RESULTS AND DISCUSSION

CHAPTER 5

RESULTS AND DISCUSSION

Galactooligosaccharide (GOS) is a prebiotic majorly present in mammalian milk. Prebiotics are proven to increase the colonization of beneficial microflora including Bifidobacterium and Lactobacillus which are known to produce short chain fatty acids (SCFA) that act as anti-inflammatory markers for various diseases including diabetes, obesity etc. Regular intake of prebiotics has proven to reduce the colonization of pathogenic microflora including *E.coli*. GOS may prove to be beneficial for its potential benefits in conditions like functional constipation (FC), depression etc. and improve the gut brain axis.

The present study 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"** has been undertaken with the primary objective to determine if intake of 10g GOS for a period of one month can improve the constipation profile and gut health of the teaching staff of The M.S. University of Baroda. The results are presented and discussed in this chapter.

The three phases according to the objectives of the study are stated under the following heads:

Phase I – Screening and identification of subjects suffering from 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

Phase I: Screening and identification of subjects suffering from functional constipation in University teaching staff

5.1.1 General information of the respondents and its association with presence of FC

5.1.2 Medical history of respondents and its association with presence of FC

5.1.3 Family history of constipation of respondents and its association with presence of FC

5.1.4 Personal habits of respondents and its association with presence of FC

5.1.5 Physical activity of respondents and its association with presence of FC

5.1.6 Perceptions and practices of respondents about constipation and its association with presence of FC

5.1.7 Constipation profile of respondents and its association with presence of FC

5.1.8 Chrononutrition profile of respondents and its association with presence of FC

5.1.9 Baseline information on dietary intake of constipated subjects and its association with presence of FC

5.1.10 Frequency of consumption of selected food groups and processed food of respondents and its association with presence of FC

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

5.2.1 Sensory evaluation with respect to standardisation of gummies

5.2.2 Substitution of sugar with varying concentration of GOS

5.2.3 Physico chemical properties of GOS gummies

5.2.4 Recovery of galactooligosaccharide (GOS) in GOS added gummies

5.2.5 Shelf life studies of GOS gummies

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

5.3.1. General information of the respondents for the clinical trial

5.3.2. Constipation profile of subjects before and after supplementation with GOS gummies

5.3.3. Gut microbiota profile of constipated subjects before and after supplementation with GOS gummies

5.3.4. SCFA profile of constipated subjects before and after supplementation with GOS gummies

5.3.5 Depression profile of constipated subjects before and after supplementation with GOS gummies

5.3.6. Quality of life profile of constipated subjects before and supplementation with GOS gummies

5.3.7. Association of various factors with constipation profile at baseline

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

Functional constipation (FC) is infrequent bowel movements or difficult passage of stools that persists for several weeks or longer. Constipation is generally described as having fewer than three bowel movements a week. Though occasional constipation is very common, some people experience chronic constipation that can interfere with their ability to go about their daily tasks, reducing their productivity and quality of life.

Therefore this phase of the study was planned to identify and validate the risk factors and parameters which are related to functional constipation. A total of 364 subjects were enrolled for this phase from The M. S. University of Baroda to study the presence of functional constipation and study the associations between them.

The results of this phase are divided into following sections:

5.1.1 General information of the respondents and its association with presence of FC

5.1.2 Medical history of respondents and its association with presence of FC

5.1.3 Family history of constipation of respondents and its association with presence of FC

5.1.4 Personal habits of respondents and its association with presence of FC

5.1.5 Physical activity of respondents and its association with presence of FC

5.1.6 Perceptions and practices of respondents about constipation and its association with presence of FC

5.1.7 Constipation profile of respondents and its association with presence of FC

5.1.8 Chrononutrition profile of respondents and its association with presence of FC

5.1.9 Baseline information on dietary Intake of constipated subjects and its association with presence of FC

5.1.10 Frequency of consumption of selected food groups and processed food of respondents and its association with presence of FC

Section 5.1.1 General information of the respondents and its association with presence of functional constipation

A pre-tested validated semi-structured questionnaire was developed and 364 teaching staff from the University responded. The background information of the study participants revealed that most of the respondents were females (59.1%), married (73.1%) and stayed in nuclear families (62.1%) with a family monthly income of >INR 123,322.00 (42.3%) and their age ranged between 35-64 years. Females were found to have a significant positive association with the presence of FC ($p<0.05$). However, other factors weren't significantly associated with the presence of FC.

Table 5.1.1: General information of the respondents and its association with presence of functional constipation (N= 364)

Parameters	Total Subjects N (%)	χ^2
Age:		
20-34	112 (30.8%)	
35-49	126 (34.6%)	**-0.006** ^{NS}
50-64	126 (34.6%)	
Sex		
Female	216 (59.3%)	**-0.12***
Male	148 (40.7%)	
Type of family		
Nuclear	226 (62.1%)	
Joint	108 (29.7%)	**0.11** ^{NS}
Extended nuclear	30 (8.2%)	
Marital status		
Married	268 (73.1%)	
Unmarried	90 (24.7%)	**0.034** ^{NS}
Divorced	6 (1.6%)	
Family monthly income		
>123,322	154 (42.3%)	
61,663- 123,321	116 (31.9%)	
46,129- 61,662	42 (11.5%)	**0.041** ^{NS}
30,831- 46,128	28 (7.7%)	
18,497- 30,830	24 (6.6%)	

Levels of Significance * $p < .05$, NS= Not significant

Section 5.1.2: Medical history of the respondents and their association with presence of functional constipation

The respondents were questioned for the presence of any existing medical conditions including insomnia or non-communicable diseases or menopause (females) and if they were associated with functional constipation.

Table 5.1.2 depicts the self-assessed medical history profile where in 16%, 11%, 6.5% and 9% of the respondents suffered from obesity, hypertension, locomotor and dental problems for past 3 years respectively. The presence of obesity for >one year showed a significant positive association with the presence of FC. Presence of NCDs including hypertension, Diabetes and insomnia did not show any significant association with constipation. However, presence of obesity, locomotor problems and dental problems were seen to be positively associated with the presence of FC among the subjects ($p<0.05$).

Table 5.1.2: Number (%) of subjects suffering from medical illnesses and their association with presence of functional constipation (N= 364)

Medical History	Total Subjects N (%)				
	No	**>3 years**	**1-3 years**	**<1 year**	χ^2
Obesity	152 (41.04%)	58 (15.66%)	71 (19.17%)	83 (22.41%)	**0.126***
Hypertension	296 (81.4%)	42 (11.5%)	20 (5.5%)	6 (1.1%)	**0.088^{NS}**
Diabetes	338 (92.6%)	13 (3.6%)	9 (2.5%)	4 (1.1%)	**0.081^{NS}**
CHD	360 (99%)	2 (0.5%)	-	2 (0.5%)	-
Locomotor problems (Knee, Joint and back pain)	272 (74.8%)	25 (6.5%)	26 (7.1%)	41 (11.2%)	**0.137***
Dental Problems (Dry mouth and **Cavities)**	284 (78.1%)	34 (9.3%)	28 (7.7%)	18 (4.9%)	**0.104***
Hypothyroidism	334 (91.85%)	15 (4.13%)	5 (1.38%)	10 (2.75%)	-
Insomnia	348 (95.7%)	5 (1.38%)	7 (1.93%)	6 (1.1%)	**0.037^{NS}**

Levels of Significance * $p < .05$, NS= Not significant

The respondents were also categorized as per their calculated BMI with respect to Asia Pacific classification. Fig 5.1.1, depicts that 44% of the subjects were obese, among which 29% respondents fell in the obese grade I category (BMI 25-29.9 kg/m^2), 13% subjects were in Obese II and 2% subjects were in Obese III category.

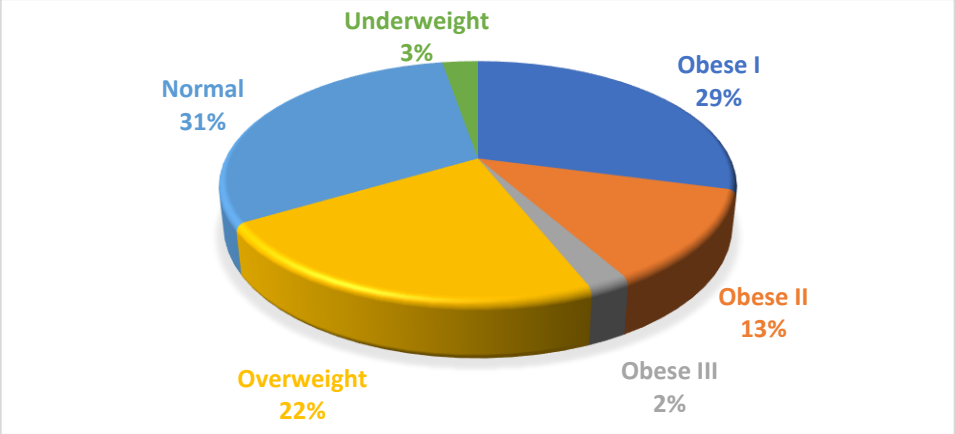

Fig 5.1.1: Obesity profile of the respondents as per BMI

A total of 216 female respondents were screened for their menstrual profile. Forty percent, 35% and 1% females in the age group of 20-34, 35-49 and 50-64 years had regular menstrual cycles respectively. Literature review has indicated presence of symptoms of constipation prior to the menses and menopause. Twenty four percent females among these female respondents had already attained menopause (Fig 5.1.2). No significant association was observed between menopause and presence of constipation.

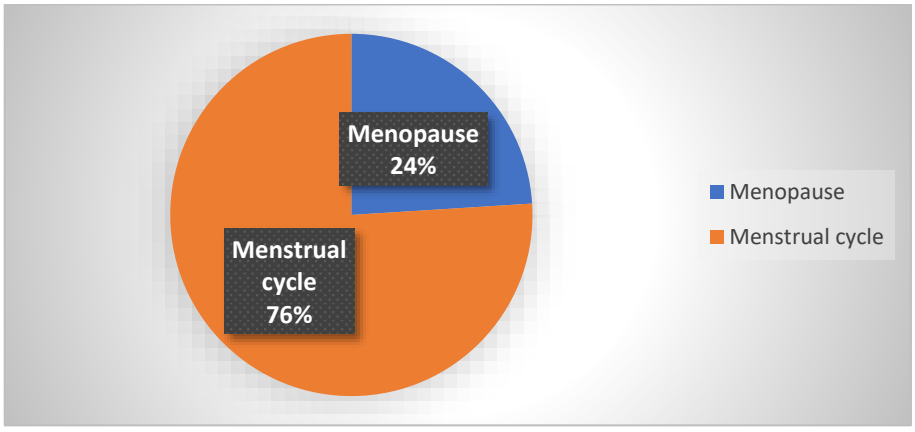

Fig 5.1.2: Menstrual profile of the respondents

Section 5.1.3: Family history of constipation profile of the respondents and its association with presence of Functional Constipation

The subjects were screened for their family history of FC. Table 5.1.3 revealed that 13% of the either of the parents were suffering from constipation for >3 years. However, not enough information was obtained for the grandparents with regards to constipation. Presence of FC in the mothers of the respondents showed a positive significant association with the presence of FC ($p<0.05$) among the subjects. However, presence of FC in the father and siblings did not significantly associate with the presence of FC in the respondents.

Table 5.1.3: Number (%) of subjects with a family history of constipation and its association with presence of Functional Constipation (N= 364)

	Total Subjects N (%)						
Family Members	**No**	**>3 years**	**1-3 years**	**<1 year**	**Others**		**r value**
					Do not know	Not Applicable	
Parents							
Father	294 (80.77)	44 (12.09%)	8 (2.2%)	6 (1.65%)	12 (3.3%)	-	**0.018^{NS}**
Mother	292 (80.22%)	50 (13.74%)	8 (2.2%)	6 (1.65%)	8 (2.19%)		**-0.111***
Siblings							
Brother	318 (87.36%)	6 (1.65%)	5 (1.37%)	5 (1.37%)	12 (3.3%)	18 (4.95%)	**0.011 ^{NS}**
Sister	314 (86.26%)	8 (2.19%)	4 (1.1%)	6 (1.65%)	10 (2.75%)	22 (6.04%)	**0.023 ^{NS}**
Grandparents							
Grandmother	294 (80.77%)	12 (3.3%)	- 2 (0.55%)	4 (1.1%)	40 (10.99%)	14 (3.85%)	-
Grandfather	308 (84.62%)	-		-	40 (10.99%)	14 (3.85%)	

Levels of Significance * $p < .05$, NS= Not significant

Section 5.1.4: Personal habits profile of the respondents and its association with presence of functional constipation

Table 5.1.4 showed most subjects never consumed alcohol, tobacco and drugs and hence no significant association was reported with the presence of FC. A Daily consumption of tea accounted for 29% of the respondents which was recorded to be the daily habit among other habits, which was seen to have a significant positive association with the presence of FC ($p<0.05$). However, occasional consumption was recorded of alcohol and colas accounting for 13% and 22% respectively.

