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## Development and standardization of prebiotic nutri-bar

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### Abstract

The food we eat plays an important role in balance of gut micorbiota and keeping the body in good health. The present study was carried out at College of Food Science and Technology, Rudrur, Nizamabad Dist, Telangana, with the main objective to develop a nutri-bar with prebiotic properties of ingredients. The availability of nutrient rich prebiotic snack foods are less in market, and the bars are finger food which is a preferred snack food by all irrespective of the age and gender. List of foods with prebiotic properties was prepared based on the information available in the secondary sources and flax seeds, ragi, oats, almonds were selected as core ingredients and studied for prebiotic properties, further used for the preparation of prebiotic nutri-bar. Different formulations of nutri-bars were prepared and based on the organoleptic evaluation scores by nine-point hedonic rating scale, the final composition with flax seeds 20 g, ragi 10 g, oats 10 g, jaggery 30 g, olive oil 10 g, almonds 10 g and coconut powder 10 g was standardized. The nutritional profiling of the final developed prebiotic nutri-bar composed with moisture of  $8.0 \pm 0.5\%$ , carbohydrates of  $55.01 \pm 3.25\%$ , fiber of  $7.65 \pm 2.3\%$ , protein  $18.5 \pm 1.25\%$ , fat  $14 \pm 0.2\%$ , and ash  $5.2 \pm 0.9\%$ . Iron and calcium content was estimated as 16 mg/100 g and 26 mg/100 g respectively. This study indicates the prospects for development of prebiotic nutri-bars with enhanced nutritional quality and sensory attributes. The developed product is not only good source of prebiotics, but also a good source of other nutrients protein, iron and calcium, which will be maintained the gut micorbiota of human beings.

**Keywords:** Nutri Bar, *Lactobacillus casei shirota*, prebiotics, flax seed, oats, ragi

### Introduction

Prebiotic foods are capable of selectively stimulating the growth of one or more desirable or health-enhancing gut bacteria. Gibson *et al.*, (2004) <sup>[10]</sup> defined prebiotics as “a selectively fermented ingredient which allows specific changes both in composition or activity in the gastrointestinal micro flora that confers benefits upon host well-being and health”. *Lactobacillus* and *Bifidobacteria* are one of the important group of probiotics. These are Gram positive organisms and represent the major part of intestinal microbiota in human beings (Christine K, *et al.* 2010) <sup>[9]</sup>.

Finger millet (Ragi, *Eleusine coracana*) is the principal food grain of the Indian rural population. It is a very good source of minerals, dietary fiber and essential amino acids. Ragi provides highest level of calcium including antioxidants and phytochemicals amongst all cereals. The total dietary fiber in it is relatively higher than that of most of other cereal grains (S.S. Swami & S.B. Swami 2010) <sup>[19]</sup>.

Oats contain beta-glucan, which was found to reduce the blood cholesterol and maintain blood glucose levels in normal ranges with several other health benefiting properties. It is reported that consumption of 3 g of beta- glucan per day can significantly reduce abnormal cholesterol levels (US Food and Drug Administration, 2006).

Flax seed is popular for its health promoting properties and excellent nutrient profile. Baked products are generally supplemented with whole flaxseed in order to achieve an attractive and appealing form with enhanced texture (Ankit Goyal *et al* 2014) <sup>[11]</sup>.

Dutcosky *et al.*, (2006) developed bar by adding inulin (I), oligofructose (OF) and gum acacia (GA) as prebiotic ingredients (13.5% w/w) to cereal and fruits. They optimized formulations (50% I + 50% OF + 0% GA and 8.46% I + 66.16% OF + 25.38% GA), showed that blends of fibres imparted, to the bars and 8 better textural characteristics.

Supriya V *et al.*, (2012) <sup>[18]</sup> developed a formulation of anti-oxidant rich nutri- bar supplement for athletes with dehydrated carrots, pumpkin seeds, rolled oats, wheat germ, almonds which have high anti-oxidant content, the nutri bar was prepared and organoleptically evaluated.

Zahra *et al.* (2014) <sup>[20]</sup> developed an easy to digest, instantly low cost and stable nutri-bars for IDPs (Internally displaced people).

Maximum moisture content (16.35%) was observed in dried apricot paste 12g, dry milk powder 1g, coconut 0.5g, cinnamon 0.5g, cardamom 0.5g, pepper 0.25g, fennel 0.25g, apricot kernel 0.5g, jaggery 2g and chocolate 2g and maximum crude protein content (9.45%).

