International Journal of Applied Sciences & Engineering (IJASE) 1(1): April, 2013 2013 29-37

Utilization of *Moringa* Leaves as Valuable Food Ingredient in Biscuit Preparation

Sanjukta Kar, Atrayee Mukherjee, M.Ghosh and D.K.Bhattacharyya

School of Community Science and Technology, Bengal Engineering and Science University, Shibpur, Howrah, West Bengal, India

Email: Sanjuktakakhat@yahoo.in

Abstract

This work deals with the studies on the composition of the constituents of Moringa leaves and scope for their utilization as food ingredient. Tests were performed to analyze the potential of *Moringa oleifera* leaves and scope for its food uses which have been carried out (after drying the collected leaves partially) using some common analytical methods revealing percentages of moisture, ash, protein, fat, carbohydrates and fibre that are 15%, 12.9 %, 13.66%, 4.58%, 42.57% and 10.95% respectively whereas antioxidant activities of the methanolic extract (concentration 0.1mg /ml) of moringa in terms of DPPH radical scavenging activity was 65% .The analytical tests on the extract shows absence of gum, steroid, saponin and presence of tannin and flavonoids. Moringa leaf enriched Biscuit was formulated utilizing this natural resource for analyzing the effects of processing on it. The biscuits prepared by using moringa leaf's powder shows percentages of moisture, ash, protein, fat, carbohydrates and fibre that are 5.5%, 2.41%, 11.2%, 15.4%, 63.92% and 1.52 % respectively indicating the promising scope for using moringa leaves as a major food ingredient.

Keywords: Moringa leaf, Bioactive compounds, Antioxidant, Moringa leaf enriched Biscuit

Introduction

Moringa oleifera (drumstick tree), also known as 'mother's best friend' and commonly known as sajina or moonga, is considered as the miracle tree due to its marvelous nutritional and medicinal values from ancient time.

India is the largest producer of moringa. Every part of the drumstick tree is enriched with varieties of ingredients that contribute to its magical health benefits. *Moringa* leaves are round shaped with wild leafy flavour and slight bitter taste. They are the significant source of calcium, iron, vitamin C, fibre, protein and β–carotene. Due to its antibacterial and anti-inflammatory action it can be used as a treating agent for diarrhoea, urinary disorder and gastric ulcer. *Moringa* leaves can successfully purify blood along with lowering of blood glucose and cholesterol level (Ramachandran C *et al.*,1980). 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). Moringa leaves apart from

oxalate and negligible amount of tannin (Bukar A. *et al.,* 2010), do not contain any anti nutritional factor. Moreover it can be kept for a long time due to its anti microbial property (Anwar F.*et al., 2003*) ensuring its safety.

The leaves are a rich source of essential amino acids such as methionine, cysteine, tryptophan, and lysine. The alcoholic extract of *Moringa oleifera* reduced a toxicity of some drugs in rats. *Moringa oleifera* leaves increased breast milk production among young mothers. In southern India, village people use the fresh leaves to prepare cow and buffalo ghee from butter fat. It has been found that there is a significant increase in the shelf life of ghee and that *Moringa* leaves can be a good source of natural antioxidants (Pari L *et al.*, *2007*).For all these benefits *Moringa* leaf's powder is used as Nutritional supplement, Antioxidant rich tea, Fortifying agent in different foods and in Capsule preparation. Fresh leaves are used as Vegetable in cookery, soups, salads, fried foods, curd etc (Yameogo CW.*et al.,* 2011).

Incorporation of moringa leaf in foods can be considered cost effective as it is abundantly available plant resource, once dried can be kept for a longtime requiring low storage and processing costs, available throughout the year and as it is cultivated in hot climate, can tolerate poor soil, the cost of production is very less.

