Processing effects on bioactive components and functional properties of moringa leaves: development of a snack and quality evaluation

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Abstract The effect of alkali pre-treatment on the nutritional, anti-nutritional and functional properties of moringa (*Moringa oleifera*) leaf flour (MLF), and sensory assessment of MLF-based snack product was investigated. The pre-treatment reduced the content of anti-nutrients, but improved the functional properties of MLF. The MLF-based ready-to-eat puffed snack exhibited high protein (21.6 g/100 g) and dietary fiber (14.8 g/100 g) contents while it contained a low fat content of 3.7 g/100 g. The HPLC analysis of phenolics revealed that chlorogenic and gallic acids were the predominant phenolic acids present in the raw leaf flour, whereas p-coumaric, caffeic and gallic acids were the major phenolic acids in the pre-treated leaf flour. Flavonoids such as catechin, kaempferol, rutin and luteolin were present in both MLFs and the prepared snack. Overall sensory quality indicated that the snacks had acceptable textural attributes and improved nutritional profile at the 20 % level of substitution. It is possible to develop a ready-to-eat convenience food product with good functional and nutritional properties using pre-treated moringa leaf.

Keywords Moringa leaf · Pre-treatment · Nutrients · Antinutrients · Functional properties · Ready-to-eat snack

Introduction

Moringa oleifera is the widely cultivated species of the *Moringaceae* family in several Asian and African countries (Sreelatha and Padma 2009). As most parts of this tree (leaves, flowers, fruits, and immature pods) are used in various traditional food formulations, medicines and for industrial purposes, it is now considered to be one of the most useful trees; the leaves are consumed as fresh/cooked in soups, salads and porridges (Lockett et al. 2000), ready-to-eat chutney. The leaf of this multi-purpose tree is rich in vitamins, and phenolic compounds including phenolic acids and flavonoids (Makkar and Becker 1996; Coppin et al. 2013). The anti-oxidative functionality of these phytochemicals, in part, addresses the use of moringa as a promising synergistic natural plant that can be applied in foods to improve human nutrition. The powdered leaves are used by pregnant women and lactating mothers with nutrient deficiencies to enhance the child nourishment (Lockett et al. 2000). The extensive use of moringa leaf in the traditional method of treatment of various diseases has inspired the isolation and characterization of different bioactive components especially with anti-oxidant and hypotensive activities. These include carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolics (Verma et al. 2009). Although moringa is sometimes used in the traditional foods, information on its use in commercial products is limited. Furthermore, it is underutilized due to its least inclusion in ready-to-use or ready-to-eat (RTE) convenience food products. Although snack foods are one of the fastest growing segments of the food industry, the delivery of nutrition using fruits, vegetables

Highlights • Pre-treatment of moringa leaf (ML) offered desirable functional properties

- Reduction in antinutrient content was observed in pre-treated ML
- Snack prepared from pre-treated ML contained high protein and dietary fibre
- Pre-treated ML could be used successfully in making ready-to-eat health snack

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and leafy vegetables in RTE snacks is relatively a new concept. Therefore, usage of moringa leaf as a functional food will help in increasing its consumption among diverse populations.

However, moringa leaf contains various anti-nutritional factors (Makkar and Becker 1996); these may hinder efficient utilization, absorption and digestion of nutrients, and thus reduce the nutrient bioavailability and nutritional status (Lestienne et al. 2007). Besides, astringency, aftertaste bitterness and dark green colour are also the serious limitations for the use of moringa leaf in food formulations. Sengeev et al. (2013) prepared wheat bread supplemented with moringa leaf flour up to 5 % and indicated that despite a high nutrient content in leaf flour, bread produced was unacceptable. Further, Nwakalor (2014) prepared wheat cookies and showed that a maximum up to 10 % moringa flour can be added to have an acceptable product. Therefore, the present study is carried out to elucidate the types of nutritional and anti-nutritional compounds, and to quantify their contents in moringa leaf flour; the functional properties of the leaf flour and its snack making quality are also evaluated.

