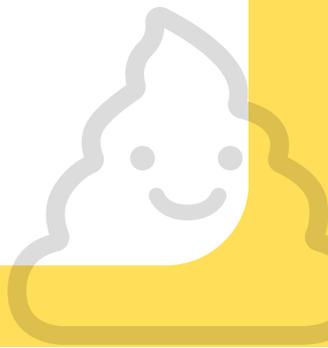
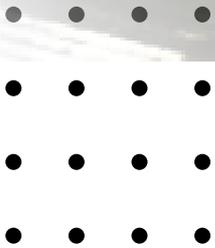


# SUMMARY AND CONCLUSION





## CHAPTER 6

### SUMMARY AND CONCLUSION

Prebiotic intake have shown to promote the colonization of good bacteria like Lactobacillus and Bifidobacterium, which are also known to generate short-chain fatty acids (SCFA) that act as anti-inflammatory markers for a variety of disorders like diabetes and obesity. It has been demonstrated that regular use of prebiotics reduces the colonization of pathogenic microorganisms. Galactooligosaccharide (GOS) may be helpful for both the above mentioned functions along with conditions like functional constipation (FC), depression, and other similar ailments.

Limited literature is available regarding the presence of FC among sedentary teaching staff and impact of GOS consumption on their constipation profile. Therefore the present study was undertaken which is entitled “**Presence of Functional Constipation in the Teaching staff of The M.S. University of Baroda and Impact Evaluation of Supplementation of Galactooligosaccharide (GOS) added Gummies on their Gut Health and Constipation Profile - A Randomized Double blind Placebo Control Trial**”. The study was divided in following three phases:

Phase I - Screening and identification of functional constipation in University teaching staff

Phase II- Development of Galactooligosaccharide (GOS) added gummies and study their acceptability Trials and shelf life studies

Phase III- Impact evaluation of supplementing GOS gummies to subjects suffering from FC on their constipation profile, gut microflora, SCFA profile, depression status and quality of life

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

### **6.1 Phase I – Screening and identification of Functional Constipation in University teaching staff**

In this phase of the research, cross-sectional design was used to screen the university teaching staff for the presence of FC and identify the subjects to be selected for the clinical trial. A pre tested validated structured questionnaire was used as a screening tool along with WHO criteria, Bristol stool chart and Rome IV criteria to assess the presence and severity of FC.

#### **Salient Features of Phase I**

##### *6.1.1. Baseline Information of subjects*

- The background information of the study participants revealed that most of the respondents were females (59.1%), age ranged between 35-62 years, married (73.1%) and stayed in nuclear families (62.1%) with a family income of >INR 123,322.00 (42.3%).
- FC was observed in 19.2% subjects (n=70), with 15.4%, 3.3% and 0.5% in mild, moderate and severe categories respectively.
- Female subjects (n=50) were more constipated than the male respondents and the results were statistically significant at  $p<0.05$ .
- Obesity in various degrees was observed among 43.9% subjects (n=160) and statistically significant correlation was observed between obesity and FC.
- Presence of FC in the mothers of the respondents was significantly associated ( $p<0.05$ ) however presence in the fathers and siblings did not significantly correlate with FC.
- Physical activity of the study subjects indicated 83% and 17% were in the sedentary and mildly active categories respectively and it was significantly negatively correlated with presence of FC among the university teaching staff ( $p<0.05$ ).

##### *6.1.2. Perceptions and practises of subjects*

- Consumption practice of tea >2 cups/day showed a positive association with FC ( $p<0.05$ ).

- Dietary practices such as chewing food and water intake was significantly associated with the presence of constipation ( $p < 0.05$ ;  $p < 0.01$ ).
- Seventy eight percent of the respondents is using a western toilet style where in 64% subjects are defecating in the straight position and it was significantly correlated with constipation ( $p < 0.05$ ). Many subjects (11%) felt constipation is a social taboo.
- Fifty two percent subjects felt constipation is a minor health issue. This aspect among the subjects were positively associated with FC ( $p < 0.05$ ).
- All the six aspects of chrononutrition profile on a free day was significantly associated with FC ( $p < 0.05$ ).

#### *6.1.3 Dietary intake of subjects*

- Type of diets (vegetarian, non-vegetarian and other diets) was not significantly associated with the presence of FC.
- Intake of total dietary fiber ( $p < 0.05$ ) and soluble fiber ( $p < 0.05$ ) showed significant negative correlations with the presence of FC.
- Significant negative associations were recorded with the frequency of consumption of whole and split pulses ( $p < 0.05$ ) and jaggery ( $p < 0.01$ ) with FC.
- Low intake of salt ( $p < 0.01$ ) and high intake of sugar ( $p < 0.05$ ) had significant negative correlations with FC among the respondents.

