paths from the ground level $|1\rangle$ to the excited level $|3\rangle$ (involving, for example, the direct transition amplitude $|1\rangle \rightarrow |3\rangle$ and the alternative transition amplitudes $|1\rangle \rightarrow |3\rangle \rightarrow |2\rangle \rightarrow |3\rangle$… and so on in higher-order virtual cycles). The injected microwave noise connecting level $|2\rangle$ to level $|1\rangle$ can, however, cause the electron to make a real transition $|2\rangle \rightarrow |1\rangle$, and thus take it out of the above coherent (virtual) cycles. Such a noise-induced real transition necessarily causes dephasing of the otherwise coherent interference of the virtual alternatives, thereby degrading the EIT. This effect can be seen in Figure 7. Our calculation explicitly takes into account this effect of the injected classical noise within the Lindblad formalism, using the Novikov theorem.

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ACKNOWLEDGEMENTS. We thank Andal Narayanan for fruitful discussions. R.V. thanks the Raman Research Institute, Bangalore for support during the course of this work.

Received 12 May 2011; revised accepted 16 August 2011

Development of non-dairy, calcium-rich vegetarian food products to improve calcium intake in vegetarian youth

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Vegetarians are prone to high risk of mineral deficiencies. The aim of the present study was to examine dietary habits of vegetarian children and adolescents for calcium adequacy and devise recipes to improve their calcium intake. In a cross-sectional survey in 236 Indian school children (89 boys) aged 7–19 years, diet was assessed by 24-h recall on three random days. Using plant foods, i.e. finger millet, soybean, leafy vegetables and sesame seeds, 14 non-dairy-based, calcium-rich products (NDBCRP) and 12 dairy-based calcium-rich products (DBCRP) were developed. Calcium content of all products was analysed using atomic absorption spectrophotometer. Mean calcium content per 100 g cooked weight of NDBCRP (337.5 ± 107.4 mg) and DBCRP (259 ± 88 mg) was similar (P = 0.12). Calcium intake was found to be low in boys (507 ± 267 mg/day) and girls (421 ± 184 mg/day), which can be enhanced by NDBCRP supplement. Thus NDBCRP products have the potential to alleviate calcium deficiency in Indian adolescents.

Keywords: Calcium intake, dietary habits, non-diary products, vegetarians.

APPROXIMATELY 99% of total body calcium is found in the skeleton[1]. Bone mass accrual continues from infancy through to early adulthood until peak bone mass is achieved by the second decade of life[2]. Therefore, adequate calcium intake is important for bone health throughout the lifespan and for the prevention of osteoporotic fractures in later years.

Calcium intake of children and adolescents in Asia, especially in India, is relatively low in comparison to their Western counterparts[3]. Various studies from India report low calcium intake and hypocalcaemia among young boys and girls, emphasizing the importance of increasing calcium intake in children and adolescents[4–7]. This could be partly attributed to the non-milk-based diets, poor dietary habits, inadequate information and knowledge about calcium-rich food and poor calcium absorption from plant foods[6,8]. Thus, there is a need to analyse the dietary intake and food choices of Indian children and adolescents to improve their calcium intake.

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Milk and milk products are a rich source of calcium in the diet. However, intake of milk and milk products in majority of Indian children and adolescents is reported to be meagre[9,10], whereas plant food like cereals, pulses and green leafy vegetables contributes to about 36–50% of daily calcium intake[6,11]. Non-dairy-based food such as calcium-fortified soy milk[12], kale and broccoli[13] has been identified as a good source of calcium in *in vivo* studies. Indigenous Indian food rich in calcium such as millets, nuts and oil seeds, and green leafy vegetables are yet to be identified and tested for their potential in calcium enrichment of daily diet of youth. This information will certainly increase the choice of calcium-rich food in Asian diets. It is therefore necessary to utilize food-based approaches and interventional strategies to promote bone mass and prevent osteoporosis in Asia.

