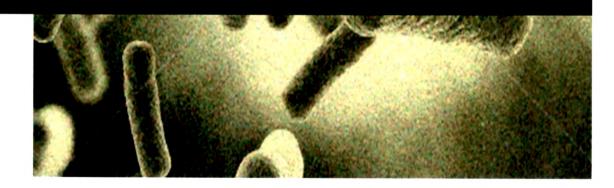# Summary and Conclusions

CHAPTER 6

SUMMARY AND CONCLUSIONS

In recent years, India has witnessed a rapidly exploding epidemic of Diabetes. Indeed, India today leads the world with its second largest number of diabetic people. In order to control the growing epidemic of type 2 diabetes, the scientific community is prepared to develop or identify new therapeutic agents that can effectively control diabetes and prevent the development and progression of its complications without compromising on safety. Fructooligosaccharide (FOS) is an upcoming prebiotic with several health benefitting properties. Recent evidence has indicated that FOS influences the functioning of gut microbiota, glycemic index, gut incretins, blood lipid and could help to assist diabetic control. Therefore, the present study was undertaken to study **"Acceptability trials of fructooligosaccharide (FOS) substituted food products and impact evaluation of FOS supplementation in type 2 diabetic adults in terms of their glycemia, gut incretin (GLP-1) and gut microbiota".** The study is divided into three phases with the following objectives-

PHASE I – Development of FOS incorporated food products and studying their various organoleptic attributes, overall acceptability and the recovery of FOS during processing of these products using HPLC technique.

PHASE II – Collection of baseline data of type 2 diabetic subjects attending health clinic of M.S. University of Baroda in terms of anthropometry, dietary, biophysical, glycemic, lipemic, GLP-1 and gut microbiota (*LAB*, *bifidobacteria* and enteric pathogen) and understanding the correlations between various parameters.

PHASE III- Effect of Fructooligosaccharide (FOS) supplementation on Glycemic, lipemic parameters, Gut incretin (GLP-1) and Gut Microflora in type 2 diabetic adults.

The results and major highlights of all the phases of the study are summarized below-

6.1   PHASE I

This phase of the research was carried out to study the physical and organoleptic characteristics of the FOS substituted popular Indian food products. The possibilities of incorporating FOS in the foods products were studied by the method of substitution. The products included *chapati, thepla, dhokla,* and *patra* which were incorporated with various levels of FOS. FOS was substituted in the base material of *chapati* and *thepla* at 4 different levels i.e. 6%, 10%, 16% and 20%, whereas base material of *dhokla* and *patra* was substituted with 3 different levels i.e. 6%, 10% and 20%. Twenty five semi trained panel judges evaluated these food products using ten point Numerical scoring test and Difference test. These food products were selected for FOS substitution as they are consumed on regular basis in the Gujarat region.

Salient Features of Phase I

6.1.1 Physical properties of FOS substituted food products

▥   As the level of FOS increased total dough weight of *chapati* and *thepla* decreased from 200 g(std.) to 175 g(20% FOS) and 250 g (std.) to 200 g(20% FOS) respectively.

▥   Time required for the cooking of *chapati* also decreased at the higher percent substitution from 38.3 seconds to 31.3 seconds. Puffing of *chapati* was reduced after 10% FOS substitution.

▥   Time required for the cooking of the *thepla* also reduced at higher percent substitution from 1.5 minute to 1 minute.

▣ The water absorption power varied from 92% (6% level of FOS incorporation) to 68% (20% level of FOS incorporation) in both *chapatis* and *thepla*.

▣ Batter weight of the *dhokla* and *patra* reduced from 200 g (std.) to 168 g (20% FOS) and 215 g (std.) to 185 g (20% FOS) respectively. Water had to be minimized as to avoid thinning of batter.

▣ The bulk density of *dhokla* varied from 3.26 g/cc (std.) to 2.70 g/cc (20% FOS substitution).

▣ The water absorption power varied from 84% (6% FOS) to 68% (20% FOS) for *dhokla* and 93% (6% FOS) to 81% (20% FOS) for *patra*.

6.1.2 Organoleptic properties of FOS substituted food products

▣ The most affected attributes for *chapati* were chewability, breakability and overall acceptability according to numerical scoring test. However, difference test revealed that almost 67% panel judges rated *chapatis* with 6% and 10% FOS substitution as equal and superior to the standard and 47% judges accepted *chapatis* at even 20% substitution.

