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Vitamin-A Enrichment in *dhokla* with *Murraya koenigi* : An Underutilized Medicinal Plant in India

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ABSTRACT

Murraya koenigi, locally known as curry leaves is the richest source of pro vitamin-A, packed with very pleasant aroma however, generally discarded from dishes due its slightly hard texture. *Dhokla* is a widely accepted and popular snack in India. Fermentation and steaming are the best cooking methods to enhance the bioavailability of nutrients. Dim vision is the first sign of vitamin A deficiency and women and children are at greater risk. Pro vitamin-Aenriched *dhokla* was developed by supplementing 5,7.5 and 10 % of *Murraya koenigi* leaves powder. Leaves were blanched for 15 seconds and freeze dried. Natural fermentation was carried out during *dhokla* preparation. *Dhokla* as analyzed for sensory acceptability, proximates, dietary fiber, antinutrients, total and *in vitro* avail able minerals and β-carotene. *Murraya koenigi* leaves contained excellent amounts of β- carotene (104100 µg/100g), total dietary fiber (53.68%), soluble dietary fiber (9.63%), insoluble dietary fiber (41.22%), calcium (2147.30 mg/100g), iron (21.30 mg/100g)and zinc (3.59 mg/100g). *Dhokla* developed with 7.5% supplementation of leaves powder scored maximum for overall sensory acceptability and contained 7,994 µg/100g of β-carotene. Per day portion size of 50-60g of developed *dhokla* can meet the 100% per day requirement of β-carotene of children and women of reproductive age. Additionally, a tremendous increase in calcium by 244% and iron by 64.5% was observed in *Murraya koenigi* supplemented *dhokla*.

Keywords Curry leaves, *Dhokla,* Sensory, β-carotene, *Murraya koenigi.*

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INTRODUCTION

Murraya koenigi, the spice leaf is a tropical to sub-tropical tree belonging to the family Rutaceae. It is commonly known as curry-leaf tree, a native of India, Sri Lanka and other Asian countries and available round the year, at very low cost.Out of fourteen global species belongs to the genus of *Murraya,* only *Murraya koenigi* (*S*preng.) and *Murraya Paniculata* (Linn.) are available in India. *Murraya koenigi* has small spreading shrub which is about 2.5 meters in height, the stem is dark green to brownish in colors one of the fantastic aromatic herbs used as an essential ingredient in Indian cuisine. Curry leaves possess strong spicy and seasoning flavor with distinctive aroma. Culinary value of curry leaf is linked to the organoleptic properties e.g. color, odor and flavor. The major constituent responsible for the aroma and flavor has been reported as pinene, sabinene, caryophyllene, cadinol and cadinene. It is primarily used in curries, snacks, pickles, soups as well as meat preparations. It is naturally packed with abundant of vitamins, minerals and antioxidants and has promising amount of β-carotene (preformed vitamin A) (Khoo *et al.* 2011, Roop 2018).

Murrayakoenigi leaves have slightly stiff texture therefore generally discarded from dishes during eating and hence the nutritional potential remains underutilized. The best way to ensureits maximum utilization is toincorporatein dried form in regular consuming food products (Drisya *et al.* 2015,Chelliah *et al*. 2016, Laland Kaur 2017). However, it was observed that the dehydrated curry leaves contain considerable amounts of oxalates and phytate phosphorous i.e. 501.55 and 86.52 mg/100g, respectively (Laland Kaur 2019) that might be a constraint in its utilization in products development.Blanching and drying of green leafy vegetables have been found quite effective in reducing anti-nutrients. Green leafy vegetables blanched for 5 min and dried in shadow showed the highest reduction for oxalate and phytates. This can be attributed to the fact that the concentrations of anti-nutritional factors are higher in the superficial layer of vegetables and blanching may rupture this layer. Freeze drying preserve vitamins, minerals and antioxidants in better amounts and equally good to preserve color, fragrance and aroma. It is evident that dried leaves contain 3 to 4 time higher active nutrients than the fresh leaves(Babuet al. 2018).

