Utilization of *Moringa olifera* Leaves as a Functional Food Ingredient in Bakery Industry

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Abstract: ***Since, Moringa olifera leaves contained considerable amount of essential micro nutrients, this study was conducted to improve nutritional property of bakery products. Fresh leaves were harvested from same location and dehydrated with and without steam blanching. Proximate composition of the blanched and un-blanched dehydrated leaves were determined and compared. Four biscuit samples were prepared from both leaves types following two factor factorial design and most preferred sample pertaining to five sensory attributes of appearance, aroma, texture, taste, overall acceptability using five point hedonic scale. Nutritional value of Dehydrated Moringa Leaves (DML) added sample was compared with a control. Results revealed, obtaining no significant difference between blanched and un-blanched leaves in ash, crude fat, crude fiber, carbohydrate contents except crude protein ($P \geq 0.05$). Further, obtained a significant improvement in beta-carotene content and slight reduction in mineral content of blanched over un-blanched leaves. 95:5, wheat flour: blanched DML incorporated biscuit was best considering all sensory attributes. Under proximate analysis of control and DML incorporated biscuits, obtained a significant different in ash, crude protein, crude fiber, carbohydrate and mineral contents except moisture and crude fat ($P \leq 0.05$). Hence, incorporating Moringa leaves into biscuits can improve nutritional profile and reduce calorie value.***

Keywords: *Moringa*, Dehydration, Blanching, Beta-carotene, Biscuits

1. Introduction

The plant *Moringa oleifera* is the most widely cultivated species among the 13 known species [1] of *Moringaceae* family [3], [5] that is native to the Sub-Himalayan part of North West India [3], which is now indigenous to many sub-tropical regions in Africa, tropical America, Sri Lanka, Mexico, Malaysia, the Phillipine Islands etc. [3], [5].

Moringa is known in different regional names such as Benzolive tree (Haiti), Horseradish tree (Florida), Nébéday (Senegal), Drumstick tree (India), Kelor, Marango, Mlonge, Mulangay, Saijihan, Sajna etc [2]. In Sinhala it is called "Murunga" and in Tamil it is called "Murungakai" [6]. *Moringa* grows mostly in dry zone in Sri Lanka such as Jaffna, Kalpitiya, Mannar, Puttalam and Hambantota [7]. In Sri Lanka there is not a large scale commercial application of *Murunga* and therefore this crop can be considered as an underutilized crop [7].

Moringa Oleifera is considered as "The Miracle Tree" because it is a multipurpose and exceptionally nutritious vegetable tree with variety of potential uses. All parts of the tree are useful, especially due to their pharmacological, nutritional, water purifying properties (seeds) and also as an alley crop in the agro-forestry industry, livestock feed, vegetable dyes, foliar spray, cosmetics and oil production [8] etc.

M. oleifera leaves can be used successfully in its dried state or powdered form for the purpose of making different types of meals and porridge diets mostly aiming pregnant expectant mothers, nursing mothers, infants and young children, as well as adults of all age groups [22] mostly due to their nutritional and medicinal properties. Usually the dried leaves can be stored for a long time and can be used regularly without refrigeration, and reportedly without loss of nutritional value [4].

Micronutrient deficiencies are now recognized as a major contributor of most of the diseases spread throughout the world. According to WHO, (2003) 19% of the 10.8 million child deaths globally a year are attributable to iodine, iron, vitamin A, and zinc deficiencies [9]. Lots of recent studies have proved that *Moringa olifera* is an excellent source of beta carotene and other vitamins as well as minerals and amino acids that can combat the effects of malnutrition [1], [10], [11]. *Moringa* leaves also contains α-linolenic acid (all-cis-9,12,15-octadecatrienoic acid) [10], [27] which can be converted for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) within human body [28]. And also *Moringa* is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce [12].

Drying can be considered as the most commonly used method for preservation, packaging, transportation and distribution [22] of leafy vegetables. The principle of preservation by dehydration process is to remove the moisture content of a material to a level where microorganism may not be able to grow and spoil it [14].

During the drying process there can be lots of losses takes place like nutritional, physical and chemical composition of leaves [15]. Therefore, to minimize drying losses various pretreatments like blanching can be used.

Blanching is important mainly for the purpose of inactivating enzymes that can cause undesirable changes to reduce the quality of the final product; modifying texture; preserving color, flavor, and nutritional value of the product (retain certain nutrients such as vitamins) and removing trapped air [14].

