



## **Shelf Life of Millet Based Diabetic Mix**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors KG and GMY designed the study, wrote the protocol and managed the analyses of the study. Authors BMV and NH performed the statistical analysis and wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

Millet is a good source of nutrients such as fiber, minerals and B-complex vitamins and their regular consumption helps in reducing non-communicable diseases. Hence, millets were used in the preparation of diabetic mix and a study was conducted to evaluate the shelf life of millet-based diabetic mix. Parameters such as moisture, free fatty acid, peroxide value and microbial load were assessed for a period of 90 days. Significant increase in moisture, free fatty acid and peroxide value was observed at different storage periods, however free fatty acid and peroxide values were in the acceptable range. Bacterial count throughout the storage period was within the safe level, whereas presence of mold and *E. coli* was not detected during the storage period. Above findings revealed that the developed diabetic mix can be stored up to 90 days.

**Keywords:** *Shelf life; free fatty acid; peroxide value; E. coli.*

## **1. INTRODUCTION**

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin (a

hormone that regulates blood sugar) or alternatively, when the body cannot effectively use the insulin it produces [1]. Adult-onset diabetes is non-insulin-dependent form. Insulin

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may be produced by pancreas but action is impaired. This form occurs mainly in the person who is usually overweight and due to lifestyle factors [2]. Millets are good source of nutrients including fiber, minerals and B complex vitamins. Studies have shown that regular consumption of millet grains and their products is associated with reduced risk of developing chronic diseases such as diabetes, cardiovascular disease, cancers, and all-cause mortality [3]. In spite of having nutritional benefits, consumption of millets remains low. Therefore, it is necessary to make them part of our daily diet through processing and value addition. Any food developed is subject to deterioration, which is associated with spoilage, development of off flavors due to microbial contamination and auto-oxidation by natural enzymes present in foods. This may lead to development of health hazards in the consumers. Hence, food storage and its safety becomes an integral part of food processing and product development. With this background, millet based diabetic mix was developed and further evaluated for its shelf life.

## 2. MATERIALS AND METHODS

### 2.1 Development of Millet Based Mix

Millet based diabetic food mix was developed by using the ingredients viz., finger millet (*Eleusine coracana*), little millet (*Panicum sumatrense*), defatted soya (*Glycine max*) flour, whole green gram (*Vigna radiata*), fenugreek seeds (*Trigonella foenum-graecum*), flax seeds (*Linum*

*usitatissium*), curry leaves (*Murraya koenigii*), bitter gourd (*Momoradi cacharantia*) and skimmed milk powder. All the ingredients used for the study were procured from local market of Bengaluru. Fresh bitter gourd and curry leaves were washed thoroughly, blanched for one minute and oven dried. Further finger millet, little millet, whole green gram, fenugreek seeds and roasted flax seeds were cleaned and made into flour. Millet based mix was developed by mixing the flour with skimmed milk powder, defatted soya flour as presented in Fig. 1.

### 2.2 Shelf life Study of Millet Based Diabetic Mix

Five hundred grams of millet based diabetic mix was stored in low density polythene cover (350 gauge) upto 90 days at room temperature (25-30°C) to evaluate its shelf life. Samples were drawn in triplicates for evaluation (fresh, after 15, 30, 45, 60, 75 and 90 days of storage). Sample was evaluated for storage quality parameters such as moisture, free fatty acid, peroxide value and microbial load.

### 2.3 Estimation of Moisture

Moisture was determined by taking 10 g of sample in petri dish and dried in an oven at 105°C till the weight of the petri dish with its content was constant. Each time before weighing, the petri dish was cooled in desiccator. Moisture content of the sample was expressed in g/100 g of sample [4].

