ISSN No: - 2456 – 2165

Effect of Flaxseed Flour and Stevia Incorporation on Microbial Quality of Multigrain Bar

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Abstract :- Flaxseed is a premium food delivering high performance and is one of the richest sources of ω-3 fatty acids. Stevia is a natural, non energetic sweetener. These two ingredients are having antimicrobial activity and fungistatic activity. This study shows the effect of microbial quality with the addition of flaxseed and stevia. The flaxseed (15%) and stevia (25,50,75,100% - replacement with sugar) were added to the sample. The consumer acceptability of the cereal bars were carried out using 9-hedonic scale, the sample with 1.12% replacement of stevia was selected. The selected bars were assessed for proximate, mineral and microbial quality. When compare to the control, the samples with flaxseed and stevia were found to be low for susceptibility to microbial growth in terms of total plate count and yeast/mold count, due to antimicrobial activity and fungi static activity of flaxseed and stevia. The foods containing flaxseed and stevia could be used without any worry about food borne disease and can be used as antimicrobial agents.

KEYWORDS: *multigrain bar, flaxseed, stevia, microbial activity.*

I. INTRODUCTION

Flax (*Linum usitatissimum)* is considered a functional food or source of functional ingredients, because it contains alpha-linolenic acid, lignans and polysaccharides. Flaxseed also provides dietary fiber and protein (flax primer) an was singled out as one of six neutraceuticals (Oomah *et al*,1995). Flaxseed has anti inflammatory, anti microbial activity.

The crude Stevia leaves and herbal powder (green) are reported to be 10-15 times sweeter than table sugar. Stevia has various properties such as antibacterial, anticandidal, antifungal, antiviral, cardiotonic (tones, balances, strengthens the heart), diuretic,hypoglycemic, vasodilator. High-purity zero-calorie natural stevia extract is of great interest to the global food industry because its natural source appeals to many consumers.

There have been growing interests recently on the part of consumers, the food industry, and researchers into food and the ways in which it may help maintain human health. Cereal bars stand out among fast foods due to their balanced nutritional content and convenience. Cereal bar, "*granola"* is a dry granulated cereal product which has a lower water activity (Macedo *et al*.,2013). Generally, the glucose syrup is the aggregator element of the bar ingredients providing quick energy absorption (Silva *et al*., 2013). 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.

II. MATERIALS AND METHODS

A. Collection of Raw Material

The investigation was carried out at the department of Food Engineering, VNMKV University, Parbhani. Raw material (wheat, barley and ragi seeds, oats, puffed rice flour, sugar,fat, chocolate syrup, GMS, cardamom, liquid glucose, etc.) were procured from a local market of Parbhni, Maharashtra(India).

B. Formulation of Multigrain Bar With the Addition of Flaxseed And Stevia

The formulations of multigrain bar prepared from different proportions of malted wheat, barley and ragi flour are given in Table 1. Other ingredients like hydrogenated vegetable oil (vanaspati), sugar, corn flour, dry nuts, chocolate syrup, ammonium bicarbonate and cardamom were added to each of these formulations of multigrain bar preparation.

- The flaxseed flour was added in different proportions i.e. 5,10,15,20% to the control sample. The sample with 15% flaxseed flour was selected, based on organoleptic evaluation.

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Table 1: Formulation of multigrain bar with incorporation of Roasted flaxseed flour and stevia:

Ingredients (g)	**Formulation**				
	C	**S_1**	**S_2**	**S_3**	**S_4**
Sugar	38	28.5	19	9.5	-
Stevia powder	-	1.12	2.25	3.37	4.5
Whole wheat flour	10	10	10	10	10
Roasted flaxseed flour	15	15	15	15	15
Barley flour	10	10	10	10	10
Ragi flour	10	10	10	10	10
Rolled oat flour	05	05	05	05	05
Corn flour	05	05	05	05	05
Puffed rice flour	05	05	05	05	05
Fats	05	05	05	05	05
Chocolate syrup	2.5	2.5	2.5	2.5	2.5

Source: Deshmukh *et al*., (2014)

C. Preparation of Multigrain Bars

The selected nutritionally rich ingredients like wheat, barley and ragi were powdered (ASTM 30) and kept. All the remaining dry ingredients (oat flour, corn flour, puffed rice flour, ammonium bicarbonate, almond and ground nut pieces) were mixed with those ground flours. Simultaneously, the sugar syrup was made by using sugar. Liquid glucose was added and the concentration of sugar syrup was maintained as 62^0 Brix. The GMS paste, fat, chocolate syrup and grinded cardamom powder were also added to the syrup to improve the flavor. The prepared composite flour was added to sugar syrup with continuous stirring and heating for 2-3 min. Then the composite flour was allowed to pour into buttered molder tray while hot. It was then pressed lightly for uniform spreading and thickness, then cut into rectangular shape using knife. After that it was baked (160^0C for 15-20 min) and cooled to room temperature. Cooled multigrain bars will be packed in polyethylene bag and stored at ambient temperature.