Present dietary scenario necessitates exploring the possibility of incorporating novel ingredients in commonly consumed foods rather than developing new food product [16]. Hence, this study was done to incorporate *Moringa olifera* leaves for the purpose of value addition of existing bakery products, to reduce wheat flour usage and people become more health conscious regarding their food etc.

2. Materials and Method

2.1 Sample preparation

Fresh plant materials (leaves with the stalks) were taken from the same plant located in Panadura, Western Province, Sri Lanka for the whole study.

Thereafter, fresh, undamaged, leaves were selected while discarding the bruised, discolored, decayed and wilted leaves. Fresh leaves were cut from the main branches and wash with distilled water. The washed leaves were then spread out on a stainless steel mesh racks for 15 min to drain out the water. Thereafter, the leaves were divided into two portions and one portion was steam blanched for four minutes and the other was kept un-blanched. Both portions of leaves were dehydrated at 48±2°C for three hours in hot air oven. Well dried leaves were milled using a grinder with a stainless steel blade. After milling, the content was sieved to get *Moringa* leaves powder at 250 µm particle size.

2.2 Development of biscuits by adding *Moringa olifera* leaves cuts.

Four biscuits samples were prepared by blending Dehydrated *Moringa* leaves (DML) with wheat flour at two different proportions according to two factor factorial experimental design as given below.

Sample number 152 = Biscuit sample with 95:5 of wheat flour: blanched DMLC ratio

Sample number 327 = Biscuit sample with 93:7 of wheat flour: blanched DMLC ratio

Sample number 451 = Biscuit sample with 95:5 of wheat flour: un-blanched DMLC ratio

Sample number 217 = Biscuit sample with 93:7 of wheat flour: un-blanched DMLC ratio

2.3 Sensory evaluation

The sensory evaluation was performed by evaluating five major sensory attributes such as appearance, aroma, texture, taste and overall acceptability using a five point hedonic scale. A semi trained sensory panel with forty members were used for this study.

2.4 Proximate analysis

Proximate analysis were carried out for two types of dehydrated *Moringa* leaves samples and two biscuit samples as *Moringa* leaves incorporated biscuit sample that was selected from the sensory analysis along with an ordinary biscuit sample of same recipe without adding DML.

Moisture content, crude ash, crude fiber and crude protein were determined according to standard methods (AOAC 1999) [17]. Crude fat content and carbohydrate content were determined according to the method described by Offor, I.F et al.,[18] and the energy values were calculated based on the Atwater formula where fat, protein, and carbohydrate supplied were 9, 4, 3.75 kcal/g respectively [16].

Beta carotene content of the dehydrated *Moringa* leaves was determined by High Performance Liquid Chromatography (HPLC) according to the method described by Ahamad et al., [19].

Mineral content (Calcium, Potassium and Iron) of the samples were determined according to the standard AOAC1999 [17] method and the each mineral content were determined using Atomic Absorption Spectrophotometer (AAS).Wavelengths, Band pass and lamp current used for the determination of three elements were 422.7 nm, 0.5 nm, 100% (calcium); 248.3 nm, 0.2 nm, 75% (iron) and 766.5 nm, 0.5 nm, 100% (potassium) respectively. The results for mineral contents were expressed as mg/L.

2.5 Statistical analysis

The collected data pertaining to the non parametric were analyzed according to Kruskal-Wallis and Mann Whitney U test and parametric data according to two sample t test using Minitab 17 Statistical Software package.

3. Results and Discussion

3.1 Nutritional analysis of dehydrated *Moringa olifera* leaves (DML)

Nutritional compositions of blanched and un-blanched dehydrated Muringa leaves are given in table 1.

Table 1: Proximate analysis of the two dehydrated leaves samples

Nutrient	Dehydrated without blanching	Dehydrated with blanching
Moisture content (g)	8.00 ± 0.626^{a}	7.15 ± 0.439^{a}
Energy (kcal)	280.97 ± 2.62^{a}	289.59 ± 4.19^{a}
Ash (g)	10.87 ± 0.137^{a}	10.62 ± 0.284^{a}
Crude protein (g)	35.42 ± 1.77^{a}	40.61 ± 1.628^{b}
Crude fat (g)	2.60 ± 0.01587^{a}	2.91 ± 0.3010^{a}
Crude fiber (g)	12.20 ± 0.405^{a}	12.59 ± 0.404^{a}
Carbohydrate (g)	30.91 ± 1.535^{a}	26.12 ± 2.58^{a}

^{a,b} *Values in the same row with different superscripts are significantly different at 0.05 significant level.*

The moisture content of blanched and un-blanched sample is not significantly different ($P \geq 0.05$). However, moisture content of blanched leaves (7.15%) is somewhat lower than that of un-blanched leaves (8.00%). This finding is in conformed with the findings of Greve et al. [20] and Waldron et al. [21] According to those finding, cells lose their wall integrity when blanched with steam and thus tent to lose more water.

