NUTRITIONAL ENHANCEMENT OF VEGAN BISCUITS USING MORINGA AND FINGER MILLET

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Abstract
The biscuit market is being driven by major factors like growing demand for wholesome on-the-go satiating options, low fat, gluten free, organic, high fiber variants. The main objective of this study was to develop a biscuit by replacing refined flour and sugar with beneficial alternatives and addition of nutritionally rich Moringa leaf powder, also comparing the nutritional content with a commercial digestive biscuit. The formulated Vegan Multigrain Bikis provide good amount of energy, are enriched with flaxseeds, rolled oats & date syrup. A fibre based product can help normalize bowel movements, help control blood sugar levels, reduce risk of obesity. The formulated product was tested and analysed for its physiochemical and organoleptic properties. The product provides energy-455 kcal, carbohydrates-61.83g, proteins-8.7±0 g, fats-19.2±1.3 g, ash content-2.64%, moisture content-3.52±0.16 g, crude fibre-3.01g. As compared to the market product it was found to have lesser carbohydrates and fats per 100g. The organoleptic analysis score indicated it was liked moderately by the panelists (overall score-7.2). The qualitative estimation of phytochemicals tests indicated presence of phenolic compounds, alkaloids and flavonoids. Presence of active component Quercetin present in moringa was also checked using HPTLC, findings indicated that the Rf values of standard, moringa powder and product were similar. Conclusively the developed product was organoleptically acceptable, vegan and a variant packed with nutrients.

Keywords: fiber, moringa powder, phytochemicals, vegan.

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INTRODUCTION

Baked products are gaining popularity because of their availability, ready-to-eat and reasonably good shelf life. Among ready-to-eat snacks, biscuits possess several attractive features including wider consumption base, relatively longer shelf-life, more convenience and good eating quality (Hooda and Jood, 2005). *The availability of innovative bakery products containing multi-grains and whole wheat with low trans-fat and calorie content has further facilitated their consumption. India is the second largest producer of biscuits(cookies) in the world.*

The proportion of individuals choosing to follow a vegan diet has increased in recent years. Veganism is currently defined as a way of living that attempts to exclude all forms of animal exploitation and cruelty, be it from food, clothing, or any other purpose. Its potential health effects, for example, plant-based diets may reduce the risk of heart disease, type 2 diabetes, cancer, and premature death, lowering intake of animal products may likewise reduce risk of Alzheimer's disease or cancer or heart disease(www.healthline.com). In this product formulation we haven't used any animal-based product, so it can be readily consumed by people looking out for vegan biscuits.

Snack products with functional ingredients have been reported by different scientists from the blends of wheat/soybean (McWatters et al., 2003), wheat/cashew-apple-residue (Ebere et al., 2015)and moringa leaf powder/wheat flour (Gashaw et al., 2015). Use of composite flour based on wheat and other cereals including minor millets in bakery products is becoming popular because of the economic and nutritional advantages (Dasappa, Sai Manohar, Jyotsna, & Venkateshwara Rao, 2004; Eneche, 1999).

Finger millet Eleusine coracana commonly known as Ragi is an important minor millet. It is a rich source of dietary fibre, calcium and phytochemicals with nutraceutical potential

(Malleshi & Hadimani, 1993). The seed coat of the millet, which forms about 15% of the kernel, is a rich source of calcium, dietary fibre and polyphenols (Chethan & Malle-shi, 2007).*It contains important amino acids viz isoleucine, leucine, methionine. Finger millet is also known to have several health benefits like anti-diabetic, atherosclerogenic effect, antioxidant effects attributed to its polyphenol and dietary fiber contents (Gull et al., 2014)*