Plant foods like cereals, green leafy vegetables and nuts contain phytins and oxalates[14,15] that bind with calcium forming insoluble salts and decrease calcium absorption. Food-processing methods like roasting and malting are shown to enhance calcium extractability from wheat, barley and green-gram preparations[16]. Malt pretreatment of sorghum flour has been shown to decrease phytin content of the flour and increase calcium extractability[17]. Weaver *et al.*[18] have shown that calcium absorption from leavened wheat flour product is more than that from unleavened products. Thus, using food-processing methods, absorption of calcium from plant foods can be enhanced.

For achieving optimum bone mass at skeletal maturity and for prevention of osteoporotic fractures, Indian recommended dietary allowance (RDA) for calcium intake has recently been revised[19]. However, strategies to meet these recommendations need to be devised taking into account dietary habits of the Indian population. Therefore, the objective of this study was to: (i) assess dietary habits and calcium intake in children and adolescents, and (ii) develop non-dairy-based plant food products with high calcium content and use processes like malting and leavening for increasing calcium absorption. The acceptability of these products was also tested to judge their potential for adopting them in dietary practice.

A cross-sectional survey was conducted from November 2008 to February 2009 encompassing students from private schools and colleges in affluent areas of Pune city, India (i.e. areas without slum clusters and low-income housing schemes, but including areas with high land prices as published by Government agencies (Ministry of Urban Development, Lands Division))[20], who voluntarily participated in the study. In all, 236 apparently healthy children and adolescents (89 boys) aged 7–19 yrs were assessed for their age, weight, height and diet. Ethical approval was granted by the ethics committee of Hirabai Cowasji Jehangir Medical Research Institute and Jehangir Clinical Development Centre, Pune. A letter of information was given to all participants in the study, and an informed written consent from parents and assent from children were obtained.

All measurements were recorded in the morning between 8 and 10 am for all subjects. Standing height was measured to the nearest 1 mm using a stadiometer (Leicester Height Meter, Child Growth Foundation, UK, range 60–207 cm). Weight was measured using an electronic weight scale to the accuracy of 100 g (Salter). Height and weight for age *Z* scores were calculated using Indian reference charts[21].

Dietary intake was assessed by 24-h recall on three random days (non-consecutive) of a week, including Sunday. Each child was asked about the intake of food items during the day at breakfast, lunch, dinner and snacks, using standard cups and spoons by trained investigators through a face-to-face interview. The recipes of food items were also recorded. The portion size was obtained by the average of actual weights of one serving of each food item from their households. This was done for each of the food items consumed such as rice, vegetables, chapatti (unleavened wheat pancake), etc. Daily nutrient intake was calculated by applying nutritive values of cooked foods[22]. For the consumption of raw food such as fruits and salads, the nutritive value tables of the National Institute of Nutrition (NIN), India, were applied[23]. Percentage contribution of calcium from milk was calculated separately.

From the cohort of study subjects, 51% of the total food intake was in the form of cereals and pulses (Figure 1). Thirty-six per cent boys and girls had no intake of milk (including milk from tea) in their diet and 86.2% were not consuming any milk products such as curd or cheese. Mean milk intake in boys was 45% of RDA (500 m/day), which was significantly more than in girls (30%; $P < 0.05$)[24]. Therefore, the main source of dietary calcium in these children and adolescents was plant foods. Thus, based on the dietary food habits, to improve

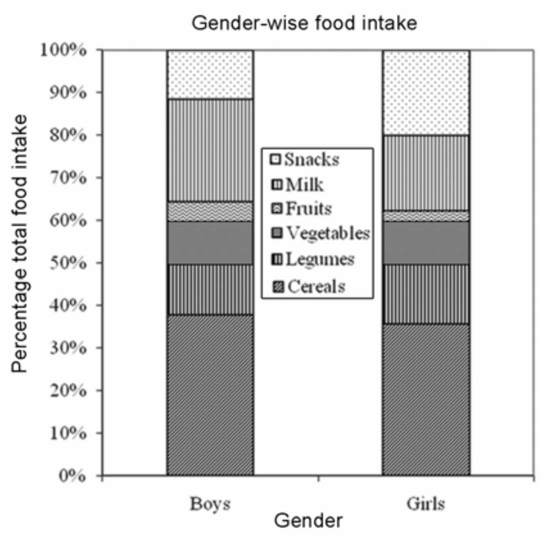

Figure 1. Gender-wise percentage distribution of food.