Materials and methods

Materials

Moringa leaves were procured from the local market of Mysore, India and cleaned to remove foreign materials, if any. All the chemicals were purchased from Sigma-Aldrich (St. Louis, USA); the reagents used were of analytical grade, and high-performance liquid chromatography (HPLC) grade solvents were used for HPLC analysis.

Pre-treatment of moringa leaves

The moringa leaves were dried in a tray drier (INDLAB, Industrial & Laboratory Tools Corporation, Chennai, India) at 45 °C for 4 h. A portion of the leaf samples was heated with 0.5 % (w/w) calcium oxide solution for 25 min; latter, they were washed thoroughly with water followed by drying at 45 °C for 6 h. These pre-treated and raw, dried leaves were ground to fine powder by employing a laboratory grinder (Universal mill, model # M20, IKA, Germany) to pass through 250 µm British Standard sieve. The leaf flour obtained were packaged in polyethylene pouches and stored below 4 °C until used.

Functional properties

The soluble nitrogen content of the raw and pre-treated moringa leaf flours was determined in distilled water (pH 7) and in 0.5 N NaCl (pH 7) (Boye et al. 2010). Protein content in the supernatant was determined using bicinchoninic acid

protein assay kit (Pierce, Rockford, Illinois, USA) and was expressed as mg/g of flour sample.

Water absorption capacity (WAC) and oil absorption capacity (OAC) were determined following the procedures of Elhardallou and Walker (1993) and Sosulski et al. (1976), respectively. These were reported as g of water or oil absorbed per 100 g of the dry flour.

Preparation of snack

Pre-treated MLF was blended with black gram and corn flour in the proportion of 20:60:20 and the required quantity of water were added to make workable dough having a moisture content of 42 %. This condition was selected based on the preliminary trials to obtain an acceptable ready-to-eat snack. The dough was extruded through a pasta making machine (La Prestigiosa, Villaveria, Italy) to obtain a ribbon shaped product and were cut into strips of 1 cm length. Later, they were hot air toasted at 220 °C for 2 min to obtain puffed snacks. They were cooled for 5 min and packaged in polyethylene pouches, sealed and stored below 4 °C until used.

Nutrient composition

The raw, pre-treated and puffed snack samples were analysed for moisture, protein, fat and ash contents following the methods of AOAC (2002). The total dietary fibre (TDF) was estimated by rapid enzymatic assay according to the method of Asp et al. (1983). The non-structural carbohydrate content was obtained by the weight difference from protein, fat and ash contents. The analytical values reported were the mean of three determinations.

Anti-nutritional factors

The phenolic compounds from defatted raw, pre-treated and snack flours were extracted with methanol containing 1 % HCl (1:20 w/v) followed by centrifugation. The total phenolic (TPC) and proanthocyanidin contents (PC) were measured by the method of Chandrasekara and Shahidi (2010), and expressed as mg gallic acid equivalents/g and mg catechin equivalents/g, respectively. The total flavonoid content (TFC) was determined by aluminium chloride calorimetric assay (Kim et al. 2003), and expressed as mg catechin equivalents/g of defatted flour.

The α -Amylase inhibitor activity (AIA) was assayed according to Shobana et al. (2009). Enzyme activity was quantified by determining the maltose equivalents released from starch at 540 nm. Enzyme inhibitory reactions for all the phenolic extracts were carried out on triplicates.

Trypsin inhibitor activity of MLF phenolic extracts was determined according to the method described by Sreerama et al. (2008). One trypsin activity unit was defined as an

increase of 0.1 unit of absorbance at 410 nm and expressed as trypsin units inhibited per mg of sample.

Total saponin (triterpinoid and steroidal) content was determined using a spectrophotometric method (Hiai et al. 1976). Sample (0.5 g) was extracted with 80 % aqueous methanol, and the results were expressed as mg diosgenin equivalent/g using diosgenin in 80 % aqueous methanol as standard.

Megazyme phytic acid assay kit (Cat. No. K-PHYT, Megazyme International, Ireland) was used to determine the phytic acid content of the sample after extracting the flours with 0.66 M hydrochloric acid and calculated using standard phosphorus.

