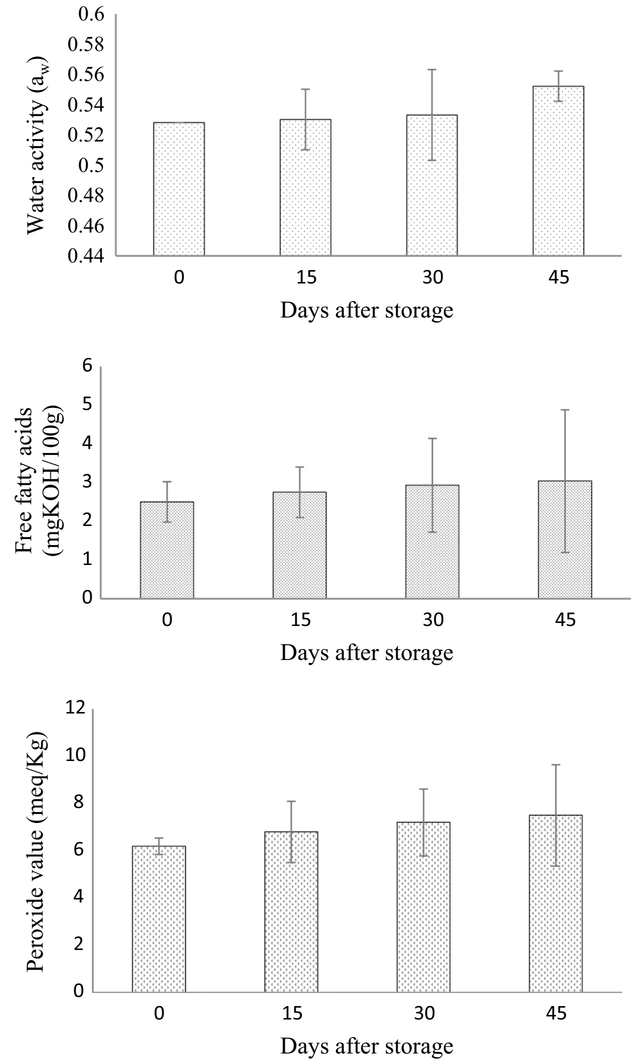

Fig. 1 Shelf life study of multigrain premix for a period of 45 days: water activity, free fatty acid value, and peroxide value

Peroxide value

Lipid oxidation is chiefly responsible for the deterioration of fats and oils. Therefore it becomes important to store the food sample under controlled conditions to minimize the release of primary metabolites such as peroxide, free radical hydroxyl group, and other reactive oxidative species (Potočnik et al. 2018). Peroxide value is used for the determination of lipid oxidation rate where peroxides are the primary products released from the breakdown of unsaturated fatty acids (Shahidi and Wanasundara 2002). The peroxide value of multigrain premix is shown in Fig. 1). Initially the peroxide value was found to be 6.22 milliequivalent/Kg and gradually it increased up to 7.5 milliequivalent/Kg over a storage period of 45 days. But again increase in peroxide value was not much significant to cause breakdown of fatty acids in large amount. Thus it showed value < 10 milliequivalent/Kg which is under the acceptable limit as per the FSSAI (FSSAI 2011). Such reduced changes could also be due to presence of mango seed powder which is a good precursor of polyphenolic compounds and retard the oxidation rate conditionally (Abdel-Aty et al. 2018). Overall change in peroxide value of multigrain premix may also be due to storage conditions such as temperature, oxygen availability, storage containers and chemical reactions occurs under unfavorable conditions. High temperature also accelerate the oxidation rate thus increases the peroxide value and causes rancidity.

Sensory analysis

Sensory analysis is one of the scientific ways to analyse and measure the quality of food product. A change in sensory attributes of multigrain premix has been shown in Table 5. Multigrain premix showed a significant difference in its overall acceptability during storage. It was reported that the product quality found to be reduced from 8.5 (0th day) to 2.5 (45th day) as per the 9 hedonic scale. Such reduction in quality could be due to the presence of lipase enzymes in millets which starts degrading the fat present in it over a long storage time and results in rancidity which leads to development of offlavor. Incorporation of fruits and vegetable seeds also enhances the rate of rancidity and leads to reduce in quality after storage.