Present dietary scenario necessitates exploring the possibility of incorporating novel ingredients in commonly consumed foods rather than developing new food product. With the current status of nutritional quality of biscuits and growing demand of nutritious foods, it seems worthwhile to take efforts in enhancing the nutritional value of biscuit (Zaker A. *et al*., 2012). This work aims to analyze the nutritional composition and antioxidant activity of moringa leaves and their incorporation in formulating cost effective nutritionally enriched biscuits and also the analysis of the viability of the products in terms of nutritive value, microbial safety and sensory qualities.

Materials and Methods

Collection of sample

Fresh leaves of *Moringa oleifera* were collected from Shibpur, Howrah, West Bengal in the month of February for analysis and product formulation.

Chemicals and reagents

Wheat flour, Sugar, Butter (Amul), Vanilla essence were purchased from local market (Kolkata, India). Sodium bicarbonate, Ammonium bicarbonate and all other chemicals used are from Merck, India.

Preparation of sample

At first the leaves were sorted to reject unwanted, over matured and insect affected portions of the leaves. Then they were thoroughly washed with potassium permanganate solution and dried in a shade at around 25°-30°C. Then the dried leaves were ground to fine powder and kept at 25°C in an airtight container.

Extraction of Moringa leaves

A portion of the ground sample was then taken for extraction. Petroleum ether extract was prepared using Soxhlet apparatus at 60°C. The remaining portion was extracted in the same way with methanol at 60°C.

Analysis of Petroleum ether extracted Moringa leaves

Proximate composition

The analysis of the samples for moisture, fibre and ash content were carried out in triplicate using standard methods (*AOAC, 1995*). Carbohydrate was determined according to Anthrone reaction method (*Southgate, 1976*) and determination of protein was done by Folin Lowry method. Energy values were obtained using the Atwater formula where fat, protein, and carbohydrate supplied were 9, 4, 3.75 Kcal/g respectively (*Manzi et al. 2001*).

Determination of Fat content

Fats were determined by Soxhlet method. 50g of the direct dried leaves were placed into a cellulose paper cone and extracted using petroleum ether in a Soxhlet extractor for 8 h. The petroleum ether was distilled off and finally removed completely by applying vacuum. The weight of the recovered oil was taken.

$$\text{Fat content (\%)} = \frac{\text{Amount of fat extracted (g)}}{\text{Weight of original sample (g)}} \times 100$$

Fatty acid composition (%w/w) of the oils isolated from Moringa leaves:

The Petroleum ether extracted fat was saponified to remove the unsaponifiable matter. After acidification of the soap, the hexane extracted layer was taken. Methyl ester was prepared by adding BF_3 methanol and refluxed. The final Petroleum ether extracted layer was taken .The extracts were evaporated in water bath. The sides of the tube were washed with sufficient GLC grade n-hexane to redissolve the methyl esters for GLC analysis.

Test for Minerals present in the Moringa leaves

Test for Iron: About 1ml. of acidic (HCl) solution of the ash was taken and Ammonium thiocyanate solution was added to it. Formation of blood red precipitate confirms the presence of iron.

Test for Magnesium: To 1ml. of acidic solution of ash, NaOH and titan yellow solution were added. Formation of red colouration confirms the presence of magnesium.

Analysis of Methanol extracted (extracted at 60^{o}C) Moringa leaves:

Tests for detection of Bioactive components in methanolic extract of Moringa leaves:

Determination of Antioxidant Activity

Radical scavenging activity of methanolic extract of moringa leaves were determined by DPPH radical scavenging activity (Oktay *et al*., 2003) by using the following formula-

$$\text{Antioxidant activity (\%)} = \frac{[(\text{Control OD} - \text{sample OD}) / \text{control OD}]}{(\text{Optical Density at 517nm})} \times 100$$

Test for Steroid (Salkowsky test): 1ml. of conc. H_2SO_4 was added to 10 mg of extract dissolved in 1ml. of chloroform. Formation of a reddish blue colouration at the chloroform layer and a green fluorescent colouration at the acid layer detect the presence of steroid.