Glucosinolates (GLS) and phenolic compounds

Glucosinolates and phenolic compounds in raw, pre-treated MLF and snack samples were simultaneously separated and identified by binary gradient HPLC analysis according to the method of Bennett et al. (2003) by taking 150 mg of sample extracted with 4 ml of 70 % methanol. Chromatographic analyses were carried out on the Luna C18 column at flow rate of 1 ml/min in a linear gradient. The chromatograms were recorded at 280 nm for phenolic acids, 320 nm for flavonoids and 227 nm for glucosinolates with a photodiode array detector. Glucosinolates and phenolic compounds were quantified using sinigrin (sinigrin monohydrate from PhytoPlan, Diehm and Neuberger GmbH, Heidelberg, Germany) and standard phenolic acids, respectively.

Colour, texture and sensory assessment of snack

The colour of MLFs and snack was measured in triplicate by employing the CIE Lab method using Hunter colour meter (Model # LABSCAN XE, Hunter Associate Laboratory, Virginia, USA) to obtain L*, a*, b* and ΔE values. A texture analyzer (Model TA-HDplus, Stable Micro Systems, Surrey, UK) was used to determine the peak force of snack during compression. A flat bottom probe (35 mm diameter) was employed to compress the sample up to 50 % at a crosshead speed of 1 mm/min. Five samples were examined each time.

Sensory assessment of the moringa snack was conducted using a 9-point hedonic scale (9: like extremely, 1: dislike extremely) with 5 as the limit of acceptance. A panel of twenty trained evaluators assessed the quality attributes of the snack in terms of appearance, texture, colour and overall acceptability (Bhattacharya and Narasimha 2008).

Statistical analysis

Multiple comparisons were made for all experiments employing Duncan's multiple range test (DMRT) at the 5 % level of significance. All statistical analyses were performed using the software Statistica'99 (StatSoft, Tulsa, OK, USA).

Results and discussion

Functional properties

The raw and pre-treated flours show distinct variation in their nitrogen solubility in both water and 0.5 M NaCl (Table 1). Raw leaf flour exhibits 120.7 and 109.4 mg/g solubilities in water and 0.5 M NaCl, respectively, whereas pre-treated flour shows lower solubilities (14.8 and 16.4 mg/g, respectively) possibly due to the leaching of soluble proteins during the pre-treatment with calcium oxide.

Water absorption capacities (WAC) of the raw and pre-treated MLF are 434.3 and 308.5 g/100 g, respectively (significant at $p \leq 0.05$) (Table 1). The WAC value for raw MLF in the present study is higher than the values reported by Singh and Prasad (2013); the variation may be attributed to the variation of location, time and position of leaves on the tree during collection. The polar amino acid residues of proteins have an affinity to water molecules (Kinsella 1976), and differences in WAC may be due to the change in contents of these amino acids in pre-treated leaf flour arising from the loss of hydrophilic amino acids during treatment. Zayas (1997) have noted that food ingredients with a high WAC may dehydrate other components of product formulation. Therefore, pre-treated MLF with lower WAC may find applications in food formulations. Water solubility indices of the raw and pre-treated MLF are 2.53 and 1.34 g/100 g, respectively showing significant reduction in pre-treated leaf flour.

Oil absorption capacity (OAC) values of raw and pre-treated MLF are 177.4 and 121.1 g/100 g, respectively (Table 1). Similar to WAC, the OAC also decreases significantly ($p \leq 0.05$) upon treatment. Kinsella (1976) has established that the hydrophobic proteins have better ability for binding of lipids. Oil binding capacity of proteins is an index to express the ability to absorb and retain oil, which in turn influences their behaviour in food products. This is an important parameter for flours intended for the development of baked and aqueous foods where the ability to hydrate and the presence of shortening are desirable. The WAC is important for flours intended for use in viscous foods as soups, gravies, dough and baked products, while OAC decides the texture and mouth feel of the flour, and is desirable in extenders in meats and baked foods (Igene et al. 2005). The raw MLF to be used in bakery food formulations requires a high oil binding activity, whereas pre-treated flour may be used in product formulations where low oil binding activity is desired. Therefore, pre-treated MLF is best suited for making low-fat snack products.