Approximately, one third of the world's preschool-age population is estimated to be vitamin A deficient in Africa and South-East Asia where highest burden of VAD, reflected by deficient concentrations of the vitamin in circulation. In India the prevalence of sub-clinical vitamin A deficiency is around 62%in preschool children. Studies from developing regions suggest that pro-vitamin A rich food sources (carotenoids) provide up to 80 % of the dietary intake of vitamin A (Laxmaiah *et al.* 2011). Considering the easy availability, low cost and high β- carotene content of *Murraya koenigi, dhokla* (a fermented and steamed snack) was developed to utilize its maximum potential and enhance sensory and nutritional quality. Regular consumption of such products which can be easily prepared at home may reduce the vitamin A deficiency in children and women of reproductive age.

MATERIALS AND METHODS

Procurement of materials

Fresh leaves of *Murraya koenigi* were procured in a single lot from Medicinal, Aromatic and Underutilized Plants Section, Department of Genetics and Plant Breeding, CCSHAU, Hisar. Other ingredients required for the development of *dhokla* such as chickpea flour, curd and spices were purchased from Goyals supermarket, Hisar in a single lot.

Study design

Control *dhokla* was prepared using chickpea flour (100%) as a basic ingredient which was replaced with five, seven and half and ten percent of curry leaves powder in T_1, T_2 and T_3 *dhokla*. Other ingredients remained same in control as well as treated dhokla. Nutritional analysis for various parameters was done in triplicate except sensory acceptability where 10 responses were collected from semi-trained panellists and results were reported as mean ± standard deviation.

Preliminary analysis

Fresh *Murraya koenigi* leaves were blanched for 15 seconds and freeze dried. Developed curry leaves powder (CLP) was analyszd for proximate composition, dietary fiber constituents, minerals, antioxidants, aninutrients and β-carotene. Proximate composition (moisture, crude protein, crude fat, crude fibre and ash) was analyzed using standard methods of AOAC (2010). Moisture was analyzed using automatic moisture analyser. Nitrogen content was digested and distilled using Kjeldahl Kel Plus, ether extraction to analyse fat content was done using Socs Plus, crude fiber was analyzed as acid and alkali resistant and dietary fibre constituents using enzymatic method as earlier mentioned by Sonia *et al.*(2020) and was estimated in Fibra plus and ash was estimated in muffle furnace. Total carbohydrate was calculated by difference. The acid digested (HNO3:HClO4; 5:1 v/v) samples were estimated for total calcium, iron and zinc by Atomic Absorption Spectrophotometer 240 FS (Australia) using the method earlier mentioned by John *et al*.(2020).

Determination of antioxidants

Antioxidants were extracted in 80% methanol. Total phenol in methanolic extracts was determined by the Folin-Ciocalteau colorimetric methodas earlier

mentioned by Vinita (2018). Phenol present in plant extract reacted with specific redox reagent (Folin-Ciocalteau reagent) to form blue chromophore constituted by a phosphor-tungstic phosphor-molybdenum complex which was measured at 750 nm uv-vis spectrophotometer (Systronics, DB 2203, India). Total flavonoids content in methanolic extract was calculated by aluminium chloride colorimetric method. Natural flavonoids present in methanolic extract reacted with sodium nitrite; the pink colored flavonoids-aluminium complex was developed with aluminium chloride in alkaline condition which was measured at 510 nm. The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable DPPH free radical, was calculated by the method described earlier (Vinita 2018) with minor modification. Different known samples aliquots were taken and volume was made up to 1 ml with methanol. Then added 3 ml of DPPH reagent and mixed the contents properly and incubated for 20 minutes at 37 ºC. Absorbance of the resulting oxidized solution was read at 517 nm against methanol as blank. Total antioxidant capacity of the methanolic extracts was determined by using ferric reducing antioxidant power (FRAP) assay as described earlier (Vinita 2018).

Determination of β-carotene

Sample of β-carotene was extracted as described earlier (Chandra-Hioe *et al.* 2017).

HPLC analysis

The HPLC was equipped with a photo diode array detector (SPD-M20A). Carotenoids were separated using a C18 column in a 25 minutes run. The flow rate and injection volume were 1.2 ml/min and 15 µl, respectively. The chromatogram was monitored at visible wavelengths and the signal intensities detected at 450 nm were used for quantification.

Determination of antinutrients

Oxalic acid was analyzed by titration method and phytic acid was extracted with 20 ml of 0.5 M HNO3 and estimated as per the method described earlier (John *et al.* 2020).