Table 5.1.4: Number (%) of subjects with specific personal habits its association with presence of functional constipation (N= 364)

Personal Habits	***Total Subjects N (%)***				
	Never	**Daily**	**Weekly**	**Occasionally**	**r value**
Alcohol	302 (82.97%)	12 (3.3%)	2 (0.55%)	46 (12.64%)	**0.02 NS**
Cigarette	330 (90.66%)	24 (6.59%)	2 (0.55%)	8 (2.2%)	**0.023 NS**
Tobacco	336 (92.31%)	18 (4.95%)	-	10 (2.75%)	-
Tea >2cups/Day	200 (54.95%)	105 (28.85%)	22 (6.04%)	38 (10.44%)	**0.102***
Coffee >2cups/day	204 (56.04%)	35 (9.62%)	15 (4.12%)	56 (15.38%)	**0.041 NS**
Colas	208 (56.16%)	10 (2.75%)	38 (4.95%)	108 (22.53%)	**0.011 NS**
Drugs	364 (100%)	-	-	-	-

Levels of Significance * $p < .05$, NS= Not significant

Section 5.1.5: Physical activity profile of the respondents and its association with presence of functional constipation

Table 5.1.5 depicts the exercise and physical activity patterns of the subjects, which revealed that 32% respondents were brisk walking >30 minutes on a daily basis followed by 19% subjects who were following a daily yoga routine. Regular physical activity showed a significant negative correlation with the presence of FC among the teaching staff ($p<0.05$). It also revealed that 70% subjects were sitting for more than 8 hours in a day, which showed a positive significantly association with the presence of functional constipation among the respondents ($p<0.01$).

Table 5.1.5: Number (%) of subjects' physical activity profile and its association with presence of functional constipation (N= 364)

Physical Activity	***Total Subjects N (%)***				
	Never	**Daily**	**Weekly**	**Occasionally**	χ^2
Cycling >30 Mins	254 (69.85%)	24 (6.6%)	34 (9.35%)	52 (14.3%)	
Brisk walking >30 mins	113 (31.08%)	117 (32.18%)	62 (17.05%)	72 (19.8%)	
Jogging/Running	220 (60.44%)	43 (11.81%)	38 (10.44%)	61 (16.76%)	
Yoga >30 min	175 (48.08%)	69 (18.96%)	53 (14.56%)	67 (18.41%)	**-0.113***
Gym >30 min	294 (80.77%)	29 (7.97%)	9 (2.47%)	32 (8.79%)	
Dance/Zumba >30 min	306 (84.07%)	11 (3.02%)	10 (2.75%)	37 (10.16%)	
No. of Hours sitting in a Day?					
<8 Hours			106 (29.1%)		
8-12 hours			222 (60.99%)		**9.09****
>12 hours			36 (9.89%)		

Levels of Significance: * $p < .05$, ** $p < 0.01$

Note: For the entirety of the Physical activity profile, the subjects received a total score out of 24. To determine the relationship between FC and physical activity profile, the subjects were categorized as Good, Fair, and Poor, and the data was then subjected to chi square analysis.

Section 5.1.6: Perceptions and practices of the respondents about constipation and their association with presence of functional constipation

The subjects were screened for their perceptions, general and dietary practices related to constipation, which are considered important as they are seen to be correlated with the presence of FC in the available literature. The results were presented in number percent and chi square associations with appropriate significance levels.

Table 5.1.6.1 shows the respondents with specific perceptions regarding FC. Eleven percent considered FC to be a social taboo and 57% subjects have suffered from FC at least once in their lifetime. Fifty two percent subjects considered FC to be a minor health issue and this showed a positive significant association with the presence of FC among the respondents ($p<0.05$).

Table 5.1.6.1: Number (%) of subjects with perceptions related to constipation and their association with presence of functional constipation (N=364)

Specific questions	Total Subjects N (%)		χ^2
	Yes	No	
Do you consider constipation as a "social taboo"?	41 (11.26%)	323 (88.74%)	**0.03** NS
Do you consider constipation as a minor health issue?	191 (52.47%)	173 (47.53%)	**0.12***
Have you ever suffered from constipation once in this lifetime?	206 (56.59%)	158 (43.41%)	**0.8** NS
Will you feel shy to seek medical help for constipation?	20 (5.49%)	344 (94.51%)	**0.04** NS
Are you able to digest milk and milk products?	346 (95.05%)	18 (4.95%)	**0.023** NS
Rating of personal gastrointestinal function (Scale of 1-5, 1 being best and 5 being worst)			
1	102 (28.02%)		**0.5** NS
2	147 (40.38%)		
3	84 (23.08%)		
4	31 (8.52%)		

Levels of Significance * p < .05, NS= Not significant

Table 5.1.6.2 depicts the general practices related to constipation which revealed that 66% subjects had a set daily regime for defecation; 70% of respondents used western toilet and 64% subjects defecated in the straight position. The presence of FC was significantly negatively associated with subjects not having a fixed defecation regime ($p<0.05$) or using a western toilet ($p<0.05$) and defecating in straight position style ($p<0.05$). Forty five percent subjects considered a wait period of 1-2 weeks before consulting any doctor for FC. Eleven percent of the survey participants occasionally used laxatives.

Table 5.1.6.2: Number (%) of subjects with general practices related to constipation and their association with presence of functional constipation(N= 364)

Specific questions	***Total Subjects N (%)***	χ^2
What is your "wait period" before seeking medical help, if you ever/will ever have constipation/ irregular stool pattern?		
Immediately	136 (37.36%)	
1-2 weeks	165 (45.33%)	**0.041** **NS**
15 days- 1 month	34 (9.34%)	
>2 months	29 (7.97%)	
What will you do first, if you ever have/whenever you had constipation?		
Treat with Home remedies	303 (83.24%)	
Seek medical help	42 (11.54%)	**0.05**[NS]
Self-medication	19 (5.22%)	
Do you have a fixed daily schedule for defecation?		
Almost always	242 (66.48%)	**-0.118***
Usually	103 (28.3%)	
Not fixed	19 (5.22%)	
What toilet style are you using currently?		
Indian Style	78 (21.43%)	**-0.107***
Western style	286 (78.57%)	
What position for defecation are you following currently? (For western Style : N= 286)		
Straight	183 (63.99%)	**-0.119***
Front bend	103 (36.01%)	
Do you take Laxatives?		
No	305 (83.79%)	
Sometimes	43 (11.81%)	**0.035**[NS]
Often	14 (3.85%)	
Daily	2 (0.55%)	

Levels of Significance * $p < 0.05$, NS= Not significant

The specific dietary habits or practices of the respondents are presented in Table 5.1.6.3.

Table 5.1.6.3 Number (%) of subjects with dietary practices related to constipation and their association with presence of functional constipation (N= 364)

Specific questions	Total Subjects N(%)	χ^2
Do you take supplements?		
No	241 (66.21%)	
Multivitamins	75 (20.6%)	**12.56^{NS}**
Calcium	37 (10.16%)	
Iron	11 (3.02%)	
How many time do your chew your food/bite?		
>20 times	62 (17.03%)	
10-20 times	96 (26.37%)	**-9.44***
<10 times	84 (23.1%)	
Unknown	122 (33.52%)	
How much water do you drink in a day?		
>2 liters	25 (6.87%)	**-17.78****
1-2 liters	141 (38.74%)	
<1 liter	198 (54.4%)	
Do you have any triggering food for constipation?		
Yes	90 (24.73%)	-
No	274 (75.27%)	
Do you follow any home remedies for Constipation?		
Yes	182 (50%)	-
No	146 (40.11%)	
Sometimes	36 (0.89%)	
If yes, what are they?		
Warm water/ Lukewarm water	154 (42.3%)	
Isabgol	116 (31.9%)	-
Banana	42 (11.5%)	
Buttermilk/ Curd	28 (7.7%)	
Ajwain	24 (6.6%)	
Which food groups might cause constipation for you?		
Milk	227 (62.36%)	
Cereals (Maida, Bread)	96 (26.37%)	
Tea/coffee	3 (0.82%)	-
Less water	36 (9.89%)	
Pulses	2 (0.55%)	

	Breakfast	**Lunch**	**Snacks**	**Dinner**	
Do you eat out?					
No	305 (83.79%)	274 (75.27%)	246 (67.58%)	124 (34.07%)	
Sometimes	45 (12.36%)	54 (14.84%)	74 (20.33%)	144 39.56%)	**10.12^{NS}**
Often	14 (3.85%)	26 (7.14%)	44 (12.09%)	94 (25.82%)	
Daily	-	-	-	-	

Levels of Significance: *p<0.05; ** p <0.01, NS= Not significant

Fifty four percent subjects consumed less than 1 liter of water each day, which was seen to have a strong significant negative association with the presence of FC ($p<0.01$). Dietary practices such as chewing food also showed a significant negative association with the presence of FC ($p<0.05$). Twenty five percent subjects used home remedies for relieving from FC. Among the natural treatments for constipation, warm water (42%) and isabgol or psyllium husk (32%) are frequently used by the subjects. The respondents said that milk (62%) and cereals (26%) were the top most foods of concern which might be a cause of FC for them. Most subjects didn't practice eating out regularly and hence no significance was reported with presence of FC among the respondents.

Section 5.1.7: Constipation profile of the respondents

The subjects were screened for the presence of functional constipation on the basis of Rome IV Criteria, Bristol stool chart type and WHO based constipation definition (<3 stools/week). A total of 8 questions was administered on a scale of 20. The scoring method is explained in detail in the Materials and Methods chapter.

The constipation profile (consisting of eight parameters) of all of the sedentary teaching staff is presented in table 5.1.7. As per the WHO criteria, 24% of the subjects had less than three bowel movements per week. Those who reported having Bristol stool type 1 and type 2, both of which are regarded to be indicative of FC, made up 5% and 12% of the total sample, respectively. According to the Rome IV criteria, which consists of six parameters, 40% subjects experienced incomplete bowel movement sometimes, 25% individuals strained while defecating sometimes, 10% participants suffered from extreme flatulence always, 7% had to practice manual removal of stool sometimes, 10% felt a blockage in their intestines sometimes and 16% of the respondents reported occasional suffering from abdominal pain while defecating.

Table 5.1.7 also showed that majority of the respondents reported medium (42%) and weak (42%) smell in their stool. The stool volume was not of concern for the respondents, as 92% reported it to be normal.

Table 5.1.7: Number (%) of subjects suffering from constipation and its symptoms (N=364)

	Parameters	Total Subjects N (%)
WHO criteria	**1. Frequency of Stools/ week**	
	<3	90 (24.73%)
	3 and more	190 (52.2%)
	Daily	84 (23.08%)
Bristol stool chart	**2. Stool Type (As per Bristol stool chart)**	
	Type 1	17 (4.67%)
	Type 2	45 (12.36%)
	Type 3	39 (10.71%)
	Type 4	179 (49.18%)
	Type 5	41 (11.26%)
	Type 6	34 (9.34%)
	Type 7	9 (2.47%)
Rome IV criteria	**3. Do you experience incomplete bowel movement?**	
	No	189 (51.92%)
	Sometimes	147 (40.38%)
	Always	28 (7.69%)
	4. Do you Need to apply Straining?	
	No	266 (73.08%)
	Sometimes	91 (25%)
	Always	7 (1.92%)
	5. **Do you experience Excessive Gas/ Flatulence?**	190 (52.2%)
	No	120 (32.97%)
	Sometimes	34 (9.34%)
	Always	
	6. Do you practice Manual maneuvers of stool removal?	
	No	333 (91.48%)
	Sometimes	27 (7.42%)
	Always	4 (1.1%)
	7. Do you experience Blockage in the intestine (anorectal blockage)?	
	No	318 (87.36%)
	Sometimes	36 (9.89%)
	Always	10 (2.75%)
	8. Do you experience abdominal pain while passing stools?	
	No	303 (83.24%)
	Sometimes	58 (15.93%)
	Always	3 (0.82%)

Other questions	**What is the odor of your stool?**	
	Strong	6 (1.65%)
	Medium	152 (41.76%)
	Weak	154 (42.31%)
	No smell	52 (14.29%)
	What is the Stool Volume?	
	Normal	336 (92.31%)
	Less than Normal	28 (7.69%)

From the above profile, the subjects suffering from FC were selected. Of the 364 subjects, 20% subjects (n=70) were suffering from functional constipation. Among them, 15% (n=55) subjects was categorized as mildly constipated, 3% (n=12) as moderately constipated and 0.5% (n=3) was severely constipated (Fig 5.1.3).

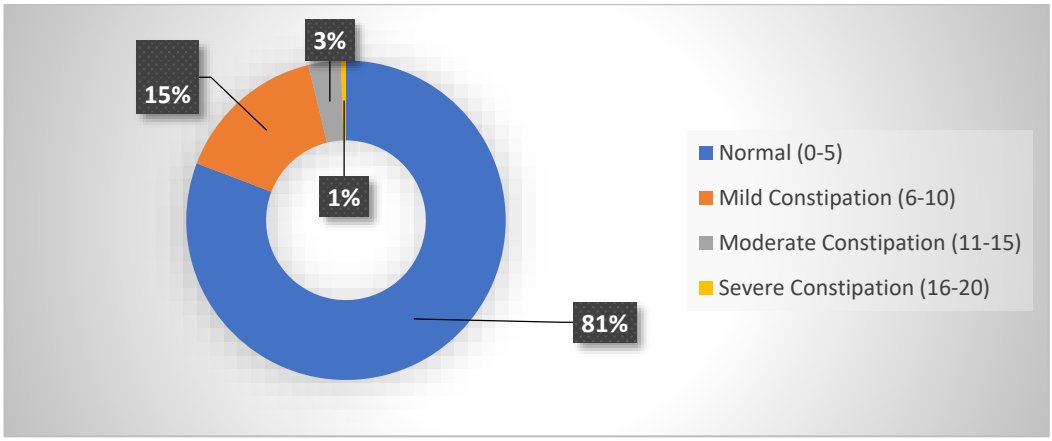

Fig 5.1.3: Constipation categorization of the respondents

Of the 70 subjects suffering from functional constipation, 26 subjects (37%) belonged to the age group 20-34, 16 subjects (23%) were in 35-49 years and 28 subjects (40%) were in 50-64 age category (Fig 5.1.4). As age of the subjects increased, the presence of FC was seen to be significantly increased ($p<0.05$).