calcium intake in the diets of children and adolescents, non-dairy-based, calcium-rich products (NDBCRP) were developed with emphasis on: (i) selection of commonly available calcium-rich food from the nutritive value tables[23]; (ii) food-processing methods that would enhance calcium absorption, like malting and leavening; (iii) comparison with dairy-based, calcium-rich products (DBCRP) to ensure high calcium content, and (iv) acceptability of the developed food products.

A list of commonly consumed 70 non-dairy food items in the study group as also from the Indian adolescent population at large was prepared. With the help of nutrient composition tables[23], calcium content in these food items was calculated. It ranged from 40 to 1450 mg of calcium per 100 g raw weight. The NDBCRP were devised using food items from the list. Calcium-rich food items like finger millet flour (*Elusine coracana*), soybean (*Glycine max*), sesame seeds (*Sesamum indicum*) (with hull), dry coconut (*Cocus nucifera*), poppy seeds (*Papaner somniferum*), dried cauliflower leaves (*Brassica oleracea* var. *botrytis*), curry leaves (*Murraya koenigii*), cumin seeds (Cuminum *cyminum*), niger seeds (Guizotia *abyssinica*) and garden cress seeds (*Lepidium sativum*) were selected to develop the recipes. Food-processing methods like malting and leavening were used to enhance calcium absorption. For malting, whole finger millet was soaked overnight in water for 8 h. It was allowed to germinate for two days. The germinated finger millet was then sun-dried for one day. The dried roots were then broken manually and the millet was milled into fine flour for use. Leavening processes like overnight fermentation, incorporation of yeast and steam were used.

Twelve DBCRP commonly consumed in India were selected, such that except for dairy products other major ingredients were similar in both the groups. DBCRP were analysed for their calcium content for comparison with NDBCRP.

All the 26 cooked food products were homogenized and analysed in duplicate for nutrient content in the laboratory using various techniques described in NIN manual[25]. Protein content of the food samples was estimated using the micro Kjeldahl method and fat content by Soxhlet extraction. Ash was estimated by igniting the food sample in a muffle furnace at 550°C. The calorific value of food products was estimated using proximate principles and Atwater's factors. The ash was dissolved in 6 N 1 : 1 hydrochloric acid and calcium concentration was analysed using atomic absorption spectrophotometry[25]. Oxalate, phytin and fibre content was estimated using the nutritive value table for raw foods[23], after adjusting for moisture content of the cooked product.

Sensory evaluation was carried out for acceptability of the newly developed NDBCRP. The panel consisted of 10 members for sensory evaluation. The panel members were isolated from each other. Samples were presented in plastic dishes or bowls coded with random numbers and served in a randomized order to all the panel members. The samples were served at room temperature and analyses were performed under normal lightening conditions. One sample was provided at a time at an interval of 5 min. The panel members rinsed their palates with water before and between tasting. A nine-point hedonic scale for acceptance (with tick-boxes 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely) was used for the independent hedonic rating of appearance, texture, taste and overall acceptability of each sample[26]. Evaluation was performed at the Hirabai Cowasji Jehangir Medical Research Institute under satisfactory conditions.

Difference in mean calcium content of DBCRP and NDBCRP was tested using Student's *t*-test. *P* value <0.05 was considered to be statistically significant. Calcium density of the diet/products was calculated using the formula

$$\text{Calcium content per 1000 calories} = \frac{1000 \times \text{calcium content of the recipes}}{\text{caloric content of the recipes}}.$$

Table 1 describes the anthropometric parameters and nutrient intake in boys and girls. Height and weight for age *Z* scores were within 2 SD for both boys and girls, indicating that the children were apparently healthy for their age. Energy, protein, calcium and phosphorus intake was significantly more in boys than in girls ($P < 0.01$). Mean calcium intake was 63% and 53% of the Indian RDA for boys and girls respectively (800 mg/day). Seventy-seven boys (87%) and 139 girls (95%) had calcium intake less than the RDA (800 mg/day)[19]. However, calcium density and calcium-to-phosphorus ratio was not significantly different in the two groups ($P > 0.1$). Calcium intake from milk was 46% and 35% of the total calcium intake in boys and girls respectively.