Development of *dhokla*

Chickpea flour was sieved and weighed amounts of CLP were added in the experimental *dhokla*. Salt, turmeric powder and curd was added to sieved flour and made a smooth batter with addition of water. All the control and experimental batters (T_1, T_2 and T_3) were kept in BOD incubator at 37°C temperature for 8 h to carry out natural fermentation. Fermented batter was poured in the greased *dhokla* maker and steam cooked for 30 minutes. A commonly prepared seasoning was poured over control and experimental *dhokla* before sensory analysis and same samples were taken for nutritional analysis.

Sensory evaluation

Developed *dhokla* were evaluated for color, appearance, aroma, texture, taste and overall acceptability using 9 point hedonic rating scale by a panel of 10 semi-trained judges. Rating of *dhokla* on a 9 to 1 point rating scale was expressed as liked extremely, liked very much, liked moderately, liked slightly, neither liked nor disliked, disliked slightly, disliked moderately, disliked very much and disliked extremely, respectively. *Dhokla* scoring an overall acceptability of 6 or above were considered acceptable and further evaluated for nutritional parameters.

Nutritional evaluation

Developed *dhokla* were analyzed for proximate composition, dietary constituents, total and *in vitro* available minerals, β-carotene and anti-nutrients. *In vitro* bioavailability of calcium, zincand iron was analyzed using enzymatic method as earlier mentioned by John *et al.* (2020).

Statistical analysis

Statistical analyses were conducted using SPSS for windows version 19.0. One way ANOVA with LSD test was performed to analyze the results. Results were expressed as a means ± SD and means were accepted as significantly different at 95% confidence interval; $p<0.05$. *In vitro* bioavailability of minerals was expressed in percent.

Table 1. Nutritional composition of *Murrayakoenigi* powder (per 100g, DM). Values are mean ± SD of three independent determinations; *Fresh weight basis.

Nutritional Parameters	Quantity
Moisture (g)	3.91±0.03
Crude fat (g)	3.19±0.03
Crude protein (g)	11.44±0.10
Crude fiber (g)	9.19±0.04
Ash (g)	10.79±0.09
Carbohydrates (g)	61.49±0.09
Soluble dietary fiber (g)	9.63±0.05
Insoluble dietary fiber (g)	41.22±0.06
Total dietary fiber (g)	53.68±0.06
DPPH scavenging activity* (mg TE)	51.67±0.57
Total flavonoids* (mg RE)	155.6±0.57
Total phenols* (mg GAE)	624.3±1.32
FRAP *(mg TE)	650.4±0.54
Calcium (mg)	2147 ±3.48
Iron (mg)	21..23±0.06
Zinc (mg)	3.59±0.06
Oxalates (mg)	497.52±0.18
Phytic acid (mg)	119.41±0.14
β- Carotene (µg)	104100 ±4.16

RESULTS AND DISCUSSION

Results of nutritional composition of *Murraya koenigi* leaves powder used in the development of β-carotene rich *dhokla* indicated that dried powder contained excellent amounts of total dietary fiber (53.68%), soluble dietary fiber (9.63%) and insoluble dietary fiber (41.22%) (Table 1). A many folds increase in soluble and insoluble dietary fiber was observed upon dehydration of *Murraya koenigi* leaves (Igara *et al.* 2016, Longvah *et al.* 2017). In a previous study, soluble and insoluble dietary fiber content of curry leaves powder was found to be 4.4 g/100g and 55.6 g/100g, respectively, which was in close proximity of the fiber content of present study (Igara *et al*. 2016).

A quite high content of β-carotene in dried *Murraya koenigi* leaves (104100 µg/100g) was observed in present study than the cited literature (Longvah *et al.* 2017, Lal and Kaur 2019, Chaudhary 2020). A wide variation in β-carotene content might be due to the differences in extraction method, drying method, analytical procedure, soil health, rainfall and also the maturity stage of leaves. The 70-80 % retention of β-carotene was observed in various thermal drying methods whereas, 95 % retention was found in freeze drying and considered better than the air drying method.