Nutrient composition

The nutrient composition of raw and pre-treated MLF and snack is shown in Table 2. The protein content of the leaf

Table 1 Functional properties of moringa raw leaf flour (RLF) and treated leaf flour (TLF)

	Nitrogen solubility (mg/g)		WAC (g/100 g)	WSI (mg/100 g)	OAC (g/100 g)
	Water	0.5 M NaCl			
RLF	120.8±0.4 ^b	109±1.1 ^b	434.3±6.8 ^b	25.3±3.7 ^b	177.4±5.2 ^b
TLF	14.8±0.2 ^a	16.4±0.4 ^a	308.6±4.4 ^a	13.4±0.7 ^a	121.1±2.8 ^a

Data are mean±standard deviation (SD)

Means with different superscripts in the same column are significantly different at $p \leq 0.05$

Symbols: *WAC* water absorption capacity, *WSI* water solubility index, *OAC* oil absorption capacity

flours is 28.4 and 28.9 g/100 g for raw and pretreated MLF, respectively. The protein content of raw MLF is comparable to the reported values for moringa leaf (Teixeira et al. 2014). Lower content of protein observed in the pre-treated flour ($p \leq 0.05$) may be due to leaching of protein during alkali treatment. The fat and ash content values of the raw and pre-treated leaf flours (4.2, 4.8 g/100 g and 7.9, 10.3 g/100 g, respectively) are in agreement with the values reported by Teixeira et al. (2014). No significant variation ($p > 0.05$) is observed in the total dietary fiber (TDF) content of raw (31.2 g/100 g) and pre-treated MLF (31.6 g/100 g). The non-structural carbohydrate content of the raw and pre-treated MLF is 27.2 and 25.5 g/100 g, respectively; a similar value of the raw MLF is also reported by Sánchez-Machado et al. (2010). The protein (21.6 g/100 g) and dietary fibre contents (14.8 g/100 g) of the snack are higher compared to the extruded snack prepared from a blend of oats and moringa leaf flour (Liu et al. 2011).

Antinutritional factors

The phenolic content (TPC) of raw flour is 33.6 mg/g while it is 9.5 mg/g for the pre-treated MLF (Table 3) indicating that the alkali treatment drastically decreases the phenolic content. Total flavonoid content (TFC) shows a significant decrease ($p \leq 0.05$) upon treatment from 27.9 mg/g (raw) to 14.1 mg/g (pre-treated). The TPC and TFC values of raw MLF values are comparable to the reported values on moringa leaf (Sreelatha and Padma 2009; Makkar and Becker 1996). The proanthocyanidin content (PC) of raw and pre-treated MLF is 19.3 and 16.0 mg/g, respectively, which is higher than the reported values for raw moringa leaf (Makkar and Becker

1996). Phenolics impair the starch and disaccharide assimilation and interact with proteolytic enzymes inhibiting their activities. The pre-treated MLF with reduced levels of polyphenols may be a suitable ingredient in the development of functional food products.

The amylase inhibitor activity (AIA) of the raw and pre-treated MLFs is 6.1 and 3.9 $\mu\text{mole/g/min}$, respectively showing significant decrease ($p \leq 0.05$) in the pre-treated flour (Table 3) while it is 3.2 $\mu\text{mole g}^{-1} \text{min}^{-1}$ for the snack prepared from the pre-treated leaf flour. Amylases hydrolyse the dietary starch to form different sugars. The inhibition of amylase activity leads to antinutrient effects while helping the weight loss. Certain plant phenolics can partially inhibit the activity of α -amylase and hence demonstrate therapeutic benefit like hypoglycemic effect to become useful in the dietary management of Type II diabetes (Chethan et al. 2008). Therefore, moringa leaf-based snack has the therapeutic potential to be termed as a health food.

Trypsin inhibitor activities of the raw and pre-treated MLF are 12.2 and 7.4 TIU/mg, respectively to show a significant reduction ($p \leq 0.05$) in pre-treated leaf flour, while the snack shows 5.9 TIU/mg (Table 3). The presence of protease inhibitors in food decreases the apparent nutritional quality of proteins and thus limits the intake of amino acids. The pre-treated MLF with lower TIA may thus be used in the food preparation.