Table 2 describes the composition of DBCRP and NDBCRP. Fourteen NDBCRP (NDBCRP 1–NDBCRP 14) were matched with 12 DBCRP (DBCRP1–DBCRP12). Finger millet, soybean and sesame seeds were the main source of calcium in NDBCRP. All the DBCRP had either buffalo milk or cottage cheese made from buffalo milk. Malting and leavening were used as processing methods for five products. Overnight fermentation was used as a method of leavening for making finger millet pancake (dosa). Yeast was used as the method of leavening for making all three types of cauliflower bread. Overnight fermentation and steam was used as a method of leavening for finger millet dhokla with cauliflower leaves. Malting was used as a processing method for making finger millet-wheat pancake (thalipeeth), finger millet–cumin biscuits and mix flour–sesame ladoo. Six products

Table 1. Anthropometric and dietary intake in Indian boys and girls

	Boys (n = 89)	Girls (n = 147)
Age (yrs)	16.3 ± 3.8	15.7 ± 3.9
Weight (kg)	48.0 ± 13.4*	43.8 ± 11.4
Weight for age Z score	–0.4 ± 1.0	–0.3 ± 1.2
Height (cm)	158.3 ± 15.5*	151.2 ± 13.3
Height for age Z score	–0.6 ± 0.9	–0.5 ± 1.0
Energy (kcal/day)	1932 ± 545*	1651 ± 488
Protein (g/day)	45 ± 13*	38 ± 12
Calcium (mg/day)	507 ± 267*	421 ± 184
Calcium density (mg/1000 kcal)	267 ± 129	263 ± 113
Phosphorus (mg/day)	1020 ± 281*	834 ± 259
Calcium : phosphorus ratio	0.50 ± 0.2	0.51 ± 0.2

Data represented as mean ± SD. $P < 0.01$ for comparison between boys and girls.

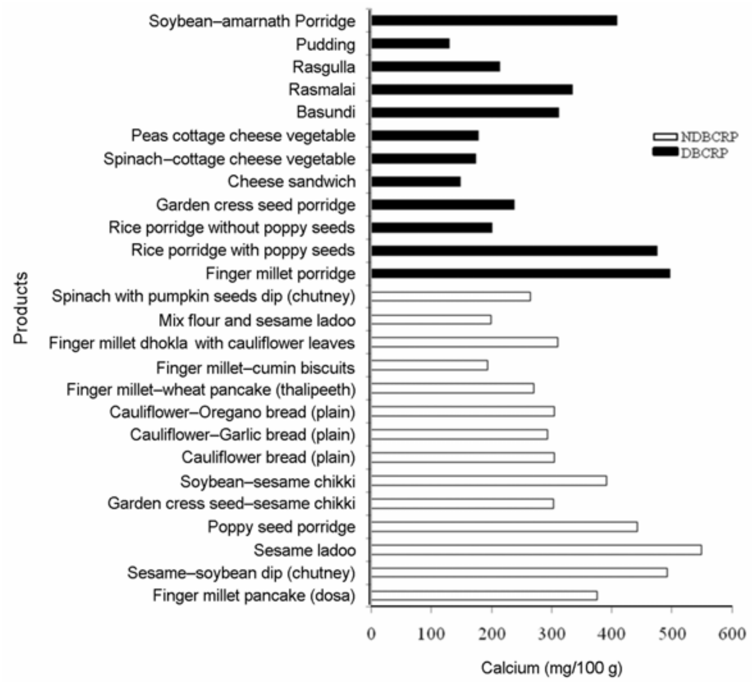

Figure 2. Calcium content of NDBCRP and DBCRP (mg/100 g).

which were high in calcium, namely ladoos, biscuits and dips (chutneys), were prepared using traditional cooking methods like roasting and grinding.

