5

SUMMARY AND CONCLUSION

Lifestyle changes and urbanization which are the key determinants of health cause detrimental effects on the quality of life of individuals and is therefore of concern. In India cardiovascular health status has seen an upward trend in the recent years affecting India's productive population. Hyperlipidemia as a strong risk factor for coronary heart disease is well established and it is characterized by elevated lipid fractions which can precipitate as elevated cholesterol or elevated triglycerides or both. An integrated approach combining lifestyle modification with pharmacologic treatment is important to reduce cardiovascular risk factors to improve vascular health and reducing costs associated with the disease.

The role of diet in cardiovascular diseases has focused mostly on dietary fats. However protective effects of other nutrients as part of entire food intake are also protective against CVD.Changing the trends in the eating habits and food production will take a pivotal role to promote health. Increased energy uptake, unbalanced diets and highlyprocessed foods are huge problems of concern as these are contributory factors causing lifestlyle diseases which are often linked to modern society.

Prebiotics exert a myriad of effects of health promotion and inulin is one. Inulin is a fructan compound extracted from the chicory root with a fully proven prebiotic function. Inulin is considered to be a fat substitute and is used as food additive along with functional probiotics. It is an important challenge for current and future research is to utilize the dual benefitting property of prebiotic ingredient such as inulin to bring metabolic changes to concrete health benefits.

Incorporating inulin as functional food ingredients in the normal diet can stem the tide of cardiovascular risk factors and surmount the rising morbidity and mortality rates. The study was carried out with the primary objectives to study the consumption pattern of prebiotic foods, develop a prebiotic foodand find out its

effect on hyperlipidemic subjects. The secondary objectives were to study the knowledge, awareness and practice of prebiotic foods among population groups, formulate a common food with prebiotic ingredient and standardize, assess the developed food product in terms of acceptability, nutrient content and microbial analysis, supplement the food product to selected hyperlipidemics and study the impact on their lipid profile.

The methodology for the study carried out in four phases. In phase I, assessment of knowledge, awareness and practice of prebiotic food consumption pattern was studied among 1400 subjects using a standard questionnaire.The area selected for the study was urban areas of Coimbatore city. The subjects were taken from various households using random sampling near Saibaba colony and Ramalingam colony. Adolescents in the age group of 10-19 years, adults in the age group of 20-60 years and elderly in the age of 60-70 years are included in the study. The subjects were literates and both genders were selected for the study. Adolescents comprised of 173 males and 275 females. In the adult group 278 males and 463 female subjects participated in the study while the elderly group comprised of 98 males and 113 females.

Background details such as education, occupation and income status was elicited from the subjects. Knowledge, awareness and frequency of consumption of prebiotic foods were recorded among the selected population. A 24 hr recall method and food frequency was used to obtain information on prebiotic food consumption pattern among the subjects. Based on the results that majoritywere unaware of prebiotic foods, an effort was taken to develop a product with prebiotic functional quality.

In Phase II, formulation and standardization of prebiotic food was done. Market survey on the availability of prebiotic food products was carried out in four well known department stores of Coimbatore to find out the availability of common food products incorporated with prebiotic ingredient. The labels were studied in about four convenience food products and mainly looked for inulin as it is a prebiotic ingredient. A diet survey was carried out among 50 adults (33 females

and 17 males) working in a government sector using purposive sampling technique to understand their diet behavior so that a suitable product can be decided. A 24 hour recall survey was administered for 3 consecutive days to find the snacking pattern of the subjects. The findings of the survey showed a snacking behavior of fried foods among the subjects and hence incorporating a prebiotic food in a snack was decided. The product concept was developed on the basis that the product can be commonly consumed by the target groups i.e. the working adults and does not deviate much from their diet. A product which can be consumed daily during their work time and is cost effective, product which can lend itself for cooking without fat and can be enriched with inulin.

Taking into consideration the product concept a baked prebiotic product was developed using inulin as an adjunct to incorporate the prebiotic functionality into the product.