Hawaz, *et al.*, (2014) [12] isolated and identified a total of nine strains of lactobacillus, i.e. *Lactobacillus leichmannii*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus coagulans*, *Lactobacillus acidophilus*, *Lactobacillus lactis* and *Lactobacillus rhamnosus*, from curd, and evaluated *in vitro* growth inhibition activities against pathogenic bacteria.

Ayyapan *et al.* (2015) carried out experiments for the development of wheat based cookies incorporated with xylooligosaccharides at 5%, 10%, and 15% levels. Moisture levels increased with increase in the percentage of xylooligosaccharides incorporated. Cookies with 5% xylooligosaccharides were found most acceptable.

Binita Subedi and Nawaraj Upadaya (2019) [8] developed bars incorporated with flaxseed rich in nutritional and sensory characteristics which are convenient to carry and consume as a snack. Full fat roasted flaxseed flour was incorporated along with commercially available instant oat flakes *viz.*, 5, 10, 15 and 20% to prepare cereal (oat) bar. Effect of flaxseed flour incorporation was studied in proximate composition, calcium, iron and phosphorus content of cereal bar.

Some common principles available from dietary fibers that act as prebiotics are inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), pyrodextrins and maltooligosaccharides (MOS).

## Materials and Methods

### Selection and procurement of ingredients

The ingredients which has capability of prebiotic properties *viz.*, ragi, flaxseed and oats, were selected for preparation of prebiotic bar and procured from the local supermarket, Bodhan, Nizamabad District.

### A. Preparation of ingredients for Prebiotic Nutribar

The ingredients flax seeds, almonds, ragi and finger millet were washed with clean water and dried. All the ingredients were roasted slightly and made into flours by passing through 52 mesh in order to maintain the particle size in the range of 100-250  $\mu\text{m}$  for studying the prebiotic properties of the ingredients and to prepare the nutribar.

### B. Isolation of *Lactobacillus spp.* from milk and curd samples in NA and MRS Medium

Prepared the *Lactobacillus* MRS agar medium with composition *viz.*, Protease peptone-10g, HM Peptone B-10g, Yeast extract-5g, Dextrose (Glucose)-20g, Tween 80-1ml, Ammonium citrate-2, Sodium Acetate-5g, Magnesium sulphate-0.1g, Manganese sulphate-0.05g, Dipotassium hydrogen Phosphate-2g, Agar Agar-18g, Final pH - 6.5 $\pm$ 0.2) and poured in sterile plates. Prepared the Nutrient Agar medium (NaCl (5 g), peptone (5 g), Beef extract (3 g), agar agar (18 g) / 1 liter, Final pH-7) and sterilized in autoclave and poured in the plates for isolation of common lactobacillus bacteria. Probiotic milk Yakult was used as source for the isolation of *Lactobacillus spp.* on selective medium MRS agar and Nutrient Agar plates by using spread plate method. An aliquot of 0.1 ml of the diluted samples from 10<sup>-3</sup> to 10<sup>-5</sup> were spread plated on MRS agar and Nutrient agar plates with the replications and incubated at 37°C for 24-48 hrs. After

incubation the colonies were enumerated from all the diluted plates. The individual common colonies were selected and purified with streak plate technique. The purified bacteria were identified based on colony morphology and presence of common load in the sample and further used for experimentation.

### C. Preparation of Minimal media (MM) with ingredients

The ingredients which were finalized for preparation of nutribar were taken into consideration for checking the prebiotic nature through solubilization with *Lactobacillus casei shirota* bacteria. All the ingredients *viz.*, oats, ragi, flaxseeds, almonds were used for the preparation of Minimal Media (MM) with the solidifying agent agar and sterilized in autoclave. After sterilization process, poured MM in the petriplates and incubated at 30°C for 24 hours to cross check the contamination. The following ingredients were used for preparation of five minimal mediums

**Table 1:** Minimal Media composition

| S.No. | Ingredient      | MM-1 | MM-2 | MM-3 | MM-4 | MM-5 |
|-------|-----------------|------|------|------|------|------|
| 1.    | Oats            | 1g   |      |      |      |      |
| 2.    | Ragi            |      | 2g   |      |      |      |
| 3.    | Almonds         |      |      | 1g   |      |      |
| 4.    | Flaxseeds       |      |      |      | 1.2g |      |
| 5.    | All ingredients |      |      |      |      | 5g   |
| 6.    | Agar            | 2g   | 2g   | 2g   | 2g   | 2g   |

### D. Different Formulation for Prebiotic nutribar

All the selected ingredients, whose prebiotic properties were studied, were used for preparation of prebiotic nutribar. A total of four formulations were used as detailed in the table 2 below.