There is a significant decrease ($p \leq 0.05$) in the saponin content in pre-treated MLF (14.8 mg/g) compared to raw leaf flour (16.1 mg/g) while the snack contains 11.5 mg/g (Table 3). Saponins are secondary plant metabolites present in plants, containing a sugar units linked to a triterpene or a

Table 2 Nutrient composition of moringa raw leaf flour, treated leaf flour and snack (%db)

	Protein	Fat	Ash	TDF	Nonstructural carbohydrates*
Raw leaf flour	28.9±0.2 ^c	4.8±0.3 ^c	7.9±0 ^a	31.2±1.1 ^b	27.2 ^b
Treated leaf flour	28.4±0.2 ^b	4.2±0.1 ^b	10.3±0.2 ^c	31.6±0.9 ^b	25.5 ^a
Snack	21.6±0.3 ^a	3.7±0.1 ^a	8.2±0 ^b	14.8±0.4 ^a	51.7 ^c

Means with different superscripts in the same column are significantly different at $p \leq 0.05$

TDF total dietary fibre

* By difference

Table 3 Antinutrient factors and phenolic content (mg/100 g) of moringa raw leaf flour (RLF) and treated leaf flour (TLF) and snack

Constituent	RLF	TLF	Snack
Antinutrient factors			
TPC (mg GA/g)	33.6±1.3 ^c	9.5±0.2 ^b	2.6±0.1 ^a
TFC (mg catechin/g)	27.9±1.3 ^c	14.1±0.9 ^b	1.5±0.1 ^a
PC (mg catechin/g)	19.3±0.2 ^c	16.0±0.1 ^b	3.4±0 ^a
AIA (μmole/g/min)	6.1±0.2 ^c	3.9±0.1 ^b	3.2±0.1 ^a
TIA (TIU/mg)	12.2±0.1 ^c	7.4±0.2 ^b	5.9±0.2 ^a
Phytic acid (mg/g)	3.5±0.1 ^b	2.1±0.2 ^a	0.5±0.1 ^c
Saponins (mg/g)	16.1±0.6 ^c	14.8±0.4 ^b	11.5±0.5 ^a
Phenolic acids			
Gallic acid	26.98±5.6 ^c	1.66±0.21 ^b	1.34±0.08 ^a
2,3-DHBA	8.49±0.26 ^c	0.23±0.01 ^a	1.61±0.06 ^b
Vanillic acid	8.33±0.19 ^c	0.88±0.02 ^b	0.42±0.09 ^a
Chlorogenic acid	80.29±0.20 ^c	0.69±0.02 ^a	1.16±0.03 ^b
Caffeic acid	8.32±0.78 ^c	1.96±0.09 ^a	4.97±0.03 ^b
p-coumaric acid	4.53±0.01 ^a	4.49±0.40 ^a	4.81±0.46 ^a
Ferulic acid	4.50±0.01 ^c	1.10±0.01 ^a	3.28±0.02 ^b
Sinapinic acid	2.83±0.24 ^c	0.80±0.03 ^a	2.14±0.06 ^b
Flavonoids			
Catechin	448.61±8.68 ^c	46.22±0.66 ^a	24.94±2.24 ^b
Kaempferol	717.15±13.9 ^c	10.67±1.16 ^a	19.60±0.51 ^b
Rutin	396.19±8.89 ^c	4.47±0.18 ^b	9.90±0.14 ^a
Naringenin	5.09±0.36 ^b	2.84±0.12 ^a	2.98±0.05 ^a
Luteolin	17.00±0.80 ^c	9.03±0.15 ^b	8.07±0.45 ^a
Myricetin	8.65±0.71 ^c	2.56±0.18 ^a	3.03±0.02 ^b
Genistein	11.46±0.86 ^c	1.56±0.04 ^a	1.91±0.04 ^b

Means with different superscripts in the same row are significantly different at $p \leq 0.05$

Symbols: TPC total phenolic, TFC total flavonoid, PC proanthocyanidin, AIA amylase inhibitor activity, and TIA trypsin inhibitor activity

steroid aglycone. Due to the presence of hydrophobic steroid and hydrophilic sugar moiety in saponin structure, saponins have excellent foaming and emulsifying properties. However, these saponins have a bitter taste and possess antinutritional effects. These are known to cause haemolysis of red blood cells and adverse effects on the growth of animals (especially non-ruminants) (Baumann et al. 2000). However, saponins in small quantity have beneficial effects on human health by reducing plasma cholesterol (Oakenfull and Sidhu 1990). In addition, some plant saponins have been reported to act as anticancer agents (Man et al. 2010).

