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Enhancement of the Nutritional Value of Whey Drink by Supplementing with Leaves of *Moringa oleifera*

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Abstract: The effect of supplementing *Moringa oleifera* leaf powder (MOLP) on the nutritional and sensory characteristics of whey drink was investigated. Whey drink was supplemented with MOLP at four different concentrations i.e., 1% MOLP (T_1), 2% MOLP (T_2), 3% MOLP (T_3), 4% MOLP (T_4) and compared with a control (T_0). The addition of MOLP at any level did not have a negative effect on pH and acidity of whey drink. Iron content of T_4 increased from 0.17 to 115 mg/100 mL, total phenolic content of MOLP was 7.4 g/100 g on dry weight basis (gallic acid). Vitamin C increased from 1.46 to 2.20 mg/100 g in T_4. The overall acceptability score of T_4 was 6.9 out 9 (total score) which was more than 76%. These results suggest that nutritional value of whey can be increased by supplementing with 4% dry leaves of *M. oleifera* in the form of a whey based drink with acceptable sensory characteristics.

Keywords: *Moringa oleifera,* leaf powder, whey drink, iron, malnutrition

Introduction

Malnutrition results in retarded physical and mental growth as well as lack of resistance against diseases, whereas, protein deficiency results in poor health and reduced capacity for physical work (NNS, 2004). Plants have natural antioxidants and polyphenolic compounds that have anticarinogenic, cardiac and hepatic protective activity (Jeong *et al.,* 2004; Kris-Etherton *et al.,* 2002; Middleton *et al.,* 2000). *M. oleifera* is fast growing tree and its flowers, leaves and pods are consumed as vegetable and tender roots are preserved in the form of tasty pickles (Anwar *et al.,* 2007). It has high contents of protein and vitamins (Juliani *et al.,* 2009; Quarcoo, 2008; Fuglie, 1999).

Whey is the by-product of cheese, containing nearly half of the total solids present in milk (Walzem *et al.,* 2002). Cheese manufacturing units of Pakistan throw thousands of liters of untreated whey into the drain on daily basis which must be treated before discharge into the environment (Marwaha and Kennedy, 1998). Soft drinks may also be manufactured from whey by making use of different types of stabilizers and fruit concentrates of orange, apple, banana, mango and lemon (Niketic and Marinkovic, 1984). Keeping in view the utilization of whey, the present investigation was aimed to prepare nutritious whey drink by exploiting the massive nutritional potential of *M. oleifera*.

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Materials and Methods

Materials. Dried *M. oleifera* leaves were ground in a laboratory scale grinder (Moulinex), sieved through mesh size 50, stored in amber colour glass bottles, sealed and stored at -40 °C till further usage in this study. All the chemicals used in this study were HPLC grade and obtained from Sigma Chemical Co. (St. Louis MO).

Treatments. *Moringa oleifera* leaf powder (MOLP) was incorporated into whey drink at four different concentrations i.e., 1% MOLP (T_1), 2% MOLP (T_2), 3% MOLP (T_3), 4% MOLP (T_4), and compared with a control (T_0). The formulation of control comprised of banana pulp 5%, sugar 5%, citric acid (20% solution) 0.5%, carageenan 0.25% and whey to make volume up to the mark, colour and flavour 1 mL/L. All the ingredients were dissolved in the whey, pasteurised in the beakers at 65 °C for 30 min, immediately cooled down, filled in clean sanitised pet bottles and stored at 4 °C for further analysis. Each treatment was replicated three times.