▣ The most affected attributes for *thepla* were color and appearance, Texture and after taste. According to difference test, almost 70% panel judges rated *theplas* as equal and superior to the standard *theplas* upto 20% level of FOS substitution.

▣ None of the organoleptic attributes altered significantly for *dhokla* upto 20% level of FOS substitution according to numerical scoring test, indicating that *dhokla* were very well accepted at all the 3 levels of FOS substitution. It was rated equal and superior by almost 73% panel judges upto 20% FOS substitution.

▣ There was no significant difference observed in scores of *patra* in terms of color and appearance, texture, taste and mouthfeel, after taste and overall acceptability. Rather, the scores for all the attributes increased as the level of FOS substitution increased indicating a high acceptability of

the product. Almost 88% panel judges rated *patras* as equal and superior to the standard upto 20% level of substitution.

6.1.3 Recovery of FOS in food products during processing

▩ The FOS content of wheat flour used for preparation of *chapati* was 5.2 g%. *Chapatis* prepared with 6% and 20% FOS addition revealed a percent recovery of 117 g% and 109 g% respectively.

▩ FOS content of raw *thepla* flour without addition of FOS was 6.6 g%. *Theplas* prepared with 6% and 20% FOS addition revealed a percent recovery of 97.7 g% and 91.8 g% respectively.

▩ The percent recovery of FOS from *dhoklas* after addition of 6% and 20% FOS was 83.2 g% and 80.9 g% respectively.

▩ With respect to percent recovery of FOS in *patra*, 6% and 20% FOS added *patras* resulted in 81.1 g% and 86 g% FOS recovery respectively.

▩ Roasting of *chapati* revealed maximum percent recovery of FOS followed by shallow frying and steaming.

▩ As the addition of FOS increased in *chapati*, *thepla* and *dhokla* percent recovery of FOS decreased. However, in case of *patra* percent recovery increased with higher addition of FOS.

Hence, it can be concluded that FOS incorporated dhokla and patra were the most acceptable products at all the three levels of substitution according to both numerical scoring and difference test. However, chapati was most accepted at 6% and 20% of FOS substitution as per the numerical scoring test and difference test respectively. According to difference test thepla was also acceptable at 20% level of FOS substitution. Recovery of FOS ranged from 81-117 g% for all the four products after processing. Therefore, these food products can serve as a healthy choice for consumption on regular basis.

6.2 PHASE II

In this phase, a cross-sectional study was designed to enroll 120 known type 2 diabetic subjects from the Health clinic of The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat. Relevant data was obtained through patient medical records, face to face interview and direct measurements. Biophysical parameter like blood pressure, biochemical parameters like fasting and glycemic parameters, lipemic parameters and gut incretin (GLP-1) and microbial parameters in terms of *lactobacillus, bifidobacteria* and enteric pathogen were examined from the stool samples of the subjects.

Salient Features of Phase II

6.2.1 Baseline information of type 2 diabetic subjects

▨ Socio economic data of type 2 diabetic subjects revealed that majority of the subjects surveyed in the study were Hindus (95%) with 43% male and 57% female subjects. All the subjects were literate. More than 45% subjects lived in nuclear family.

▨ All the subjects were on oral hypoglycemic drugs- metformin and sulfonylurea.

▨ Most subjects (88%) had family history of diabetes with 56% subjects having single parent as family history of diabetes. About 76% subjects had family history of hypertension and 44% had family history of chronic heart disease.

▨ With regards to secondary complication of diabetes 71% subjects had hypertension, 25% subjects had dyslipidemia, stroke or CVD. Almost 11% had complications of neuropathy, nephropathy and retinopathy.

▨ With regards to the duration of diabetes, 22% subjects were recently diagnosed as diabetic for 5 years. About 21% subjects diagnosed for diabetes for 5-15 years and 56% subjects had diabetes for more than 15 years.

6.2.2 Physical activity pattern and Anthropometric profile of type 2 diabetic subjects

▣ GPAQ questionnaire for assessing physical activity pattern developed by WHO revealed that almost half (58.3%) subjects had low physical activity (MET minutes <60). Around 32.5% had moderate physical activity level whereas only 9.2% subjects had high physical activity level.