### **6.2 Phase II- Development of Galactooligosaccharide (GOS) added gummies and study their acceptability Trials and shelf life studies**

This phase of research was carried out to study the organoleptic, physico chemical characteristics and shelf life studies of GOS added gummies. Standard gummies were prepared with different proportions of the ingredients (sugar, water, citric acid, and agar) and then evaluated in triplicates. The possibilities of incorporating GOS in the gummies were studied by the method of substitution after obtaining the standard gummy at 3 different levels i.e. 60%, 80%, and 100%. A ten point numerical scoring test and difference test were used to evaluate the standard and GOS gummies by a panel of ten trained judges in triplicates. The most suitable gummy (100% GOS gummies) underwent shelf life testing and physicochemical analyses.

## **Salient Features of Phase I**

### *6.2.1 Standardization of gummies (For a batch of 24 gummies)*

The various ingredients used for the preparation of standard gummies were water, sugar, agar and citric acid. The trained panel found fewer significant differences in the organoleptic qualities of the gummies prepared using varying levels of ingredients. Gummies with 75ml of water yielded best results. However, F value showed no significant change with the varying levels of water.

- Gummies with 60g of sugar produced best results. However, F value indicated no significant change with varying levels of sugar.
- Gummies with 2g of agar produced best results. However, F value indicated no significant change with varying levels of agar.
- The most acceptable gummies could be prepared with the addition of 1.9g citric acid

### *6.2.2 Sugar substitution with GOS at varying levels (60%, 80%, 100%) (For a batch of 24 gummies)*

- Most acceptable gummies were prepared with 60% GOS. Further substitution of sugar with GOS reduced the sweetness of the gummies.
- In order to produce 100% GOS gummies sugar was totally replaced with GOS along with an addition of 5.5g sucralose which improved the overall acceptability of the gummies

### *6.2.3 Physicochemical properties*

- The physico chemical properties of 100% GOS gummies formulation recorded 24.8% moisture, 3.37 pH, natural yellow color and optimum springiness and gumminess with minimal adhesiveness.

### *6.2.4 Recovery of GOS in gummies*

- The 100% GOS gummies recorded a final retention of 95% GOS using HPLC which indicated minimal prebiotic loss even when subjected to higher temperatures.

#### 6.2.5 Shelf life analysis of GOS gummies

- Six months of storage at accelerated temperatures of 37°C resulted in considerable improvement in chewiness with no significant differences in the organoleptic properties.
- Microbiological parameters revealed no significant differences in *E.coli*, total plate count, yeast, and mold count.

#### **Phase III-Impact evaluation of supplementing GOS gummies to subjects suffering from FC on their constipation profile, gut microflora, SCFA profile, depression status and quality of life**

In this phase subjects suffering from FC were asked to participate further in the study for the double blind placebo control clinical trial. This phase was registered with CTRI, ICMR. Based on their willingness, 48 subjects were divided into control (n=24) and experimental (n=24) randomly according to the computer generated random numbers with the help of a senior professional in the Department. Due to poor compliance 13 subjects were dropped out from the groups. Final sample size in the control group was 18 and experimental group was 17. This phase was undertaken to examine the effect of daily intake of 4 gummies loaded with 10g GOS for 4 weeks on objective and subjective criteria. Objective criteria included determination of gut microflora in terms of *lactic acid bacteria*, *Bifidobacteria*, *Clostridium*, *Bacteroides* and phyla Bacteroidetes, firmicutes and SCFA profile in terms of acetic acid, propionic acid and butyric acid. Subjective criteria included constipation profile, quality of life and depression status.

#### **Salient Features of Phase III**

##### 6.3.1. Baseline information of constipated subjects

- The demographic details of the participants selected for the clinical trial. It showed that most of the respondents were females (74%), married (71%) and stayed in nuclear families (63%) with a normal BMI having a family income of >INR 123,322.00 (49%) and their age ranged between 25-45 years.