The blanched dried leaves had lower ash content (10.62%) than un-blanched dried leaves (10.87%). This finding is in compliance with the findings of Fellows, (1990). Those findings described that blanching may cause separation and losses of water soluble nutrients including minerals. However, according to the statistical analysis, the crude ash content between the two blanched and un-blanched samples had not a significant different ($P \geq 0.05$).

In the case of crude protein content this in the blanched sample (40.61%) is significantly higher than that of un-blanched sample (35.42%) ($P \leq 0.05$). This finding is in agreement with the study done by Titi Mutiara K et al., [13]. Reason for this consequence is concentrating of protein in the blanched leaves due to loosing of moisture during blanching process that imparts in reduction of cell wall integrity. Further, results obtained for the dehydrated *Moringa* leaves are compatible with the protein contents obtained by Liyanage et al., [6] for the dehydrated *Moringa olifera* leaves collected from different locations in Sri Lanka.

The level of crude protein content is of particular nutritional significance as it may meet animal's protein and energy requirements and boost the immune system against diseases [10]. Fuglie, [9] stated that the protein quality of *Moringa oleifera* rivals that of meat and eggs and protein digestibility is high (85% to 90%), with its amino-acid composition corroborating with the FAO reference protein for a growing child. According to some researches, *Moringa olifera* leaves contain all the essential amino acids [12], [10], [29]. Another special of this leaves is that they contain sulphur containing amino acids namely methionine and cystine which are often in short supply in most legumes [30] those are powerful antioxidants that help in the detoxification of harmful compounds and protect the body from radiation [31].

Crude fat content of the two leaves samples were 2.60% and 2.91% for un-blanched and blanched samples respectively. When comparing these two findings, the two treatments had not shown a significant difference ($P \geq 0.05$). However, the resulted crude fat content in un-blanched sample is closer to the values obtained at 50°C in the study done by Alakali et al., [22].

The crude fiber contents of un-blanched and blanched dehydrated leaves were 12.20% and 12.59% respectively. And also there is no significant different between the fiber content of blanched and un-blanched leaves ($P \geq 0.05$). Therefore, it can be concluded from this study that the blanching did not have a significant impact on the fiber content. The fiber content obtained in un-blanched sample is much lower than the results obtained by Alakali et al., [22]. Current study shows that the fiber content in blanched sample is slightly greater than that of un-blanched sample. Whereas, Singh and Prasad, [25] had also obtained similar observation but with much more fiber content than this study.

According to the calculations, the carbohydrate content in un-blanched and blanched dehydrated leaves was 30.91% and 26.12% respectively. And also, there is no significant different between carbohydrate content of blanched and un-blanched seed ($P \geq 0.05$). The values obtained by this study are much lower than the results obtained by Alakali et al. [22] for *Moringa* leaves dehydrated at 50°C (46.34%±0.33) and also lower than the carbohydrate content stated by Fuglie, [9].

The caloric values obtained for the un-blanched and blanched samples were 280.97 kcal/100g and 289.59 kcal/100g respectively, that values are not significantly different ($P \geq 0.05$).

Beta carotene content of blanched and un-blanched dehydrated *Moringa* leaves are given in table 2.

Table 2: Beta carotene content of the two dehydrated Moringa leaves samples

Nutrient	Dehydrated without blanching	Dehydrated with blanching
Beta carotene (mg/100g)	20.01±0.04^{a}	75.00 ±0.09^{b}

^{a,b} *Values in the same row with different superscripts are significantly different at 0.05 significant level.*

Beta-carotene is the most potent precursor to vitamin A. Beta-carotene can be converted into vitamin A (retinol) within the animal body [10], [27]. As given in table 2, beta carotene content in the blanched leaves (75 mg/100g) is significantly higher than un-blanched leaves (20 mg/100g) ($P \leq 0.05$). This is mainly due to the inactivation of certain enzymes during blanching process that can oxidize beta carotene. Apart from that, Francis [26] stated that blanching does not destroy β-carotene (Since β-carotene is heat stable, it not destroyed by most methods of cooking). However the results are in compliance with the study done by Titi Mutiara K et al., [14]; where the steam blanched leaves had higher beta carotene content than un-blenched samples. So according to the results obtained, dehydrated *Moringa* leaves can be used as a rich beta carotene source because it can exceed the recommended daily allowance for both men (600µg/day) and women (working; 600µg/day, pregnant; 600µg/day and lactating; 960µg/day), Children in all ages (400-600µg/day) according to food labeling and advertising regulations act of Sri Lanka, 2005 [24].