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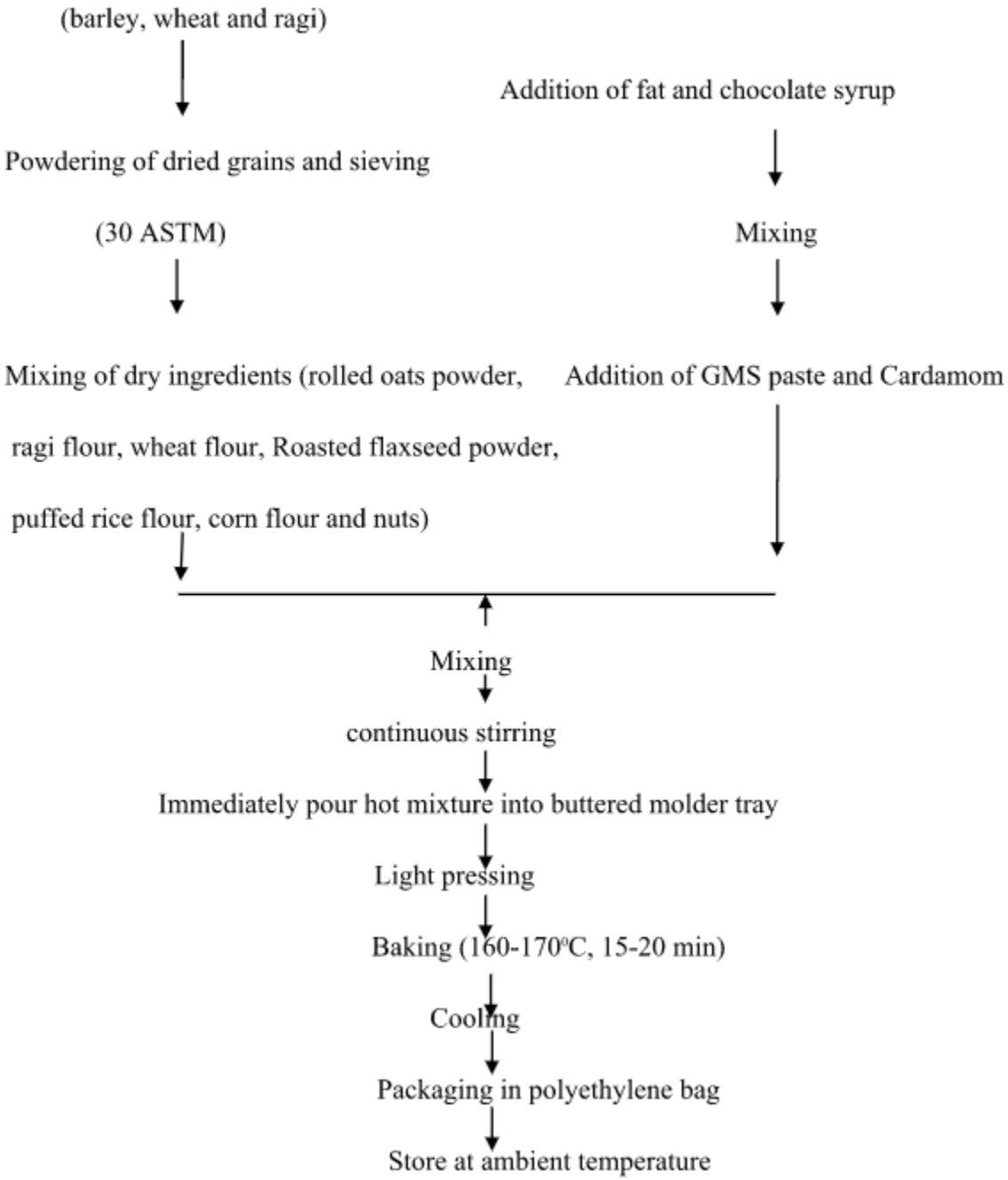
Flow sheet 5: Preparation of multigrain bars:



III. CHEMICAL ANALYSIS

Chemical analysis of multigrain bars was carriedout. Carbohydrate was determined by phenol-sulfuric acid method, crude fibre (AOAC, 2000), protein by Kjeldhal method, fat (AOAC, 2000) and minerals (Ranganna, 1986).

A. Microbial analysis of multigrain bar

Prepared multigrain bar sample selected on the basis of sensory quality were store 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). The results expressed in cfu/g. The pour plate technique was followed.

- *Total plate count*

Serial dilutions (up to 10^2) were prepared in 0.1 % saline water. 1 ml of each serial dilution were

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inoculated in petri plates containing 15-20 ml sterilized nutrient agar and incubated at 37ºC to 24-48 hrs.