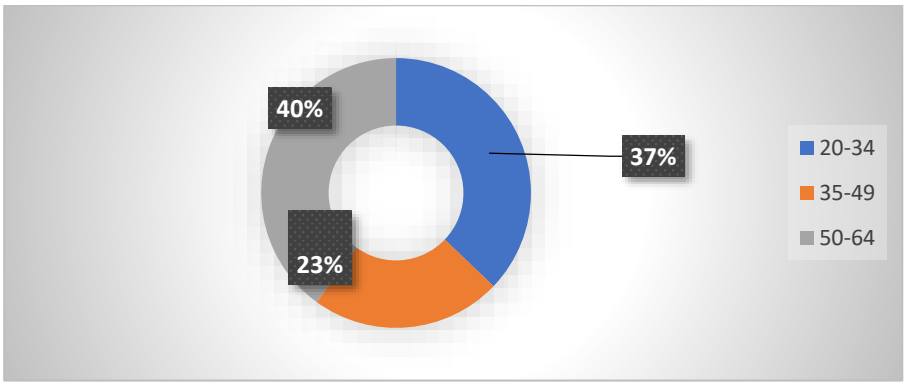

Fig 5.1.4: Age group Distribution of Constipated respondents

Of the 70 subjects suffering from FC, 71% (n=50) were females and 20 subjects (29%) were males (Fig 5.1.5) which stated that females were comparatively more prone to suffer from FC than the males.

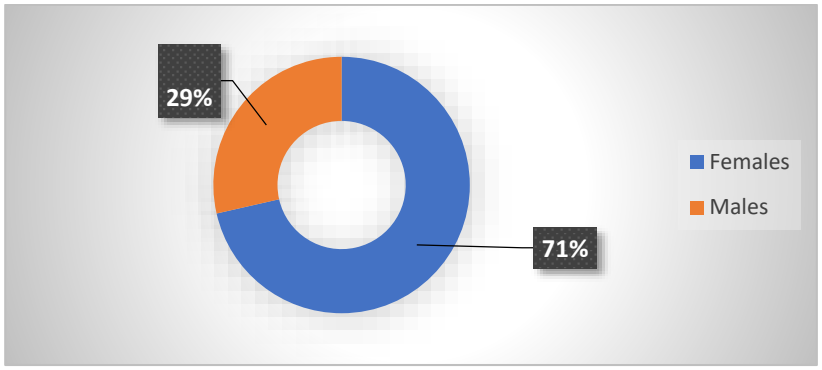

Fig 5.1.5: Gender Distribution of constipated respondents

The constipated subjects were further categorized on the basis of their BMI (Fig 5.1.6). Of the 70 subjects suffering from functional constipation, 26% subjects were obese I category, 9% were in obese II category and 3% were in obese III category

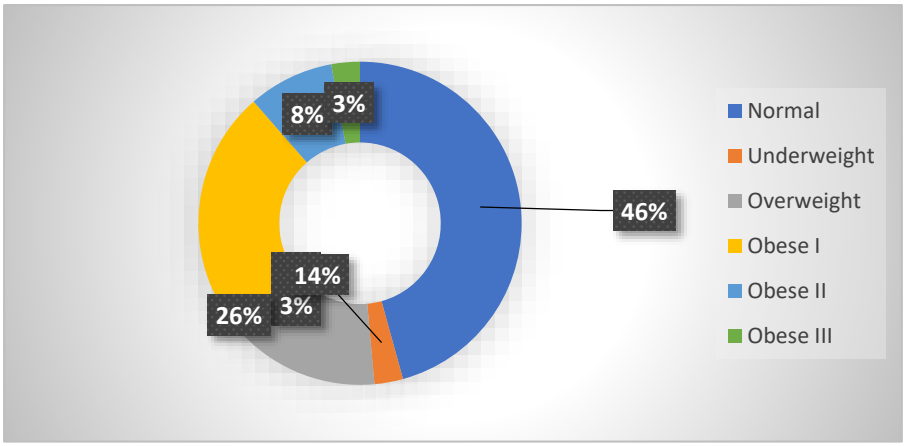

Fig 5.1.6: BMI categorization of constipated respondents

Section 5.1.8: Chrononutrition profile of the respondents and their association with presence of functional constipation

The subjects were screened for chrononutrition profile on a working day and on a free day (Table 5.1.8.1;Table 5.1.8.2) which comprised of 6 questions. Appropriate marks were assigned as per the responses. Correlation with the constipation profile was assessed further to check for any associations. The results are expressed in terms of number percent.

According to the findings in Table 5.1.8.1, 17% of the subjects skipped their breakfast daily. The majority of respondents (50%) indicated that lunch is their largest meal of the day whereas 17% subjects stated breakfast to be their largest meal. No significant associations were recorded between any parameters with the presence of FC as well as the chrono nutrition profile on a working day with the presence of FC.

Table 5.1.8.1 : Number (%) of subjects' chrono nutrition profile and its association with the presence of constipation on a working day (N=364)

Parameters	Presence of Constipation (N%)		
	Yes (N=70)	No (N=294)	$\chi 2$
Do you skip Breakfast?			
No	40 (57.14%)	230 (78.43%)	**3.49**^{NS}
Sometimes	16 (22.86%)	40 (13.61%)	
Often	3 (2.86%)	20 (6.8%)	
Daily	11(17.14%)	4 (1.36%)	
Largest meal of the Day			
Breakfast	16 (22.86%)	46 (15.75%)	**2.89**^{NS}
Lunch	32 (45.71%)	148 (50.34%)	
Snacks	-	2 (0.68%)	
Dinner	22 (31.43%)	98 (33.33%)	
Duration between First and last Meal?			
<12 hours	42 (60%)	166 (56.46%)	**1.9** ^{NS}
12-14 hours	26 (37.14%)	100 (34.01%)	
>14 hours	2 (2.86%)	28 (9.52%)	
Duration between last Meal and sleep Onset?			
>6 hours	6 (8.57%)	38 (12.93%)	**1.13** ^{NS}
2.01-6 hours	34 (48.57%)	168 (57.14%)	
<2 hours	30 (42.86%)	88 (29.93%)	
Time range for Last Meal of Day?			
Before 8.00 PM	14 (20%)	74 (25.17%)	**1.78**^{NS}
8-10.59 PM	54 (77.14%)	210 (71.43%)	
11.00 PM and after	2 (2.86%)	10 (3.4%)	
Do you wake up at night to eat?			
Yes	4 (5.62%)	4 (1.36%)	**5.95** ^{NS}
Sometimes	2 (2.86%)	24 (8.16%)	
Rare	4 (5.62%)	14 (4.76%)	
No	60 (85.71%)	252 (85.71%)	
Chrononutrition Profile on a working Day	**-0.159** ^{NS}		

Levels of Significance * p <0.05, NS = Not significant

According to Table 5.1.8.2, 35% subjects skip their breakfast on a working day from which 23% was shown to have the presence of FC. Ninety five percent subjects considered lunch to be the largest meal of the day from which 45% was reported to have the presence of FC. Eighty three percent subjects reported a time gap of < 2 hours between their last meal and sleep onset which showed a significant positive association with the presence of FC ($p<0.05$).

Table 5.1.8.2 : Number (%) of subjects' chrono nutrition profile and its association with the presence of constipation on a free day (N=364)

Parameters	Presence of Constipation Subjects (N%)		
	Yes (N=70)	No (N=294)	$\chi 2$
Do you skip Breakfast?			
No	40 (57.14%)	230 (78.43%)	**3.49** ^{NS}
Sometimes	16 (22.86%)	40 (13.61%)	
Often	3 (2.86%)	20 (6.8%)	
Daily	11 (17.14%)	4 (1.36%)	
Largest meal of the Day			
Breakfast	16 (22.86%)	46 (15.75%)	-
Lunch	32 (45.71%)	148 (50.34%)	
Snacks	-	2 (0.68%)	
Dinner	22 (31.43%)	98 (33.33%)	
Duration between First and last Meal?			
<12 hours	46 (65.71%)	172 (58.5%)	**1.25** ^{NS}
12-14 hours	22 (31.43%)	98 (33.33%)	
>14 hours	2 (2.86%)	24 (8.16%)	
Duration between last Meal and sleep Onset?		38 (12.93%)	**0.93***
>6 hours	8 (11.43%)	158 (53.74%)	
2.01-6 hours	28 (40%)	98 (33.33%)	
<2 hours	34 (48.57%)		
Time range for Last Meal of the Day			
Before 8.00 PM	6 (8.57%)	58 (19.73%)	**0.59***
8-10.59 PM	60 (85.71%)	224 (76.19%)	
11.00 PM and after	4 (5.71%)	12 (5.08%)	
Do you wake up at night to eat?			
Yes	4 (5.62%)	4 (1.36%)	**5.95**^{NS}
Sometimes	2 (2.86%)	24 (8.16%)	
Rare	4 (5.62%)	14 (4.76%)	
No	60 (85.71%)	252 (85.71%)	
Chrononutrition Profile on a Free Day		**-0.13***	

Levels of Significance * $p<0.05$, NS = Not significant

Note: For the entirety of the chrono nutrition profile, the subjects received a total score out of 30. To determine the relationship between FC and chrono nutrition profile, the subjects were categorized as good, fair, and poor, and the data was then subjected to chi square analysis.

Eighty five percent subjects who reported to have FC had their meals after 8pm and this was seen to be significantly associated with the presence of FC among the subjects ($p<0.05$). Mostly the subjects never woke up at night for snacking hence no significant association was reported with the presence of FC. However, the chrononutrition profile on a free day was shown to have a significant negative association with the presence of FC among the sedentary university teaching staff ($p<0.05$) which suggested subjects tend to deviate from their working day lifestyle on a free day which result in poor chrono nutrition status or lifestyle pattern and can be a contributing factor for FC.

Section 5.1.9- Baseline information on dietary intake of constipated subjects and their association with presence of functional constipation

The subjects were assessed for their dietary profile in terms of their type of diet consumed and macronutrients and dietary fiber measure. Fig 5.1.7 shows the % of subjects consuming different types of diets. Sixty two percent of the respondents followed a vegetarian diet, 26% adhered to a non-vegetarian diet, and 41% subjects followed other diets, which included Jain food, ovo-vegetarian food, or lacto-ovo vegetarian food. Table 5.1.9.1 reveals 57% subjects suffering from FC were vegetarians, 21% were non-vegetarian and 11% followed other diets. No statistically significant association between the various types of diet consumed and the presence of FC.

Baseline data was collected for the dietary intake of the constipated subjects using 24 hours recall method for 3 days (n=40). Macronutrients such as carbohydrate, protein, fats, total dietary fiber, soluble and insoluble fiber were measured before supplementation trial. Table 5.1.9.2. presented the amounts of the nutrients expressed as % Estimated Average Requirements (EAR) and % Recommended Daily Allowance (RDA) of females as that is the predominant gender among this population. The respondents were having 68%, 110% and 141% of their energy, protein and carbohydrate intake respectively as per EAR. Total dietary fiber and soluble fiber was shown to have significant negative correlation with the presence of FC ($p<0.05$).

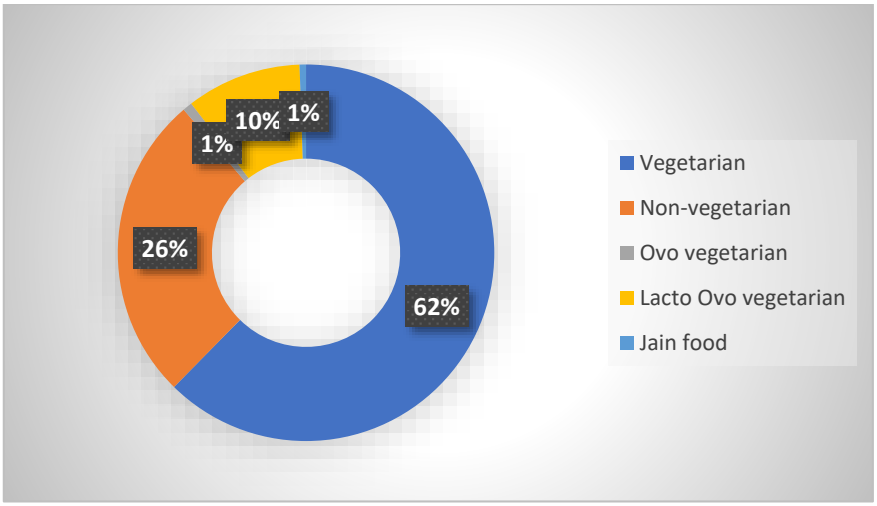

Fig 5.1.7: Eating practices of subjects

Table 5.1.9.1: Number (%) of subjects' type of diet consumed and its association with the presence of constipation (N=364)

Type of Diet consumed	Presence of Constipation (N%)		
	Yes (N=70)	No (N=294)	$\chi 2$
Vegetarian Non-vegetarian Other diets (Ovo vegetarian, Lacto-Ovo vegetarian, Jain food)	40 (57.14%) 22 (31.43%) 8 (11.43%)	182 (61.9%) 74 (25.17%) 38 (12.92%)	**0.089**^{NS}

NS= Not significant

Table 5.1.9.2: Percent calorie and nutrient intake of RDA and EAR by constipated subjects before supplementation (N=40)

Nutrients	Results	%RDA (n=40)	% EAR (n=40)	'r' value
Energy (kcal/day)	1130±160.4	-	68.07%	**0.081**^{NS}
Protein (g/day)	39.8±5.41	86.5%	110.56%	**0.037**^{NS}
Carbohydrate(g/day)	141.1±28.5	117.5%	141%	**0.47**^{NS}
Fats (g/day)	42.5±8.71	85%	-	**-0.085**^{NS}
Total dietary Fiber (g/day)	18.33±4.41	73.32%	-	**-0.137***
Soluble fiber (g/day)	2.92 ±1.49	46.72%	-	**-0.126***
Insoluble Fiber(g/day)	11.55 ±6.94	61.6%	-	**-0.088**^{NS}

*p<0.05, NS= Not significant

Section 5.1.10: Food frequency of the respondents and its association with constipation status

Table 5.1.10 shows the frequency of consumption of various food groups and its association with the presence of FC.