▣ According to the Asia Pacific Classification of BMI, about 20.8% and 61% subjects were overweight and obese respectively.

▣ Comparing between male and female BMI, around 30% females were overweight as compare to 10% overweight male counterparts.

6.2.3 Biophysical and biochemical profile of type 2 diabetic subjects

▣ According to JNC VII 2003 classification, 67% subjects were borderline hypertensive and 22% subjects were having poor control of hypertension.

▣ Mean fasting serum blood glucose and post prandial blood glucose of the subjects was 143 mg/dl and 219 mg/dl respectively. Mean glycated hemoglobin was 9.0%.

▣ Mean gut incretin value for GLP-1 was 0.3 pmol/L.

▣ According to ADA, 2007 classification 86% subjects had poor control of diabetes.

▣ The outcomes of the lipid profile elicited that mean cholesterol levels of the subjects was 200.3 mg/dl.

▣ About 58.3% subjects had good control for serum total cholesterol levels whereas 28.3% and 13.4% had borderline and poor control on TC levels.

▣ Almost 64% subjects had good control over triglyceride levels with TG levels <150 mg/dl, whereas 15.9% subjects had TG >200 mg/dl.

▣ About 86% males and 60% females showed good control on TC/HDL ratio i.e. <6.4 and <5.6 respectively.

6.2.4 Dietary profile of type 2 diabetic subjects

- General food habits revealed that, 77.4% subjects were vegetarian followed by 3.3% and 19.3% non-vegetarian and ovo-lacto vegetarian respectively.

- 24 hr dietary recall method elicited that the mean energy intake was 43.6% and 49.3% higher than the prescribed RDA for females and males respectively.

- Protein intake was 14-20% lower in both the groups and fat intake was 98% and 119% higher in males and females respectively.

- Iron intake was 60% and 56% lower than the RDA in females and males respectively and almost all the micronutrients were below the normal range.

- Subjects were consuming a diet which was high in saturated fatty acids (SFA) and lower in omega 3 and omega 6 fatty acids.

- Total fiber intake of the subjects was almost 60% lower than the RDA.

- Only 12.5% subjects were consuming green leafy vegetables on frequent basis and only 20% subjects were consuming fruits frequently.

- Almost 33% subjects consumed snacks like khakhara, bhajiya and fried papad on regular basis. Sweets like kheer, icecream and peda was consumed by 30% subjects on less frequent basis, rest of the subjects consumed sweets rarely.

- Almost 18% subjects consumed probiotic and prebiotic rich foods on most frequent basis, 71% consumed on frequent basis and 11% subjects had less frequent consumption of these foods.

6.2.5 Gut microbiota of type 2 diabetic subjects

- The mean log values in terms of CFU/g of stool sample for *Lactobacillus*, *Bifidobacteria* and Enteric pathogen were 6.34, 6.34 and 4.47 respectively.

- There was no significant difference between the mean log counts of the gut microbiota in male and female subjects.

▣ Subjects with >6.5 log10 CFU/g of *bifidobacteria* had lower FBS (133.2mg/dl), PP2BS (203.7 mg/dl), HbA₁c (8.5%) and higher total fiber (16.5 g) as compared with the subjects with *bifidobacteria* <6.5 log10 cfu/g, had higher values for FBS (145 mg/dl), PP2BS (221.8 mg/dl), HbA₁c (9.1%) and lower total fiber values (12.2 g).

6.2.6 *Association of lifestyle factors of the subjects with anthropometric, dietary, biochemical and gut microbial parameters*

▣ Pearson's correlation values revealed that there was a positive correlation between fasting blood sugar, family history and CHO intake ($p < 0.05$).

▣ PP2BS was significantly positive correlated with physical activity and negatively correlated with fiber intake ($p < 0.05$).

▣ GLP-1 was significant negatively correlated with WHR and fat intake ($p < 0.05$). *Bifidobacteria and LAB* were significant positively correlated with total fiber intake whereas *bifidobacteria* was negatively correlated with WHR and fat intake ($p < 0.05$).

▣ TC was inversely correlated with physical activity and fiber and positively correlated with WC, WHR, energy and fat intake ($p < 0.05$). However, TG, LDL, and TC/HDL showed a significant negative correlation with fiber intake ($p < 0.05$).