- *Yeast and mold count*

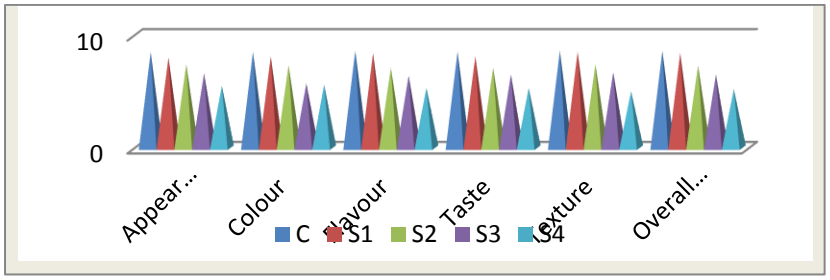
Serial dilutions (up to 10^2) were prepared in 0.1 % saline water. 1 ml of each serial dilution were inoculated in petri plates containing 15-20 ml sterilized potato dextrose agar and incubated at 30ºC to 3-5 days.

- *Sensory Analysis*

The multigrain bar samples were prepared with the addition of varying levels of roasted flaxseed flour and stevia. The sensory evaluation of flaxseed bar was 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 was made through rating products on a 9 points Hedonic Scale.

IV. RESULTS AND DISCUSSION

Table 2: Sensory evaluation of flaxseed bar replaced with stevia



C - Multi grain bar with 15% flaxseed; S_1- Multi grain bar with 15% flaxseed + 25% replacement with stevia; S_2-Multi grain bar with 15% flaxseed + 50% replacement with stevia**;** S_3-Multi grain bar with 15% flaxseed + 75% replacement with stevia; S_4 - Multi grain bar with 15% flaxseed + 100% replacement with stevia.

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) and Sadafi *et al*.,(2014).

Table 2: Chemical composition of multigrain samples

Parameters	**Control (A)**	**C**	**S_1**
Moisture (g/100g)	5.56	5.32	5.36
Fat (g/100g)	8.31	10.2	9.7
Protein (g/100g)	10.8	12.7	12.71
Crude fibre (g/100g)	5.06	6.1	6.24
Ash (g/100g)	2.04	2.17	2.2
Carbohydrate (g/100g)	70.15	66.5	60.41
Energy (kcal/100g)	398.59	408.6	379.78
Calcium (mg/100g)	82.74	162.7	167.5
Magnesium (mg/100g)	67	131.19	132.20
Iron (mg/100g)	4.70	6.81	6.90
Potassium (mg/100g)	168.05	289	294.8

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Chemical composition of multigrain bars was performed and tabulated in Table 2. Protein content of F3 sample was found to be 12.7 g/100g. There was increase in protein content on addition of flaxseed. It was seen that there was marked effect of flaxseed addition on the proximate and mineral content of the samples. Flaxseed enhances the quality of nutrients and bioactive compounds and thereby increasing the content in proteins, amino acids, sugars, and vitamins.

A. 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 Table17.

Table 3: 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	C	S_1	A	C	S_1
Fresh	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	$2.3x10^3$	$2.0x10^3$	$2.0x10^3$	ND	ND	ND
90	$4.6x10^3$	$3.7x10^3$	$3.2x10^3$	$3X10^2$	$1x10^2$	ND

A- (control) Multigrain bar; C - Multi grain bar with 15% flaxseed; S_1- Multi grain bar with 15% flaxseed and 25% stevia

In the present investigation, the susceptibility of fresh multigrain bar to microbial growth by comparing the colony-forming units (CFU) on Petri dishes inoculated with diluted multigrain bar suspensions. Selective growth media were used to differentiate between bacterial and mold/yeast growth. It was observed that the total plate count for control sample was found to be $2.3x10^3$on 75^{th} days of storage at room temperature and yeast and mold count were not detected up to 75 but it was appeared to be 3 $X10^2$ on 90^{th} days of storage. When compare to the control, flaxseed enriched sample was found to be low for susceptibility to microbial growth in terms of total plate count and yeast/mold count. Such difference in the microbial counts between control sample and flaxseed enriched sample is due to flaxseed antimicrobial activity and fungistatic activity (Shin *et al.,* 2007; Xu *et al.,* 2008; Barbary *et al*., 2010). The findings of present investigation were close resemblance with the observations reported by Girma *et al*.,(2013) and Xu *et al*.,(2008).

In case of stevia added sample, the total plate count ($3.2x10^3$) on 90^{th} days of storage and yeast - mold count was not detected. It was also noticed that the stevia added sample shown the less microbial growth than that of flaxseed enriched and control samples. It might be due to the addition of stevia and flaxseed flour to the sample. The foods containing Stevia could be used without any worry about food borne disease and can be used as antimicrobial agent (Gasmalla *et al*., 2014).

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V. CONCLUSION

From the present investigation, it can be concluded that the stevia added sample was shown the less microbial growth, because of its antimicrobial activity. And the flaxseed also has antimicrobial and fungi static activity. So, these ingredients can increase the shelf life and decreases the spoilage of foods.

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