Analysis. Fat, pH, acidity, protein, lactose, ash content and total solids were determined by following the respective methods AOAC 7.093, AOAC 7.094 and AOAC 7.095 (AOAC, 2000). Ca, K and Mg were analysed on atomic absorption spectrophotometer (Model: AA240, Varian, Australia) briefly, 5 g sample was taken into a conical flask, 10 mL of concentrated nitric acid was added and contents of the flask were heated for 20 min. After cooling, 5 mL perchloric acid was added and again heated vigorously till the volume was reduced to 2-3 mL followed by cooling. The contents were then diluted in 50 mL volumetric flask by using distilled water. Absorbance of each mineral was determined on the respective wave length, also the unknown concentration determined by constructing calibration curve using 8 standards of each mineral (R^2= 0.9891 to 0.9961). Concentration of iron was determined by using the standard method AOAC 999.11 of AOAC (2000). Vitamin assay was performed on HPLC by following the respective methods AOAC 971.30, AOAC 948.26, and AOAC 975.42 (AOAC, 2000). Free fatty acids and peroxide values were determined by using standard methods cd 8-53 and cd 1-25 of Firestone (1997). For the determination of total phenolic contents of MOLP, 1 mL (methanolic extract of leaf powder) was poured into 11 mL screw capped test tube, 2 mL (0.2-molar) folin ciocalteu, 2 mL sodium carbonate (7.5%) added, mixed and stored in dark for 20 min at ambient temperature and absorbance measured at 765-nm in visible region on a double beam spectrophotometer (Shidmadzu, Japan). The concentration of phenolic compounds was measured on a standard curve constructed by using gallic acid as standard (R^2= 0.9943) by following the method of Anwar *et al.* (2007). The sensory evaluation of whey drink was conducted by a panel of 10 trained judges performed on a 9 point hedonic scale (1- the worst; 9- the best) as prescribed by Larmond (1987). The samples were evaluated for texture, taste, smell and overall acceptability. Samples were presented in glasses, coded with three digit random numbers and all servings were completely randomized. The data of triplicate experiments designed completely randomized (CRD). All data expressed as Mean±SD, the significant difference among the treatments calculated by using Duncan's Multiple Range Test SAS 9.1 Statistical Software.

Results and Discussion

Composition of whey drink supplemented with *M. oleifera* leaves. The results of chemical composition of whey drink show that fat, protein, lactose, ash and total solids content were 0.42%, 0.65%, 4.38%, 0.58% and 6.11%. Addition of MOLP at all the four levels did not have any negative effect on pH and acidity of supplemented whey drink (Table 1).

As the concentration of MOLP increased to 2%, fat content increased significantly ($P<0.05$). The highest fat content 0.64% observed in T_4 which was 52% over the control. Protein content of the supplemented whey drinks increased in a concentration dependent manner. Increase in protein content was 164% in T_4 as compared to the control. The increase in fat and protein content was due to the presence of higher concentration of these constituents in MOLP. Addition of MOLP at all levels increased the ash content of whey drinks significantly ($P<0.05$), T_1 and T_2 comparison with each other ($P>0.05$), increased to 52 and 65% in T_3 and T_4, respectively, as compared to the control. Increasing trend was observed in total solids with increased level of supplementation. The highest total solids recorded in T_4 (16.93%) followed by T_3 (15.84%) and T_2 (14.89%).

Total phenolic contents: Total phenolic content of MOLP was 7.4 g/100 g on dry matter basis (GAE). The total phenolic contents of *Sesamum indicum* cake extract was 1.94% on dry matter basis (Mohdaly *et al.,* 2011). In *M. olifera* concerentration of phenolic compounds was considerably higher than *S. indicum* (Mohdaly *et al.,* 2011) and canola hull (Naczk and Shahidi, 1998).

Minerals content of whey drink supplemented with *M. oleifera* leaves. Calcium content of all the treatments

Table 1. Effect of MOLP supplementation on composition of whey drink

Treatments	pH	Acidity	Fat	Protein	Lactose	Ash	TS
				(%)			
T_0	6.43 ± 0.99^a	0.23 ± 0.10^a	0.42 ± 0.08^c	0.67 ± 0.13^e	4.65 ± 0.21^a	0.60 ± 0.14^c	12.75 ± 0.21^e
T_1	6.37 ± 0.83^a	0.24 ± 0.06^a	0.45 ± 0.16^c	0.94 ± 0.11^d	4.56 ± 0.15^a	0.71 ± 0.22^b	13.80 ± 0.18^d
T_2	6.38 ± 0.66^a	0.24 ± 0.09^a	0.51 ± 0.14^b	1.19 ± 0.34^c	4.39 ± 0.11^b	0.85 ± 0.12^b	14.89 ± 0.35^c
T_3	6.35 ± 0.77^a	0.25 ± 0.14^a	0.55 ± 0.11^b	1.46 ± 0.29^b	4.31 ± 0.08^b	0.91 ± 0.18^a	15.84 ± 0.42^b
T_4	6.38 ± 1.02^a	0.24 ± 0.12^a	0.64 ± 0.19^a	1.73 ± 0.43^a	4.25 ± 0.14^b	0.99 ± 0.24^a	16.93 ± 0.29^a