Table 5.1.10: Number (%) of subjects' food frequency of various foodstuffs and its association with constipation status (N=364)

Food Groups	Total Subjects N (%)						χ^2
	Daily	2-3 times/week	Weekly once	Fortnightly	Monthly once	Never	
Fruits (1serving)	169 (46.43%)	147 (40.38%)	29 (7.97%)	7 (1.92%)	12 (3.3%)	-	**-5.29** ^{NS}
Milk and Milk products (>200ml)	246 (67.58%)	54 (14.84%)	35 (9.62%)	9 (2.47%)	16 (4.4%)	4 (1.1%)	**7.04** ^{NS}
Whole pulses (1 katori)	93 (23.55%)	187 (51.37%)	67 (18.41%)	8 (2.2%)	7 (1.92%)	2 (0.55%)	**-10.64***
Split Pulses (1 katori)	81 (22.25%)	189 (51.92%)	77 (21.15%)	14 (3.85%)	3 (0.82%)	-	**-11.47***
Green leafy vegetables (1 katori)	59 (16.21%)	199 (54.67%)	80 (21.98%)	18 (4.95%)	8 (2.2%)	-	**-0.54** ^{NS}
Vegetable A (Gourd family/ parwal) (1 katori)	66 (18%)	178 (48.63%)	89 (24.45%)	12 (3.3%)	12 (3.3%)	8 (2.2%)	**-0.25** ^{NS}
Vegetable B (Onion/carrot etc) (1 katori)	167 (45.88%)	153 (42.03%)	36 (9.89%)	3 (0.82%)	2 (0.55%)	3 (0.82%)	**-10.29**^{NS}
Non veg foods (Eggs/ Fish/ Chicken)	20 (5.49%)	48 (13.19%)	42 (11.54%)	11 (3.02%)	14 (3.85%)	229 (62.91%)	**-6.09** ^{NS}
Natural Sweeteners (Jaggery/ Honey)	117 (32.14%)	75 (20.6%)	48 (13.19%)	57 (15.66%)	10 (2.75%)	57 (15.66%)	**-15.24****
Artificial Sweeteners	12 (3.3%)	22 (6.04%)	10 (2.75%)	2 (0.55%)	10 (2.75%)	302 (84.62%)	**0.66** ^{NS}
Processed foods							
Chips/French fries	5 (1.37%)	37 (10.16%)	52 (14.29%)	52 (14.29%)	112 (30.77%)	106 (29.12%)	**0.14** ^{NS}
Biscuits/Cookies	45 (12.36%)	74 (20.4%)	69 (18.96%)	50 (13.74%)	66 (18.13%)	60 (16.48%)	**0.54** ^{NS}
Burgers/pizza	-	8 (2.2%)	27 (7.42%)	44 (12.09%)	187 (51.37%)	98 (26.92%)	-
Ready to eat foods	3 (0.82%)	13 (3.57%)	27 (7.42%)	36 (9.89%)	116 (31.87%)	169 (46.43%)	**0.54** ^{NS}

Intake/day	Total Subjects (N%)	$\chi 2$
What is your sugar Intake/day?		
I don't take sugar	86 (23.63%)	
<2 tsp	146 (40.11%)	**9.29***
2-4 tsp	112 (30.77%)	
>4 tsp	20 (5.49%)	
What is your salt Intake/day?		
I don't take salt	8 (2.2%)	
<1 tsp	200 (54.95%)	-
1-2 tsp	128 (35.16%)	**12.27****
>2 tsp	28 (7.69%)	

* p < .05, ** p < .01, NS= Not significant

Milk and milk products (68%) were the most commonly consumed food group on a daily basis followed by fruits (46%) and vegetables including carrots onions. The other food groups consumed upto 55% 2-3 times/ week was whole (55%) and spilt pulses (52%) and green leafy vegetables (55%). Least consumed food group by the subjects were processed foods and artificial sweeteners. The population under study was predominantly vegetarians hence they almost never took nonveg (63%).

Chi square test was applied to determine the association between consumption of various food groups on a daily basis, 2-3 times/week and weekly with the constipation status of the subjects. Significant negative associations was recorded with the consumption of whole pulses ($p<0.05$), split pulses ($p<0.05$), natural sweeteners (jaggery) ($p<0.01$) with the presence of FC.

Majority of respondents consumed sugar <2tsp (40%) which was seen to have a positive association with presence of FC ($p<0.05$). Low intake of salt was recorded in the subjects (55%). Intake of salt showed a significant negative correlations with the presence of constipation among the respondents which indicated a lower intake of salt benefits from FC ($p<0.05$).

RESULT HIGHLIGHTS (PHASE I)

- 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 comparatively 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 ($p<0.05$) with the presence of FC in the respondents. However presence of FC in the fathers and siblings did not significantly correlate with FC among the respondents.
- 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 subjects ($p<0.05$).
- Consumption of tea >2 cups/day showed a positive association with FC ($p<0.05$).
- Significant association was observed between the chrononutrition profile on a free day with the constipation profile ($p<0.05$).
- Seventy eight percent of the respondents were using a western toilet style where in 64% subjects were defecating in the straight position and these practices was significantly correlated with the presence of FC ($p<0.05$).
- Fifty two percent subjects felt constipation is a minor health issue which showed a positive association with presence of FC ($p<0.05$).
- Dietary practices such as chewing food and water intake was significantly associated with the presence of constipation ($p<0.05$; $p<0.01$).
- 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 consumption of whole and split pulses ($p<0.05$) and jaggery ($p<0.01$) with the presence of FC.
- Low intake of salt ($p<0.01$) and high intake of sugar ($p<0.05$) had significant negative correlation with the presence of constipation among the respondents.

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

Oligosaccharides are an essential component in our daily diets which is largely utilized for their various health benefits. However, they are gaining worldwide popularity for their physiological effect on the gut microbiome. Galactooligosaccharide (GOS), a prebiotic with the ability to provide health advantages, has the potential to penetrate the market. In addition to health benefits, oligosaccharides can be integrated into food products to improve their taste and texture owing to their physicochemical properties (Collins and Rastall, 2008). Hence, this phase of the study focused on feasibility and acceptability of GOS added gummies.

Standardization of gummies included using different concentration of the ingredients namely water, sugar, agar and citric acid to obtain the most acceptable product. Sugar was further replaced with varying proportions of GOS from 60%-100%. All the products were tested by the trained panel in triplicates to minimize error. The accepted GOS gummy was then subjected to physico chemical, recovery and shelf life analyses.

The results of this phase are presented under the following heads:

5.2.1- Sensory evaluation with respect to standardisation of gummies

5.2.2- Substitution of sugar with varying concentration of GOS

5.2.3- Physico chemical properties of GOS gummies

5.2.4- Recovery of galactooligosaccharide (GOS) in gummies

5.2.5- Shelf life studies of GOS gummies

Section 5.2.1.Sensory evaluation with respect to standardisation of gummies

According to Sessler, Weiss and Vodovotz (2013), confectionery such as gummies are food matrices which, due to their popularity among consumers, are suitable for the addition of functional ingredients such as vitamins, antioxidants, fiber and probiotics microorganisms. Hence gummies were selected as the vehicle for clinical trial.

The mean scores, standard deviation, and F test results for sensory evaluation of gummies prepared with different concentrations of water, sugar, agar, citric acid, and

GOS are presented in tables 5.2.1.1–5.2.1.4 respectively. The trained panel did not find any significant differences in the sensory properties of gummies prepared with different concentrations of water, sugar, and agar. In terms of aftertaste, overall taste, acceptability, and total score, F value revealed a significant difference in the gummies with varied quantities of citric acid. As the levels of citric acid increased from 1/8th tsp to 1tsp, a significant increase in after taste and flavor was observed.

The standard gummies were formulated with 75 ml water, 2 g agar, 1.9 g citric acid, 60g sugar which yielded 24 gummies in one batch.

Table 5.2.1.1 Mean values for sensory qualities of gummies standardized with varying levels of water

		SENSORY QUALITIES								
Levels of Water		Color and Appearance	Mouthfeel	Texture	Overall Taste	Flavor	After taste	Chew ability	Overall Acceptability	Total Score
Maximum Score		(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(80)
55ml	Mean	8.77	7.85	8.08	7.69	7.46	7.62	7.69	8	63.15
	SD	±1.09	±0.8	±0.95	±0.94	±1.2	±0.87	±1.32	±0.91	±6.82
	Range	7-10	7-9	7-10	6-9	6-9	6-9	6-9	7-10	54-73
65ml	Mean	8.61	7.85	7.61	7.38	7.23	7.38	7.69	7.69	61.46
	SD	±1.33	±0.9	±0.96	±1.19	±1.59	±1.33	±1.32	±1.38	±8.77
	Range	6-10	7-9	6-9	6-9	5-10	6-10	6-10	6-10	51-76
75ml	Mean	8.69	8.38	8.15	7.77	7.92	7.92	7.85	8.15	64.85
	SD	±1.11	±0.87	±1.07	±1.42	±1.19	±1.19	±1.41	±1.41	±8.36
	Range	7-10	7-10	6-10	6-10	6-10	6-9	6-10	6-10	53-77
85ml	Mean	8.23	7.38	7.38	6.92	7.08	7.38	7.15	7.46	59
	SD	±1.3	±1.04	±1.12	±1.44	±1.38	±0.96	±1.14	±1.19	±8.66
	Range	6-10	6-9	6-9	5-9	5-9	6-9	6-9	6-10	46-73
	Anova (F)	**0.51**	**2.64**	**1.67**	**1.19**	**0.97**	**0.7**	**0.71**	**0.81**	**1.21**
	P value	0.68 ^{NS}	0.06 ^{NS}	0.19 ^{NS}	0.32 ^{NS}	0.41 ^{NS}	0.56 ^{NS}	0.56 ^{NS}	0.5 ^{NS}	0.32 ^{NS}

NS= Not significant

Table 5.2.1.2 Mean values for sensory qualities of gummies standardized with varying levels of sugar

Levels of Sugar		*SENSORY QUALITIES*								
		Color and Appearance	Mouthfeel	Texture	Overall Taste	Flavor	After taste	Chew ability	Overall Acceptability	Total Score
Maximum Score		(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(80)
50g	Mean	8.38	7.38	7.69	6.92	6.77	7.23	7.46	7.46	59.31
	SD	±1.45	±1.04	±1.11	±1.32	±1.42	±1.09	±1.05	±1.27	±8.52
	Range	6-10	6-9	6-9	5-9	5-9	6-9	6-9	6-10	46-73
60g	Mean	8.69	8.08	8	7.77	7.69	7.69	7.46	8.15	63.54
	SD	±0.85	±0.86	±0.91	±1.01	±1.1	±0.95	±1.13	±0.99	±6.67
	Range	7-10	7-9	7-9	6-9	6-9	6-9	6-9	7-10	55-73
70g	Mean	8.38	7.69	7.69	7.62	7.31	7.46	7.54	7.85	61.54
	SD	±1.19	±0.85	±1.03	±0.87	±1.03	±0.97	±0.88	±0.99	±6.5
	Range	6-10	6-9	6-9	6-9	6-9	6-9	6-9	7-10	52-73
80g	Mean	8.31	7.77	7.62	7.69	7.38	7.62	7.46	8	61.85
	SD	±1.44	±1.01	±0.96	±0.95	±1.12	±0.87	±1.13	±0.91	±7.31
	Range	6-10	6-9	6-9	6-9	6-9	7-9	6-9	7-10	52-73
	Anova (F)	**0.24**	**1.17**	**0.37**	**1.79**	**1.37**	**0.57**	**0.02**	**1.05**	**0.74**
	P value	0.87 ^{NS}	0.33 ^{NS}	0.77 ^{NS}	0.16 ^{NS}	0.26 ^{NS}	0.64^{NS}	0.9 ^{NS}	0.38 ^{NS}	0.53 ^{NS}

NS= Not significant

Table 5.2.1.3 Mean values for sensory qualities of gummies standardized with varying levels of agar

