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Fortification of cookies using *Nelumbo nucifera rhizome* (*Lotus stem*) to meet the nutritional requirement of toddlers

Khushboo[1], Sheetal Thakur[2], Davinder Singh[3]
[1] Student, Maharishi Markendeshwar (Deemed to be) University, Mullana (Ambala), Haryana
[2] Professor, Maharishi Markendeshwar (Deemed to be) University, Mullana (Ambala), Haryana
[3] Assistant Professor, Chandigarh University, Mohali, Punjab

Abstract
Micronutrient deficiencies are very common issue in toddlers due to unhealthy eating pattern. Fortification is one of the most promising method to overcome barriers of malnutrition. So, in this study *Nelumbo Nucifera rhizome* (*Lotus stem*) which is a pack of micronutrients, is fortified in cookies so that it can replace the monotonous healthy snacks which toddlers usually avoid eating. Cookies were fortified by dried lotus stem powder by replacing refined flour at proportions *viz*. 0 percent (control), 10 percent, 20 percent and 30 percent. Physical and proximal analysis was done of dried lotus stem powder and cookies. Sensory analysis of cookies was performed by 20 panellists (students and faculty) by using nine point Hedonic scale rating system. Statistical analysis was done in triplicates, using statistical package for the social sciences (SPSS ver. 23) software. Macronutrients showed no significant difference on addition of dried lotus stem powder in cookies expect protein which decreased slightly in Sample-3 (1.43^{a} ±0.05g) when compared to control (1.70^{b} ±0.10g). Significant improvement was observed in micronutrients including calcium, iron and total dietary fiber (TDF) on fortification with dried lotus stem powder. Water absorption power (WAP) increased significantly with increase in concentration of dried lotus stem powder. There is an improvement in calcium, iron and TDF in cookies on addition of dried lotus stem powder when compared to control. Cookies fortified with 30% dried lotus stem powder were found more acceptable as compared to control and other samples. So, cookies can be included in supplementary feeding programmes being run in country to decrease the impact of micronutrient deficiencies in toddlers.

Keywords: lotus stem, cookies, fortification, iron, calcium, fibre

1. Introduction
To meet the nutritional requirement of the population fortification is one of the most magnificent intervention. The most important is to choose the vehicle food which should be either a staple food or consumed widely by the population. Fortification of iron, iodine and zinc were the most cost effective and ensures large benefit: cost ratios [7].
One of the most under-used crop named *Nelumbo nucifera gaertn* rhizome or Lotus stem (also called as Kamal kakdi in local language) was explored in this study. The length of Lotus stem varies from 60-140 cm and diameter 0.5-2.5 cm. Its physical characteristics include – yellowish white to yellowish brown color, smooth surface with longitudinal striations and brown patches along with nodes and internodes [21]. It has antibacterial effect against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *B. pumilis* and *Pseudomonas aeruginosa* and the ethanol extract have hypoglycemic properties [22]. Methanol extract of lotus stem has the diuretic activity at the dose of 300mg/kg, 400mg/kg and 500mg/kg [23].
In yeast-induced pyrexia methanol extract of lotus stem showed anti-pyretic activity in rats [31]. Ethanol extract of lotus stem was hypotheses to be anti-obesity in humans with pre-adipocytes as it supressed the lipid accumulation and attenuate the expression adipogenic transcription factors. There was a considerable reduction in the weights of adipose tissues in high fat fed rats on administration of lotus stem extract [24].

Inadequate weaning practices and starch based diets can lead to a number of nutritional deficiencies like protein-energy deficiency, anaemia, altered gut motility, under development of bones and muscles, ultimately failure to thrive. Early years of the child growth are the most crucial ones, as they lay the foundation of lifelong health. If the nutrition is not restored in early age, then there are chances of comorbities in later stage of life. When the child's diet is shifted from exclusive breast milk to a well-balanced nutritionally adequate diet, then there are a number of challenges that need to be overcome like behaviour problems, rejection to the new foods and so on [27]. By the age of 19-24 months i.e. 1.5years to 2 years, preference of baked products is 62% [10]. Therefore, the objectives of this paper were to study the nutritive value of lotus stem and to enhance mineral and fibre content of the cookies.