Means of triplicate experiment; means with same letters in same column are statistically non-significat ($P>0.05$); TS = total solids; T_0 = control without any addition of MOLP**;** T_1 = 1% MOLP; T_2 2% MOLP; T_3 3% MOLP; T_4 4% MOLP increased in a dose dependent manner. The results showed the increasing level of calcium content of fortified whey drink by 10%, 19%, 31% and 47%, respectively, as compared to the control, while the effect on potassium content of the drinks was non-significant (Table 2). Potassium content ranged from 1226 to 1245 mg/L among different treatments and control. The elevation in calcium content of the experimental samples was due to the higher concentration of calcium in MOLP. The addition of MOLP at 4% level increased the iron content of the whey drink by 675% over the control.

The addition of MOLP at 1% level (T_1) increased the iron content from 0.17 mg/L to 44 mg/L which was 257% higher than the control (Aney *et al.,* 2009) while, studying the nutritional value of *M. oleifera* reported that the calcium, magnesium and iron content of 100g dried MOLP was 2000 mg, 1328 mg and 28.2 mg, respectively. As recommendations of FAO, WHO 400 mg and 1200 mg calcium is required on daily basis for the children of 1-3 years age and nursing women. Half of the total calcium requirement for nursing women may be easily and economically fulfilled by consuming just two glasses of 250 mL of whey drink supplemented with 4% MOLP, almost 75% of the calcium requirements of the children can be met by 250 mL of the fortified drink. *M. oleifera* leaves demonstrated higher vitamin A than carrots, more calcium and potassium than milk and banana with superior amino acid profile resembling to egg proteins (Juliani *et al.,* 2009). Milk is not a complete food because of its lower iron and vitamin C contents, fortification of milk with iron is usually practiced in poor and malnourished countries. Most of the strategies involve the usage of sulphates of iron, which are unnatural and accelerate the oxidative breakdown (Fox and McSweeney, 2003). The iron requirement of 10 and 15 mg per day for children and nursing women can be efficiently fulfilled by 250 and 300 mL of supplemented (T_4) whey drink. Feeding *M. oleifera* supplemented diet significantly improved the health status of school going babies and pregnant women in Senegal who gave birth to healthy babies (Juliani *et al.,* 2009).

Table 2. Effect of MOLP supplementation on mineral content of whey drink

Treatments	Calcium	Potassium	Magnesium	Iron
		(mg/100 g)		
T_0	268 ± 0.93^{e}	1245 ± 6.13^{a}	35 ± 0.43^{b}	0.17 ± 0.03^{e}
T_1	297 ± 0.81^{d}	1231 ± 2.48^{a}	38 ± 1.24^{b}	44 ± 0.09^{d}
T_2	320 ± 0.54^{c}	1226 ± 1.05^{a}	42 ± 0.06^{b}	75 ± 0.34^{c}
T_3	353 ± 0.77^{b}	1238 ± 0.78^{a}	45 ± 0.31^{a}	99 ± 0.52^{b}
T_4	394 ± 1.06^{a}	1241 ± 3.16^{a}	48 ± 0.19^{a}	115 ± 0.05^{a}

Means of triplicate experiment; means with same letters in a column are statistically non-significant; refer Table 1 for the detail of treatments.

Feeding 10 g leaves to pregnant women may provide adequate concentration of essential micro nutrients to save them from becoming anemic, physically and mentally weak (Fahey, 2005). This could be the simple, economical and efficient way of preventing and correcting the anemic conditions of millions of children and women of the poor nations who cannot afford medication and expensive nutritional supplements.

Vitamins content of whey drink supplemented with *M. oleifera* leaves. The addition of MOLP at all levels significantly ($P<0.05$) increased the vitamin B_5, B_2, B_6 and vitamin C content (Table 3). The increase in vitamins B_5, B_2, B_6 and C was 158%, 372%, 193% and 50%, respectively, in T_4. The increase in vitamin content was due to the chemical composition of the MOLP which resulted in higher concentrations of these vitamins in the whey drinks. The addition of MOLP did not have any effect on free fatty acids and peroxide value of whey drink at all levels. The recommended daily allowance of vitamin B_2 is 0.8 and 1.8 mg for children

Table 3. Effect of MOLP supplementation on vitamin content and stability of whey drink