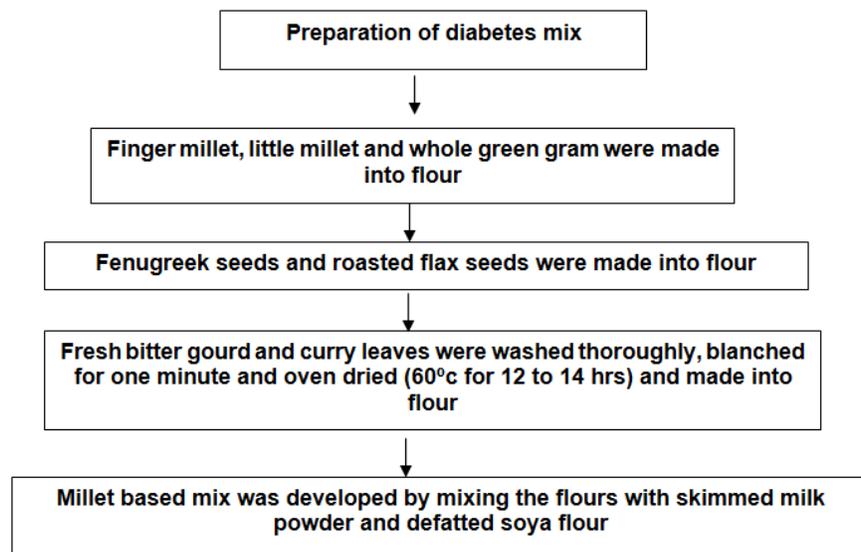


Fig. 1. Development of diabetes mix

## 2.4 Free Fatty Acids and Peroxide Value

About 10 gm of the oil was weighed accurately into a 250 ml conical flask to which was added 50 ml of a mixture of equal volume of alcohol and ether previously neutralised after the addition of 1 ml of phenolphthalein solution. The contents were warmed in a water bath until the substance is completely dissolved. The solution was titrated with 0.1 N KOH with constant shaking until a pink colour persists for 15 sec. The titer value in ml (a) was noted [5].

$$\text{Acid value} = \frac{a \times 0.00561 \times 1000}{\text{Weight in g of substance}}$$

Point five to one gram of clear melted fat was weighed accurately in the boiling flask. To this, 30 ml of acetic acid- chloroform mixture was added and fat was dissolved. 1 ml of saturated potassium iodide was added. After 5 min 100 ml of distilled water was added. The liberated iodine was titrated against N/1000 ml sodium thiosulphate. When the end point is approached 1ml of freshly prepared starch was added and titration was completed till the blue colour disappears. Blank was carried out using all the reagents without the oil [5].

$$\text{Peroxide value of oil (meq/kg of sample)} = \frac{(\text{Titre-blank}) \times N \times 1000}{\text{Wt of oil (g)}}$$

## 2.5 Microbial Quality

Microbial load was assessed by pour plate method [6]. Ten grams of each sample (different variations) was mixed in 90 ml sterile water blank to give  $10^{-1}$  dilution. Subsequent dilutions up to  $10^{-4}$  were made by transferring serially 1 ml of the dilution to 9 ml of sterile water blanks. The populations of bacteria, molds and yeasts were estimated by transferring 1 ml of  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  dilutions respectively to a sterile petri dish and approximately 20 ml of media viz., nutrient agar, Martins Rose Bengal Agar and Davis Yeast Extract Agar for bacteria, molds and yeasts respectively were poured into plates. The plates were rotated twice in clockwise and anticlockwise direction for uniform distribution of the inoculums. After solidification of the media, plates were kept for incubation in an inverted position at  $30 \pm 1^\circ\text{C}$  for two to four days and emerged colonies were counted.

## 2.6 Statistical Analysis

Data are shown as means with their standard deviations. One way analysis of variance (F-test) was applied to assess the statistical significance.

## 3. RESULTS AND DISCUSSION

In foods, lipid peroxidation and enzymatic hydrolysis are the main factors which affect shelf life of the food/product [7]. Peroxide value usually used as an indicator of deterioration of fats, as peroxidation takes place the double bonds in the unsaturated fatty acid breakdown to produce secondary oxidation products which indicate rancidity [8].

Initial moisture content of the millet based diabetic mix was  $8.77 \pm 0.06\%$  which increased significantly upto  $10.03 \pm 0.13\%$  at the end of storage period (90 days) as indicated in Table 1. Similar results were observed by [9], who found that the moisture content of composite flour varied from 8.41 to 10.20 in the polyethylene bags for the period of 3 months. [10] observed gradual increase in moisture content (10.93 to 12.4 percent) of composite flour with increase in storage period due to hygroscopic nature of flour and change in the relative humidity during storage.