**Table 2:** Nutribar Formulation composition

| Ingredients    | Control sample(g) | Formula-1(g) | Formula-2(g) | Formula-3(g) |
|----------------|-------------------|--------------|--------------|--------------|
| Oats           | 40                | 10           | 10           | 10           |
| Ragi           | -                 | 15           | 10           | 25           |
| Flaxseeds      | -                 | 15           | 20           | 5            |
| Jaggery        | 30                | 30           | 30           | 30           |
| Olive oil      | 10                | 10           | 10           | 10           |
| Almonds        | 10                | 10           | 10           | 10           |
| Coconut powder | 10                | 10           | 10           | 10           |

### E. Organoleptic evaluation

Organoleptic evaluation was done for prebiotic nutribars by a group panel members from the Institute using 9-point hedonic scale (Amerime *et al.*, 1965) [3].

### F. Chemical and nutritional properties

The chemical and nutritional properties of the prebiotic nutribar were studied using standard procedures as detailed below.

#### a) Estimation of Moisture

The amount of moisture present in the food sample was determined by using standard methods, given by AOAC, (2005) [4, 5].

#### b) Estimation of Carbohydrate

The carbohydrate content of prebiotic nutribar was estimated by Anthrone method. According to Hedge, (1962) [13].

**c) Estimation of Crude Fat**

The amount of fat was determined by using Soxhlet apparatus method by AOAC, (2012) [6, 7].

**d) Estimation of Total Ash**

The total ash content of given food sample was determined by using AOAC, (2012) [6, 7] method.

**e) Protein estimation by micro- kjeldahl method:**

Nitrogen is the major element next to carbon, hydrogen and

oxygen found in living things given by AOAC, (2005) [4, 5].

**f) Estimation of crude fiber**

Crude fiber was estimated by acid and subsequent alkali treatment according to Maynard, A. J. (1970) [15].

**g) Estimation of Calcium**

Calcium was estimation was done by titration method (Ranganna, 2010) [16].

**Results and Discussion**

**A. Isolation of *Lactobacillus casei shirota* on NA and MRS plates**



**Fig 1:** Isolation of *Lactobacillus casei shirota* from Probiotic milk sample

Isolated the *Lactobacillus casei shirota* strain from the Yakult Probiotic milk. The total viable count of bacteria was recorded on MRS medium after incubation at 37°C for 24 hrs. Then colony morphology of common growth of *Lactobacillus casei* recorded and purified on selected medium plates (Fig.1).

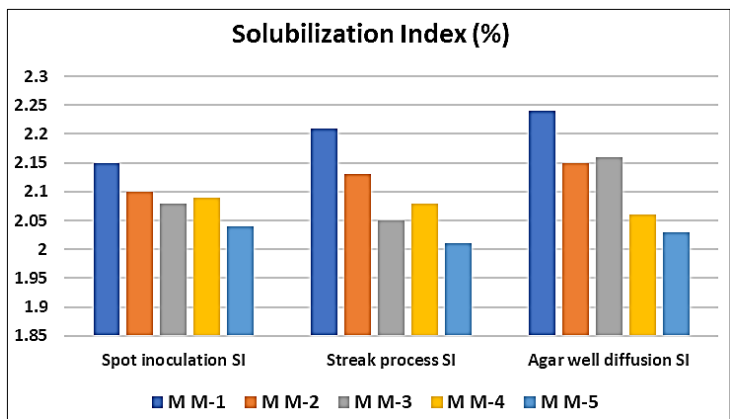
**Spot inoculation on minimal medium (MM) plates**

The spot inoculation of purified probiotic bacteria was done on MM petri plates at 9 spots and incubated at 37°C. Among five MM with the prebiotic bar ingredients, MM-1 having flax

seeds ingredients shown more solubilization index (2.15) and this leads to the more consumption by *Lactobacillus casei* followed by MM-2(2.1) having ragi ingredient, MM-3(2.08) having oats ingredient, MM-4(2.09) having almonds ingredient and lowest solubilization index was observed in MM-5(2.04). However, with this solubilization method, it is proved that all the ingredients were consumed by *L. casei* more or less. It is confirmed that all the ingredients act as prebiotic and further used for product preparation (Table.3 and Fig.2 &3)