As shown in Figure 2, in the NDBCRP group, sesame ladoo was the richest source of calcium (549.1 mg/100 g) followed by sesame–soybean dip (chutney) (491.5 mg/100 g) and poppy seed porridge (442.2 mg/100 g). Finger millet–cumin biscuits (193.2 mg/100 g) and mix flour–sesame ladoo (199.7 mg/100 g) were the lowest sources of calcium in NDBCRP group. In DBCRP group, finger millet porridge (496.6 mg/100 g), rice porridge with poppy seeds (475.5 mg/100 g) and soybean–amaranth porridge (407.7 mg/100 g) were the richest sources of calcium, whereas spinach–cottage cheese vegetable (173.2 mg/100 g) and peas–cottage cheese vegetable (177.8 mg/100) were the lowest sources of calcium.

Table 3 represents the average nutrient composition of NDBCRP and DBCRP. The mean calcium content of NDBCRP was 337.5 ± 107.4 mg, whereas that of DBCRP was 274.3 ± 127.8 mg/100 g cooked weight. There was no significant difference in calcium content of the two groups (P = 0.12). However, there was significant difference in the energy, protein, fat, oxalate, phytin and fibre content of NDBCRP and DBCRP. Nevertheless, even after adjusting for calorie content, there was no significant difference in the calcium content of the two groups (P = 0.547).

Figure 3 shows the mean sensory scores of NDBCRP. Ten out of fourteen products were well accepted by the panel members. Finger millet pancake (dosa) and sesame–soybean dip (chutney) was the most acceptable product. Seventy per cent of the panel member reported that they ‘liked extremely’ the finger millet pancake (dosa) with sesame–soybean dip (chutney). Finger millet–wheat pancake (thalipeeth) was the second most liked product, with 50% of the panel members reporting that

Table 2. Food composition of non-dairy based, calcium-rich products (NDBCRP) and dairy-based, calcium-rich products (DBCRP)