Microbiological analysis

The shelf life analysis of multigrain premix was done to avoid any microbiological contamination. The results are shown in Table 6. The microbiological analysis of the premix was done on 0th and 45th day (last day) during storage. The results revealed that the obtained multigrain premix was devoid of *E. coli*, *Salmonella sp., S. aureus,* Yeast & mold Count. The total plate count and coliform count were within the acceptable limits as per the FSSAI (2011). On the other hand, Tangariya et al. (2018) has reported 5.5×10^2 cfu/g total plate count in chappati multigrain flour after 2 months of storage whereas Ntuli et al. (2013) has also observed total plate count of 0.679 cfu/g in whole wheat flour. From several studies it was found that ready to eat products which contains enterobacteriaceae colony less than 10^2 would be considered safe for consumption (Khanom et al. 2016). In addition to yeast and molds, the considerable limit was mentioned as 10^5 (World Food Programme 2012)· Some research studies have showed that microbial growth of dried food can be inhibited up to several months probably if stored under air tight containers, 3–4 ply laminates, metallic containers etc.

Table 6 Microbiological analysis of multigrain premix

Micro-organisms	No. of colonies	Method
Total Plate count, cfu/g	< 10	IS-5402
Coliform count, cfu/g	< 10	IS-5402 (P-1)
E. coli, /g	Absent	IS-5887 (P-1)
Salmonella sp. cfu/g	Absent	IS-5887 (P-3)
S. aureus, cfu/g	Absent	IS-5887 (P-2)
Yeast & Mold Count, cfu/g	< 10	IS-5403

Conclusion

The study revealed that incorporation of multiple millets along with seeds from fruits and vegetable increased the nutritional value of the premix. As the premix was formulated using millets hence it resulted in non-gluten forming protein which could be suggested to gluten intolerant patients. As millets are considered to be good source of insoluble starch hence it can also be suggested to diabetic patient due to low glycemic index. Since the multigrain premix is being prepared without frying method, therefore, it can be effectively consumed as regular breakfast by mixing with milk and can also use for making various snacks like savory, flat bread, nutrition bar etc. The product was found to be satisfactory for all the parameters such as water activity, free fatty acids, and peroxide value after storage period of 45 days. Hence, it can be recommended as healthy and tasty snack premix especially to those combating with obesity as it contains a good amount of fibre. The idea of incorporation of multigrain to form a premix which renders improved protein quality, minerals, dietary fiber etc. leads to new

Table 5 Sensory analysis of Multigrain premix

Storage days	Appearance	Colour	Texture	Flavour	Overall acceptability
0	8.5 ± 0.53^a	8.2 ± 0.79^a	8 ± 0.81^a	8.1 ± 0.74^a	8.5 ± 0.53^a
15	7.9 ± 0.74^a	8.0 ± 0.82^a	7.8 ± 0.79^a	7.2 ± 0.79^a	7.3 ± 0.67^a
30	4.6 ± 0.79^b	4.3 ± 0.88^b	4.4 ± 0.82^b	4.3 ± 0.74^b	4.6 ± 0.79^b
45	2.4 ± 0.84^c	2.2 ± 0.79^c	2 ± 0.82^c	1.9 ± 0.88^c	2.5 ± 0.53^c

All the values are mean ± standard deviation for the obtained readings. Means and standard deviation with different superscript in same column show significant difference at $p \leq 0.05$ (n = 2) combination in the development of nutrient rich products with variety. Moreover, it could also be a better replacement to commercial starch source with improved essential nutritional composition due to the gluten free nature of pearl millet.

Acknowledgements The author would like to acknowledge the Department of Food Technology, Bhaskaracharya College of Applied Sciences, and University of Delhi for providing basic amenities for performing various experiments in the laboratory.

Authors' contributions Authors contributed to this study have been included in the manuscript.