In present study, *Murraya koenigi* leaves powder contained excellent amounts of calcium iron and zinc i.e. 2147.30, 21.30 and 3.59 mg/100g, respectively (Table 1). Dehydration generally increases the amounts of minerals. Dehydrated curry leaves

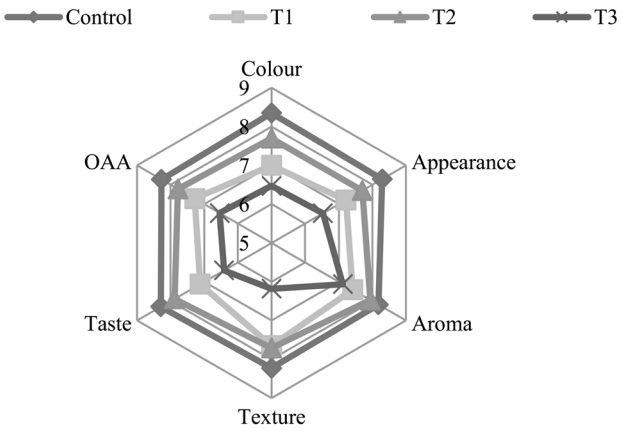

Fig. 1. Mean sensory scores of *Murraya koenigi* supplemented *dhokla.*

Table 2. Nutritional composition of *Murraya koenig*i supplemented *dhokla* (per 100g, DM). Values are mean ± SD of three independent determinations; *Fresh weight basis.

	Control	T_1	T_2	T_3
Moisture*	58.8±0.1^{d}	57.6±0.1^{c}	56.5±0.1^{b}	55.7±0.2^{a}
C. fat	5.2±0.01^{d}	4.9±0.02^{c}	4.7±0.0^{b}	4.6±0.0^{a}
C. protein	16.8±0.0^{a}	17.2±0.0^{b}	17.4±0.0^{c}	17.5±0.0^{d}
C. fiber	2.5±0.1^{a}	3.0±0.01^{b}	3.3±0.00^{c}	3.48±0.0^{d}
Ash	2.3±0.1^{a}	2.7±0.02^{b}	3.2±0.00^{c}	3.57±0.0^{d}
CHO's	14.5±0.1^{a}	14.6±0.1^{a}	14.8±0.1^{b}	15.1±0.1^{c}
SDF	4.2±0.1^{a}	4.5±0.05^{b}	4.6±0.0^{c}	5.19±0.1^{d}
IDF	8.5±0.1^{a}	10.3±0.1^{b}	11.6±0.1^{c}	12.7±0.1^{d}
TDF	12.8±0.1^{a}	14.8±0.1^{b}	16.3±0.1^{c}	17.9±0.1^{d}
Ca	48.4±0.1^{a}	153±0.1^{b}	207±0.0^{c}	263±0.0^{d}
Iron	3.1±0.03^{a}	4.1±0.02^{b}	4.6±0.0^{c}	5.1±0.0^{d}
Zinc	1.1±0.04^{a}	1.3±0.08^{a}	1.4±0.1^{b}	1.5±0.1^{b}
Oxalates	7.20±0.1^{a}	32.2±0.0^{b}	44.5±0.1^{c}	56.7±0.1^{db}
Phytic acid	383±0.1^{b}	376±0.1^{ab}	368±0.1^{a}	357±0.1^{a}

had 10.44 mg/100g of iron and 2111.70 mg/100g of calcium as compared to 0.93 and 819 mg/100g of iron and calcium, respectively in fresh leaves (Lal and Kaur 2017). It was observed that dehydration increased 3 to 4 folds of calcium concentration in leafy vegetables.The results of antioxidants of present study were found in close proximity with the previous findings of those who observed that total phenolic conten of GLVs lied between 20 to 2200mg GAE/100g (Sreeramulu *et al*. 2013) with minimum observed range of 20 to 113 mg while maximum observed range was 260 to 2200 mg. Usually the high amounts of total phenols and total flavonoids are correlated with high DPPH and FRAP values which indicate the dominating contribution of polyphenols towards antioxidant activities.