The phytic acid contents of the raw and pre-treated MLF are 3.5 and 2.1 mg/g, respectively (Table 3) showing a significant decrease ($p \leq 0.05$); further reduction is observed in snack product (0.5 mg/g). The antinutrient effects of phytic acid include lowering the bioavailability of minerals, and inhibiting the digestibility of proteins besides while offering

several beneficial effects like acting as an antioxidant and anticancer agent (Turner et al. 2002).

Glucosinolates (GLS) and phenolic compounds

The contents of identified phenolic acids and flavonoids in raw and pre-treated MLFs, and snack product are shown in Table 3. The phenolic acids are gallic, 2,3-dihydroxybenzoic, vanillic, chlorogenic, caffeic, *p*-coumaric and ferulic acids. Chlorogenic acid (80.3 mg/100 g) and gallic acids (27.0 mg/100 g) are major phenolic acids in the raw leaf flour (Fig. 1a) though substantial amounts of other phenolic acids are also present. Alkali pre-treatment decreases the phenolic acid content significantly ($p \leq 0.05$) compared to the raw leaf flour. The major phenolic acids present in the pre-treated leaf flour are *p*-coumaric acid (4.5 mg/100 g), caffeic acid (2.0 mg/100 g) and gallic acid (1.7 mg/100 g) (Fig. 1b). However, the snack possesses higher content of phenolic acids than the pre-treated leaf sample (Table 3). Other ingredients like black gram and corn flour in the snack product formulation may have contributed to increasing in phenolic acid content. Caffeic acid (5.0 mg/100 g), *p*-coumaric acid (4.8 mg/100 g) and ferulic acid (3.3 mg/100 g) are the abundant phenolic acids in the snack (Fig. 1c). The phenolic acid *p*-coumaric acid appears to be stable during alkali treatment as its contents in raw, treated and snack are not statistically different ($p \leq 0.05$).

The major flavonoids in MLFs are catechin, kaempferol, rutin, naringenin, luteolin, and myricetin (Table 3). Similar to phenolic acids, significant ($p \leq 0.05$) reduction in flavonoid content occurs in the pre-treated leaf compared to the raw sample (Fig. 2a). The content of flavonoids is of the order of raw leaf > pre-treated leaf > snack. Kaempferol (717.2 mg/100 g) is the predominant flavonoid in raw leaf flour followed by the catechin (448.6 mg/100 g) and rutin (396.2 mg/100 g) (Fig. 2a). The major flavonoids in the pre-treated flour are catechin (46.2 mg/100 g), kaempferol (10.7 mg/100 g) and luteolin (9.0 mg/100 g) (Fig. 2b), while catechin, kaempferol and rutin are the principal flavonoids detected in the developed snack (Table 3; Fig. 2c). Compared to other flavonoids, higher retention of catechin is noted in snack; other ingredients in the snack formulation may have contributed to the higher content of catechin in the snack. Alkali treatment of moringa leaves shows significant ($p \leq 0.05$) reduction in both the phenolic acids and flavonoids (Table 4) possibly due to the oxidative degradation of phenolic acids including enzymatic browning as for the extracts of *Hsian-tsao* (Yen and Hung 2000). A decrease in flavonoids such as quercetin and kaempferol during blanching, boiling or microwave cooking of onions has been reported (Lombard et al. 2005). Phenolics such as caffeic acid, coumaric acid, catechin and quercetin are found to possess strong α -glucosidase inhibitory activity,

Fig. 1 Phenolic acid profiles of raw (a) pre-treated moringa leaf (b) and snack (c). Phenolic acids detected at 280 nm. 1, gallic acid; 2, 2,3-DHBA; 3, vanillic acid; 4, chlorogenic acid; 5, caffeic acid; 6, p-coumaric acid and 7, ferulic acid

which in turn helps in the control of diabetes (Kwon et al. 2006). However, at higher levels, these phenolics exhibit antinutritional effects (Shahidi 1997). Therefore, pre-treated MLF with a low level of phenolics may have potential health benefits and may be used as ingredients in the therapeutic snack.

