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## Nutritional, microbiological and organoleptic evaluation of multigrain bar enriched with flaxseed and stevia for antidiabetic purpose

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### Abstract

Flaxseed is one of the richest sources of omega-3 fatty acids, protein and dietary fibre. Stevia is a non-energetic, natural sweetener. These two substances have antibacterial and fungi static properties. With the addition of flaxseed and stevia, the microbiological load can be decreased. The multigrain bar was supplemented with flaxseed (15%) and stevia (25,50,75,100% sugar substitution). The multigrain bar's consumer acceptance was assessed using a 9-hedonic scale, with the sample containing 1.12% stevia substitution was chosen. The proximate, microbiological and organoleptic quality of the selected bars were evaluated. The flaxseed and stevia samples were compared with the control sample. Susceptibility to microbial growth was observed, a decrease in overall plate count and yeast/mold count was due to antibacterial and antifungal properties of stevia. Flaxseed and stevia containing foods are safe to use for diabetic patients and maybe beneficial and can be utilized without fear of pathogenic spoilage and food born illness.

**Keywords:** Flaxseed, stevia, multigrain bar, microbial and sensory evaluation

### Introduction

Flaxseed is the richest source of alpha-linolenic acid, lignans and other nutritional components. Flaxseed contains functional components such as dietary fibre, oil, protein and phenolic compounds, which are responsible for a number of health benefits. Flaxseeds contain approximately 28% fiber, soluble and insoluble. The insoluble fiber helps to relieve constipation, improve colon health and may prevent colon cancer. It also contains dietary fibre and protein (flax primer) and it acts as nutraceutical (Oomah *et al.*, 1995) [17]. Flaxseed is anti-inflammatory and anti-microbial. Addition of flaxseed flour increases the hardness, cohesiveness and springiness of the products and lead to an improved overall acceptability (Sharma *et al.*, 2014) [21]. Flaxseed decreases the glucose levels in the body and improves the insulin sensitivity. So, the flaxseed containing foods are more beneficial for diabetes.

Stevia (*Stevia rebaudiana bertonii*) is a natural sweet herb. Its white crystalline compound (stevioside) is the natural herbal sweetener with no calories and is over 100-300 times sweeter than table sugar. Stevia has antibacterial, anticandidal, antifungal, antiviral, cardiogenic (tones, balances, and strengthens the heart), diuretic, hypoglycemic, and vasodilator characteristics, among others. Because its natural source appeals to many customers, high-purity zero-calorie natural stevia extract is of tremendous interest to the worldwide food business. Food and the ways in which it may help sustain human health have piqued the interest of consumers, the food business, and researchers in recent years. High-purity zero-calorie natural stevia extract is of great interest to the global food industry because its natural source appeals to many consumers. Stevia is safe for diabetic patients to use as a substitute for sugar and other sweeteners.

Multigrain bars are unique among fast snacks since they are well-balanced, having good nutritional value and ease of use. The Granola bar is a dry granulated cereal with a low water content (Macedo *et al.*, 2013) [13]. Glucose syrup is used in a variety of ways and it is the component of the bar that acts as an aggregator and provides quick binding results (Silva *et al.*, 2013) [23]. The various varieties of bars available in the global market with good organoleptic properties and consumer appeal are referred by names such as chewy cereal granola bars, organic bar, choco bar, muffin bar, fruit filled bars and so on.