2. Materials and Methods
2.1 Raw material preparation
The procurement of raw material was done concomitantly. Fresh lotus stems were purchased from Juggy Veggies (Ludhiana). Rice bran oil (Adani wilmer Ltd, Ahemdabad), milk powder (EveryDay, Nestle India Ltd., Moga, India), ground sugar (Jagraon Co-Op Sugar Mill Ltd., Jagraon, Punjab), salt (Tata Chemicals, Mumbai, India), baking powder (Ajanta Food cookies, New Delhi, India) were used. Lotus stem were washed, peeled and dried at 60°C for 24 hours in hot air oven (Swastik Scientific Company,

Mumbai). Then finally dried Lotus stem was grounded in commercial grinder (Bajaj Electricals, Mumbai) and sifted through 60-mesh sieve. The fine powdered raw material was stored in an air tight container for further use.

2.2 Batter and cookies preparation

Cookies' dough was prepared by using AACC method [04] and the proportion of ingredients is given in Table 1. Fat and sugar was creamed by mixing manually. Dextrose containing sodium chloride was added to the resulting cream, and mixed for 5 min to obtain a homogeneous mixture. Finally, flour containing various proportion of lotus stem powder, which had been sieved twice with sodium bicarbonate was added and mixed. The required amount of water is added to obtain the desired texture and kept in freezer for 5-10 minutes. Lastly the dough was divided into 25g each and rolled to make the shape of cookies, added to the pre greased cookie moulds and baked for 10 minutes at 400ºF. Four batches of cookies were prepared i.e. fortified with 0% (Control), 10 %, 20 % and 30 % of lotus stem. Total weight of the dough was 200g and 8 cookies were made from each dough. Finally, the cookies were cooled on the cooling trays after taking them out from the moulds.

Table 1: Preparation of cookies with the variation of refined flour and lotus stem

Ingredients	Control	Sample-1	Sample-2	Sample-3
Refined flour (g)	100	90	80	70
Lotus stem (g)	0	10	20	30
Sugar (g)	30	30	30	30
Salt (g)	1	1	1	1
Sodium Bicarbonate (g)	1	1	1	1
Rice bran oil (g)	28	28	28	28
Dextrose (ml)	13.80	13.80	13.80	13.80

2.3 Physical characteristics of cookies

After 24 hours of baking time, cookies were analysed for their physical attributes. AACC (1995) method: 10-50D (200) was used to determine cookies width and thickness. Cookie width (W) was measured by placing 6 cookies edge-to-edge to get the average width in millimetres by using vernier calliper. Cookie thickness (T) was measured by calculating the average of 6 cookies, by stacking 6 cookies on top of each other and measuring it by using vernier calliper. Volume was determined by Millet-seed displacement method and was expressed as specific volume (mL/g). Water Absorption Power (WAP) was determined with the method given by Gill B S *et al.* [15].

2.4 Proximal analysis of cookies

Fat content of cookies was calculated by Soxhlet method (AOAC 934.01), amount of protein by Kjeldahl method (AOAC 988.05), Ash percent by dry ashing method (AOAC 900.02A) and calcium and iron by using spectrophotometer [05]. Total Dietary Fiber (TDF) of cookies was determined and reported as g/100 g dry weight (dw) of the sample, using total dietary fibre assay kit following AOAC Method 991.43 [05]. Moisture content was analysed by oven drying method [01]. The data obtained was in triplicates. Carbohydrates were calculated using the formula [30].

$$Carbohydrates\ (\%) = 100 - [Moisture\ (\%) + Protien\ (\%) + Lipid\ (\%) + Mineral\ (\%)]$$

Total calories were estimated by converting the major nutrients with their coefficient into energy and then finally adding up them to get the total calories.