A thorough search on the availability of inulin was sourced from ingredient suppliers with product specifications in India and Generally Recognized As Safe (GRAS) certified Fructafit inulin was obtained.

A baked prebiotic product namely cake was formulated where four variations of cakes were made using inulin to replace baking fat on a weight basis at 0% (standard) 25% (T1), 30% (T2), 75% (T3) and 100% (T4) replacement levels. Traditional creaming method of preparation was used for mixing of ingredients. Sensory evaluations were carried out to measure overall acceptability, detect differences between formulations and measure intensity attributes. The sensory evaluation and the quality of the products was assessed by 15 panelists and based on the scores and overall acceptability the cake variation was not acceptable.Hence another baked product namely biscuits was developed. Biscuits were formulated with three variations using inulin as fat and sugar replacer. A standard biscuit and three variations namely P1(fat and sugar removed), P2 (62% fat replaced and 100% sugar removed) and P3 (37% fat replaced). The formulations were prepared by standard creaming method of biscuit preparation and the developed biscuit was assessed for sensory

evaluation by 15 judges using a 9 point Hedonic scale and was compared with control biscuits.

Nutrient analysis for inulin powder separately and for the P1, P2 and P3 was conducted for nutrients such as energy, carbohydrate, protein, fat, inulin, crudefibre and total antioxidant activity using standard procedures.Tests for antimicrobial activity of the developed biscuits was done for 0 and 7^{th}day using standard plate count method.

The total cost incurred in the preparation of the biscuits based on the market prices prevalent during the specific time was computed. The cost of unit weight of food ingredients was calculated and the economic feasibility was assessed.

In Phase III, A government sector namely Bharat Sanchar Nigam Limited in Saibaba colony, Coimbatore was selected to identify the target groups i.e. hyperlipidemics through purposive sampling technique. Two hundred and four subjects were screened for their lipid profile. Inclusion criteria was that they should be in the age group of 30-60 years with no known disease. Those who are willing to take part in the study and were willing to take the prebiotic supplement were included in the study. Subjects with chronic diseases and those having lipid lowering drugs were excluded.

Demographic details such as age and gender distribution, the education status, occupation and income status, type of family was elicited using an interview schedule. Using standard procedures, anthropometric measurements such as height, weight, waist and hip circumference were recorded. Blood pressure was measured for the subjects and lipid fractions like total cholesterol, triglycerides, HDL Cholesterol, LDL Cholesterol and VLDL were estimated using standard procedure. The values were compared against reference values of National Cholesterol Education Programme and hyperlipidemic subjects were identified.

In Phase IV, the efficacy of prebiotic food on lipid profile was studied. Experimental group was supplemented two prebiotic biscuits of 16gm each

(11gm of inulin) for 60 days as a snack. The dietary intake for all the group participants was monitored and recorded using a daily food chart. The diet pattern and consumption pattern of fats/oil, consumption of coffee and prebiotic foods were elicited from the subjects.

An informed consent was taken from all the subjects and the serum lipid profile was assessed for pre intervention (0 day), mid intervention period (30^{th} day) and post intervention (60^{th}day) and the results were inferred.

The findings of the study are

PHASE I

Details on awareness, knowledge and practice of prebiotic food consumption pattern

- Among 1400 subjects, 61 per cent were females and 39 per cent were males. Fifty three per cent were adults who predominated the group while 32 per cent were adolescents and 15 per cent were elderly subjects.

- Fifty one per cent females and 53 per cent males were post graduates. Twenty one per cent males and 33 per cent females were graduates. Seven per cent and nine per cent of the male and female subjects under took professional courses.

- Except for 36 per cent of total subjects all the others were employed. Thirty two per cent were adolescents and hence were studying and the rest four per cent were in elderly group. Fifty two per cent of the subject were employed in government sector.

- Majority of the subjects (89 per cent) were in high income group (HIG). The economically weaker section earning income of less than Rs.5000 was noted among one per cent each of male and female subjects.