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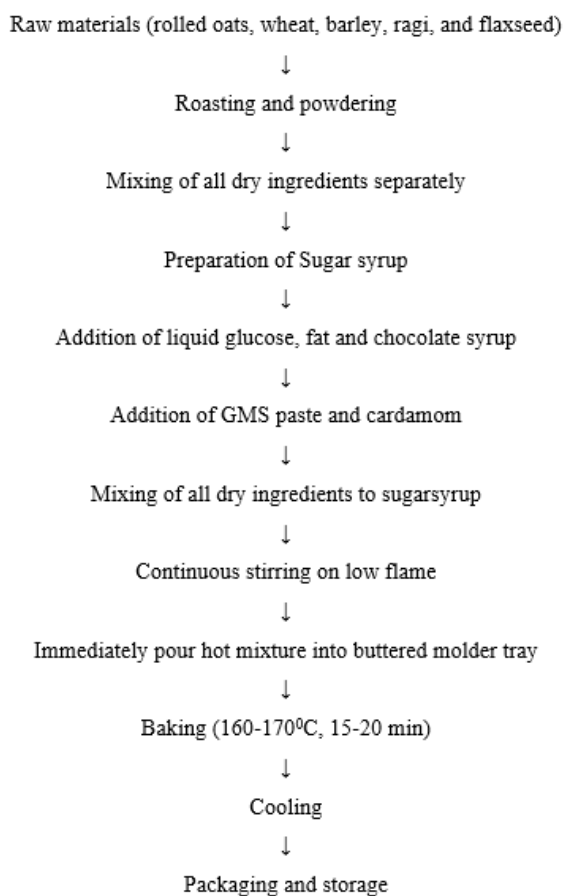
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## Materials and Methods

Raw materials like rolled oat, *ragi*, whole wheat flour, barley flour, corn flour, sugar, fat, Other ingredients *viz.* stevia (Vedika-Herbal green powder), liquid glucose, GMS and Vanaspati (Dalda) and puffed rice etc. were purchased from local market of V.N.M.K.V., Parbhani.

Chemicals of analytical grade and sufficient glassware required are available in the laboratory, The equipment such as Baking oven, molding trays and others were obtained from Department of Food process technology, Department of Food Engineering, Department of Food chemistry and nutrition and Department of Food and Industrial Microbiology, College of Food Technology. V.N.M.K.V., Parbhani.

## Methods for preparation of nutritional bars



## Estimation of protein

Protein content was determined by Micro-kjeldhal method (A.O.A.C.1990) [2].

% Nitrogen was calculated by using following formula and % protein was calculated by multiplying 6.25.

$$\% \text{ Nitrogen} = \frac{\text{CBR} \times \text{Normality of H}_2\text{So}_4 \text{ acid} \times \text{Moles of Nitrogen} \times \text{Dilution factor}}{\text{Wt. of sample (g)}} \times 100$$

## Where

CBR=SBR-BBR

(Corrected burette reading = Sample burette reading- Blank burette reading)

Normality of (H<sub>2</sub>So<sub>4</sub>) acid = 0.01N

Moles of Nitrogen = 14/100

Total protein = % Nitrogen x 6.25

## Estimation of Crude fiber

About 2-5 g of moisture and fat free sample was weighed into 500 ml beaker and 200 ml boiling 0.25 N (1.25w/v) Sulfuric acid was added. The mixture was boiled for 30 min keeping the volume constant by addition of water at frequent intervals. At the end of this period the mixture was filtered through a filter paper and residue was washed with hot water till it becomes free from acid. The material then transferred to the same beaker and 200ml of boiling 0.313N NaOH solution was added. After boiling for 30 min the mixture was filtered through filter paper. The residue was washed with hot water till free from alkali followed with some alcohol. It was then transferred to crucible, dried overnight at 80-100°C and weighed. The crucible was heated in muffle furnace at 550-600°C for 4hrs and cooled and weighed again. The difference in weights represents the weight of crude fiber (AOAC, 2000) [3].

## Estimation of total Carbohydrates

The sample was weighed (0.5 g) accurately in test tube and kept in ice water bath for few minute followed by the addition of cold H<sub>2</sub>SO<sub>4</sub> (72 per cent) with gentle stirring The viscous paste was diluted with distilled water to 36 obtain final concentration 2 N with respect to acid. It was then refluxed at 98°C for 3 - 4 hours to achieve complete hydrolysis. The sugar content was estimated by Phenol-H<sub>2</sub>SO<sub>4</sub>method, using glucose as standard. The orange yellow color was read at 480 nm on spectrophotometer. From the calibrated curve the concentration of sugar in hydrolysate was calculated and per cent total sugar in the sample was quantified (AOAC 1990) [2].