▣ FBS, PP2BS and HbA₁c were significant positively correlated with establishment of beneficial bacteria (*bifidobacteria and LAB*). Whereas higher values of glycemic parameters were negatively associated with establishment of beneficial bacteria.

▣ Multiple linear regression analysis revealed that family history, dietary fiber and *bifidobacteria* were the most prominent predictors for FBS. Similarly, dietary fiber, LDL-C and GLP-1 were the predictors which might have maximum effect on the criterion variable PP2BS and for HbA₁c the most significant predictors were LDL-C, *bifidobacteria* and WHR.

Hence, this phase concludes that type 2 diabetic subjects under the study had poor glycemic control. Their lipemic values were also borderline high with high prevalence of hypertension. Subjects consumed an unhealthy and unbalanced diet which was high in fat and low in dietary fiber. Establishment of beneficial gut microflora was also lower in the gut than compared to the gut microflora of healthy individuals. Therefore, supplementation of prebiotic rich foods to the subjects may enhance the beneficial bacteria in the gut which might help in reducing the glycemic and lipemic levels of the subjects.

6.3 PHASE III

In this phase diabetic subjects were asked to participate further in the study and based on their willingness, 65 subjects were divided into control (n=20) and experimental group (n=45) according to the computer generated randomized tables. Due to poor compliance and poor glycemic response as a lifestyle factor 5 subjects were dropped out from the experimental group. Final sample size in the experimental group was 40. This phase was undertaken to examine the effect of daily intake of 10g of FOS for 8 weeks on clinic-biochemical parameters in terms of FBS, PP2BS, HbA1c, GLP-1, lipid profile, atherogenic indices and blood pressure and gut microbiota in terms of *lactic acid bacteria, bifidobacteria* and enteric pathogen of the diabetic adults.

Salient Features of Phase III

6.3.1 Anthropometric, Biophysical and dietary profile of the subjects before and after supplementation trial

▣ No changes in the anthropometric measurements were observed in both control and experimental group. A significant reduction ($p<0.05$) in systolic blood pressure was observed as a result of FOS supplementation

in the experimental group. However, diastolic blood pressure remains unchanged.

- Almost 2.37% reduction in waist circumference in female experimental group and 1.42% reduction in males was noticed. The experimental group showed a shift towards a better health profile with a significant reduction in systolic blood pressure ($p<0.05$, $p<0.01$) in both male and female experimental group and a marginal non-significant reduction in diastolic blood pressure

- Both energy and carbohydrate intake was increased in experimental group as well as in control group after supplementation. The fat intake remained similar in control and experimental group where as protein intake was slightly reduced after supplementation. Fiber intake of experimental group was significantly higher (84%) after supplementation compared to the initial values at baseline ($p<0.001$).

6.3.2 Glycemic response and GLP-1 values of type 2 diabetic subjects before and after FOS supplementation

- After supplementation, the glycemic response of the subjects reduced by 23.9%, 21.2% and 9.41% in terms of FBS ($p<0.001$), PP2BS ($p<0.001$) and HbA_{1c} ($p<0.001$). About 52% increase in GLP-1 values were also observed post supplementation ($p<0.05$).

- Both controlled diabetic ($HbA_{1c}<8$) and uncontrolled diabetic ($HbA_{1c}>8$) had a significant reduction in glycemic response after supplementation. However the percent reduction in PP2BS, HbA_{1c} was higher in uncontrolled diabetics ($HbA_{1c}>8$) and percent increase in GLP-1 was also higher in uncontrolled diabetes.

- Diabetic subjects with lower (<0.3) and higher (≥0.3) GLP-1 values had almost similar percent reduction in glycemic values.

6.3.2 Lipemic response of type 2 diabetic subjects before and after FOS supplementation

▨ FOS supplementation resulted in a significant reduction in serum TC, TG and LDL levels by 10%, 5.4% and 6.8% respectively. However, no significant reduction was observed for HDL and VLDL levels.

▨ FOS supplementation resulted in 11.9% and 8.13% reduction in TC in males and females respectively. About 10.9% and 11% significant reduction was observed for LDL in both the groups. In terms of TG only females had significant percent reduction (8.5%) after supplementation. However, no significant reduction was seen in HDL for both males and females.