- Eighty eight per cent of the subjects were in nuclear type and 73 per cent had a family size of 3-4 members.

- Twenty five per cent of the subjects were aware of prebiotics while majorit(75 per cent) were unaware of prebiotics. Nineteen per cent males and 76 per cent females adolescents were aware of prebiotics comparatively while the elderly were mostly unaware. Females were more aware than the males.

- The awareness of prebiotic information was mainly from magazines (32 per cent), internet (27 per cent), newspapers(21 per cent) however the information on prebiotics foods from medical fraternity namely doctors and dietitian was only 17.5 per cent.

- Twenty eight per cent of the adolescents, 58 per cent adults and of 62 per cent elderly age group did not have a knowledge about the meaning of the term prebiotic food.

- The consumption of prebiotics foods even among those who were aware of it was only 58 per cent but on the contrary whether aware or unaware everyone was consuming wheat, onion, garlic tomato and banana on a regular basis which are the common prebiotic foods.

- The association of gender and education with awareness showed no significant relationship.

- Occupation and income showed a significant relationship with awareness at one per cent level.

- Education and occupation was found to have significant relationship with perception of prebiotics and was found to be significant at one per cent level.

- Prebiotic foods in the cereal food groups like wheat, barley were consumed daily and weekly basis. Chicory added in coffee powder was consumed daily by all subjects

PHASE II

Formulation and standardisation of a prebiotic food

- Prebiotic food ingredients such as fructo-oligosaccharides (FOS), inulin, mannanoligosaccharides (MOS) are prebiotic foods commonly incorporated in biscuits and biscuits

- Evaluation of the snacking pattern of the subjects showed that fried foods like vada, bonda, bajji, puffs and were the common snacks consumed. The contribution of energy from these snacks towards the total calorie intake was found to be 6 -28 per cent.

- The biscuits were developed and standardised through trial and retried three times for all the variations to get consistent final products It was noted that addition of inulin changes the physical characteristics of the biscuits.

- The biscuits were circular in shape with a crumbly texture. Moisture content was found to be less than 4 per cent.

- Among the biscuits P1, P2 and P3, fat content was found to be highest in P3 (25.88gm) which can be attributed to the fat added in the formulation. The protein content of control biscuits (6.06gm) was found lower than that of the three biscuit variations (11gm, 7.08gm and 6.26gm). Crude fiber content of standardbiscuits (0.45gm) was observed to be lowest.

- The inulin content in P1, P2 and P3 was 25.7gm, 34.5gm and 19.1 gm respectively.The difference in the values may be attributed to the amount of inulin used in the formulation. Antioxidant activity was also observed in all the biscuits where activity was highest in P2 (229.54 µg). P2 contributed the lowest calories compared to the other variations P1 and P3.

- It can be observed that P2 was acceptable with a high score for overall acceptability (7.45±0.39) among the three. The attributes for appearance, taste and texture was comparatively high. Hence P2 (Fat replaced and

sugar removed) presented similar results to the standard and was accepted.

- Each serving of 16gm biscuits provided 86.8 kcal, 5.52 gm of inulin, 3.28gm of fat and 0.17 gm of fibre.

- The total viable count for the biscuitsat room temperature was within the standard limits on initial and 7 days.

- Therefore based on the results of sensory evaluation, nutrient content and microbial safety of the prebiotic biscuits, P-2 (Fat replaced and sugar removed) was selected for the study.

Total cost in the preparation of the biscuits was Rs.36 per 100g and the cost of 32g of prebiotic biscuit was Rs.11.5. Hence, the formulated biscuits are economical and affordable.