## Estimation of ash

5 g of finely ground sample was weighed in pre-weighed silica crucible and ignited till smokeless. Then it was transferred to muffle furnace and heated at 550°C for 4hrs for complete oxidation of organic matter and resultant ash content was calculated (AOAC, 2000) [3].

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

## Microbial analysis of multigrain bar

Prepared multigrain bar sample selected on the basis of sensory quality were stored at room temperature (27-32°C) and analyzed at the interval or 15 days up to 90 days for TPC and yeast and mold count according to the procedure given by Ranganna (1986) [19]. The results expressed in cfu/g. The pour plate technique was followed.

## Total plate count

Serial dilutions were prepared in 0.1% saline water. 1 ml of each serial dilution were inoculated in petri plates containing 15-20 ml sterilized nutrient agar and incubated at 37°C to 24-48 hrs.

## Yeast and mold count

Serial dilutions (up to 10<sup>2</sup>) were prepared in 0.1% saline water. 1 ml of each serial dilution were inoculated in petri plates containing 15-20 ml sterilized potato dextrose agar and incubated at 30 °C to 3-5 days.

## Sensory analysis

The sensory evaluation of prepared samples were carried out

a 10 member trained panel comprised of postgraduate students and academic staff members of the faculty who had some previous experience in sensory evaluation. The panel members were requested in measuring the terms identifying sensory characteristics and in use of the score. Judgment were made through rating products on a 9 points Hedonic Scale with corresponding descriptive terms ranging from 9 'like extremely' to 1 'dislike extremely'. The format for sensory evaluation card is given in Appendix-1 (Meilgaard *et al.*, 1999)<sup>[15]</sup>.

### Theoretical determination of energy value of multigrain bars

Energy value of nutritional bars were computed from

proximate composition of the bars by taking the value of 4, 4 and 9 kcal for carbohydrates, protein and fat respectively.

### Statistical Analysis

The obtained data in the present investigation was statistically analysed. The analysis of variance of the data obtained was done by using completely Randomized Design (CRD) for different treatments as per the method given by Panse and Sukhatme (1984)<sup>[18]</sup>. The analysis of variance revealed at significance of  $P < 0.05$  level, S.E. and C.D. at 5% level were mentioned whenever required.

### Results and Discussion

**Table 1:** Organoleptic evaluation of selected multigrain bars incorporated with flaxseed flour

Samples	Appearance	Colour	Flavour	Taste	Texture	Overall acceptability
C	8.1	8.0	7.86	7.9	8.0	8.1
F <sub>1</sub>	8.08	8.1	8.0	8.0	8.1	8.0
F <sub>2</sub>	8.12	8.1	8.1	8.15	8.1	7.8
F <sub>3</sub>	8.2	8.3	8.1	8.2	8.2	8.3
F <sub>4</sub>	7.8	7.7	7.8	7.9	8.3	8.0
S.E. +	0.11246	0.081281	0.085674	0.046691	0.075425	0.074207
C.D at 5%	0.35378	0.25571	0.26952	0.14689	0.23728	0.23345

\*Each value is an average of ten determinations

C - Multigrain bar prepared with malted grains (control); F<sub>1</sub> - Multi grain bar with 5% flaxseed; F<sub>2</sub> - Multi grain bar with 10% flaxseed; F<sub>3</sub> - Multi grain bar with 15% flaxseed; F<sub>4</sub> - Multi grain bar with 20% flaxseed

Table 1 indicating the findings of present investigation related to the enrichment of multigrain bar with flaxseed flour in different proportions. By sensory evaluation it was found that sensory scores for taste, flavour, texture and after taste were more with F<sub>3</sub> sample. The highest score for colour of multigrain bar (F<sub>3</sub>) was 8.2. The flavor of multigrain bar was influenced by addition of cardamom, ghee and flaxseed flour. It was also noticed that samples F<sub>2</sub> and F<sub>3</sub> were rated high for flavor (8.1) whereas sample F<sub>4</sub> scored least value for flavor (7.8). The addition of flaxseed flour with other ingredients improves the neutral flavor in the prepared products (Carter.,1993).