Moringa oleifera (drumstick tree), is considered as the miracle tree due to its marvelous nutritional and medicinal values. Moringa leaves are a significant source of calcium, iron, vitamin C, fibre, protein and β–carotene. Moringa, along with antioxidant properties, shows high protein content with all the essential amino acids and micronutrient composition indicating its potential to be used as food (Foid N. et al.,2001). Quercetin, a potent antioxidant, exhibiting multiple therapeutic properties is found in high concentrations in moringa leaves. There is mounting evidence that incorporating M. oleifera into food and food products improves the physicochemical and organoleptic characteristics and also extends the shelf life of food. [Food and Nutrition Bulletin 39(1)]

Oat remains an important cereal crop in the developing world. Oat is well accepted in human nutrition and is an excellent source of β-glucan, contains relatively high levels of protein, lipids, vitamins and phenolic compounds. As a functional food it has physiological benefits like hypoglycemic effect, hypocholesteromic effect (Wani et al.,2014).

Jaggery commonly known as Gur in India, is a natural sweetener obtained on concentrating the sweet juices of sugarcane. Jaggery is far complex than sugar, hence, it is digested slower than sugar and releases energy slowly and not spontaneously. This provides energy for a longer time. Jaggery contains 28g/kg of mineral salts, as against only 300mg/kg is found in refined sugar (Rao et al., 2007).

Keeping in view the above points, in this study an attempt has been made to replace sugar with date syrup and jaggery, avoiding use of butter or milk, use of composite flours in the biscuits.

MATERIAL AND METHODS

Raw materials

The following ingredients were used to formulate the biscuits viz sunflower oil, flour blend, rolled oats, jaggery powder, black dates, flaxseeds, cinnamon powder, baking powder, vanilla essence were acquired from local grocery store. The Moringa oleifera leaves were procured from vegetable market area and were then cleaned, dried and powdered.

Method of preparation

All the measurements were done using measuring cups and spoons sourced from retail shop.

Processes before mixing: The flour blend (Wheat flour, Ragi flour, Sorghum flour) was dry roasted for 5 minutes, cooled and sieved. Black dates soaked overnight were de-seeded and ground into a fine paste using small amount of water. Flaxseeds were also dry roasted for 2 minutes, cooled and ground to a fine powder. Edible Sunflower oil was heated until warm.

Formation of dough: To a bowl measured quantities of the dry ingredients including jaggery powder, moringa powder (12%), flaxseed powder (1.5%) were added and mixed properly. Followed by addition of date syrup and vanilla essence, the ingredients were again mixed, warm oil was poured onto it and blended using electric blender. Once all the ingredients started binding well, rolled oats were added and then by manual kneading a dough of desirable consistency was made. It was allowed to rest for 15 minutes and then small balls of the dough were rolled, flattened and cut into round shapes using a cookie cutter and then arranged on a baking tray lined with butter paper. The biscuits were baked for 20 minutes in a furnace at 210^{o}C. The baked biscuits were then allowed to cool and stored in a dry, airtight container.

Proximate analysis

All the analysis of the sample (Vegan multigrain Bikis) were carried out in triplicates. For moisture, ash and crude fiber content

Figure 1 Final product – Vegan Multigrain Bikis

estimation, respective methods specified in FSSAI manual (FSSAI Manual of Cereal and cereal products 2012) were followed with certain modifications in procedure for crude fiber. The protein content was estimated using Biuret method and the Carbohydrate content was calculated by weight difference method. Energy values were obtained using the Atwater General formula wherein experimental values obtained for fats, protein and carbohydrate were multiplied by factors 9, 4, 4 Kcal/g respectively.

Estimation of fat content

The determination of fat content of the biscuit was performed using Soxhlet method (Vijay Kothari et.al, 2012) with some modification. After extraction final yield was calculated using formula, on complete evaporation of solvent.

% Fatcontent= [Weight of dried extract (g) / weight of sample (g)] x 100

Qualitative estimation of phytochemicals

1 g of powdered sample was dissolved in methanol and water separately and subjected to extraction on rotary shaker at 34 rpm (rotations per minute) for 1 hour. After extraction the obtained solvent was decanted, filtered using Whatman filter paper no.1 and used further for respective phytochemical tests.