▨ Uncontrolled diabetics ($HbA_{1c} > 8$) had higher percent reduction in TC, LDL and VLDL values by 10.8%, 8.6% and 12.1% respectively. Whereas controlled diabetic subjects showed 10.4% higher percent reduction in TC values by 12.6%

▨ Subjects with higher BMI (≥ 23) had higher significant percent reduction in TC and LDL levels by 9.2% and 11.6% respectively. However no changes were observed for TG, HDL and VLDL as per BMI status.

▨ Significant reductions were observed in TC/HDL, LDL/HDL and non-HDL by 10.4%, 7.6% and 13% respectively ($p<0.05$, $p<0.001$). However, no significant changes were seen for TG/HDL after FOS supplementation.

6.3.3 Gut microflora counts of the subjects before and after FOS supplementation

▨ The fecal log counts of *Lactic acid bacteria* and *bifidobacteria* showed a significant increase by 9.3% and 10.9% respectively ($p<0.001$) post supplementation.

▨ There was a significant reduction by 4.8% ($p<0.001$) of fecal log counts of Enteric pathogen in diabetic subjects after FOS supplementation.

▣ Both controlled diabetics (HbA$_{1c}$<8) and uncontrolled diabetics (HbA$_{1c}$>8) had a significant increment in *Lactic acid bacteria* counts and *bifidobacteria* counts after supplementation (p<0.01, p<0.001).

▣ Percent decrease in enteric pathogen counts was significantly higher in subjects with their initial glycated hemoglobin levels more than 8 (uncontrolled diabetics).

6.3.4 *Association amongst anthropometric, biophysical, glycemic, GLP-1 and lipemic parameters and gut microbial parameters of type 2 diabetic subjects*

▣ GLP-1 showed significant negative correlation with WC (p<0.01) and WHR (p<0.05). Among glycemic and lipemic parameters GLP-1 was significant negatively correlated with PP2BS (p<0.01) and with TC and HDL (p<0.05).

▣ TC revealed a significant positive correlation with DBP, HDL, VLDL-C (p<0.05), TG and LDL-C (p<0.001). LDL-C was significant positively correlated with WC, SBP, TG, VLDL-C (p<0.05) and TC (p<0.01).

▣ *Lactobacillus* was significant negatively associated with FBS, HbA$_{1c}$ (p<0.001) and VLDL-C (p<0.05). A non-significant negative association was also found amongst *LAB*, SBP and LDL-C.

▣ *Bifidobacteria* was significant negatively correlated with DBP, PP2, HbA$_{1c}$ (p<0.01). *Enteric pathogen* was significant positively associated with DBP, PP2 (p<0.001), SBP, FBS and HbA$_{1c}$ (p<0.05) and inversely associated with GLP-1.

▣ Linear multiple regression analysis revealed that reduction in FBS (criterion variable) was affected most by *Bifidobacteria* followed by WC and WHR (predictor variables). Reduction in PP2BS (criterion variable) was affected maximum by *Bifidobacteria* followed by SBP and TG (predictor variables).

▣ Reduction in HbA1c (criterion variable) is effected most by *Bifidobacteria* followed by WC and *Enteric pathogen* (predictor variables) and reduction

in GLP-1 (criterion variable) was affected maximum by WC. *Bifidobacteria* and *Enteric pathogen* accounted almost similar effect in increase in the plasma GLP-1 values (predictor variables).

From the above results it can be concluded that FOS is an attractive therapy for the management of type 2 diabetic subjects. As 8 weeks FOS supplementation resulted in reduction in the blood glucose (FBS, PP2BS and HbA₁c), blood lipid and GLP-1. Supplementation also helped in colonization of beneficial gut microorganisms. Hence FOS incorporated food products which were prepared in the phase I of the study can be recommended in the daily diets for glycemic control in type 2 diabetic adults.

MAJOR CONCLUSIONS

▨ Acceptability trials of FOS substitution in the selected food products viz. *chapati, thepla, dhokla* and *patra* can be consumed on regular basis as *chapati* was accepted at 10% FOS substitution and rest of the products were fairly acceptable at 20% substitution.

▨ The results of the recovery of FOS in the food products under the study can be used for quantifying FOS content of these food products and thereby determining the total FOS intake by the individuals consuming these products.

▨ The hypothesis stated in the present study that- FOS improves glycemic and lipemic response of type 2 diabetes and increased establishment of beneficial gut microbiota (*LAB, bifidobacteria* and enteric pathogen) has been authenticated.