Alpaslan and Hayta (2006) claimed that the addition of flaxseed provides the good texture to the food products. On comparison of these results, it was concluded that multigrain

bars enriched with 15% flaxseed rated high in all organoleptic attributes and further it was significantly overall acceptable over other bars. Mridula *et al.*, (2011)<sup>[12]</sup> and Hussain *et al.*, (2008)<sup>[11]</sup> reported that 15% flaxseed enriched food products were having more acceptability. The similar results were found with findings revealed by Mridula *et al.*, (2011)<sup>[12]</sup> and Hussain *et al.*, (2008)<sup>[11]</sup>. The F<sub>3</sub> was selected finally, by comparing all the sensory parameters, further experiments were carried out F<sub>3</sub> as a control sample.

**Table 2:** Organoleptic evaluation of multigrain bars incorporated with flaxseed and Stevia

The multigrain bars were prepared by addition of flaxseed (15%) and stevia in different proportions (25, 50, 75 and 100%) for the replacement of sugar with low calorie sweetener and to improve the product characteristics were evaluated for organoleptical properties by a group of ten semi – trained panel members.

**Table 2:** Organoleptic evaluation of selected multigrain bars with flaxseed by using stevia in different proportions

Samples	Appearance	Colour	Flavour	Taste	Texture	Overall acceptability
C	8.5	8.5	8.6	8.51	8.6	8.6
S <sub>1</sub>	8.0	8.1	8.4	8.1	8.5	8.4
S <sub>2</sub>	7.4	7.3	7.1	7.1	7.4	7.3
S <sub>3</sub>	6.6	5.7	6.4	6.5	6.7	6.5
S <sub>4</sub>	5.5	5.6	5.3	5.3	5.0	5.2
S.E. +	0.10567	0.18708	0.19365	0.19235	0.18708	0.15864
C.D at 5%	0.33244	0.58855	0.60921	0.60513	0.58855	0.49907

\*Each value is an average of ten determinations

C - Multi grain bar with 15% flaxseed; S<sub>1</sub>- Multi grain bar with 15% flaxseed + 25% stevia; S<sub>2</sub>-Multi grain bar with 15% flaxseed + 50% stevia; S<sub>3</sub>-Multi grain bar with 15% flaxseed + 75% stevia; S<sub>4</sub> - Multi grain bar with 15% flaxseed + 100% stevia

The data pertaining to the results of sensory evaluation of

flaxseed bar was presented in the Table 2. It could be observed from the table that in case of flaxseed bar replaced with stevia the sensory score for all parameters were found to be decrease drastically. The visual appreciation is the first sense used in the choice and acceptance of the product. The color is an essential attribute in evaluating the quality of a

food. The color was found to be dark green linearly with replacement, which resulted drastic reduction of color scores by consumers. More or less the similar results were depicted by Giri *et al.*, (2014)<sup>[8]</sup> who monitored the color changes in kulfi prepared by the addition of different proportions of stevia. At the level where concentrations of above 25% replacement, the color became nearly unacceptable by the consumer. These sensorial scores for color summarized that replacement of stevia has marked negative effect on colouring parameter of multigrain bar.

As it was observed, by increasing the amount of diet sugar (stevia), the score of overall acceptance of the product was dramatically decreased which was statistically significant. Up to 25% replacement of sugar by stevia did not affect body and texture and flavour score significantly. Hence judges

adjudged the 25% sugar replaced multigrain bar on par with the control. More or less the similar results were reported by Giri *et al.*, (2014)<sup>[8]</sup> and Sadafi *et al.*, (2014)<sup>[20]</sup>.