Code	Recipe	Description of recipe (ingredients in grams)
NDBCRP1	Finger millet pancake (dosa)	Leavened pancake made from finger millet flour (28), soybean flour (10), sesame seeds (10), cumin seeds (2) and vegetable oil (2). Process used: malting and leavening.
NDBCRP2	Sesame–soybean dip (chutney)	Dip made from fresh coconut (50), roasted soybean (25), sesame seeds (25), cumin seeds (8) and curry leaves (5).
NDBCRP3	Sesame ladoo	Sweet balls made from sesame seeds (20), niger seeds (20), refined palm sugar (20) and clarified butter (2).
NDBCRP4	Poppy seed porridge	Sweet made from poppy seeds (100), rice (30), refined palm sugar (100) and fresh coconut (50).
NDBCRP5	Garden cress seed–sesame chikki	Sweet made from garden cress seeds (10), sesame seeds (5), dried coconut (10), refined palm sugar (25) and clarified butter (2).
NDBCRP6	Soybean–sesame chikki	Sweet made from soybean (15), sesame seeds (10), refined palm sugar (25) and clarified butter (2).
NDBCRP7	Cauliflower bread (plain)	Bread made from finger millet flour (10), soybean flour (10), refined flour (10), dried cauliflower leaves (10), sesame seeds (5) and clarified butter (5). Process used: malting and leavening.
NDBCRP8	Cauliflower–garlic bread (plain)	Bread made from finger millet flour (10), soybean flour (10), refined flour (10), dried cauliflower leaves (10), sesame seeds (5), garlic (10) and clarified butter (5). Process used: malting and leavening.
NDBCRP9	Cauliflower–oregano bread (plain)	Bread made from finger millet flour (10), soybean flour (10), refined flour (10), dried cauliflower leaves (10), sesame seeds (5), oregano (5) and clarified butter (5). Process used: malting and leavening.
NDBCRP10	Finger millet–wheat pancake (thalipeeth)	Unleavened pancake made from finger millet flour (36), wheat flour (8), jowar flour (*Sorghum vulgare*) (7), bengal-gram flour (7), radish leaves (15) and vegetable oil (5). Process used: malting.
NDBCRP11	Finger millet–cumin biscuits	Biscuits made from finger millet flour (30), refined flour (10), cumin seeds (5) and clarified butter (25). Process used: malting and leavening.
NDBCRP12	Finger millet dhokla with cauliflower leaves	Leavened pancake made from finger millet flour (25), soybean flour (10), cauliflower leaves (5) and sesame seeds (5). Process used: malting and leavening.
NDBCRP13	Mix flour and sesame ladoo	Sweet balls made from finger millet flour (10), soybean flour (10), bengal-gram flour (10), sesame seeds (5), dry coconut (5), sugar (15) and clarified butter (20). Process used: malting.
NDBCRP14	Spinach with pumpkin seeds dip (chutney)	Dip made from spinach (42), coriander (17), gingelly seeds (9), pumpkin seeds (8), garlic dry (8) and chillies green (8).
DBCRP1	Finger millet porridge	Sweet made from finger millet flour (5), coconut fresh (9), poppy seeds (17), buffalo milk (52) and sugar (17).
DBCRP2	Rice porridge with poppy seeds	Sweet made from rice (5), coconut fresh (9), poppy seeds (17), buffalo milk (52) and sugar (17).
DBCRP3	Rice porridge without poppy seeds	Sweet made from rice (6), coconut fresh (10), buffalo milk (63) and sugar (21).
DBCRP4	Garden cress seed porridge	Sweet made from garden cress seeds (9), dry coconut (9), buffalo milk (56), clarified butter (2) and sugar (23).
DBCRP5	Cheese sandwich	Sandwich made from white bread (70), cheese (16), green chillies (8) and coriander leaves (10).
DBCRP6	Spinach–cottage cheese vegetable	Vegetable made from cottage cheese (16), spinach (53), onion (11), tomato (11), Dry garlic (1), cumin (1) and oil (3).
DBCRP7	Peas–cottage cheese vegetable	Vegetable made from cottage cheese (46), peas (20), onion (9), tomato (14), cumin (1) and oil (5).
DBCRP8	Basundi	Sweet made from buffalo milk (90) and sugar (9).
DBCRP9	Rasmalai	Sweet made from cottage cheese (25), buffalo milk (50) and sugar (25).
DBCRP10	Rasgulla	Sweet made from cottage cheese (66) and sugar (33).
DBCRP11	Pudding	Sweet made from white bread (10), buffalo milk (70), egg (hen) (10) and sugar (15).
DBCRP12	Soybean–amaranth porridge	Sweet made from soybean flour (5), amaranth flour (3), buffalo milk (85), sugar (8) and clarified butter (7).

Table 3. Comparison of nutrient composition of NDBCRP and DBCRP

Nutrient per 100 g cooked weight	NDBCRP	DBCRP	*P* value
Calcium (mg)	337.5 ± 104.4	274.3 ± 127.8	0.199
Energy (kcal)	364 ± 115	259 ± 88	0.018
Protein (g)	10.8 ± 4	6.6 ± 2.9	0.008
Fat (g)	19.3 ± 10.1	11.4 ± 4.1	0.020
Carbohydrates (g)	36.8 ± 12.3	30.1 ± 13.3	0.206
Oxalate (mg)*	177.8 (240.3)	1 (11.0)	0.000
Phytin (mg)*	162 (253.1)	4.5 (45.2)	0.000
Fibre (g)*	5.7 (7.1)	0.5 (1.9)	0.006

Data represented as mean ± SD. *Estimated using tables of nutritive value of Indian foods after adjusting for moisture content and data presented as median (IQR) as they were not normally distributed.