Test for Tannins (Ferric chloride test): 5ml. of the extract solution was allowed to react with 5% ferric chloride solution. Greenish black colouration confirms the presence of tannin.

Test for Gum (Molisch's test): 2ml. conc. HCl was added to 2 ml extract solution. Then it was treated with 15% ethanolic solution of alpha napthol (Molisch's reagent). Formation of a red violet ring at the junction of layers confirms the presence of Gum.

Test for Saponin: 1ml. solution of the extract was diluted with distilled water to 20 ml and was shaken for 15 minutes. Development of stable foam indicates the presence of Saponin.

Test for Flavonoids: 5ml. of the extract solution was hydrolyzed with 10% v/v H_2SO_4 and cooled. Then it was extracted with diethyl ether and divided into 3 parts in 3 test tubes.3 portions

of the test tubes were treated with 1ml. dilute ammonia, 1ml. dilute sodium bi carbonate and 1ml of 0.1(N) sodium hydroxide solutions respectively. Formation of yellow colour in test tube indicates the presence of flavonoids.

Preparation of Moringa leaf enriched Biscuit

Ingredient composition

50 g Wheat flour, 25 g Sugar powder, 18.25 g Butter, 5 g Moringa leaf powder,1g Ammonium bicarbonate powder, 0.75 g Sodium bicarbonate powder, 2 ml. Vanilla essence, 10 ml. water.

Preparation procedure

All dry ingredients were sieved through 85 mesh screens and were mixed together to obtain a uniform blend. Fat for shortening, sugar and vanilla essence were mixed together to obtain sweetened shortening cream. Then dry flour was added to shortening cream with addition of water to prepare dough. The dough was prepared by manual kneading of all the dry and liquid ingredients to attain uniformity with desirable visco-elastic characteristics. When dough was ready it was kept for 10-15 minutes as it is and then used for sheeting. Sheets were prepared by rolling balls of dough on wooden platform and they were cut by hand operated metal dye, arranged on butter coated tray and were kept for baking. Baking takes place in three successive stages in electric oven. In the beginning structural changes take place due to heating of dough. In second stage greatest loss of moisture take place. In third stage the colour of biscuit changes to desired color of finished biscuit. Each lot requires 20 minutes at 160^0C for baking. Then the baked Biscuits were cooled to room temperature and packed and stored in dry airtight container at 25^0 C.

Analysis of products

Proximate composition Analysis

The analysis of the samples for moisture, fibre and ash content were carried out in triplicate using standard methods (*AOAC, 1995*). The material was defatted by successive solvent extraction in a centrifuge with chloroform and methanol in 1:2, 1:1, 2:1 ratio. Carbohydrate was determined according to Anthrone reaction method (*Southgate, 1976*) and determination of protein was done by Folin Lowry method. Energy values were obtained using the Atwater formula where fat, protein, and carbohydrate supplied were 9, 4, 3.75 Kcal/g respectively (*Manzi et al., 2001*).

Microbiological Analysis

The total microbial loads of the market Biscuit (control) and the Moringa leaf enriched Biscuit samples were enumerated in freshly prepared zero and 7 days of storage at 25°C as described by APHA (2005). Microbiological quality of market Biscuit and Moringa leaf enriched Biscuit samples made were evaluated by enumerating total viable organisms which include total aerobic count of bacteria, *E.coli*, total coliforms, yeast and molds.

Ten grams of Biscuit samples were homogenized using CM 101 CYCLO MIXER (REMI) vortex stirrer with 90 ml sterile saline (0.85% NaCl) to obtain a 10^{-1} dilution. Further tenfold serial dilution was made using the same diluents till a dilution of 10^{-8} was obtained. The spread plate technique was used to assess the microbial population. Aliquot (0.1 ml) of suitable dilution was spread plated in duplicates onto prepared, sterile and dried petridishes of suitable media for the enumeration of different organism. Plate count agar was used for total viable count and Potato Dextrose Agar was used for the presence of yeasts and moulds. After inoculating, the plates were agitated, allowed to solidify, incubated and inverted in an incubator at 37ºC for 48 hours±2 for total viable counts and at 25°C for 3-5 days for yeasts and moulds. The number of colonies counted on the plates taken into consideration the dilution factor and expressed as $\log_{10}$cfu/ml. Microbiological examinations were carried out at 1 and 7day of intervals.