Funding Did not receive any funding.

Availability of data and material All the data obtained from the study are included in the published article.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Not applicable.

Consent to participate Sent by all the authors via mail.

Consent for publication Data mentioned in the given research paper is completely our own data. Data was not taken from any other researcher therefore, no consent is required.

References

Abah CR, Ishiwu CN, Obiegbuna JE, Oladejo AA (2020) Sorghum grains: nutritional composition, functional properties and its food applications. Eur J Nutr Food Saf 101–111

Abdel-Aty AM, Salama WH, Hamed MB, Fahmy AS, Mohamed SA (2018) Phenolic-antioxidant capacity of mango seed kernels: therapeutic effect against viper venoms. Rev Bras 28:594–601

Abdulrahman WF, Omoniyi AO (2016) Proximate analysis and mineral compositions of different cereals available in gwagwalada market, FCT, Abuja, Nigeria. J Adv Food Sci Technol 3:50–55

Aktaş N, Gerçekaslan KE, Uzlaşır T (2018) The effect of some preroasting treatments on quality characteristics of pumpkin seed oil. OCL 25(3):A301

Aneja KR (2007) Experiments in microbiology, plant pathology and biotechnology. New Age International

Angioloni A, Collar C (2011) Significance of lipid binding on the functional and nutritional profiles of single and multigrain matrices. Eur Food Res Technol 233(1):141–150

AOAC, Association of Official Analytical Chemists (2000) Official methods of analysis SM. Method 922.06, #960.0. Fat analysis and fatty acid analysis. Approved November 3, International, Gaithersburg, MD, U.S.A

AOAC, Association of Official Analytical Chemists (2005) Official methods of analysis, 18th edn. Association of Official Analytical Chemists, Arlington

Ashoush IS, Gadallah MGE (2011) Utilization of mango peels and seed kernels powders as sources of phytochemicals in biscuit. World J Dairy Food Sci 6(1):35–42

Ashwath Kumar K, Sharma GK, Anilakumar KR (2019) Influence of multigrain premix on nutritional, in-vitro and in-vivo protein digestibility of multigrain biscuit. J Food Sci Technol 56:746–753

Aslam HKW, Raheem MIU, Ramzan R, Shakeel A, Shoaib M, Sakandar HA (2014) Utilization of mango waste material (peel, kernel) to enhance dietary fiber content and antioxidant properties of biscuit. J Glob Innov Agric Soc Sci 2(2):76–81

Chauhan S, Sonawane SK, Arya SS (2017) Nutritional evaluation of multigrain Khakra. Food Biosci 19:80–84

Devi NM, Prasad R, Palmei G (2018) Physico-chemical characterisation of pumpkin seeds. Int J Chem Stud 6(5):828–831

FSSAI (Food Safety Standards Authority of India) (2011) Food Safety and Standards (contaminants, toxins and residues) regulations.

Gohari AA, Farhoosh R, Haddad KM (2011) Chemical composition and physicochemical properties of pumpkin seeds (Cucurbita pepo Subsp. pepo Var. Styriaka) grown in Iran

Handa C, Goomer S, Siddhu A (2010) Effects of whole-multigrain and fructoligosaccharide incorporation on the quality and sensory attributes of cookies. Food Sci Technol Res 17(1):45–54

Khanom A, Shammi T, Kabir MS (2016) Determination of microbiological quality of packed and unpacked bread. Stamford J Microbiol 6(1):24–29

Kittiphoom S (2012) Utilization of mango seed. Int Food Res J 19(4)

Lutterodt H, Slavin M, Whent M, Turner E, Yu LL (2011) Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. Food Chem 128(2):391–399

Mishra G, Joshi DC, Panda BK (2014) Popping and puffing of cereal grains: a review. J Grain Process Storage 1(2):34–46

Njike VY, Smith TM, Shuval O, Shuval K, Edshteyn I, Kalantari V, Yaroch AL (2016) Snack food, satiety, and weight. Adv Nutr 7(5):866–878