The preliminary qualitative screening for phytochemicals in the sample was performed for detecting secondary metabolites viz. alkaloids, phenols/tannins, flavonoids, saponins, terpenoids, glycosides (Malliga et al.,2017), (Silva et al.,2017).

Estimation of active components by HPTLC

The sample and Moringa leaf powder extracts were obtained after filtration through Whatman filter paper no.1 and micropore syringe respectively prepared using methanol as solvent. The moringa extract was further diluted with distilled water in 1:1 ratio and used for application. 10 µl of sample extract, moringa extract and standard (Quercetin)were applied as separate bands on the TLC silica gel $60F_{254}$ plate of dimension 10cm x 4cm, using Linomat 5 applicator and Hamilton syringe.

The solvent system mentioned (Thomas et.al), was modified for obtaining a better resolution and maximum number of spots; Ethyl acetate: Dichloromethane: Formic acid: Glacial acetic acid: water: 10 : 2.5 : 1.0 : 1.0 : 0.1 .The plate was then developed in the twin trough chamber. The plate was air dried and then scanned under CAMAG TLC Scanner 3 at 254 nm using winCATS software.

Estimation of Microbial load

The total microbial load of the biscuits was evaluated by enumerating the total viable count using the respective method mentioned in FSSAI manual. (FSSAI Manual on Microbiological food testing, 2012) with some modification required. 1 g of powdered sample was aseptically transferred to 9ml saline and centrifuged at 2300 rpm for 20 minutes and

then the supernatant was used to prepare serial dilutions wherein 1 ml of sample was aseptically added into a test tube containing 9ml of sterile saline and serial dilutions were made up to 10^{-5}. This was followed by pour plate technique. To determine the total viable count of aerobic bacteria, yeasts and molds count Nutrient Agar and Sabouraud's Agar were used respectively. The plates of Nutrient Agar were incubated at 37°C for 24 hours and Sabouraud's agar plate were incubated at room temperature for 5 days. The number of colonies counted on the plates were multiplied by the dilution factor and expressed as CFU / g.

Sensory Evaluation

Evaluation was done using scoring method to estimate the overall acceptability of the product in terms of color, odor, hardness, appearance, taste, mouthfeel, crunchiness. Thirty untrained panelists of age group 20-50 years were provided with a score card. According to the scale used, the scores indicated 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely.

Packaging

While taking into account the nature and composition of the product, storage conditions, inertness of the packet, economical aspect and easy availability metallized polyester film package was chosen as the most suitable material. It provides a complete barrier to light, gas, moisture and has good water vapor and oxygen permeability barrier properties.

Labelling

The label for the packaging material was designed by referring to the guidelines specified in the FSSAI (Packaging and Labelling) Regulations, 2011.

Figure 2 Front side of label

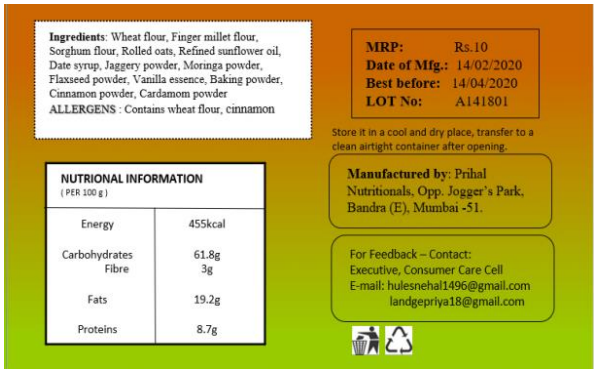

Figure 3 Back side of label

RESULTS AND DISCUSSION

The results obtained have been summarized below after calculating average of the obtained experimental values:

Proximate analysis

Parameter	Nutritional value *(per 100 g)*
Energy	455 kcal
Carbohydrates	61.83 g
Proteins	8.7±0. g
Fats	19.2±1.2 g
Ash	2.64 %
Moisture	3.52±0.16 %
Crude fiber	3.01 %

[Mean ± SD of triplicate analysis]

Table 1 Proximate analysis of Vegan Multigrain Bikis

The proximate composition of the biscuits revealed that they provide a good amount of energy and protein content. The high protein

content can be attributed to the composite effect of moringa leaf powder as studied by Sanjukta Kar et al.,(2013), finger millet has protein content 9.8% and crude fiber 3.8%, (Gull et al.,2014) and flaxseed powder (Elshehy et al.,2018).