PHASE III:

Details of the subjects screened for hyperlipidemia

- Among 204 subjects screened for hyperlipidemia, 58 per cent males and 81 per cent females were in the 51-60 years age group. Majority had a family size of 2-3 and the education status of the subject showed that 41 per cent and 30 per cent of males were undergraduates and post graduates respectively. Forty per cent of females were post graduates.Ninety two per cent and 96 per cent of males and females respectively worked in government sector and were in high income group.
- 13 per cent of males and 15 per cent of females were found to be obese with BMI.
- The WHR of the subject showed that the people at risk for CVD were higher in 33% to males (67) and 52% to females (107) were found to be at high risk with a 0.9 and >1
- Among the total of 204 screened subjects, hyperlidemia was seen among 42 per cent (86 subjects) where 49 were females and 37 were males.

- Twenty nine per cent of the subjects were categorized with borderline cholesterol level of 200-239mg/dL. Among females 13 per cent had triglyceride level > 200mg/dL which is high and in males eight per cent were in high category. Nine per cent of male subjects had high LDL cholesterol while three per cent females were observed to be in the high LDL cholesterol category.

- The association of age distribution and lipid profile of the screened subjects showed a significant relationship at one per cent level ($p<0.001$). Education and occupation of the subjects was found to be associated with lipid profile and was significant at 5 per cent ($p<0.05$) and 1 per cent level ($p<0.001$).

PHASE IV

Impact of the developed prebiotic food product on lipid profile

- Mean body mass index of the subjects showed that all the subjects were overweight with BMI between 26-29. Body mass index values did not differ significantly among the periods (0^{th} day, 30^{th} day and 60^{th} day) andit was concluded that inulin supplementation did not show any effect on the BMI values of the experiment subjects

- It was observed that all the subjects in both groups had high waist hip ratio (>0.9 for males ;>0.85 for females) and the mean values of waist hip ratio in experiment females were higher than the control females. Post intervention values of waist hip ratio showed no significant changes in the experiment subjects.

- The mean systolic and diastolic blood values in the experiment subjects showed no significant differences post intervention

- Fats like butter, ghee, coconut oil and refined vegetable oil were consumed by all the subjects in both the groups. It was noted that fats like soyabean oil, canola oil and rice bran were least consumed.

- Consumption of coffee among the subjects shows that maximum (40 per cent) of the control male subjects and40 per cent of experiment subjects consume less than 3 cups of coffee a day

- Inulin contribution from coffee was higher in experiment groupand the intake was found to be 3.4gm per day.

- The consumption pattern of prebiotic foods among the experiment and control showed that the common prebiotic foods were wheat, onion, garlic and banana. Among the experiment group onion and garlic were found to be mostly consumed as they are common spices and condiments added to almost all preparations in their daily diet. .

- The mean intake of energy, carbohydrates and fat was higher than the recommended dietary allowances. Protein intake was found to be low.

- Among the hyperlipidemics (86 subjects), the mean serum total cholesterol levels in males and females were 226.7±25.71 and 227.± 23.95 respectively. The mean level for triglycerides, LDL-c and VLDL-c in males were higher than females. HDL-c levels were lower in females (43±7.712) compared to males (45.6±11.33).

- Combined hyperlipidemia was seen among 86 per cent of the subjects with female subjects having a maximum of 59 per cent (44) and males with 41 per cent (30). Dyslipidemia which is elevation of serum cholesterol, triglycerides (TGs) or both or a low high-density lipoprotein level was noted among 14 per cent of the subjects

- Cholesterol levels of the experiment male subjects showed a decrease from 229±20.90 mg/dLto 219.07±19.07mg/dL. In the female subjects it decreased from 221.53±14.71 mg/dLat 0 dayto 214.42±14.97 mg/dLat 60thday.

- The lipid fraction triglycerides in males showed a decrease of 60 mg/dL from 299.08±173.08 mg/dL at 0 day to 239.45±60.74 mg/dL at 60th day. The

females also recorded a considerable decrease in the triglyceride levels from 279.76±88.85 mg/dL at 0 day to 268.02±86.91 mg/dLat 60th day

- HDL-c levels increased from 48.03±8.24 mg/dLat 0 day to 50.06±8.06 mg/dLat 60th day. in males subjects and in females it increased from 45.65±7.99 mg/dLat 0 day to 48.58±7.56 mg/dLat 60th day.. LDL-c levels decreased from 111.42±7.27 mg/dLat 0 day to 102.84±7.35 mg/dLat 60th day.in males and 114.81±14.07mg/dLat 0 day to 104.55±14.15mg/dL at 60th dayand VLDL-c levels did not show any significant changes.