### Proximate composition of multigrain bars incorporated with flaxseed and stevia

The present investigation had been carried out to study the suitability of flaxseed flour and stevia incorporation on quality characteristics of multigrain bars. On the basis of these results it was observed that sample containing 15% flaxseed flour and 25% stevia given best amongst there category for organoleptic and physical characteristics. On the basis of obtained results these samples were selected for further studies and the data pertaining to proximate properties of these multigrain bars are summarized in Table.

**Table 3:** Proximate composition of selected bars

Samples	Parameters (g/100gm)					
	Moisture	Protein	Fat	Crude fiber	Ash	Carbohydrates
C	5.56	10.8	8.31	5.06	2.04	70.15
F <sub>3</sub>	5.32	12.7	10.2	6.1	2.17	66.5
S <sub>1</sub>	5.36	12.71	9.7	6.24	2.2	60.41
SE ±	0.054263	0.16680	0.21759	0.088819	NS	0.91655
CD at 5%	0.18749	0.57632	0.75180	0.30689		3.1668

\*Each value is an average of three determinations

C- Multigrain bar prepared with malted grains; F<sub>3</sub> - Multi grain bar with 15% flaxseed; S<sub>1</sub>- Multi grain bar with 15% flaxseed + 25% stevia

It could be observed from Table 3 that the moisture content of all multigrain bars were more or less similar. The control sample found to be the highest (5.56%) where the lowest (5.32%) readings were observed for the flaxseed incorporated multigrain bar. Munhoz *et al.*, (2014)<sup>[16]</sup> reported that on addition of flaxseed flour the moisture content of the products were decreased linearly. The carbohydrate content decreased in the sample S<sub>1</sub> because of the replacement of stevia (25%) with sugar. Similar results were quoted by Global stevia institute and Giri *et al.*, 2014<sup>[8]</sup>. Incorporation of flaxseed significantly increased the protein content of sample while maximum score was observed for stevia replaced sample (12.71). The obtained results are in good agreement with findings of energy bar reported by Mridula *et al.*, (2011)<sup>[12]</sup>.

The crude fat content of flaxseed incorporated multigrain bar was rated higher (10.2%) to other samples. These results are consistent with the findings of Mantri *et al.*, (2012)<sup>[14]</sup> who prepared the energy bar by using oats, flaxseed, bengal gram soy protein with varying levels of sweeteners.

The stevia replacement also significantly increase the crude

fibre. It was also noticed that there is no significant difference between the samples regarding the ash content. The results for ash content of multigrain bar was similar with the results obtained by Giacomino *et al.*, (2013)<sup>[7]</sup> who incorporated flour mixes and extruded flaxseed meal in cereal-based bars. On the basis of findings related to proximate composition of flaxseed enriched, stevia replaced samples, it could be observed that incorporation of flaxseed and stevia improves the nutritional properties of multigrain bars. The addition of stevia as a low calorie sweetener, found to be decreased the calories in the prepared samples

### Measurement of theoretical energy value of selected multigrain bars

Energy value is determined by using values of crude protein, crude fat and total carbohydrate content of sample and considering that 1g of protein yields 4 Kcal energy, 1g of fat yields 9 Kcal energy and 1g carbohydrates yields 4 Kcal energy. Total energy value is calculated by adding above three energy values which gives energy value per 100g of sample. The details of computing energy values of 100g of each variation of multigrain bar are summarized in Table

**Table 4:** Theoretical energy value of selected multigrain bars

Samples	Nutrients per 100 g			Energy value of product kcal/100g
	Carbohydrate	Protein	Fat	
A <sub>3</sub> (control)	70.15	10.8	8.31	398.59
F <sub>3</sub>	66.5	12.7	10.2	408.6
S <sub>1</sub>	60.41	12.71	9.7	379.78

A<sub>3</sub>- Multigrain bar prepared with malted grains; F<sub>3</sub> - Multi grain bar with 15% flaxseed; S<sub>1</sub>- Multi grain bar with 15% flaxseed and 25% stevia

Energy value of selected bars were found to be 398.59 kcal/100g for A<sub>3</sub>, 408.6 kcal/100g for F<sub>3</sub> and 380.2 kcal/100g for S<sub>1</sub> samples.