Microbial analysis

The total viable count for bacteria & fungus was estimated to be less than 30 CFU/g that is within the range which reflected that the biscuits were safe for consumption.

Sensory analysis

The overall acceptability value obtained after collecting data from 30 untrained panelists was 7.2 which indicated liked moderately. (according to scale used '7' indicates like moderately).

While the crunchiness, hardness, odor, taste were acceptable, mouthfeel(dry) and earthy taste reduce its consumer acceptability which leads to need for further improvement. Higher concentrations of date syrup increase penetration value of cookies therefore affect firmness(hardness) of cookies, asshown in studies performed by Alsenaien et al., 2015. Also, lipid content increases palatability of the foods prepared from Moringa leaf powder (Sanjukta Kar et al.,2013).

Phytochemical Analysis

The above-mentioned phytochemicals were detected using water and methanol as solvents. Alkaloids, phenols, terpenoids and glycosides were found to be present in both solvent extracts of the sample while saponins were absent.

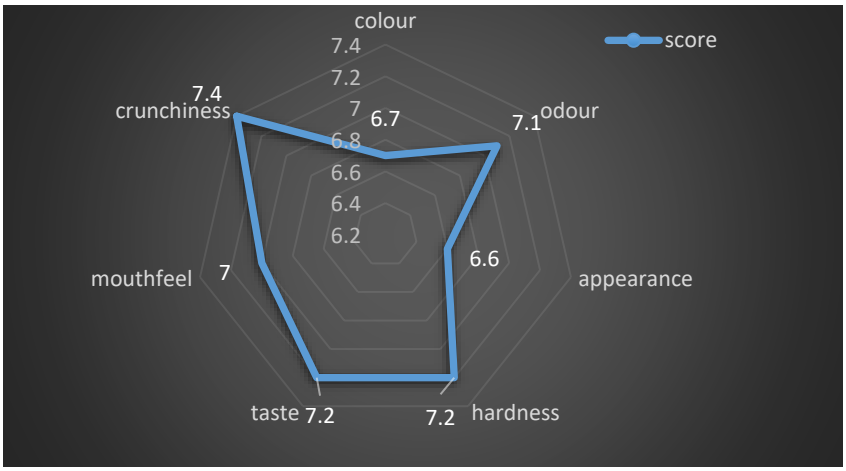

Figure 4 Radar chart for mean score of organoleptic parameters

Phytochemicals	*Water*	*Methanol*
Alkaloids	+	+
Phenols/Tannins	-	+
Flavonoids	+	+
Saponins	-	-
Terpenoids	+	+
Glycosides	+	+

Key:(+) - present; (-) – absent

Table 2. Observation table of Phytochemicals

	Rf values
Sample (Vegan Multigrain Bikis)	*0.89*
Moringa Leaf Powder	*0.88*
Standard (Quercetin)	*0.87*

Table 3. Qualitative analysis for estimation of Quercetin

The presence of these phytochemicals in M. oleifera have also been reported in another study (Malliga et al.,2017) that helps validate the results obtained.

HPTLC analysis

Qualitative analysis of quercetin was performed. The Rf values were calculated and compared.

The TLC plate after drying was observed under 254nm and the bands were studied, also chromatograms were analysed. The TLC plate was later derivatized using anisaldehyde for better observation on color development of the bands.