Sensory evaluation of the Biscuit samples

The market Biscuit (control) and the Moringa leaf enriched Biscuit were kept at 25^{0}C until evaluation. Twenty members were chosen from the department of School of Community Science and Technology, BESU, Shibpur, Howrah, West Bengal**.** Evaluation was done at Nine Point Hedonic Scale. Characteristic evaluation included odour, taste, texture and overall acceptability. The information contained on the sensory performance was indicated as 9 = like extremely,8 = like very much7= like moderately 6= like slightly,5= neither like or dislike,4= dislike slightly,3= dislike,2= dislike very much,1= dislike extremely.

Results and Discussion

The prime objective of incorporation of Moringa leaves in biscuit preparation was to enhance the nutritional value of biscuits.

Fresh moringa leaves and dried moringa leaves have been analysed for their moisture content as moisture

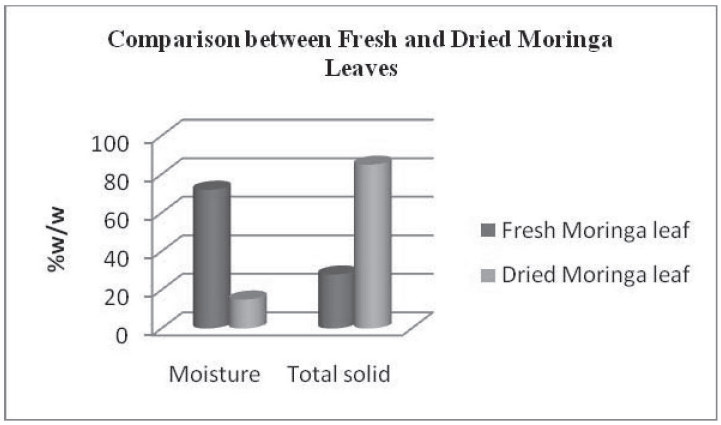

Figure 1: Comparison between Fresh and Dried *Moringa* leaves

determines perishability of the leaves.

High moisture content of the fresh leaves increase chances of microbial deterioration. High nutritional content found in the dried leaves are important nutritional indicators of the usefulness of the plant as a likely feed resource. Drying the leaves assists to concentrate the nutrients, facilitate conservation and consumption, as such, it can be used during the time when feed is scarce or can be transported to areas where it is not cultivated.

The proximate composition of *Moringa oleifera* leaves show that the leaves are rich source of protein, sugars, energy and minerals. The high ash content directly reflects high mineral content preserved in the leaves. Moringa leaves along with significant protein content show around 4.58% of lipids, which functions to increase palatability of foods prepared from Moringa by absorbing and retaining flavours.

Table 1: Proximate composition of the petroleum ether extract of Moringa leaves

Nutrient	Amount
Moisture (g/100g)	15.0 ± 0.15
Carbohydrate (g/100g)	42.57 ± 0.06
Protein (g/100g)	13.66 ± 0.19
Fat (g/100g)	4.58 ± 0.22
Fibre (g/100g)	10.95 ± 0.11
Ash (g/100g)	12.9 ± 0.10
Energy (kcal/100g)	255.55 ± 1.02

[Mean ± SD of three determinations]

The oil extracted from moringa leaves has been analysed for its fatty acid composition**.** The result shows that moringa leaf oil contains very high amount of PUFA(around 11.41% linolenic acid and 10.44% linoleic acid). PUFAs are linked to the development and functionality of the immune system. Thus Consumers