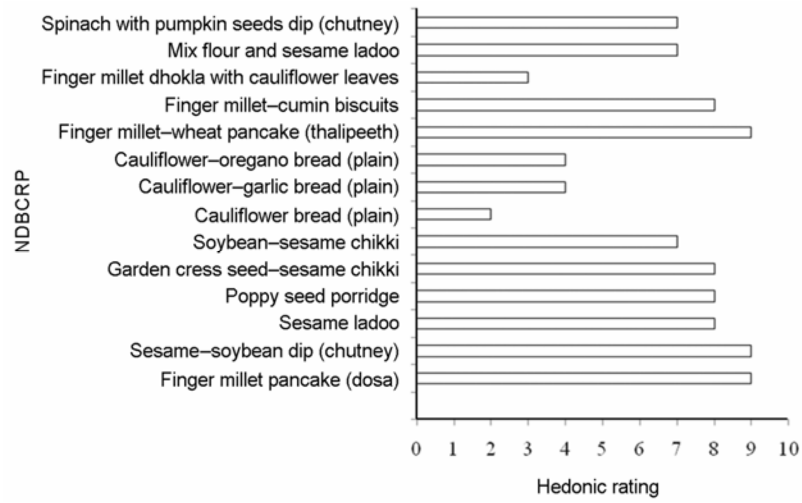

Figure 3. Sensory scores of NDBCRP based on hedonic rating scale. Data are presented as mean scores.

they 'liked extremely' thalipeeth. Plain cauliflower bread was not accepted well by the panel members. Ninety per cent of the panel members reported that they 'dislike very much' plain cauliflower bread due to its strong pungent odour and taste. Cauliflower bread with oregano and garlic was better accepted by them, as addition of garlic and oregano had reduced the pungent flavour of the bread. Like cauliflower bread, finger millet dhokla with cauliflower leaves was also 'disliked very much' by 90% of the panel members due its pungent odour and taste.

We have reported calcium intake in children and adolescents in Pune city; 92% of the study subjects had calcium intake below the RDA. We have developed 14 NDBCRP and demonstrated that they are at par with similar dairy products with respect to calcium content. We used food-processing methods like malting and leavening to increase calcium absorption of the products. We also found that 10 out of the 14 products were completely acceptable.

Several studies have reported that calcium intake[4–7,27] is below the Indian RDA in children and adolescents (800 mg/day)[19]. Similar results were seen in our study. Moreover, diets of Indian children and adolescents are mainly cereal and millet-based[6,12]. Other non-dairy based, calcium-rich sources like calcium-fortified soy milk[28], fortified orange juice and apple juice[29] are not commonly consumed by Indian adolescents. Hence NDBCRP provide an opportunity to increase calcium content in the diets of children and adolescents, which is in line with their dietary practices. One meal per day of 200 g of NDBCRP would provide 84% of the daily RDA for Indian children and adolescents[19].

Plant food sources of calcium also contain large quantities of phytates, oxalates, tannins and fibres which are known to be potent inhibitors of calcium absorption. Reports suggest that around 30% of calcium is absorbed from dairy sources as against 10% from non-dairy sources due the high phytic acid content[15,30]. Several traditional food-processing and preparation methods such as soaking, fermentation and germination/malting can be used at the household level to enhance the bioavailability of micronutrients in plant-based diets by increasing the physico-chemical accessibility[31]. Study by Navert and Sandstorm[32] has shown that the phytic acid content of bread decreases to 40% by leavening. Thus, leavening helps increase calcium absorption by degradation of phytic acid[18]. Studies have also shown that the addition of malt to sorghum increases calcium absorption from 32–35% to 102–103% (ref. 17). Hence, NDBCRP developed using malting and leavening are expected to have high calcium absorption.

In conclusion, our data have shown that dietary calcium intake of Indian children and adolescents is below the RDA, and that the 14 NDBCRP developed in our study can be incorporated in the diet of Indian adolescents to increase their calcium intake on a day-to-day basis.

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ACKNOWLEDGEMENTS. We thank our department staff for participating in the sensory evaluation of developed food products and Deepa Pandit for help during data collection. We also thank Dr Uma Diavte, Director, Hirabai Cowasji Jehangir Medical Research Institute, Pune for support, and Jagdish Sanwalka and Padmalata Ravikumar for their help. N.J.S. thanks the Department of Science and Technology, Government of India for funds.

Received 19 July 2010; revised accepted 15 July 2011