Free fatty acid and peroxide values of developed mix increased significantly from  $1.31 \pm 0.10$  to  $3.83 \pm 0.08\%$  oleic acid and  $0.31 \pm 0.05$  to  $2.01 \pm 0.10$  mEq  $\text{O}_2$  /Kg of oil respectively. According to [7], the peroxide values in the pearl millet upma ready to cook mix samples stored did not show any significant increase during the first 2 months and increased slightly, thereafter. After 6 months storage, peroxide value increased from  $2.5 \pm 0.05$  to  $17.6 \pm 0.20$  meq  $\text{O}_2$   $\text{kg}^{-1}$  fat and free fatty acids from  $0.27 \pm 0.021$  to  $0.56 \pm 0.042\%$  as oleic acid which may be due to the breaking of long chain fatty acid chains in to individual fatty acid moieties. Storage of composite flour in polythene bags showed gradual increase in free fatty acid than wheat flour alone [9]. Hence free fatty acid and peroxide values were within the acceptable limit.

Microorganisms play significant role in the determination of shelf life of food products. They are usually responsible for spoilage of many food items [11]. Hence determination of microbial load during storage is important. Table 2 shows the microbial load of stored diabetes mix. Total bacterial count in fresh sample was found to be  $1.86 \pm 0.30 \times 10^3$  CFU, which increased significantly as the storage period increased ( $5.97 \pm 0.58 \times 10^3$  CFU at 90<sup>th</sup> day of storage). (Mold and *E. coli* were not detected in both fresh and stored samples. However, increase in microbial load was within the limit of safe level

**Table 1. Storage stability of diabetes mix**

Storage duration (Days)	Moisture (%)	FFA (% oleic acid)	PV (mEq O <sub>2</sub> /Kg of oil)
Initial	8.77 ± 0.06	1.31 ± 0.10	0.31 ± 0.05
15 days	8.92 ± 0.07	1.46 ± 0.07	0.38 ± 0.07
30 days	8.98 ± 0.08	2.81 ± 0.10	0.45 ± 0.10
45 days	9.26 ± 0.04	3.08 ± 0.13	0.78 ± 0.15
60 days	9.58 ± 0.08	3.30 ± 0.08	1.04 ± 0.08
75 days	9.78 ± 0.07	3.38 ± 0.23	1.53 ± 0.05
90 days	10.03 ± 0.13	3.83 ± 0.08	2.01 ± 0.10
F value	*	*	*
SEm±	0.04	0.08	0.06
CD @ 5%	0.13	0.25	0.18

Note: FFA: Free fatty acid, PV: Peroxide value

**Table 2. Microbial load of stored diabetes mix**

Storage duration (Days)	10 <sup>3</sup> CFU/g		
	Total bacterial count	Mold	<i>E. coli</i>
Initial	1.86 ± 0.30	ND	ND
15 days	1.95 ± 0.55	ND	ND
30 days	2.12 ± 0.36	ND	ND
45 days	3.22 ± 0.55	ND	ND
60 days	4.56 ± 0.68	ND	ND
75 days	5.12 ± 0.54	ND	ND
90 days	5.97 ± 0.58	ND	ND
F value	*	-	-
SEm±	0.28	-	-
CD @5%	0.87	-	-

Note: CFU: Colony forming unit

(Bacterial count not > 10,000 per g of sample; Mold and *E. coli* absent in 0.1 g of sample (Source: FSSAI, 2011)).

The total viable counts for quality protein maize and wheat flour were  $2.2 \times 10^4$  and  $2.6 \times 10^4$  CFU/g respectively with quality protein maize flour having the lowest total viable count [12]. Ibeanu et al. [13] reported that low microbial load of the complementary mixes was due to low water activity and low pH caused by fermentation of the grains. Microbial proliferations in foods need certain conditions - namely available water (water activity), proper pH, right temperature and nutrients and time. By controlling these conditions one can prevent microbial growth and extend the shelf life of a food.

#### 4. CONCLUSION

Millets are gaining popularity due to their health benefits. They can be utilized properly for the development of diabetic mix for the management of diabetes. Study of shelf life provides information regarding keeping quality and period of usage of the product. Also packaging material

and ambient conditions required for proper storage. Millet based diabetic mix developed can be stored for 90 days without affecting its shelf life in polythene bags. Further packaging materials and techniques can be explored for improved shelf life.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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