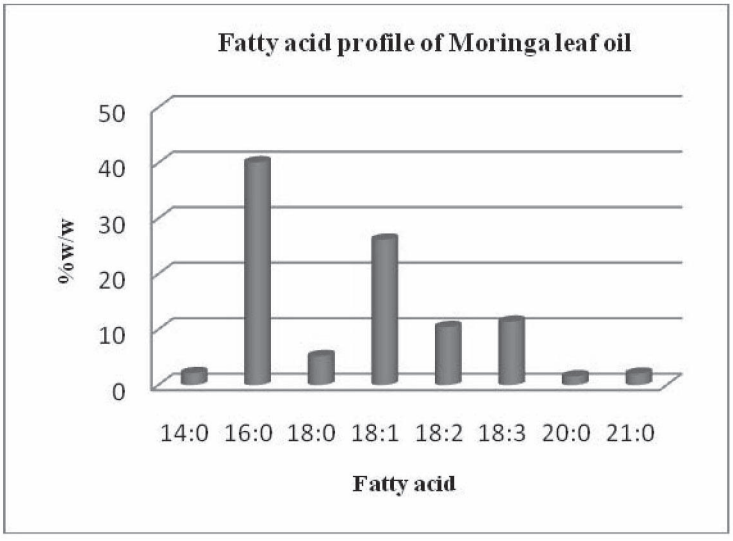

Figure 2: Fatty acid composition (%w/w) of the oil extracted from Moringa leaves

have preference of food low in saturated fatty acids (SFA) and high in polyunsaturated fatty acids (PUFA).

Table 2: Results of spot test of minerals

Spot test for Iron	Positive
Spot test for Magnesium	Negative

The results of spot test (Table 2) indicate presence of iron and absence of magnesium. The presence of iron made the leaves more significant as a nutrient supplement.

Table 3: Analysis of Methanolic extract of Moringa leaves

Experiments	Results
Salkowsky test	Negative (Steroid absent)
Ferric chloride test	Positive (Tannin present)
Molisch's test	Negative (Gum absent)
Saponin test	Negative (Saponin absent)
Flavonoid test	Positive (Flavonoids present)
DPPH radical scavenging activity	65%

From Table 3, it has been revealed that the methanolic extract of moringa leaves contain Tannin and Flavonoid whereas Gum, Saponin, Steroids are absent in the sample .The antioxidant present in the sample is very much heat stable. They have 65% scavenging activity at a concentration of 0.1mg/ml methanol only. So heat processing during formulation of nutritionally enriched biscuit may also possess a significant percentage of antioxidant activities.

The Market Biscuit (control) and Moringa leaf enriched Biscuit prepared in the present study have been analysed for proximate composition and the results are included below in Figure 3.

The Moringa leaf enriched Biscuits show high percentage of carbohydrate, fat and energy. The ash as well

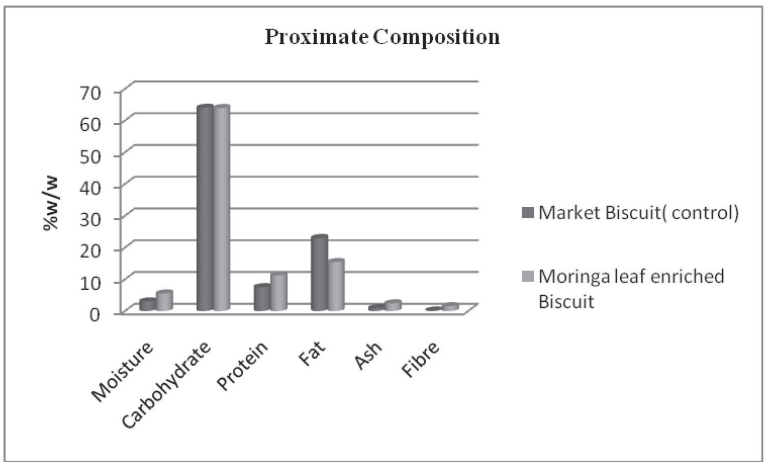

Figure 3: Proximate composition of the Market Biscuit (control) and Moringa leaf enriched Biscuit

as fibre content of the Moringa leaf enriched Biscuit are higher as compared to market biscuit(control). Moringa leaf enriched Biscuit yields around 423.10 kcal/100g whereas the market sample yields 493 kcal/ **100g of biscuit.**