Treatments	Vitamin B_5	Vitamin B_2	Vitamin E	Vitamin C	FFA%	PV (meq/kg)
	(mg/L)					
T_0	3.94 ± 0.03^{e}	2.14 ± 0.16^{e}	2.85 ± 0.05^{e}	1.46 ± 0.21^{e}	0.10 ± 0.01^{a}	0.23 ± 0.03^{a}
T_1	5.59 ± 0.14^{d}	4.16 ± 0.24^{d}	3.91 ± 0.18^{d}	1.69 ± 0.19^{d}	0.10 ± 0.01^{a}	0.23 ± 0.01^{a}
T_2	7.12 ± 0.11^{c}	6.06 ± 0.42^{c}	5.02 ± 0.45^{c}	1.83 ± 0.41^{c}	0.11 ± 0.03^{a}	0.25 ± 0.05^{a}
T_3	8.68 ± 0.31^{b}	9.03 ± 0.34^{b}	6.15 ± 0.29^{b}	2.01 ± 0.12^{b}	0.11 ± 0.01^{a}	0.26 ± 0.02^{a}
T_4	10.20 ± 0.43^{a}	10.12 ± 0.66^{a}	8.36 ± 0.45^{a}	2.20 ± 0.67^{a}	0.11 ± 0.02^{a}	0.26 ± 0.09^{a}

Means of triplicate experiment; means with same letters in same column are statistically non-significant; refer Table-1 for the detail of treatments; FFA = free fatty acids; PV = peroxide value.

and nursing women, 300 mL of supplemented whey drink at 2% MOLP can meet 100% body's requirement with respect to this vitamin. 423 and 17 mg/100 g, vitamin B_2 and C were present in Ethiopian *Moringa stenopetala* (Price, 1985). Anwar *et al.* (2007) studied the effect of *M. oleifera* addition on oxidative stability of some vegetable oils and found that blend of soybean and sunflower containing 80% (MOO) has induction time from 1.12 to 5.99 h, 1.47 to 6.22 h, recorded the increase in oxidative stability 435% and 323%, respectively.

Sensory evaluation. The results of sensory evaluation (Table 4) indicated that supplementation of MOLP up to T_2 level did not have any negative effect on texture and taste score. Texture and taste score of T_1 and T_2 were at par with the control (P>0.05). With the increasing increments of MOLP, the score for these two parameters decreased. Some panelists criticised T_3 and T_4 for relatively higher coarse texture. Level of criticism for T_4 was not adverse as the people are already habitual of adding green leaves of coriander and other herbs in the butter milk. Quarcoo (2008) studied the development of *M. oleifera* based beverage and reported that addition of increasing concentration of *M. oleifera* leaf powder tended to make the colour greener and decreased the colour score. The low colour score was attributed to the uncommon green colour of the beverage; the lower concentrations were rated higher for this parameter. This problem could have been resolved by homogenising the supplemented whey drinks at high pressure. Homogenisation is commercially carried out to make the nectars and high fruit preparations homogenous and smooth (Spreer, 2005). Score for smell of all the treatments and control was non-significantly influenced from each other. The overall acceptability score of T_4 was 6.9 out of 9, which was more than 76% of the total score.

Table 4. Effect of MOLP supplementation on sensory characteristics of whey drink

Treatments	Texture	Taste	Smell	Overall acceptability
T_0	7.6±0.11[a]	7.9±0.23[a]	7.5±0.08[a]	7.5±0.03[a]
T_1	7.3±0.08[a]	7.6±0.09[a]	7.4±0.02[a]	7.2±0.14[a]
T_2	7.4±0.14[a]	7.5±0.11[a]	7.5±0.04[a]	7.3±0.05[a]
T_3	7.0±0.03[b]	7.0±0.17[b]	7.2±0.19[a]	7.0±0.09[b]
T_4	6.8±0.06[b]	6.6±0.14[b]	7.2±0.12[a]	6.9±0.04[b]

Means of triplicate experiment; means with same letters in same column are statistically non-significant; refer Table 1 for the detail of treatments.

Conclusion

The addition of *M. oleifera* leaf powder at all concentrations improved the nutritional value of the whey drink. Protein and iron content significantly increased in the supplemented drinks. 300 mL of supplemented whey drink can fulfill 100% requirement of vitamin B_2 of nursing women. Highly nutritious whey drink can be successfully prepared by the addition of MOLP up to 4% level; whey drink supplemented with MOLP should be homogenised for better sensory characteristics.

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