2.5 Sensory analysis of the cookies

The sensory analysis of cookies were carried out according to the methodology suggested by Peryam [26]. The selection of 20 untrained panellists including students and staff members of department, who evaluated cookies on the basis of color, texture, taste, appearance and overall acceptability using 9-point hedonic scale. Water was served to rinse the mouth in between the tasting and sample were served in testing area at room temperature (25°C).

2.6 Statistical Analysis

Data were analysed statistically, in triplicates, using statistical package for the social sciences (SPSS ver. 23) software [13]. Data subjected to analysis of variance and means were compared by Duncan Multiple Range Test (DMRT) and small sample t test, respectively. Kruskal Wallis test was applied to test significance difference between samples on the basis of sensory scores. Further, Mann Whitney test was used to proform pair wise comparision of the samples.

3. Results

3.1 Proximal analysis of the raw material (dried lotus stem powder)

AACC (1935) and AOAC 985.25 were used to analyse dried lotus stem powder for nutritive attributes. Sample readings were compared to the standard nutritive values [16] of the dried lotus stem (Table 2). The moisture content of the sample was on significantly higher side than of standard ($p \leq 0.01$). Total ash was 10g/100g of the sample. There was a significant ($p \leq 0.01$) difference in carbohydrate of the sample by 7g/100g whereas total protein provided by the sample was significantly ($p \leq 0.01$) higher by 4g/100g than of standard. Therefore, total calories provided by the sample was approximately 17kcal per 100g less than standard. Fat content variation was non-significant with standard value i.e. 1.3 g/100g. The sample of dried lotus stem powder per 100g provided 217Kcal as compared to standard (234Kcal). Calcium and iron content were also comparable but non-significant variations was observed. Crude fibre was noticeably higher in sample by 2g/100g compared to the standard. Thus, sample obtained was appropriate for fortification as it contain adequate amount of micro and macro nutrients [11].

Table 2: Comparison of the standard nutritive values of lotus stem with that of sample

Nutrients	ICMR Nutritive value Tables	Sample tested	t value
Energy (Kcal)	234.00	217.50 ± 2.50	11.50**
Proteins (g)	4.10	8.20 ± 0.26	27.24**
Carbohydrates (g)	51.40	43.70 ± 0.68	19.61**
Fats (g)	1.30	1.10 ± 0.10	3.46
Moisture (g)	9.50	10.00 ± 0.02	41.56**
Calcium (mg)	405.00	400.00 ± 2.95	2.93
Iron (mg)	60.60	61.00 ± 0.94	0.73
Crude fiber (g)	25.00	27.00 ± 1.84	1.88

*Significant at 5 % level of significance
**Significant at 1 % level of significance
Data are presented as mean ± standard deviation, in triplicates

3.2 Physical analysis of the cookies

The physical analysis of the cookies was done which included Bulk Density (BD), specific volume, height, diameter, weight and WAP. Control and all the 3 samples showed similar results for all the physical properties other than WAP. BD of the cookies was observed to be 0.50g/ cm^3. Specific volume of cookies was calculated as 1.125 m^3/kg. Average height and diameter of cookie was 0.456 inches and 1.77 inches, respectively. Weight of cookie was calculated as 20g. As shown in table 3, there is a direct proportion of WAP and dried lotus stem powder concentration in cookies. The WAP of control was 130^a ± 2.00 percent with zero concentration of dried lotus stem powder. Though in sample 3 (30%), WAP was 170^d ± 3.49 percent.