*6.3.2 Gut microflora and SCFA profile of subjects before and after GOS gummy supplementation*

- The experimental group showed a significant increase in the beneficial gut microorganisms with respect to genera Bifidobacterium ( $p < 0.01$ ), Lactobacillus ( $p < 0.001$ ) by 322% and 1230% respectively. However, pathogenic microorganism belonging to genus Clostridium ( $p < 0.05$ ), firmicutes and Bacteroidetes showed a significant reduction by 63%, 73% and 85% respectively.
- The F/B ratio which is a common indicator of gut dysbiosis was estimated to be 1.23 and it improved significantly by 190% in the experimental group post supplementation ( $p < 0.01$ ). Both the phyla Firmicutes and Bacteroidetes exhibited a strong significant negative correlation ( $p < 0.001$ ) indicating gut dysbiosis in constipated individuals.
- Significant strong negative correlations were observed between the different species studied namely Bacteroides and Bifidobacterium ( $p < 0.05$ ). Significant strong negative correlations were observed between the different species studied namely Clostridium and Lactobacillus ( $p < 0.01$ ). Significant strong negative correlations were observed between the different species studied namely Bifidobacterium and Clostridium ( $p < 0.01$ ).
- Post supplementation a significant increase in the levels of butyric acid (75%) and acetic acid levels (29%) in the experimental group were recorded ( $p < 0.01$ ;  $p < 0.05$ ). At the baseline significant SCFA namely butyric acid and acetic acid ( $p < 0.01$ ) also revealed a positive correlation.

*6.3.3. Subjective criteria for subjects before and after GOS gummy supplementation*

- Post supplementation with GOS gummies resulted in significant improvement in the constipation profile ( $p < 0.01$ ) with respect to the number of stools/ week ( $p < 0.05$ ), type of stool form ( $p < 0.05$ ), sensation of incomplete stool passage ( $p < 0.01$ ), straining while defecation ( $p < 0.05$ ) and sensation of abdominal pain ( $p < 0.01$ ) by 23%, 43%, 81%, 77%, 18% and 72% respectively.
- Post supplementation, there was a significant reduction (40%) in various categories of depression viz-à-viz mild mood disturbance (31%), moderate depression (57%) borderline clinical depression (100%) ( $p < 0.01$ ).
- Post supplementation there was a significant increase in the quality of life profile in the

experimental group in comparison to the control group ( $p < 0.01$ ). All the parameters of QOL revealed a significant reduction namely reduction in physical discomfort, sensation of decreased appetite and psychological disturbances by 20%, 39% and 23% respectively.

*6.3.4. Associations between various factors such as gut flora, SCFA, depression status and QOL with presence of functional constipation at baseline*

- Significant positive correlations were recorded between beneficial gut microbes belonging to genus *Lactobacillus* and *Bifidobacterium* ( $p < 0.05$ ), *Bacteroides* and *Bacteroidetes* ( $p < 0.01$ ). At the baseline significant positive associations were recorded among SCFA namely butyric acid and acetic acid ( $p < 0.01$ ), butyric acid.
- At baseline, severity of constipation was negatively correlated with SCFA studied which included acetic acid, propionic acid and butyric acid. However, butyric acid and acetic acid showed significant negative correlation with the severity of FC ( $p < 0.01$ ;  $p < 0.05$ ).
- With respect to the gut flora, all the microbes studied and phyla were negatively correlated with the severity of FC. However, *Clostridium* showed a significant positive correlation with the severity of FC ( $p < 0.05$ ). Significant negative correlation was recorded among *Bifidobacterium*, *Lactobacillus* and F/B ratio and with the severity of FC ( $p < 0.01$ ;  $p < 0.01$ ,  $p < 0.05$ ).
- Significant positive correlations were recorded among depression status ( $p < 0.05$ ) of the subjects with the severity of FC. Depression status indicated significant negative correlations with the levels of butyric acid ( $p < 0.05$ ). Significant *Bifidobacterium* ( $p < 0.05$ ) and *Lactobacillus* ( $p < 0.05$ ). Strong significant positive associations were recorded with *Clostridium* and depression status ( $p < 0.01$ ).
- Significant positive correlations were recorded among quality of life ( $p < 0.05$ ) of the subjects with the severity of FC. Depression status indicated significant correlation with the levels of *Bifidobacterium* ( $p < 0.05$ ) and *Lactobacillus* ( $p < 0.05$ ).

**Hence, null hypothesis was rejected and following alternate or working hypotheses have been accepted:**

- Presence of functional constipation in the teaching staff of The M.S. University of Baroda was recorded to the tune of 19%.
- It was feasible to develop gummies with 100% replacement of sugar with GOS with optimum sensory attributes, shelf quality and high recovery rate of GOS.
- Ten grams GOS supplementation (4 gummies) for 4 weeks improved the constipation profile, gut health with respect to higher colonization of beneficial microflora (LAB and Bifidobacteria) and short chain fatty acid profile (acetic acid, propionic acid, butyric acid), depression status and quality of life in constipated subjects.