Levels of Agar		*SENSORY QUALITIES*								
		Color and Appearance	Mouthfeel	Texture	Overall Taste	Flavor	After taste	Chew ability	Overall Acceptability	Total Score
Maximum Score		(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(80)
2g	Mean	8.31	8.31	8.39	8.46	8.23	8.08	7.85	8.42	66.04
	SD	± 1.65	± 0.75	± 0.87	± 0.97	± 0.83	± 1.19	± 1.21	± 1.12	± 7.71
	Range	6-10	7-9	7-10	6-10	7-9	6-9	6-9	7-10	54-75
2.5g	Mean	8.62	8	7.92	8.15	7.92	8	7.92	8.35	64.89
	SD	± 1.12	± 1	± 1.04	± 0.8	± 1.12	±0.91	± 0.95	± 1.03	±7.29
	Range	7-10	6-9	6-9	7-9	6-9	6-9	6-9	7-10	52-74
3g	Mean	8.77	7.77	7.85	7.69	7.46	7.62	7.46	8	62.62
	SD	± 0.93	± 1.01	± 1.07	± 1.03	± 1.13	± 1.19	± 0.97	± 1.22	± 7.77
	Range	7-10	6-9	6-9	6-9	6-9	6-9	6-9	6-10	50-74
3.5g	Mean	8.46	8	7.85	7.77	7.67	7.77	7.31	7.85	62.08
	SD	± 1.12	± 0.71	± 0.81	± 1.01	± 1.15	±0.93	± 1.18	± 1.14	±8.09
	Range	7-10	7-9	7-9	6-9	6-9	6-9	6-9	6-10	47-73
	Anova (F)	**0.34**	**0.82**	**0.96**	**1.81**	**1.26**	**0.52**	**0.97**	**0.77**	**0.76**
	P value	0.8 ^{NS}	0.49 ^{NS}	0.42 ^{NS}	0.16 ^{NS}	0.3 ^{NS}	0.67^{NS}	0.41^{NS}	0.51 ^{NS}	0.52^{NS}

NS= Not significant

Table 5.2.1.4 Mean values for sensory qualities of gummies standardized with varying levels of citric acid

		SENSORY QUALITIES								
Levels of citric acid		Color and Appearance	Mouthfeel	Texture	Overall Taste	Flavor	After taste	Chew ability	Overall Acceptability	Total Score
Maximum Score		(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(80)
1/8tsp (0.48g)	Mean	8.31	7.54	7.69	7.77	7.39	7.54	7.15	7.77	61.15
	SD	± 1.25	± 0.78	± 0.95	± 0.73	± 1.04	±0.78	± 0.99	± 1.01	±6.59
	Range	6-10	6-9	6-9	7-9	6-9	6-9	6-9	6-10	51-73
¼ tsp (0.95g)	Mean	8.77	7.77	8.08	8	8	8.08	7.54	8.35	64.58
	SD	± 0.83	± 1.01	± 0.86	± 1.15	± 1.22	±0.86	± 1.2	± 1.03	±7.34
	Range	8-10	6-9	7-9	6-9	6-9	7-9	6-9	7-10	53-74
½ tsp (1.9g)	Mean	8.9	8.31	8.46	8.77	8.81	8.58	8.08	8.77	68.69
	SD	± 0.86	± 0.63	± 0.66	± 0.73	± 0.69	±0.64	± 1.04	± 0.93	± 4.5
	Range	7-10	7-9	7-9	7-10	7-10	7-9	6-10	7-10	56-75
1 tsp (3.8g)	Mean	8.46	7.46	8	7.85	7.92	7.92	7.65	7.65	62.92
	SD	± 1.33	± 0.97	± 0.91	± 0.9	± 0.95	±1.26	± 1.28	± 1.07	±5.98
	Range	6-10	6-9	6-9	6-9	6-9	6-9	6-10	6-9	48-70
	Anova (F)	**0.86**	**2.56**	**1.79**	**3.43**	**4.5**	**2.88**	**1.46**	**3.45**	**3.52**
	P value	0.47^{NS}	0.07^{NS}	0.16^{NS}	0.02^{*}	0.01**	0.05*	0.24^{NS}	0.02^{*}	0.02^{NS}

*Significant at p <0.05; ** $p<0.01^{NS}$= Not significant

Section 5.2.2- Substitution of sugar with varying concentration of GOS

Scores of the panel with respect to different levels of GOS are presented in Table 5.2.2.1 and graphically presented in Fig 5.2.1. The results revealed a statistically significant reduction in mouthfeel, texture, overall acceptability, and total score as the GOS concentration exceeded 60%.

To mitigate this, the sugar content in the standard gummies were substituted with various levels of sucralose along with GOS, which improved the taste and acceptability. Difference Test was performed to determine the superiority, inferiority, or similarity obtained with varying levels of sucralose added to the 100 percent GOS gummies as compared to the standard sugar gummies in terms of overall acceptability. The results showed no significant difference among the gummies with varying levels of sucralose addition. However, the GOS gummy with 5.5g sucralose was found to be superior as compared to the other variations of sucralose (Table 5.2.2.2).

Table 5.2.2.1 Mean values for sensory qualities of gummies standardized with varying levels of GOS

		SENSORY QUALITIES								
Levels of GOS		Color and Appearance	Mouthfeel	Texture	Overall Taste	Flavor	After taste	Chew ability	Overall Acceptability	Total Score
Maximum Score		(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(80)
0%	Mean	8.77	7.77	8.08	8	8	8.08	7.54	8.35	64.58
	SD	± 0.83	± 1.01	± 0.86	± 1.15	± 1.22	± 0.86	± 1.2	± 1.03	± 7.34
	Range	8-10	6-9	7-9	6-9	6-9	7-9	6-9	7-10	53-74
60%	Mean	8.42	8.17	8.25	8.17	8.25	8	7.8	8.17	65.25
	SD	± 0.79	± 0.72	± 0.75	± 0.72	± 0.75	± 0.85	± 1.11	± 0.94	± 5.85
	Range	7-9	7-9	7-9	7-9	7-9	7-9	6-9	7-10	57-73
80%	Mean	8.58	7.67	7.75	7.58	7.91	7.58	7.42	7.5	62
	SD	± 1.08	± 0.98	± 1.22	± 1.16	± 0.9	± 1.24	± 1.24	± 1.17	±8.022
	Range	7-10	6-9	6-9	6-9	7-9	6-9	6-9	6-9	51-72
100%	Mean	8	7	7.17	7.33	7.17	7.25	7.08	6.92	58
	SD	± 0.9	± 1.21	± 0.94	± 0.78	± 0.83	± 0.75	± 0.79	± 1.08	± 5.95
	Range	7-9	5-8	6-9	6-8	6-8	6-8	6-8	5-8	48-65
	Anova (F)	**1.43**	**3.22**	**3.25**	**2.41**	**3.52**	**2.06**	**1.08**	**5.22**	**3.31**
	P value	0.25 NS	0.03*	0.03*	0.08 NS	0.02*	0.14NS	0.37 NS	0.001***	0.03*

NS – the difference between the mean values within the columns is not significant.
Mean values represent the average of the scores in triplicate
Level of significance in increasing order – (*p<0.05, **p<0.01,*p<0.001)

Table 5.2.2.2 Mean values for difference test for sucralose addition in 100% GOS gummies

Level of Sucralose addition	***Equal***	***Superior***	***Inferior***	***Chi square value***
5g	8 (40%)	11 (55%)	1 (5%)	3.4 NS
5.5g	7 (35%)	13 (65%)	0 (0)	
6g	1 (5%)	3 (15%)	17 (85%)	

The GOS gummies were thus formulated with 75 ml water, 2 g agar, 1.9 g citric acid, 60g GOS and 5.5g sucralose which yielded 24 gummies in one batch.

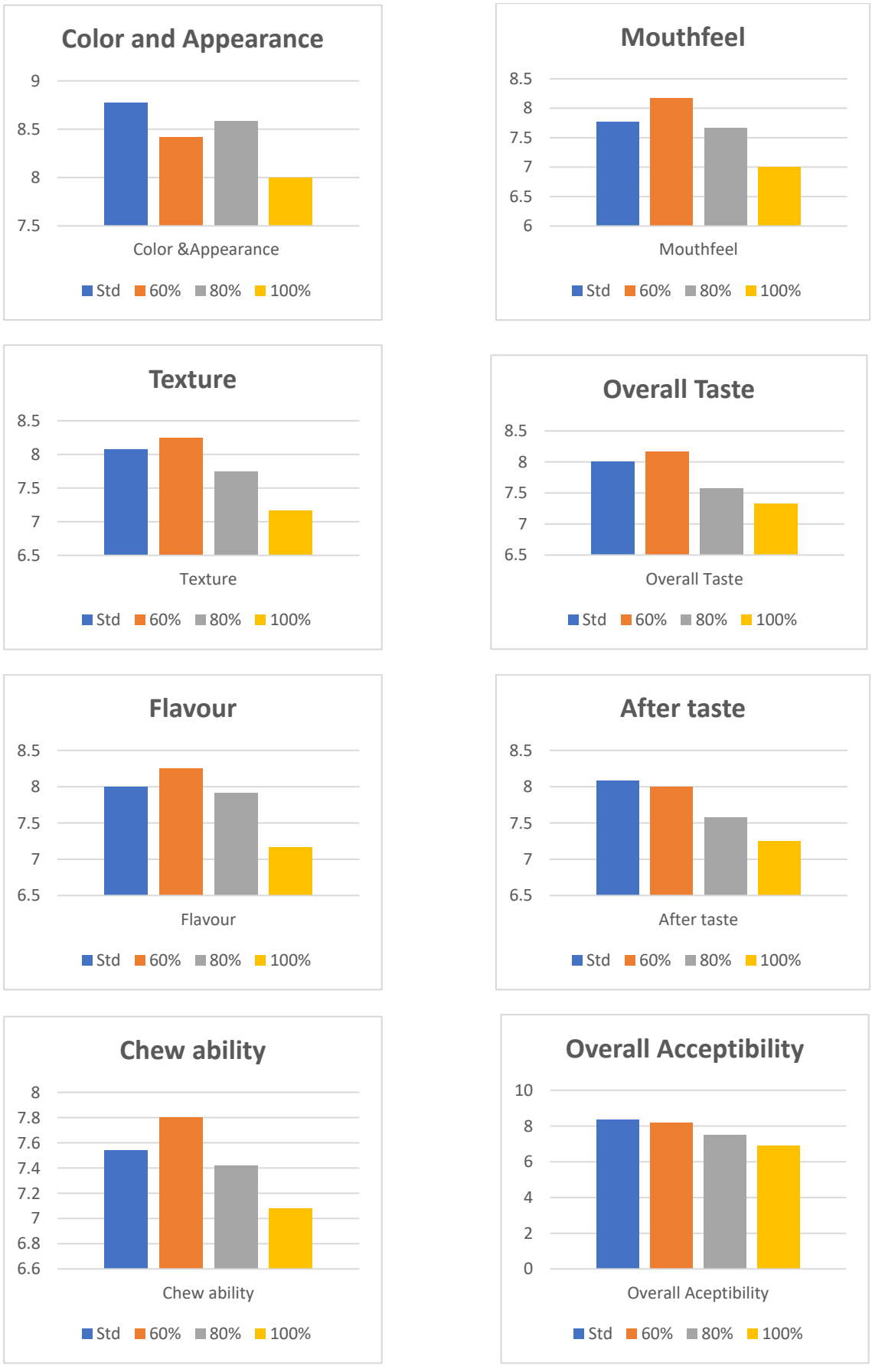

Fig 5.2.1: Sensory Scores at different levels of GOS substitution

Section 5.2.3- Physico chemical properties of GOS gummies

The physico-chemical parameters assessed for the final product (100% GOS added gummy) accepted by the trained panel included determination of colour, moisture, acidity, and texture profile analysis and is depicted in Table 5.2.3.

Color: The colour intensity was measured using a tintometer (Lovibond) and the result depicted 7 lovibond units (Table 7) which is similar to the natural color Curcumin.

Moisture: The moisture content of the gummies was recorded to be 24.8 % which is lower as compared to other sugar based jelly candies as reported in the literature.

pH: The acidity of the gummies were recorded to be 3.37 ± 0.2 which indicates an acidic pH of the gummies.

Texture: Texture profile analysis revealed that the gummies had optimum chewiness of 5.7mJ and 2.13mm springiness with minimal adhesiveness of 0.2mJ which is similar to the texture of jelly based gummies.

Table 5.2.3 Mean values of physico chemical characteristics of the 100% GOS gummies

Sl. No	*Parameter*	*Results*
1.	**Moisture**	24.8 ± 0.2 %
2.	**Acidity (pH)**	3.37 ± 0.2
3.	**Color**	7 Lovibond Units
4.	**Texture Profile**	Hardness (g) : 324 ± 10 Adhesiveness (mJ) : 0.2 ± 0.0 Resilience: 0.23 ± 0.11 Fracturability (g) : 250 ± 0 Cohesiveness : 0.85 ± 0.05 Springiness (mm) : 2.13 ± 0.05 Gumminess (g) : 275 ± 0.11 Chewiness (mJ) : 5.70 ± 0.1

The physico chemical properties of the100% GOS gummy formulation recorded 24.8% moisture, acidic pH, natural yellow color and optimum springiness and gumminess and reduced adhesiveness.

Section 5.2.4- Recovery of galactooligosaccharide (GOS) in gummies

The present study of replacing sugar with GOS also focussed to study the retention of GOS in the final product (100% GOS added gummy) using High Performance Liquid Chromatography (HPLC) technique. The chromatogram obtained is depicted in Fig.5.2.2. For determining the recovery of GOS in the gummies, retention time (RT) and peak of standard GOS was compared with the RT peak of gummies with variation of 1-2 minutes of RT. The test revealed a recovery of 95% GOS which indicated a minute loss of the prebiotic even when subjected to higher temperatures.