Table 4: Microbial analysis of the Market Biscuit (control) and Moringa leaf enriched Biscuit

Days of Storage	Market Biscuit(control) TPC	Fungal	Moringa leaf enriched Biscuit TPC	Fungal
	(X 10^8 cfu/ml)	(X 10^8 cfu/ml)	(X 10^8 cfu/ml)	(X 10^8 cfu/ml)
0 DAY	Negligible	Negligible	Negligible	Negligible
7 DAYS	1.8	2.1	1.9	2.3

Microbial study shows that the microbial counts (Total plate count, Fungal count) are within the accepted range and the Moringa leaf enriched Biscuits made are safe to consume. Coliform and *Escherihia coli* have not been detected in case of Market Biscuit (control) and Moringa leaf enriched Biscuit for both zero days and after seven days of storage.

Organoleptic score of the Market Biscuit (control) and Moringa leaf enriched Biscuit have been evaluated in terms of colour, odour, taste, texture and overall acceptability by Hedonic rating scale indicating less acceptance for the Moringa leaf enriched Biscuit than the marketed glucose biscuit. Though in terms of texture and colour, the Moringa leaf enriched Biscuits were acceptable but the typical leafy flavour and slight bitter taste of the product decreases its consumer acceptance leading to further modifications like addition of strong flavour and taste enhancing agent.

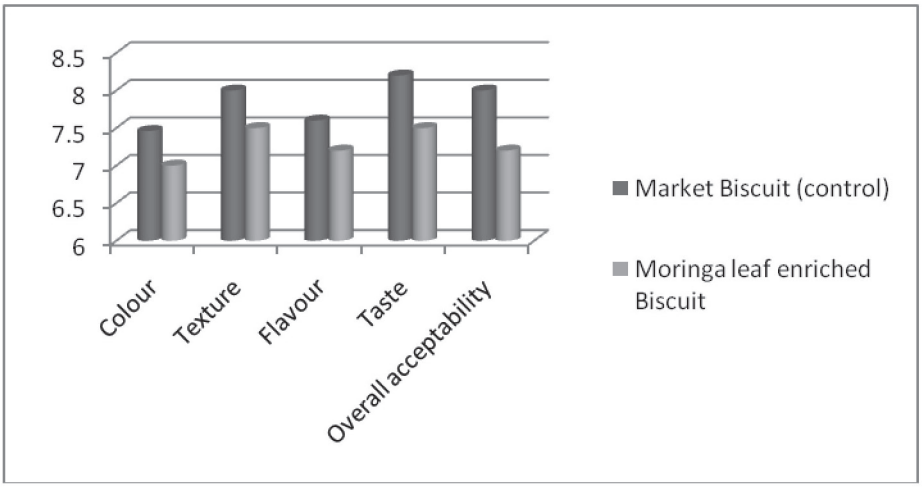

Figure 4: Hedonic rating of the Market Biscuit (control) and Moringa leaf enriched Biscuit

Conclusion

The moringa leaves can be readily selected and utilized as a remarkable food ingredient to formulate a wide range of products considering its valuable nutrient composition with noticeable amount of both macronutrients (carbohydrate, protein, desired fatty acid) and micronutrients (minerals and antioxidants). Presence of heat stable antioxidant increases the potential of the Bioactive compound based Moringa leaf to become a valuable food ingredient. Moringa has been reported to possess some medicinal properties and therefore its inclusion in the diet as nutritional supplements or in the process of fortification of foods is highly promising.

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