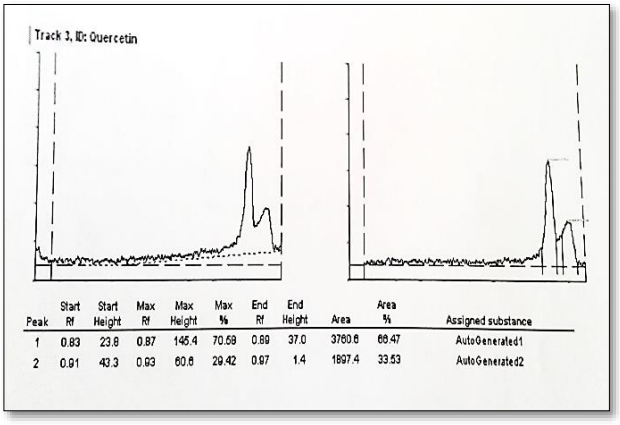

Fig. 5 Chromatogram of Standard (Quercetin)

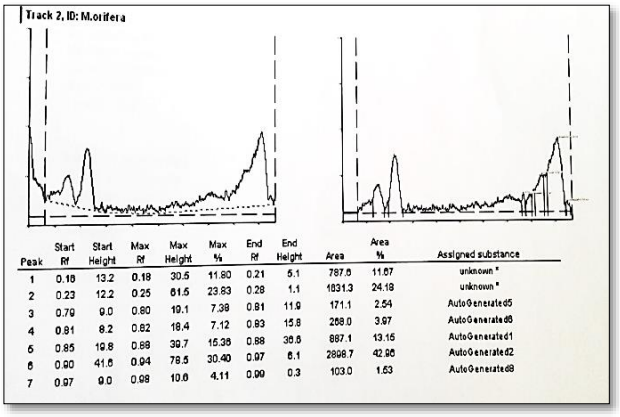

Fig. 6 Chromatogram of Moringa oliefera

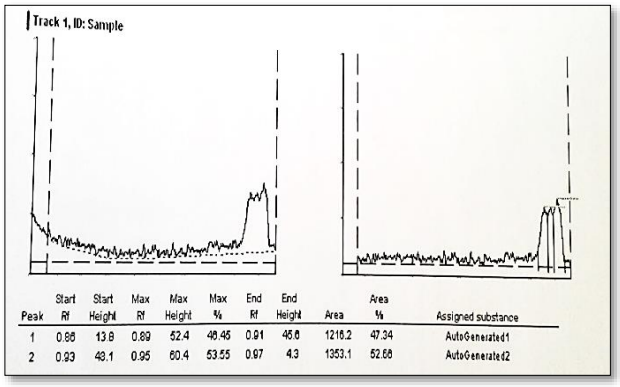

Fig.7 Chromatogram of Sample (Vegan Multigrain Bikis)

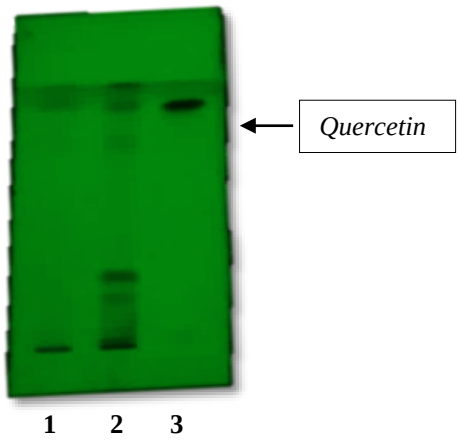

Fig.8 HPTLC plate under 254 nm

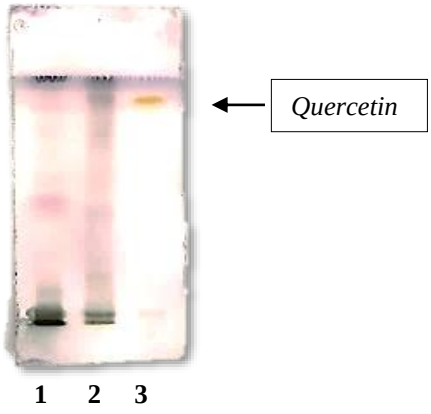

Fig. 9 Plate after derivatization (Anisaldehyde) under visible light

Key of tracks: **1** - *Sample* (Vegan Multigrain Bikis), **2** - *M. Oleifera leaf powder*, **3** - *Standard (quercetin)*