- Control subjects showed no significant changes in their lipid profile post intervention.

- The differences in mean serum lipid values at 60th day for control and experiment subjects in both males and females showed that control subjects had lesser mean lipid values than the experiment group at 60th day for all lipid fractions except for LDL-c where control subjects showed higher mean LDL-c level than experiment group in both genders at 60th day

- Analysis of Co-Variance (ANACOVA) results to find whether the serum lipid values differ significantly between control and experiment group and between males and female groups showed that the co-variates (lipid fractions at 0 day) has significant effect since the F ratio value (1099.407 for TC; 88.798 for triglycerides; 495.531 for HDL-c; 1513.303 for LDL-c and 190.504 for VLDL-c) is significant at 1 per cent level for all the lipid profiles.

- Between control and experiment groups, there was a significant difference since the F value (48.388 for cholesterol; 57.050 for triglycerides; 16.863 for HDL-c; 397.507 for LDL-c) is significant at 1 per cent level. However F-value ratio for VLDL-c was not significant.

- The F –value comparing between gender for TGL and LDL was found to be significant at 5 per cent level (4.012 for TGL; 5.852 for LDL-c) while F – value for TC, HDL-c and VLDL –c was not significant

- The ANOVA results shows that the F ratio comparing the TC, TGL and VLDL-c levels between was not significant. However, HDL-c and LDL-c levels were significant at 5 per cent level. The interaction effect testing whether the group differences exist on all the periods for TC, TGL and LDL-c which is found to be significant at 1 per cent level.

- This shows that for males the inulin induced effect had significant effect over the three periods (0day, 30th day and 60th day) for TC, TGL and LDL .

- Significant differences between the periods (0th day, 30th day and 60th day) and between the groups was observed for the female subjects. The F-ratio value comparing the TC levels between groups was 17.129 which was significant at 1 per cent level while the F-value for other lipid fractions were not significant. The interaction effect testing whether the group differences exist on all the periods (0day, 30th day and 60th day) for TC, HDL-c and LDL-c is 30.875, 14.030, 100.746 respectively which is found to be significant at 1 per cent level. Triglycerides level for females on all the three periods was found to be significant at 5 per level.

- For females the inulin induced effect had positive effect over the three periods (0day, 30th day and 60th day) for TC, TGL, HDL-c and LDL-c and was found to be significant. No significant changes on the interaction effect on the groups and periodswas observed for VLDL-c levels.

Conclusion

Hyperlipidemia is a major risk factor contributing to mortality burden of cardiovascular diseases. Prebiotics are known for their nutraceutical effects and can influence the blood lipid levels. The approach of using prebiotics in diet to modulate serum lipid level is a promising method to mitigate the attenuated effect in hyperlipidemics and the present study focus in the line of using this approach by developing efficacious prebiotic food using inulin giving the product a novel function. The results indicate that diet rich in inulin has beneficial effects on total cholesterol, triglycerides and LDL-C, as well as HDL-c concentration in the

hyperlipidemic subjects.Hence Regular intake of fermentable fibressuch as inulin may play an important role in determining the health benefits associated with prebiotic intake.

Limitations

Gut microflora changes with inulin supplementation was not possible since the participants were not cooperative in providing stool samples required for the estimation. Measuring of changes in short chain fatty acids could strengthen the results of the study.

Recommendations

Future research can focus on efficacy of prebiotics in terms of dosage and intervention period in reducing cardiovascular risks. The combination of prebiotics in development of new novel prebiotic products is important challenge for current and future research.