Table 3: WAP of the samples of the cookies

Samples	WAP (%)	t value
Control (0 %)	130^a ± 2.00	NA
Sample 1 (10 %)	147^b ± 2.26	13.02**
Sample 2 (20 %)	160^c ± 2.72	19.10**
Sample 3 (30 %)	170^d ± 3.49	19.85**

*Significant at 5 percent level of significance
**Significant at 1 percent level of significance
Data are presented as mean ± standard deviation

3.3 Proximal analysis of cookies

Proximal analysis of cookies was done which is summarized in Table 4. Duncan Multiple Range Test (DMRT) was administrated for pair wise comparison of samples, where superscript "a" represents smaller value of means. Same protein content was recorded in control (1.70g) and Sample-1 (1.6g) which was significantly more than Sample-2 & 3 (1.44g & 1.43g respectively). No significant difference was observed in carbohydrate and fat content of cookies across different levels of dried lotus stem powder concentration. Thus, difference among samples on the basis of total calories provided by the cookies remained non-significant. It can be observed (Table 4) that moisture content was inversely related to the dried lotus stem powder. Moisture content of control and Sample 3 was significantly different (2.41% and 1.96% respectively) with each other due to the difference in the TDF. Further a direct proportional relational was found between dried lotus stem powder and calcium, iron and TDF of the cookies. Calcium content of control was significantly low (14.01mg) as of Sample-3 (29.07mg). A negligible amount of iron was recorded in control (0.04mg) but noted significantly high in Sample-3 (3.04mg). Similar trend was observed in TDF as control contained negligible amount (0.01gm) but Sample-3 provided 1.03g of TDF per cookie ($p \leq 0.05$). The ash content was significantly low (1.00%) in control as compared to Sample-3 (1.14%).

Table 4: Proximal analysis of cookies (nutrients provided by 2 cookies)

Parameter	Control (0%)	Sample-1 (10%)	Sample-2 (20%)	Sample-3 (30%)
Energy (Kcal)	101.40^a ±2.23	101.53^a ±2.25	100.77^a ±1.17	99.13^a ±0.96
Protein (g)	1.70^b ±0.10	1.60^b ±0.08	1.44^a ±0.05	1.43^a ±0.05
Carbohydrate (g)	16.33^a ±0.30	15.92^a ±0.38	15.90^a ±0.49	15.55^a ±0.64
Fat (g)	3.61^a ±0.35	3.50^a ±0.16	3.50^a ±0.11	3.50^a ±0.13
Moisture (%)	2.41^c ±0.15	2.36^{bc} ±0.10	2.12^{ab} ±0.21	1.96^a ±0.05
Ash (%)	1.00^a ±0.10	1.06^{ab} ±0.01	1.12^b ±0.02	1.14^b ±0.01
Calcium (mg)	14.01^a ±0.50	20.00^b ±0.53	21.00^b ±1.00	29.07^c ±0.57
Iron (mg)	0.04^a ±0.07	1.00^b ±0.10	1.90^c ±0.10	3.04^d ±0.07
TDF (g)	0.01^a ±0.01	0.51^b ±0.05	0.74^c ±0.02	1.03^d ±0.17

Values with similar superscripts in a row do not differ significantly ($p < 0.05$).
Data are presented as mean ± standard deviation, in triplicates

3.4 Sensory Evaluation

To analyse the significance difference of the sensory score given to the samples, Kruskal Wallis test was applied. To perform further pair wise comparison of the samples, Mann Whitney test was used (Table-5). The attributes include color, texture, flavour, mouth feel and overall acceptability, on the basis of which sensory analysis of cookies was performed. All these attributes differ significantly ($p \leq 0.05$) among all the samples. Pair wise comparison of cookies showed no significant difference between control and Sample-1 i.e. control and Sample-1 were scored similar for all the sensory attributes. Sample-1 and Sample-3 were also scored same on different attributes of sensory evaluation. Sample 2 and control were significantly different for all the sensory attributes *viz.* colour, texture, mouth feel, appearance, taste and overall acceptability. Similarly, significant difference was observed in Control and Sample-3 on texture, mouthfeel and overall acceptability of cookies,

whereas, colour, appearance and taste were scored same. Sample-1 and Sample-2 differs significantly in terms of colour, texture, appearance, taste and overall acceptability but similar in terms of mouthfeel. Sample-2 and Sample-3 were same for texture and mouthfeel but significantly different for colour, appearance, taste and overall acceptability.