**Table.3:** Solubilization index (S.I) of Minimal mediums done through spot Inoculation, streak process and agar well diffusion method

| S. No | Sample          | Solubilization Index (%) |                |                     |
|-------|-----------------|--------------------------|----------------|---------------------|
|       |                 | Spot inoculation         | Streak process | Agar well diffusion |
| 1.    | Minimal Media 1 | 2.15                     | 2.21           | 2.24                |
| 2.    | Minimal Media 2 | 2.1                      | 2.13           | 2.15                |
| 3.    | Minimal Media 3 | 2.08                     | 2.05           | 2.16                |
| 4.    | Minimal Media 4 | 2.09                     | 2.08           | 2.06                |
| 5.    | Minimal Media 5 | 2.04                     | 2.01           | 2.03                |



**Fig 2:** Solubilization index by *Lactobacillus casie shirota* on all Minimal mrdiums mediums

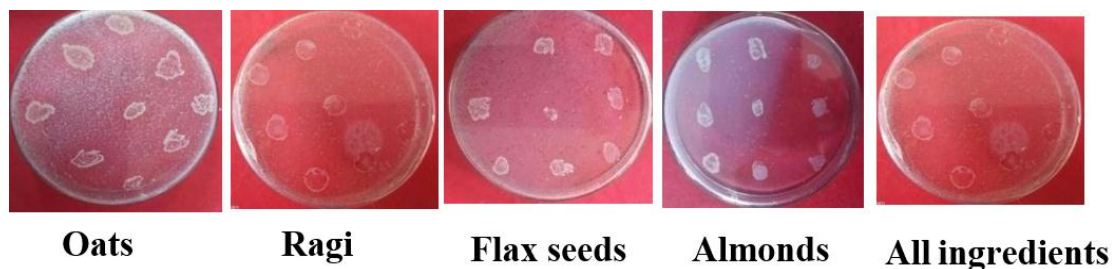


Fig 3: Spot inoculation of *Lactobacillus casei shirota* on minimal medium plates



Fig 4: Streak Process inoculation of *Lactobacillus casei shirota* on MM plates

The bacteria were inoculated through streaking on MRS petri plates and incubated at 37°C. Among five MM with the Nutribar ingredients, MM-1 having flax seeds ingredients showing more solubilization index (2.21) and this leads to the more consumption by *Lactobacillus casei* followed by MM-2(2.13) having ragi ingredients, MM-3(2.05) having oats

ingredients, MM-4(2.08) having flax seeds ingredients and lowest solubilization index was observed in MM-5(2.01). However, with this solubilization method, it is proved that all the ingredients were consumed by *L. casei* more or less. It is confirmed that ingredients act as prebiotic and further used for product preparation (Fig.4)

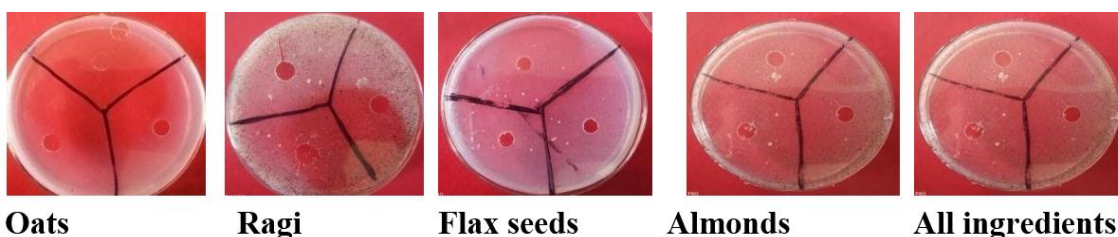


Fig 5: Agar well diffusion inoculation of *Lactobacillus casei shirota* on MM plates

The agar well diffusion of probiotic bacteria in the petri plates was poured with liquid probiotic microorganism pipetted into 3 wells of medium and incubated at 37°C. Among five MM with the prebiotic bar ingredients, MM-1 having flax seeds ingredients showing more solubilization index (2.24) and this leads to the more consumption by *Lactobacillus casei* followed by MM-2(2.15) having oats ingredient, MM-3(2.16) having ragi ingredient, MM-4(2.06) having almonds ingredient and lowest solubilization index was observed in MM-5 having ingredients (2.03). However, with this solubilization method, it is proved that all the ingredients were consumed by *L. casei* more or less. It is confirmed that ingredient act as prebiotic and further used for product preparation (Fig.5)