Most of the research studies have found that mineral content in dried *Moringa* leaves are higher than most of the plant sources [10], [32] including Calsium, Zinc, Iron, Magnesium, Potassium and Phosphorous. Mineral content obtained for both blanch and un-blanched dehydrated *Morenga* leaves in the current study are given in table 3.

Table 3: Mineral content of the two dehydrated Moringa leaves samples

Mineral	Dehydrated without blanching	Dehydrated with blanching
Ca	2493.27 ±0.53^{a}	2419.24±0.95^{b}
K	2288.11 ±0.50^{a}	2254.33±0.54^{b}
Fe	23.19 ±0.45^{a}	22.01 ±0.46^{a}

^{a,b} *Values in the same row with different superscripts are significantly different at 0.05 significant level.*

According to the table 3, Calcium (Ca) content in un-blanched leaves (2493.27 mg/100g) is significantly higher than that of blanched leaves (2419.24 mg/100g) ($P \leq 0.05$). The Ca content obtained in this study is higher than that the values reported by Liyanage et al., [6] for certain districts in Sri Lanka. Moyo et al. [10] stated that the Calcium content in the dried *Moringa* leaves are reported to be higher compared with other plant sources. Ca is reported to be required for the formation and maintenance of bones and teeth which can prevent osteoporosis. It is also considered as an essential mineral for normal blood clotting and nervous function.

When considering the level of potassium, blanched sample had 2254.33mg/100g which is significantly lower than the un-blanched leaves (2288.11 mg/100g) ($P \leq 0.05$). However, the values obtained by this study are higher than the results obtained by Liyanage et al.,[6] (except in Polonnaruwa area).

In the case of Iron (Fe) content, which is slightly lower in blanched leaves (22.005 mg/100g) comparatively un-blanched (23.19 mg/100g), however, the difference is not significantly different ($P \geq 0.05$). Nevertheless, the Fe content in the un-blanched dehydrated leaves obtained by this study is higher than the Fe content reported by Liyanage et al., [6] (except in Polonnaruwa area). Iron is an important component of Hemoglobin and Myoglobin for oxygen transport and cellular processes of growth and division. Iron is also an essential trace element for normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats. Iron also has a role in energy metabolism as it facilitates transfer of electrons in the electron transport chain for the formation of ATP [10].

The variations of the mineral content in the current study comparatively previous studies can be attributed to the variation of the soil condition, growing regions, plant variety and maturity level etc.

3.2 Organoleptic properties of the developed biscuits

Organoleptic properties pertaining to appearance, aroma, texture, taste and over all acceptability of *Moringa* leaves incorporated biscuits are given in table 4.

Table 4: The average rank of the four biscuit samples with respect to the five sensory attributes.

Sample number	*Appearance*	*Aroma*	*Texture*	*Taste*	*Overall Acceptability*
152	106.6[a]	98.0[a]	88.8[a]	111.3[a]	110.2[a]
217	64.5[b]	59.0[b]	73.1[a]	53.4[b]	53.1[b]
327	82.3[b]	93.0[a]	86.1[a]	88.3[c]	93.0[c]
451	68.6[b]	63.4[b]	74.0[a]	69.0[b]	65.7[b]

[a,b,c] *Values in the same row with different superscripts are significantly different at 0.05 significant level.*

Table 4 represents the average ranks of the four biscuit samples with respect to five sensory stimuli. Some panelists stated that the sample 217 and 451 had more bitter mouth feel after taste and that makes offensive sensation. However, it is much less in the sample 152 and also it had the better appearance. Thus, the sample 152 was recorded as the most preferable sample than the others. Moreover, Sample number 217 and 451 having had unpalatable flavour which was much less in sample number 152 and 327.

By considering all the parameters, sample number 152 was selected as the most preferred product which had the highest average ranks for all the sensory attributes and most preferred among the majority of the panelists.