Table 5: Average of sensory analysis of cookies

Sample	Colour	Texture	Mouthfeel	Appearance	Taste	Over All
Control	6.75±1.02	6.80±0.95	6.75±0.91	6.65±1.14	7.05±0.94	6.8±0.78
Sample-1	7.00±1.17	6.93±1.1	7.13±1	7.00±0.86	7.45±1.19	7.1±0.87
Sample-2	7.85±0.81	7.78±1.34	7.45±1.28	7.58±0.85	8.38±0.79	7.81±0.83
Sample-3	7.25±0.85	7.60±0.88	7.43±0.49	7.05±0.69	7.55±0.78	7.38±0.57
Chi-Square	12.45**	12.44**	9.38*	8.45*	20.22**	18.33**
Z value[#]						
Control and Sample-1	0.78	0.24	1.24	0.76	1.31	1.37
Control and Sample-2	3.31**	2.84**	2.61**	2.66**	4.27**	3.78**
Control and Sample-3	1.44	2.52*	2.51*	1.01	1.6	2.53*
Sample-1 and Sample-2	2.40*	2.38*	1.45	1.96*	2.72**	2.84**
Sample-1 and Sample-3	0.49	1.94	1.05	0.18	0.02	0.70
Sample-2 and Sample-3	2.29*	0.99	0.97	2.04*	3.17**	2.33*

Data are presented as mean ± standard deviation, in triplicates
[#]Pair wise comparison using Mann-Whitney test
*Significant at 5 % level of significance
**Significant at 1 % level of significance

4. Discussion

The method of addition of micronutrients in the porcessed food is fortificaiton. This is one of the most effective intervention to decrease the micronutrient deficiency among the population. The edible component which is to be added to the food to increase the nutritional value of the food is fortificant nd it should be locally available. Food vehicle is the food product in which fortificant is added. Numerous benefits of fortification makes it apropriate to be used in cost effective public health interventions. Proximal analysis reveled the dried lotus stem powder was high in iron, calcium and total dietary fiber. Dried lotus stem powder as a fortificant is an apropriate approach as lotus stem is easily available vegetable. Cookies were selected to be the food vehicle as it can be processed centrally and also support the bakery industry [2]. Moreover the target group was toddlers who lacks the innmate ability in chosing a balanced diet and are fussy eaters. Healthy snacks are very important to curb the micronutrient deficiencies [3].

Geology, soil, climate and temperature are the factors on which the physiological properties of crop depends which is the reason the sample of dried lotus stem differs nutritionally from the standard. Lotus stem is effective treatment for deworming, fever and vomiting [25]. Difference observed can be due to different growing climate, temperature, soil and farming techniques adopted for lotus cultivation [17]. In this study, dried lotus stem powder was prepared and fortified to the cookies. There is lack of consumption of fruits and vegetables among the toddlers which leads to the micronutrient deficiencies. The consumption pattern revealed toddlers are inclined towards sweeten things rather than cereals. So, cookies were a perfect replacer to add the nutrition in diets and which can be easily consumed by toddlers [8].

With the addition of dried lotus stem powder, there was no change in the physical attributes except WAP. Height, specific volume, bulk density, diameter and weight did not differ significantly with the incorporation of dried lotus stem powder. Similarly, Aramouni and Khouryieh [6] incorporated flaxseed flour in cookies to study physical and sensory characteristics and there was no significant difference observed in the physical characteristics except texture and spread ratio. There is a direct proportion of WAP and dried lotus stem powder concentration in cookies. The WAP of control was 130^{a}± 2.00 percent with zero concentration of dried lotus stem powder. Though in sample 3 (30%), WAP was 170^{d} ± 3.49 percent. This increase in WAP is due to the TDF present on dried lotus stem powder. Sudha [28] testified this fact by developing cookies incorporated bran blend of wheat, rice, oats and barley by replacing wheat flour and found that WAP increased proportionally with concentration of the bran blend.