**Microbial quality of selected multigrain bars stored at room temperature:** Prepared multigrain bar samples selected on the basis of sensory quality were stored at room temperature and analyzed at the interval or 15 days up to 90 days for TPC and yeast and mold count. The data pertaining to microbial examination is shown in Table 5.

**Table 5:** Microbial quality of selected multigrain bars stored at room temperature

Storage period (Days)	Microbial quality (cfu/g)					
	Total plate count			Yeast and mold count		
	A <sub>3</sub>	F <sub>3</sub>	S <sub>1</sub>	A <sub>3</sub>	F <sub>3</sub>	S <sub>1</sub>
0	ND	ND	ND	ND	ND	ND
15	ND	ND	ND	ND	ND	ND
30	ND	ND	ND	ND	ND	ND
45	ND	ND	ND	ND	ND	ND
60	ND	ND	ND	ND	ND	ND
75	2.3x10 <sup>3</sup>	2.0x10 <sup>3</sup>	2.0x10 <sup>3</sup>	ND	ND	ND
90	4.6x10 <sup>3</sup>	3.7x10 <sup>3</sup>	3.2x10 <sup>3</sup>	3X10 <sup>2</sup>	1x10 <sup>2</sup>	ND

A<sub>3</sub>- (control) Multigrain bar prepared with malted grains; F<sub>3</sub> - Multi grain bar with 15% flaxseed; S<sub>1</sub>- Multi grain bar with 15% flaxseed and 25% stevia

This study examined the colony forming units (CFU) on petri dishes injected with diluted multigrain bar solutions to investigate the susceptibility of fresh multigrain bar to microbial development. To distinguish between bacterial and mold/yeast development, selective growth medium were employed.

The total plate count for the control sample was determined to be 2.3x10<sup>3</sup> on the 75th day of storage at room temperature, and yeast and mould counts were not discovered until the 75th day, but they seemed to be 3 X10<sup>2</sup> on the 90th day. In terms of total plate count and yeast/mold count, flaxseed enriched samples were found to be less susceptible to microbial development than the control.

The difference in microbe counts between the control and flaxseed samples is striking. In terms of total plate count and yeast/mold count, the flaxseed supplemented sample was found to be less susceptible to microbial development than the control. The antibacterial and fungi static properties of flaxseed account for the difference in microbial counts between the control and flaxseed enriched samples (Shin *et al.*, 2007; Xu *et al.*, 2008; Barbary *et al.*, 2010). The outcomes of this experiment were very similar to those reported by Girma *et al.*, (2013) and Xu *et al.*, (2008).

On the 90th day of storage, the total plate count (3.2x10<sup>3</sup>) and yeast - mould count were not observed in the stevia-added sample. It was also discovered that the sample with stevia had less microbiological growth than the flaxseed supplemented and control samples. It could be because the sample contained stevia and flaxseed flour. The foods containing Stevia might be utilised without any worry about food borne disease and can be used as antibacterial agent (Gasmalla *et al.*, 2014).

## Conclusion

According to the present investigation, addition of the flaxseed and stevia were increased the nutritive value of the product with good consumer acceptability. The least microbial growth was shown in the stevia-added sample because of its antibacterial effect. High-purity zero-calorie natural stevia is used as a substitute for sugar and other sweeteners. Flaxseed also contains antibacterial and antifungal properties. It decreases the glucose levels in the body and improves the insulin sensitivity. So, the flaxseed and stevia containing foods are safe to use for diabetic patients. It can be concluded that these substances can reduce the spoilage and extends the shelf life of food products.

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