The contents of glucosinolates in raw and pre-treated MLFs as identified and quantified by HPLC using sinigrin as standard are 10.7 and 1.5 mg/100 g respectively (Table 3). The treatment shows marked reduction in the glucosinolate content; similar pattern of reduction has been observed during cooking of *brassica rapa* (Francisco et al. 2010). However, trace amounts of glucosinolates have been identified in snack. Glucosinolates are a large group of

sulphur-containing secondary plant metabolites that exhibit protective activities against different types of cancer including the induction of detoxification enzymes (phase II enzymes) and the inhibition of cancer-activation enzymes (phase I enzymes) (Mithen et al. 2003), as well as anti-proliferative mechanisms.

Sensory and instrumental assessment of the snack

Texture is the most important quality attribute for a snack; the developed snack has a high score of 7.8 (out of 9-point scale) indicating good acceptability by the sensory panellists. The product prepared from untreated leaf flour (even at 5 % level) was unacceptable to the sensory judges. The most important

Fig. 2 HPLC profiles of flavonoids in raw (a) pre-treated moringa leaf (b) and snack (c). Phenolic acids detected at 320 nm. a, catechin; b, kaempferol; c, rutin; d, naringenin; e, luteolin and f, myricetin

Table 4 Colour and texture of the moringa raw leaf flour, treated leaf flour and snack

Sample	L*	a*	b*	ΔE	Sensory texture	Peak force (N)
Raw leaf flour	49.1 $\pm$ 0.2 ^b	-1.19 $\pm$ 0.1 ^b	29.4 $\pm$ 0.1 ^b	56.7 $\pm$ 0.2 ^b	Not determined	Not determined
Treated leaf flour	49.9 $\pm$ 0.1 ^c	-6.0 $\pm$ 0.1 ^a	29.4 $\pm$ 0.2 ^b	50.7 $\pm$ 0.1 ^a	Not determined	Not determined
Snack	39.0 $\pm$ 0.4 ^a	0.59 $\pm$ 0.03 ^c	19.5 $\pm$ 0.7 ^a	64.3 $\pm$ 1.4 ^c	Good crispy	6.0 $\pm$ 0.5

Means with different superscripts in the same column are significantly different at $p \leq 0.05$

attributes accepted by the panellists are the marginal uneven light brown colour, puffy appearance, square shape, and the crispy and snappy texture. Pre-treatment increases the greenness of flour as compared to the raw flour sample, as reflected by the negative values of a^* (Table 4). The lightness (L^*) and yellowness (b^*) do not vary significantly upon pre-treatment. The snack possesses a brownish green colour as the L^* value is low and the b^* value is high. The peak force of the snack is low (6.0 N, Table 4); during compression the snack undergoes several fractures as the compression proceeds as the graph shows multi cracks (graph not included) which is a desirable feature of a good crispy snack.

Conclusions

Moringa leaves are a good source of protein and dietary fibre. However, the dark colour, astringent taste and off-flavour are the serious limitations for the incorporation of leaf flour in food products to be used as a health food. The present study revealed the alkali treatment induced changes in the contents of nutrient and antinutrient in moringa leaf flour in addition make it suitable for product formulation. Reduction in the content of antinutrients and improvement in functional properties were observed. Furthermore, this study also demonstrated the suitability of alkali-treated moringa leaf flour containing bioactive phytochemicals in the development of a acceptable ready-to-use or ready-to-eat snack. The present study reported the implications of processing on the composition and contents of phenolic compounds and glucosinolates in moringa leaf flour. These results provided useful information for effective utilization of moringa leaf flour as a functional food ingredient in health promoting snack.

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Conflict of interest The authors declare that there is no conflict of interest.

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