3.3 Comparison of the nutritional profile of the two biscuit samples.

Nutritional value of the selected biscuit sample from the sensory evaluation was compared with a control sample (An ordinary biscuit with the same recipe that did not contain dehydrated *Moringa* leaves). Results pertaining to the analysis are given in table 5.

Table 5: Proximate analysis of the two biscuit samples

Nutrient	Control sample	Moringa added biscuit sample
Moisture content (g)	1.07 ± 0.0656^a	1.14 ± 0.0376^a
Energy (kcal)	493.47 ± 0.73^a	490.04 ± 1.16^b
Ash (g)	2.05 ± 0.151^a	2.51 ± 0.135^b
Crude protein (g)	5.14 ± 0.01^a	6.68 ± 0.193^b
Crude fat (g)	25.26 ± 0.111^a	25.32 ± 0.183^a
Crude fiber (g)	0.99 ± 0.202^a	1.57 ± 0.0751^b
Carbohydrate (g)	65.49 ± 0.08^a	62.78 ± 0.15^b

[a,b] *Values in the same row with different superscripts are significantly different at 0.05 significant level*

The data given in table 5 clearly indicate that the moisture content in control and *Moringa* leaves incorporated samples were 1.04% and 1.14% respectively and moisture content of both control and *Moringa* leaves incorporated biscuits were not significantly different to each other ($P \geq 0.05$).

Further, the ash content in control and *Moringa* leaves incorporated biscuits samples were 2.05% and 2.51% respectively and the ash content in *Moringa* leaves added biscuits was significantly different to the control sample ($P \leq 0.05$) which indicates the improvement of the mineral content of *Moringa* incorporated biscuits. The crude protein content in the control sample was 5.14% and in *Moringa* leaves added sample was 6.68%. According to the statistical analysis, there was a significant different in protein content between the two samples ($P \leq 0.05$). This is due to the high amount protein content (40.61%) in dehydrated blanched *Moringa* leaves. The crude fat content in the control sample was 25.26% and in the *Moringa* leaves added sample was 25.32% that is not significantly differ ($P \geq 0.05$). There was a significant different between the crude fiber content in the control sample (0.99%) and the *Moringa* leave added sample (1.57%). The carbohydrate content in the control sample was 65.49% and in the *Moringa* leaves added biscuit sample was 62.78% which is also significantly different to each other ($P \leq 0.05$). The calorie values in the *Moringa* leaves added biscuit sample (490.04 kcal) is lower than that of the control sample (493.47 kcal) and it had also shown a significant different ($P \leq 0.05$).

Mineral content of control and *Moringa* leaves incorporated biscuits samples are given in table 6.

Table 6: Mineral content of the two biscuit samples

Mineral	Control sample	*Moringa* added biscuit sample
Ca	31.67 ± 0.67^a	61.58 ± 0.46^b
K	117.44 ± 0.29^a	145.11 ± 0.47^b
Fe	2.17 ± 0.41^a	2.18 ± 0.39^a

[a,b] *Values in the same row with different superscripts are significantly different at 0.05 significant level*

When considering the mineral content of the two biscuits samples (Table 6), the *Moringa* leaves added biscuit sample (61.58 mg/100g) had significantly higher amount of Calcium than the control sample (31.70 mg/100g) ($P \leq 0.05$). And also Potassium content in *Moringa* leaves added biscuit sample (145.11 mg/100g) was significantly higher than that of the control sample (117.44 mg/100g) ($P \leq 0.05$). Reason for this consequence is due to higher Calcium (2419.24 mg/100g) and Potassium (2254.33 mg/100g) content in blanched dehydrated *Moringa* leaves. The Fe content of the two samples (2.17mg/100g for control and 2.18 mg/100 for the *Moringa* leaves added biscuit samples) had not shown a significant difference ($P \geq 0.05$).
According to the results obtained from this study, adding *Moringa* leaves to biscuits can improve the nutritional value while cutting down of the calorie value.

4. Conclusion

Blanched dehydrated *Moringa* leaves had improved protein and beta carotene content over the un-blanched dehydrated *Moringa* leaves. When considering all the sensory characteristics of the four biscuit samples produced by incorporating of dehydrated *Moringa* leaves, 95:5 wheat flour: blanched dehydrated *Moringa* leaves ratio was the most preferred ratio as far as sensory and nutritional quality of the biscuits are concerned. Further, adding *Moringa* leaves can improve the mineral content of the biscuits while cutting down of the calorie value.

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