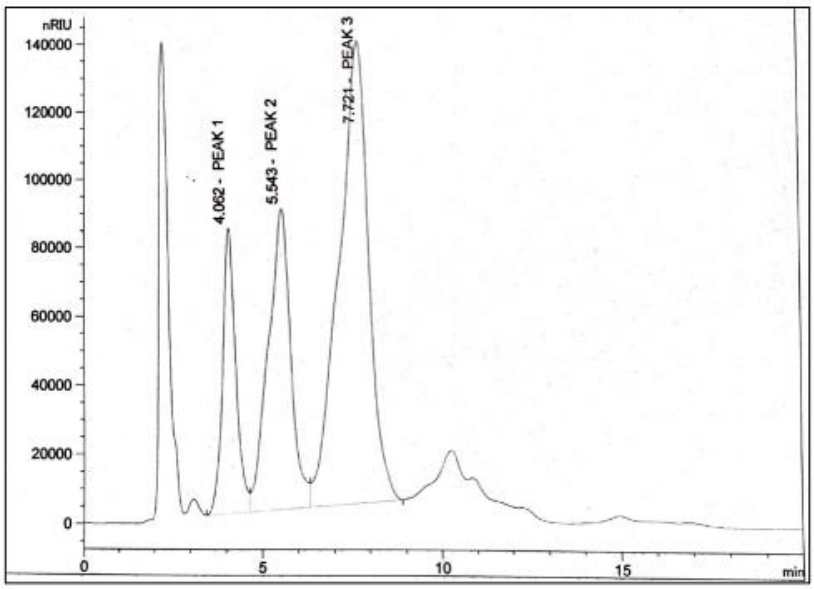

Fig 5.2.2: Chromatogram depicting GOS standard peak in one gummy

The 100% GOS gummies recorded a final retention of 95% GOS indicating minimal prebiotic loss even when subjected to higher temperatures.

Section 5.2.5- Shelf life studies of GOS gummies

5.2.5.1 Effect of storage on Microbial Growth of GOS gummies

The most prevalent microorganisms that cause deterioration in confectioneries are yeast

and molds. However, at the end of 6 months, no significant presence of yeast and mold was detected (Table 5.2.5.1). The gummies were packed in airtight HDPE bottles and contained citric acid as a flavouring and salivating agent, which may have contributed to their longer shelf life at accelerated temperatures. Several factors are known to have contributed to the increased shelf life of the gummies. Shelf life studies conducted over a six-month period (Table 5.2.5.1) revealed no de-novo growth of *E. coli*, a slight increase in TPC (Log10cfu/g), and a slight decrease in yeast and mold count. Yeast and mold count increased till day 15, after which the growth declined (2.26 Log10cfu/g), possibly as a result of the overall moisture content decreasing at day 30. Upon storage, however, no significant differences in microbiological parameters (*E. coli*, total plate count, yeast and mold count) were observed.

Table 5.2.5.1. Mean values of microbial count (Log_{10}CFU/g of sample) of GOS gummies stored for a period of 6 months (37ºC)

Duration	Total Plate Count	Yeast and Molds	E.coli
0 Day	2.12± 0.75	3.14±0.1	Nil
15 Days	3.18± 0.77	3.18±0.12	Nil
30 Days	3.0±0.52	2.26±0.9	Nil
60 Days	3.15±0.56	2.26±0.9	Nil
180 Days	3.48±0.73	2.26±0.87	Nil
F test	**1.92**	**2.38**	**-**
P value	0.12 ^{NS}	0.06 ^{NS}	-

NS= Not significant

5.2.5.2 Effect of storage on sensory parameters of GOS gummies

Sensory trials demonstrated a considerable improvement in some of the organoleptic qualities including chewiness and a subsequent reduction in colour and flavour during sensory evaluation for shelf life analysis. F test revealed no significant differences in the other organoleptic characteristics of gummies (Table 5.2.5.2). The panel members accepted the gummies after 180 days of storage at accelerated temperatures (37ºC), which indicated a successful shelf stability of the 100% GOS gummies even after six months on the shelf in terms of its sensory qualities.

Table 5.2.5.2. Mean values for sensory parameters of GOS Gummies stored for a period of 6 months (37ºC)

Duration		*Color/ Appearance*	*Mouth feel*	*Texture*	*Over all Taste*	*Flavor*	*After taste*	*Chewiness*	*Overall Acceptability*	*Total Score*
	Max score	10	10	10	10	10	10	10	10	10
0 Day	Mean	8.74	8.12	7.88	8.47	8.53	8.24	8.29	8.47	66.94
	SD	±0.75	±0.93	±0.93	±1.0	±0.94	±1.2	±0.92	±0.8	±5.09
	Range	8-10	6-9	6-10	7-10	7-10	6-10	7-10	7-10	60-75
	Percent	87.4	81.2	78.8	84.7	85.3	82.4	82.9	84.7	83.7
15 Days	Mean	8.63	7.75	7.94	7.94	8.19	8.25	8.19	8.18	65.06
	SD	±0.81	±1.06	±0.85	±1.6	±0.91	±0.9	±0.75	±0.83	±5.42
	Range	7-10	6-9	6-9	1	6-9	6-10	7-9	7-9	54-71
	Percent	86.3	77.5	79.4	5-10 79.4	81.9	82.5	81.9	81.8	81.3
30 Days	Mean	8.69	8.44	8.25	8.31	8.25	8.13	8.31	8.44	66.81
	SD	±0.6	±0.89	±0.86	±1.4	±0.86	±0.9	±0.95	±0.96	±5.11
	Range	7-9	7-10	7-9	5	6-9	6	7-10	7-10	57-75
	Percent	86.9	84.4	82.5	6-10 83.1	82.5	81.3	83.1	84.4	83.5
60 Days	Mean	9.06	8.31	8.25	7.8	8.31	8.19	8.06	8.19	66.19
	SD	0.68	0.7	1	0.9	0.87	0.98	1	0.91	5.6
	Range	8-10	7-9	7-10	6-9	6-9	6-10	6-9	6-9	55-75
	Percent	90.6	83.1	82.5	78	83.1	81.9	80.6	81.8	82.74
180 Days	Mean	9.6	8.5	8.2	9.15	9.23	8.61	9.38	9.08	71.8
	SD	0.77	0.97	1.1	0.69	0.73	1.39	0.77	0.86	5.13
	Range	8-10	7-10	7-10	8-10	8-10	6-10	8-10	8-10	63-78
	Percent	96	85	82	91.5	92.3	86.1	93.8	90.7	89.75
F test		**3.48**	**1.92**	**0.58**	**2.38**	**2.96**	**0.64**	**4.58**	**2.42**	**2.82**
P value		0.01*	0.12 NS	0.68 NS	0.06 NS	0.02*	0.63 NS	0.002**	0.06 NS	0.02*

NS – the difference between the mean values within the columns is not significant.
Mean values represent the average of the scores in triplicate
Level of significance in increasing order – (*p<0.05, **p<0.01)

The 100% GOS gummy revealed a shelf stability of more than six months with respect to its sensory qualities and microbial evaluation.

RESULT HIGHLIGHTS (PHASE II)

The result highlights of this phase of the study is divided into four parts namely results on standardization of gummies, GOS substitution and sucralose addition, physicochemical properties of standardized gummies, recovery of GOS of gummies and shelf life analysis of GOS gummies for a period of 6 months at 37ºC.

Standardization of gummies (For a batch of 24 gummies)

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

GOS substitution and addition of sucralose (For a batch of 24 gummies)

- Most acceptable gummies were prepared with 60% GOS.
- In order to produce 100% GOS gummies, addition of 5.5g sucralose yielded best results.

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.

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.

Shelf life analysis of GOS gummies

- Six months of storage at accelerated temperatures of 37ºC 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

Galactooligosaccharide (GOS) as a prebiotic has a potential to improve gut health thereby improving the depression and short chain fatty acid profile of constipated subjects. In this phase 10gm of gummies with 100% GOS per day was supplemented for a period of 4 weeks to the teaching staff of The M.S. University of Baroda suffering from functional constipation after obtaining their consent and the subjects were assessed for their constipation profile, depression score and Quality of life before and after supplementation.

The study design used for this phase was Double Blind placebo controlled Trial. The tools and techniques for undertaking this phase of the study has been detailed in the Materials and Methods Chapter. The various questionnaires used for determining the subjective parameters are appended in the Appendix. The consort for this phase of the study is depicted in Fig 5.3.1.

The results of this phase are presented under the following heads:

5.3.1. General information of the respondents for the clinical trial

5.3.2. Constipation Profile of subjects before and after supplementation with GOS gummies

5.3.3. Gut Microbiota profile of constipated subjects before and after supplementation with GOS gummies

5.3.4. Short chain fatty acid (SCFA) profile of constipated subjects before and after supplementation with GOS gummies

5.3.5 Depression profile of constipated subjects before and after supplementation with GOS gummies

5.3.6. Quality of life profile of constipated subjects (PAC-QOL) before and supplementation with GOS gummies

5.3.7. Association of various factors of subjects suffering from FC at baseline

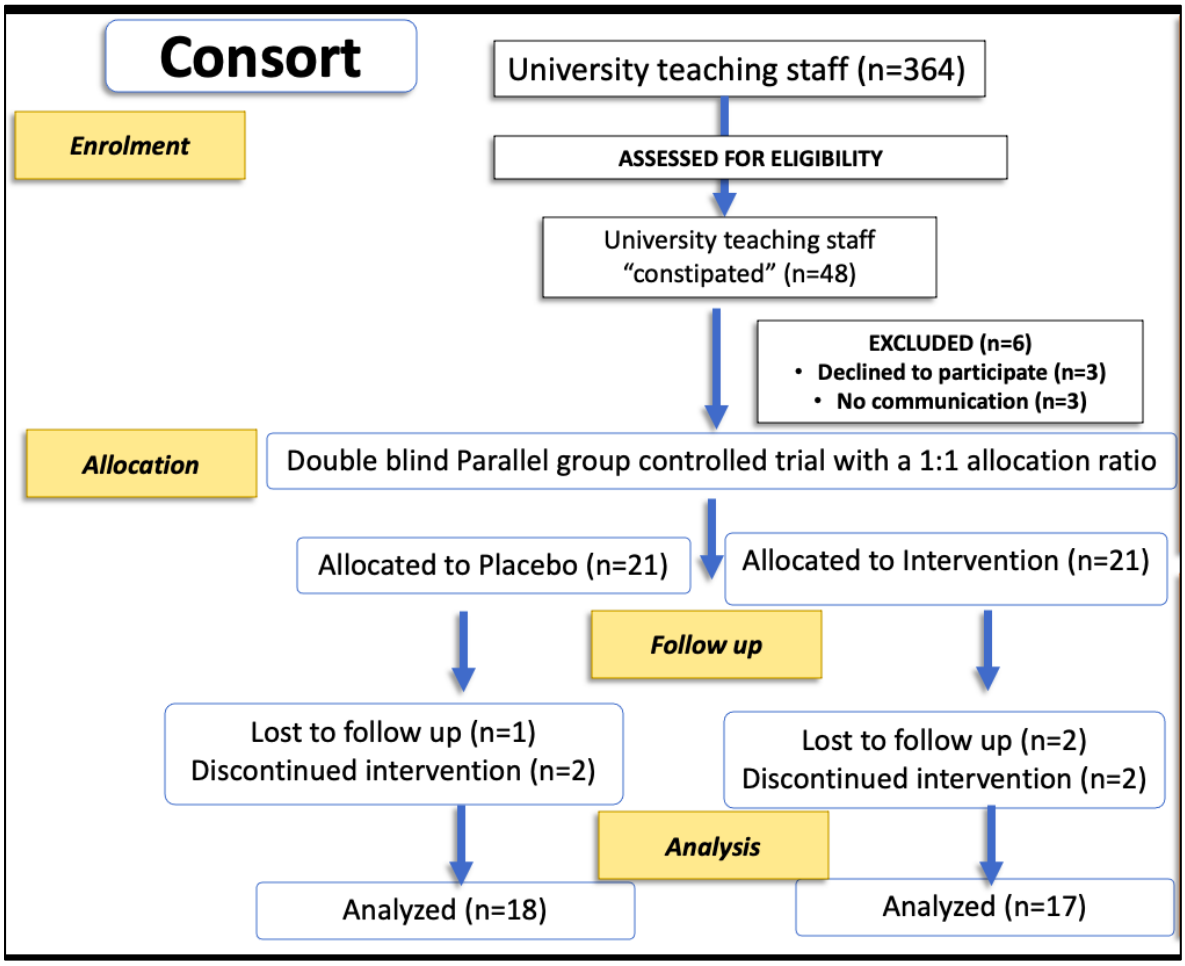

Fig 5.3.1: Consort for the Double Blind randomized clinical trial

Section 5.3.1 General information of the respondents for the clinical trial

The demographic details of the participants selected for the clinical trial are presented in Table 5.3.1. Most of the respondents were females (74%), married (71%) and stayed in nuclear families (63%) with a normal BMI having a monthly family income of >INR 123,322.00 (49%) and their age ranged between 25-45 years.