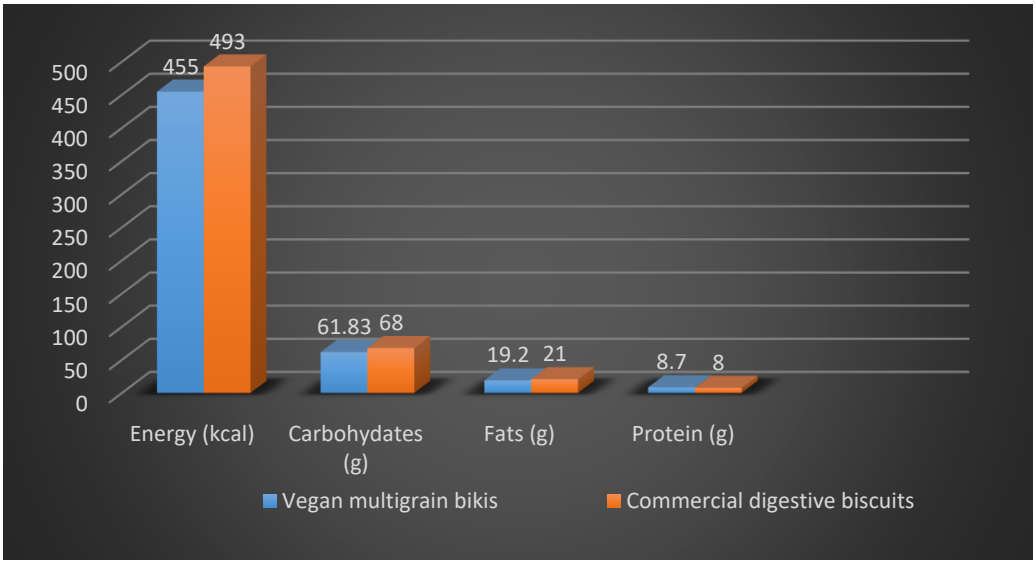

Fig.10 Bar graph representing comparative values of Nutritional content

Similar bands of the three components were marked and Rf values were calculated for arriving at a conclusion. The similar bands were found to represent quercetin.

Hence, it suggested that quercetin is present in the developed product. While developing a fixed composition of solvent system for detecting well-defined bands of M.Oleifera similar results have been reported (Thomas et.al)[13].

Product comparison:

Since the aim of the study was to formulate a commercially viable biscuit substituting sugar and refined flour unlike biscuits available in the market and to enhance its nutritional quotient using flaxseed powder and Moringa leaf powder. We selected the biscuit brand which is quite renowned, well-known and consumed by many people and has been available in the market over a long period of time.

The above graph represents the proximate composition of the biscuit developed in the present study and market biscuit. The use of date syrup and refined oil instead of palm oil attributes to an observed reduced fat content in the developed biscuits than the market biscuit.

CONCLUSION

The results obtained indicate that the biscuits serve good amount of proteins and energy. Use of functional foods, avoiding use of animal-based products, use of composite flours and oats are the

factors that highlight the biscuits as a nutritious, vegan variant also a good source of fibre. The comparison is indicative that the formulated biscuits have reduced amount of fats.

Future scope:

The results obtained in the qualitative tests further can be quantified using High Performance Liquid Chromatography for estimating the flavanoid, glycoside, terpenoid content present in the biscuits. The antioxidant activity can be analysed by DPPH method and the mineral content by Flame photometry.

The taste and dry mouthfeel can be improved by addition of plant-based milks, emulsifiers. The biscuits can be further comparatively studied for other parameters and areas of improvement can be identified in order to scale them up to a marketable level.

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