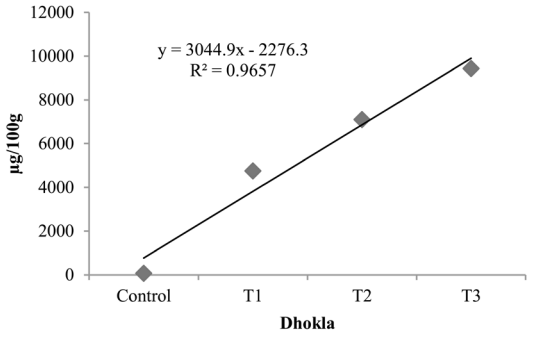

Fig. 2. Beta carotene content of *Murraya koenigi* supplemented *dhokla.*

Mean sensory scores of overall acceptability of T_1, T_2 and T_3 *dhokla* (6.54 to 7.78) (Fig. 1) indicated that all the products were adjudged within liked slightly to liked moderately by the judges and all three *dhokla* were found acceptable by the judges Results of the present study are in close agreement with those of earlier workers who also incorporated curry leaves at various levels i.e. 3, 4, 5 and 10 % in the development of *chapatti*, cooked rice and seasoned potatoes, *mathri, uttapam, idli* and lemon rice, *naan, vadiyan,bhatura, vada* (Laland Kaur 2017), buns and *shrikh and.* In our study, results indicated that T_1 *dhokla* contained maximum moisture, whereas T_3 *dhokla* contained maximum crude protein. Further it was observed that T_3 *dhokla* contained maximum ash and crude fiber. CLP supplemented *dhokla* had significantly higher ($p \leq 0.05$) contents of crude protein, crude fiber and ash than that of control *dhokla* (Table 2). Our findings are corroborated with those reported by earlier investigators (Drisya *et al.* 2015, Chelliah *et al.* 2016, Laland Kaur 2017).

Contents of insoluble, soluble and total dietary fiber were increased significantly in *dhokla* with each level of supplementation of *Murraya koenigi* in control formulations (Table 2). Other workers also found an increase in the dietary fiber content of the products prepared using curry leaves powder (Chelliah *et al.* 2016, Sudha *et al.* 2014). Dehydrated curry leaves can be successfully incorporated in food products to enhance their nutritional and medicinal value.

Incorporation of curry leaves (3-5%) enhanced the iron, calcium and β-carotene in developed products.In present study, a tremendous increase in calcium by 244% and iron by 64.5% was observed in *Murraya koenigi* supplemented *dhokla.* Oxalic acid might have hindered the per cent bioavailability of calcium and zinc however the iron bioavailability was least affected (Fig. 2). Results of present study were found in close agreement with earlier workers (Laland Kaur 2019). *Murraya koenigi* supplemented *dhokla* had 50 times higher concentration of β-carotene than control (Fig. 3). An intake of 60g portion size of T_1, the most acceptable *dhokla* prepared with 7.5 % level of incorporation of *Murraya koenigi* can meet the 100 % RDA of vitamin A.

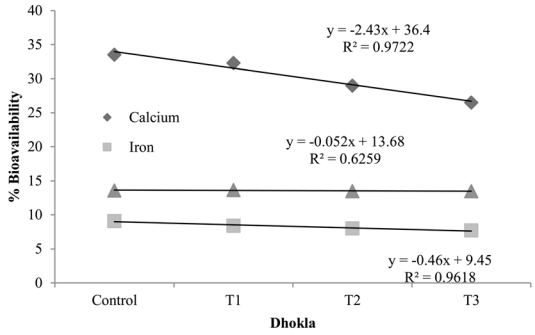

Fig. 3. *In vitro* bioavailabiliy of *Murraya koenigi* supplemented *dhokla.*

CONCLUSION

Curry leaves powder can be successfully incorporated up to 10% to develop β-carotene rich products without affecting the sensory acceptability except color. Overall sensory acceptability of *dhokla* prepared using 7.5% level of incorporation of CLP was found maximum, though all three *dhokla* were found acceptable. Beta carotene rich products can be successfully developed by incorporating curry leaves powder as its content in developed *dhokla* ranged from 5392 to 10597 µg/100g. Per day consumption of 50-60 g of developed *dhokla* can meet 100% of daily RDA of β-carotene. Besides β-carotene developed *dhokla* had significantly higher contents of calcium, iron, fiber and crude protein than the control products. Consumption of curry leaves powder supplemented products may improve the sub-clinical deficiency of vitamin A in vulnerable group.

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