Table 5.3.1: General Information of the Respondents for the clinical trial (N= 35)

Parameters	Total Subjects N (%)		
	Control (N=17)	**Experimental (N=18)**	**Total (N=35)**
Age:			
25-34	9 (52.94%)	7 (38.89%)	16 (45.71%)
35-45	8 (47.06%)	11 (57.89%)	19 (54.29%)
Sex			
Female	14 (82.35%)	12 (66.67%)	26 (74.29%)
Male	3 (17.65%)	6 (33.33%)	9 (25.71%)
Type of family			
Nuclear	9 (52.94%)	13 (72.22%)	22 (62.86%)
Joint	7 (41.18%)	3 (16.67%)	10 (28.57%)
Extended nuclear	1 (5.88%)	2 (11.11%)	3 (8.57%)
Marital status			
Married	12 (70.59%)	13 (72.22%)	25 (71.43%)
Unmarried	5 (29.41%)	5 (27.78%)	10 (28.57%)
Family monthly income			
>123,322	9 (52.94%)	8 (44.44%)	17 (48.57%)
61,663- 123,321	5 (29.41%)	9 (50%)	14 (40%)
46,129- 61,662	3 (17.65%)	1 (5.56%)	4 (11.43%)
BMI (kg/m^2)			
18.1-20	6 (35.29%)	8 (44.44%)	15 (42.86%)
20.1-22	5 (29.41%)	6 (33.33%)	11 (31.43%)
22.1-24	6 (35.29%)	4 (22.22%)	10 (28.57%)

Section 5.3.2- Constipation Profile of subjects before and after supplementation with GOS gummies

The results of this section are presented in Table 5.3.2, which shows that supplementation with 100% GOS gummies led to a significant improvement in the constipation profile (Total score: $p<0.01$) in the experimental group, whereas no significant change in the control group was observed upon consumption of placebo.

Stool frequency (<3 stools/week) was used to screen the constipated subjects. Stool frequency was observed to be <2 stools/ week at baseline for both the groups. Upon consumption of GOS gummies, a significant improvement to 3 stools/ week was noted which was considered as normal ($p<0.05$).

Bristol stool type 1 and 2 were considered for screening of FC. At baseline, the subjects were having similar stool type (Type 2). However, experimental group showed a significant improvement in the stool type (Type 2 to Type 3) which is considered to be normal ($p<0.05$) as opposed to the control group which showed no change in stool type upon consumption of placebo.

The following parameters of Rome IV criteria also showed considerable improvement upon consumption of GOS gummies. The parameters include sensation of passage of incomplete stool ($p<0.01$), straining during defecation ($p<0.01$), and abdominal pain while defecation ($p<0.05$). Sensation of blockage in the intestine was reduced, however not significantly. Excessive flatulence was noted to be significantly increased ($p<0.01$) on consumption of GOS gummies as opposed to the consumption of sugar gummies, which might be explained due to the harmless side effect of fiber consumption. Manual maneuvers was not observed in any of the groups pre and post supplementation.

Table 5.3.2- Mean scores of parameters of constipation profile before and after supplementation in experimental and control group (N=35)

Parameters	Experimental				Control			
	Pre	**Post**	**Paired t value**	**% diff**	**Pre**	**post**	**Paired t value**	**% diff**
Stool Frequency (<3 stools/week)@	0.29± 0.47	0.35± 0.49	**1.84***	↑ **20.73%**	0.22± 0.43	0.06± 0.24	**-5.12****	↓ **72.72%**
Bristol stool chart (Type1-Type7)$	2.67±0.77	3.83±0.92	**-4.75***	↓ **43.45%**	2.18± 1.51	2.12± 1.54	**0.08** NS	↓ **2.75%**
Incomplete stool sensation #	1.17±0.38	0.22±0.43	**6.27****	↓ **-81.2%**	1.65± 0.49	1.59± 0.51	**0.1** NS	↓ **3.64%**
Straining while defecating #	0.94±0.24	0.22±0.43	**5.65****	↓ **-76.6%**	0.77± 0.44	0.82± 0.53	**0.9** NS	↑ **6.49%**
Excessive Flatulence #	0.11±0.32	0.14±0.62	**-1.84***	↑ **27.27%**	0.82± 0.52	0.71± 0.47	**1.13** NS	↓ **13.42%**
Manual stool Maneuver#	0	0	-	-	0	0	0	-
Blockage sensation #	0.65±0.49	0.53±0.51	**1.46** NS	↓ **18.46%**	0.06± 0.24	0.11± 0.32	**-6.19** NS	↑ **83.33%**
Abdominal Pain #	0.78±0.43	0.06±0.24	**7.65****	↓ **92.31%**	0.29± 0.47	0.35± 0.49	**-1.7** NS	↑ **20.69%**
Total Score for constipation severity ^^ (out of 20)	6.61±0.85	1.94±1.47	**5.31****	↓ **70.65%**	8.59± 2.65	8.71± 2.6	**-0.062** NS	↑ **1.4%**

Statistically significant at (**p<0.01), (*p<0.05), NS- Not significant

Note: @ indicate number of subjects having <3 stools/ week

$ The values for Bristol stool chart indicate type of stool from Type 1-Type 7

Scores were allotted (0-2) for the questions under various parameters as 2, 1 and 0 for always, sometimes and never respectively.

^^ A total sore of 20 was allotted for 8 parameters of constipation profile

Section 5.3.3- Gut microbiota profile of constipated subjects before and after supplementation with GOS gummies

Subjects were screened for their gut microbiota profile with respect to genus LAB, Bifidobacterium, Clostridium, Bacteroides and phyla Firmicutes and Bacteroidetes. Table 5.3.5 and Fig 5.3.3 reveals a significant improvement in the microbial profile of the experimental group when fed with 100% GOS gummies for a period of 30 days as compared to the control group fed with placebo.

Microbiome composition of fecal samples before supplementation of GOS gummies

Prior to the clinical trial, the phyla that predominated in the gut microbiomes of individual constipated participants on day 0 of the placebo and GOS groups, respectively, were Bacteroidetes, Firmicutes, and genus Clostridium. The genera Bacteroides, Lactobacillus and Bifidobacterium were less in proportion in both the groups (Fig 5.3.2).

Gut microbiome composition of fecal samples after supplementation of GOS consumption

The genera that predominated in the gut microbiomes of the participants in the placebo and GOS groups, respectively revealed Bifidobacterium, Lactobacillus, and Bacteroides were the most prevalent genera which significantly improved by 1230%, 322% and 219% among the subjects. There was a considerable decrease in the phyla Bacteroidetes and Firmicutes as well as in the genus Clostridium by 85%, 73% and 63% respectively.

F/B ratio was improved in GOS group

F/B ratio also improved significantly ($p<0.01$) in the experimental group which indicated improved gut health. On day 30, we discovered that the experimental group had a higher ratio (3.57) of Firmicutes to Bacteroidetes (F/B) than the Placebo group (1.29) (Fig 5.3.3).

Table 5.3.3- Mean values of selected gut microbes analyzed in the constipated subjects (N=35)

Parameter (ng)	Control				Experimental			
	Pre	**Post**	**Paired t value**	**% increase/ decrease**	**Pre**	**post**	**Paired t value**	**% increase/ decrease**
Lactobacillus	1.75± 2.19	2.56± 1.86	-1.7 NS	↑ 46.29%	1.91± 2.78	25.4±23 .86	-3.87***	↑ 1229.84%
Bifidobacterium	5.05± 6.45	5.12± 8.47	-0.05 NS	↑ 1.39%	5.14±9.8 7	42.79±2 5.02	-4.89**	↑ 321.99%
Clostridium	12.41± 9.41	17.87±12. 92	-1.64 NS	↑ 44%	11.3±10. 22	4.2± 5.62	2.71*	↓ -62.83%
Bacteroides	3.04± 4.04	2.78± 2.69	0.36 NS	↓ 8.55%	4.26± 6.03	13.61±1 5.55	-4.33 **	↑ 219.48%
Firmicutes	33.82± 14.68	18.77± 11.53	3.62 NS	↓ -44.5%	32.18±1 4.02	8.55±6. 34	7.17**	↓ -73.43%
Bacteroidetes	44.14± 20.68	14.45± 18.78	4.34 NS	↓ -67.26%	35.63±2 1.92	5.46±5. 74	5.05**	↓ -84.68%
F/B Ratio	1.66± 6.1	1.29±0.73	-0.276 NS	↑ 22.29%	1.23± 1.14	3.57±3. 33	-4.2**	↑ 190.24%

Level of Significance (***p<0.001,**p<0.01,*p<0.05), NS- Not significant

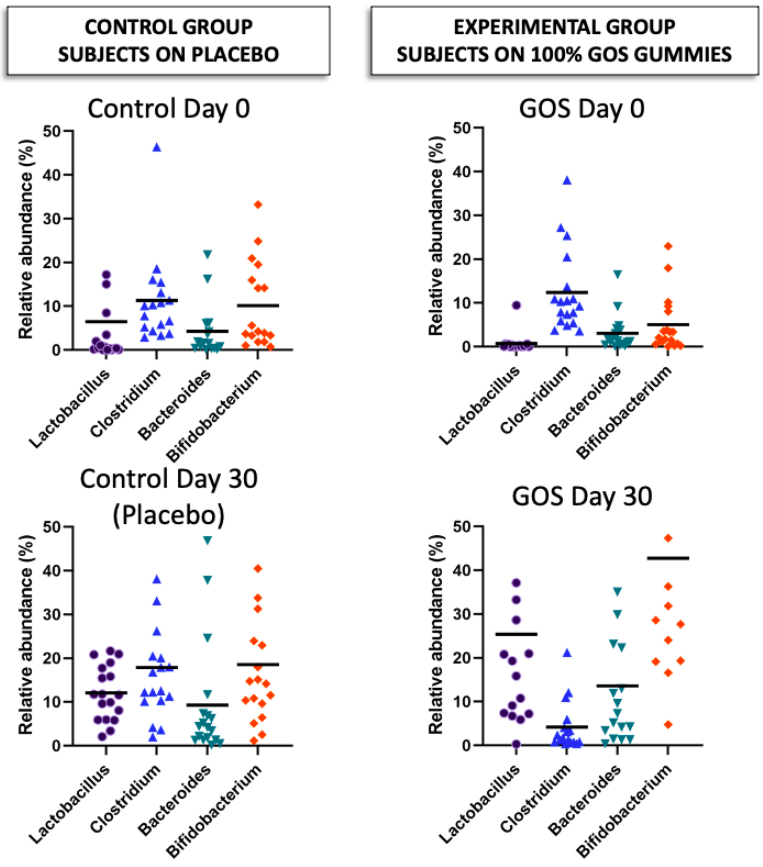

Fig 5.3.2: Gut Microbiota profile in control and experimental group

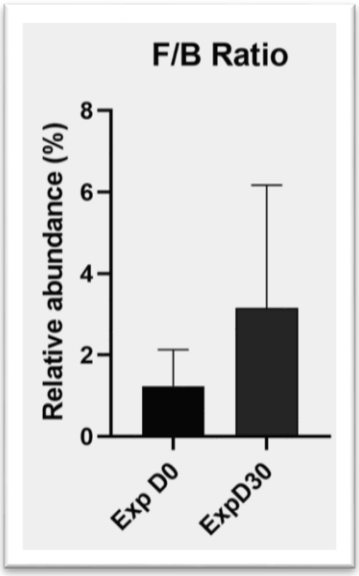

Fig 5.3.3 F/B ratio in experimental group

Section 5.3.4- Fecal SCFA profile of constipated subjects before and after supplementation with GOS gummies

Literature review has shown the fecal SCFA alter upon consumption of prebiotics. Hence the subjects were screened for their fecal short chain fatty acid (SCFA) namely acetic acid, propionic acid and butyric acid with the help of GC technique.

The levels of acetic acid and butyric acid in the experimental group improved by 29% ($p<0.05$) and 75% ($p<0.01$) respectively when fed with 100% GOS gummies for a period of 30 days as compared to the control group fed with placebo. A non-significant improvement in fecal propionic acid levels was observed by 6% in the experimental group (Table 5.3.6).

Table 5.3.4- Mean values of fecal short chain fatty acids analyzed in the constipated subjects (N=15)

	Experimental (n=8)				Control (n=7)			
Parameter (mg/ml)	**Pre**	**Post**	**Paired t value**	**% increase/ decrease**	**Pre**	**post**	**Paired t value**	**% increase/ decrease**
Acetic acid	0.69± 0.11	0.89± 0.08	-2.11*	↑ 28.9%	0.69± 0.07	0.67± 0.09	0.94 ^{NS}	↓ 2.9%
Propionic acid	0.47± 0.02	0.5± 0.02	-1.1	↑ 6.38%	0.02± 0.02	0.02± 0.03	-0.6 ^{NS}	-
Butyric acid	0.04± 0.03	0.07± 0.03	-3.12**	↑ 75%	0.02± 0.01	0.02± 0.01	-0.6 ^{NS}	-

Statistically significant at (**$p<0.01$,*$p<0.05$), ^{NS}- Not significant

Section 5.3.5- Depression profile of Constipated subjects before and after supplementation with GOS gummies

Subjects were screened for their depression profile using Beck's depression questionnaire (21 questions). Table 5.3.3 exhibits a significant improvement in the total depression score ($p<0.01$) in the experimental group when fed with 100% GOS gummies for a period of 30 days as compared to the control group fed with placebo. Post supplementation there was a significant reduction (40%) in various categories of depression in the experimental group.