Nikolić N, Radulović N, Momcilović B, Nikolić G, Lazić M, Todorovic Z (2008) Fatty acids composition and rheology properties of wheat and wheat and white or brown rice flour mixture. Eur Food Res Technol 227(5):1543–1548

Ntuli V, Mekbib SB, Asita AO, Molebatsi N, Makotoko M, Chatanga P (2013).Microbial and physicochemical characterization of maize and wheat flour from a milling company, Lesotho

Ogungbenle HN (2003) Nutritional evaluation and functional properties of quinoa (Chenopodium quinoa) flour. Int J Food Sci Nutr 54(2):153–158

Potočnik T, Cizej MR, Košir IJ (2018) Influence of seed roasting on pumpkin seed oil tocopherols, phenolics and antiradical activity. J Food Compos Anal 69:7–12

Pradeep PM, Dharmaraj U, Rao BS, Senthil A, Vijayalakshmi NS, Malleshi NG, Singh V (2014) Formulation and nutritional evaluation of multigrain ready-to-eat snack mix from minor cereals. J Food Sci Technol 51(12):3812–3820

Punia S, Kumar M, Siroha AK, Kennedy JF, Dhull SB, Whiteside WS (2021) Pearl millet grain as an emerging source of starch: a review on its structure, physicochemical properties, functionalization, and industrial applications. Carbohyd Polym 260:117776

Obadina A, Ishola IO, Adekoya IO, Soares AG, de Carvalho CWP, Barboza HT (2016) Nutritional and physico-chemical properties of flour from native and roasted whole grain pearl millet (Pennisetum glaucum [L.] R. Br.). J Cereal Sci 70:247–252

Rakcejeva T, Galoburda R, Cude L, Strautniece E (2011) Use of dried pumpkins in wheat bread production. Proc Food Sci 1:441–447

Ranganna S (2005) Handbook of analysis and quality control for fruits and vegetables products, 3rd edn. Tata McGraw-Hills

Rybicka I, Gliszczyńska-Świgło A (2017) Minerals in grain gluten-free products. The content of calcium, potassium, magnesium, sodium, copper, iron, manganese, and zinc. J Food Compos Anal 59:61–67

Sablania V, Bosco SJD, Rohilla S, Shah MA (2018) Microencapsulation of Murraya koenigii L. leaf extract using spray drying. J Food Meas Charact 12(2):892–901

Sahu U, Prasad K, Sahoo P, Sahu BB, Sarkar PC, Prasad N (2015) Biscuit making potentials of raw and roasted whole grain flours: cereals and millets. Asian J Dairy Food Res 34(3)

Saleh AS, Zhang Q, Chen J, Shen Q (2013) Millet grains: nutritional quality, processing, and potential health benefits. Compr Rev Food Sci Food Saf 12(3):281–295

Syed QA, Akram M, Shukat R (2019) Nutritional and therapeutic importance of the pumpkin seeds. Seed 21(2):15798–15803

Sharma C, Punia D, Khetarpaul N (2013) Sensory characteristics, proximate composition, dietary fibre content and storage stability of barley, wheat and chickpea composite flour biscuits. Br Food J

Shahidi F, Wanasundara PKJPD (2002) Extraction and analysis of lipids. Food Lipids Chem Nutr Biotechnol 133–168

Singh P, Raghuvanshi RS (2012) Finger millet for food and nutritional security. Afr J Food Sci 6(4):77–84

Slavin J (2003) Why whole grains are protective: biological mechanisms. Proc Nutr Soc 62(1):129–134

Taylor JR (2016) Millet pearl: overview

Tangariya P, Sahoo A, Awasthi P, Pandey A (2018) Quality analysis of composite flour and its effectiveness for Chapatti formulation. J Pharmacogn Phytochem 7(4):1013–1019

Veronezi CM, Jorge N (2012) Bioactive compounds in lipid fractions of pumpkin (Cucurbita sp) seeds for use in food. J Food Sci 77(6):C653–C657

World Food Program (WFP) (2012) Food quality control. www.wfp.org

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.