Total carbohydrate and fat content does not differ significantly with the incorporation of dried lotus stem powder whereas protein content of control was 1.70^{b} ±0.10g and protein content of Sample-3 was 1.43^{a} ±0.05g. Collectively, total calories provided by the cookies did not change significantly with the addition of dried lotus stem powder. Calcium and iron were increased with increase in concentration of dried lotus stem powder. This result was supported by Ibrahium and Hegazy [18], who incorporated germinated fenugreek seed flour by replacing wheat flour in cookies and evaluated the cookies for iron bioavailability and organoleptic properties. On analysing chemically, it was found that Ca, Fe and Zn contents were significantly elevated (p<0.05) on increasing the fenugreek seed flour concentration in biscuits as compared to the control which were wheat cookies. Fiber content of the cookies also noticeably increased with the incorporation of the dried lotus stem powder. The control cookie contained no fiber at all whereas Sample 3 with 30 % concentration of lotus stem powder had 1g of crude fiber in each cookie [09]. Also, Kohajdováa and Karovičováa [19] developed cookies by substituting wheat flour with apple fiber powder and studied its physical and sensory characteristics. It was observed that the odour became fruity and the taste became sweeter on the addition of fiber. The fiber content of sample increased which showed nutritional benefits.

Moisture content decreased significantly with the increase in dried lotus stem concentration. So, moisture content of the sample is inversely proportional to the concentration of dried lotus stem which could be due to the increase in fiber

content of the cookie with the incorporation of dried lotus stem powder. With the increase in the mineral content of the cookies on incorporation of dried lotus stem powder, the ash content was also increased [29]. Ash content increases with increase in concentration of dried lotus stem powder because total mineral and TDF content was increased with incorporation of dried lotus stem powder in cookies [12]. Sensory analysis showed that Sample-2 and Sample-3 were significantly different in attributes colour, appearance, taste and overall acceptability but texture and mouthfeel remained constant. Moudgil and Barak [20] fortified cookies with partially hydrolysed guar gum with the aim to investigate their physical and organoleptic characteristics. The overall sensory acceptability was first increased but after increasing the concentration above 2 %, the hardness increased significantly. They concluded that cookies can be incorporated with partially hydrolysed guar gum at 2 % concentration, with sensory acceptability and fiber addition.

5. Conclusion

Toddler is a phase where the foundation of good health is laid, but due to their shift in normal diet from supplementary foods and breast milk, they tend to eat less and are picky eater due to which they are prone to micronutrient deficiencies. Fortification is one of the preferred approaches to combat micronutrient deficiencies, so present study has fortified *Nelumbo Nucifera Rhizome* (Lotus Stem) in cookies as it is rich in iron, calcium and TDF. *Nelumbo Nucifera Rhizome* when fortified in cookies calcium, iron and fiber content increases with increase in the percentage of the dried lotus stem powder. WAP of the samples increases with increase in dried lotus stem powder due to absorptive property of TDF. Sample-2 (20%) was preferred likely when analysed on the basis of sensory attributes and Sample-3 was similar in texture and mouthfeel with Sample-2. But on analysing proximally, Sample-3 was the most nutritious as it contained 29.07^{c} ±0.57mg calcium, 3.04^{d} ±0.07mg iron and 1.03^{d} ±0.17mg TDF. So, cookies with 30% of the lotus stem is most appropriate with the nutrition point of view and also acceptable in desired sensory attributes for toddlers. These developed cookies can be used in as supplementary feeding programmes for iron, calcium and fibre supplementation.

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