**G. Organoleptic evaluation on different formulations**

The organoleptic evaluation was conducted with 35 semi trained panel members using 9-point hedonic scale. Based on the scores of organoleptic evaluations (Annexure 1), formulation 2 was finalized for the standardized product. The proportion of ingredients in standardized product of

formulation 2, are flax seeds 20 g, oats 10 g, ragi 10 g, jaggery 30 g, olive oil 10 g, almonds 10 g and coconut powder (10 g) (Table.4)

Table 4: Organoleptic Evaluation of Different Formulations

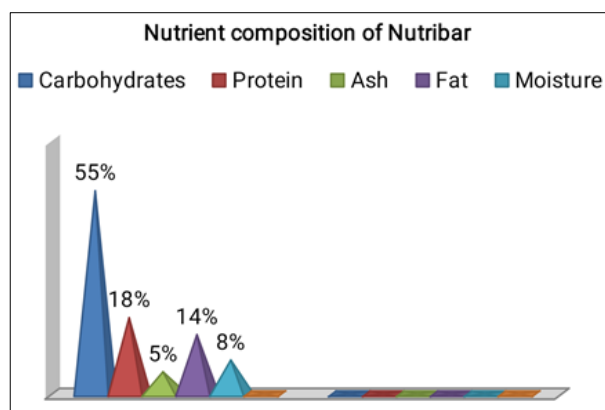
| Sample  | Color | Texture | Flavour | Appearance | Overall Acceptability |
|---------|-------|---------|---------|------------|-----------------------|
| Control | 8     | 8       | 7       | 7          | 8                     |
| F1      | 8     | 7       | 7       | 6          | 7                     |
| F2      | 8     | 8       | 8       | 8          | 9                     |
| F3      | 7     | 6       | 6       | 7          | 6                     |

**H. Proximate Composition of Standardized prebiotic nutribar (F2)**

The nutritional profiling of the developed and standardized prebiotic nutribar was done and the results showed a moisture content of 8.0±0.5%, carbohydrates of 55.01±3.25% (including fiber content was 7.65±2.3%), protein content of 18.5±1.25%, fat content of 14±0.2%, and an ash content of 5.2±0.9%. Iron and calcium content was estimated as 16 mg/100 g and 26 mg/100g respectively.

**Table 5:** Nutrient composition of prebiotic nutribar

| S. No | Parameters    | Percentage $\pm$ SD |
|-------|---------------|---------------------|
| 1     | Moisture      | 8.0 $\pm$ 0.5       |
| 2     | Fat           | 14 $\pm$ 0.2        |
| 3     | Protein       | 18.5 $\pm$ 1.25     |
| 4     | Carbohydrates | 55.01 $\pm$ 3.25    |
| 5     | Ash           | 5.2 $\pm$ 0.9       |
| 6     | Fiber         | 7.65 $\pm$ 2.3      |
| 7     | Calcium       | 26 $\pm$ 0.32       |
| 8     | Iron          | 32 $\pm$ 0.92       |

**Fig 5:** Nutrient composition of Prebiotic nutribar

According to Alla, G. U. (2018) [2] developed nutribar for the sports athletes the moisture content observed was 6.34%, carbohydrates content was 71.07%, protein content was 17.74%, fat content was 25.5%, iron content was 32 mg/100 g.

This experiment shows developing the nutrient rich product with the prebiotic ingredients which helps to support the gut and aids in digestion and also for body building. Successfully proved that the ingredients taken are prebiotic with the probiotic bacteria i.e *Lactobacillus casei shirota* strain through different methods. Hence, developed and standardized a nutribar with different ratios on the basis of sensory evaluation.

### Conclusion

A prebiotic nutribar was developed with ingredients flaxseeds, ragi, oats, and almonds. The developed nutribars showed prebiotic properties and good protein, fiber, iron and calcium content. As this product is preferred by all age groups irrespective of the gender, consumption of this nutribar on regular basis can add nutrients to the daily diet and also improves the health of gut microbiota.

### Future Scope

The present investigation was given much useful information on various aspects of Development and Standardization of Prebiotic Nutribar, yet some more work should be carried out on the nutribar can also be prepared with synbiotic. Modifying the nutritional profiling with other nutrient for rich prebiotic ingredients. Shelf life studies are required for the prepared prebiotic product.

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