Depression was observed among 31% subjects in various categories ranging from mild mood disturbance and clinical depression to moderate depression (Fig 5.3.4). Sixty seven percent subjects moved to normal category whereas 33% subjects moved from borderline clinical depression to mild mood disturbance category.

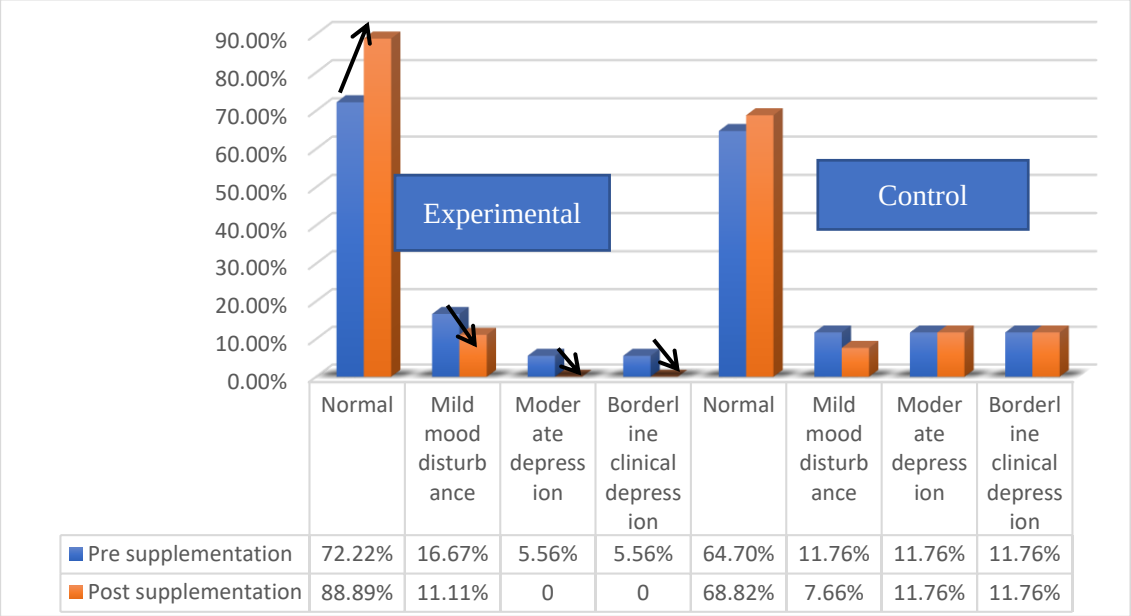

Fig 5.3.4: Depression profile categorization in control and experimental groups

Table 5.3.5- Mean scores of depression profile before and after supplementation in experimental and control group (N=35)

Parameter	Experimental				Control			
	Pre	Post	Paired t value	% diff	Pre	post	Paired t value	% diff
Normal	0.72±0.19	0.89±0.4	**1.32***	↑ **23.61%**	0.65±0.49	0.62±0.49	**0.32 NS**	↓ **4.61%**
Mild mood disturbance	0.16±0.78	0.11±0.9	**1.11***	↓ **31.25%**	0.11±0.13	0.16±0.4	**1.89 NS**	↑ **45.45%**
Moderate depression	2.56±0.91	1.1±0.49	**2.01****	↓ **57.03%**	2.11±0.42	2.11±0.2	**0.13 NS**	-
Borderline clinical depression	2.56±1.71	-	**2.12*****	↓ **100%**	2.11±0.91	2.11±0.7	**0.13 NS**	-
Total Score	5.5 ±3.16	2.27 ±1.47	**2.72****	↓ **40.55%**	5.18 ±3.72	5.12 ±4.75	**0.37 NS**	↓ **1.15%**

Statistically significant at (p<0.001,**p<0.01,*p<0.05), NS- Not significant

Section 5.3.6- Quality of life of constipated subjects before and supplementation with GOS gummies

Subjects were screened for their quality of life (QOL) with PAC-QOL questionnaire. They were scored on a the basis of 28 questions bearing a total of 127 points under various parameters such as physical discomfort, decreased appetite, psychological disturbances, life with constipation. Questions on physical discomfort included sensation of bloating, feeling heavy etc. Questions on decreased appetite included carefulness about eating pattern etc. Questions on psychological disturbances included being irritable, upset, obsessed or stressed about the FC condition among others. Subjects were also asked about their degree of satisfaction with the treatment given. Details of the questions are mentioned in the appendix (Appendix IX).

Table 5.3.4 and Fig 5.3.2 reveals a significant improvement in the total score ($p<0.05$) in the experimental group when fed with 100% GOS gummies for a period of 30 days as compared to the control group fed with placebo. All the parameters in QOL shown a significant reduction in physical discomfort, sensation of decreased appetite and psychological disturbances by 20%, 39% and 23% respectively.

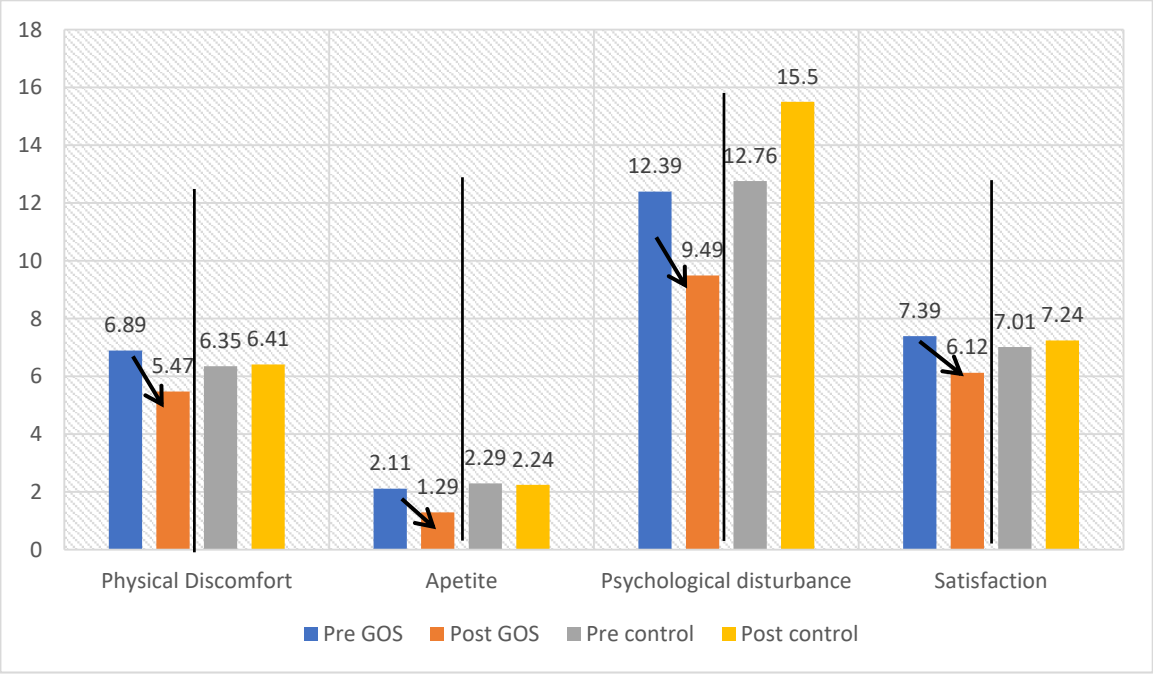

Fig 5.3.5: PAC-QOL categorization in control and experimental groups

Table 5.3.6- Mean scores of quality of life before and after supplementation in experimental and control group (N=35)

Parameter	Experimental				Control			
	Pre	**post**	**Paired t value**	**% increase/ decrease**	**Pre**	**Post**	**Paired t value**	**% increase/ decrease**
Physical discomfort	6.89±0.49	5.47±0.49	**1.32***	↓ **20.61%**	6.35±0.49	6.41±0.49	**0.32 NS**	↑ **0.95%**
Sensation of decreased appetite	2.11±0.49	1.29±0.49	**1.11***	↓ **38.86%**	2.29±0.49	2.24±0.49	**1.89 NS**	↓ **2.18%**
Psychological disturbances	12.39±0.49	9.49±0.49	**2.01***	↓ **23.4%**	12.76±0.49	15.5±0.49	**0.13 NS**	↑ **21.47%**
Treatment satisfaction	7.39±0.49	6.12±0.49	**2.12***	↓ **17.19%**	7.01±0.49	7.24±0.49	**0.13 NS**	↑ **3.28%**
Total Score	28.44± 17.47	22.0± 12.43	**2.74***	↓ **22.64%**	37.0± 28.72	45.18± 22.86	**-2.02 NS**	↑ **22.11%**

Statistically significant at (*p<0.05), ^{NS}- Not significant

Section 5.3.7- Associations between various factors such as gut flora, SCFA, depression status and QOL with presence of functional constipation at baseline

Table 5.3.7 shows the association between various parameters with constipation status at baseline among the university teaching staff suffering from FC.

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 ($p<0.05$).

Table 5.3.6 Associations between various factors such as gut flora, SCFA, depression, QOL with presence of functional constipation at baseline (N=15)

Parameters	Lactobacillus	Clostridium	Bifidobacterium	Bacteroides	Bacteroidetes	Firmicutes	F/B Ratio	Acetic acid	Butyric acid	QOL	Depression status	Severity of Constipation status
Propionic acid	0.614*	-0.374	-0.242	-0.149	-0.570*	-0.266	-0.32	0.164	-0.134	0.03	-0.056	-0.12
Butyric acid	0.376	0.02	0.6*	0.389	0.37	0.54	0.12	0.703**	-	0.153*	0.079*	-0.43**
Acetic acid	-0.089	0.031	-0.183	0.393	-0.065	0.081	0.031	-	-	0.085	0.208	-0.38*
F/B Ratio	0.062	0.021	0.422	-0.591*	-0.573*	0.229	-	-	-	0.02*	0.07	-0.391*
Firmicutes	-0.393	0.361	0.036	-0.135	0.670*	-	-	-	-	0.088	0.057	-0.135
Bacteroidetes	-0.522	0.329	-0.318	0.664**	-	-	-	-	-	-0.104	-0.067	0.15
Bacteroides	-0.568	-0.054	-0.452	-	-	-	-	-	-	0.129	-0.096	-0.376
Bifidobacterium	0.12	0.024	-	-	-	-	-	-	-	-0.164*	-0.33*	-0.089**
Clostridium	-0.327	-	-	-	-	-	-	-	-	0.142	0.452**	0.022*
Lactobacillus	-	-	-	-	-	-	-	-	-	-0.391*	-0.317*	-0.393**
Depression Status	-	-	-	-	-	-	-	-	-	-	-	0.35*
QOL	-	-	-	-	-	-	-	-	-	-	-	0.53**

Statistically significant at (*p<0.05, **p<0.01), ^{NS}- Not significant

Note: Associations were calculated among different parameters at baseline using Pearson's correlation among subjects (N=15). Subjects were allotted scores against the symptoms of constipation profile.

RESULT HIGHLIGHTS (PHASE III)

- The demographic details of the participants selected for the clinical trial were revealed in Table 5.3.1. 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.
- 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.
- 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.

RESULT HIGHLIGHTS (PHASE III)

- 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.
- Significant strong negative correlations were also observed between the different species studied namely *Bacteroides sp*. and *Bifidobacterium sp*. ($p<0.05$), *Clostridium sp*. and *Lactobacillus sp*. ($p<0.01$) and *Bifidobacterium sp*. and *Clostridium sp*. ($p< 0.01$).
- 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 ($p<0.05$).

DISCUSSION

PHASE I

Functional constipation (FC) has a multifaceted pathogenesis, with particular emphasis on genetic history, socioeconomic level, poor fibre intake, inadequate fluid intake, immobility, disruption of the hormone balance, drug side effects, or body structure (Chen et al, 2022; Rajindrajith S, 2011). FC is an increasingly prevalent concern and obstacle among the elderly (Forootan et al, 2018; Brenda et al, 2015). However, the results obtained in the study showed the presence of FC comparatively more in younger and older adults. The results also shows that as age increases the prevalence of FC increases among the study population.

Literature review suggests females are more prone to suffer from FC (Forootan et al, 2018; Papatheodoridis et al, 2010). According to a study conducted by Verkuijl et al (2020), women experienced constipation substantially more frequently than males did (19.7% versus 10.6%, $p<0.001$) which corroborated with the study's findings where frequency of constipated female subjects were more than male subjects (76% versus 25%, $p<0.0$). Potential causes could be, for instance, women may have a slower intestinal transit and often report bowel function changes due to fluctuating amounts of progesterone and estrogen during their menstrual cycles, or it could be because they are more likely to report health issues than men. Other possible of FC to be more common in women might be daily inactivity, low income, poor education, higher coffee and tea consumption, and some dietary factors, such as less fruit and vegetable consumption, according to data from the first National Health and Nutrition Examination Survey (Verkuijl et al 2020; Sandler RS et al, 1990).

Studies on the relationship between socio economic factors including education level, living in an urban or rural setting and the occurrence of constipation have produced conflicting results (Papatheodoridis GV et al, 2010). No significant difference in the prevalence of constipation among responders with different levels of education or living conditions was recorded in a study by Verkujil et al (2020). According to a studies subjects with higher education and urban residents are less likely to be impacted than those with lesser levels of education, and urban residents are less frequently impacted than those who residing in rural areas (Werth and Cristopher, 2021; Wald et al, 2007). However, in the current study no

significant association was recorded between the income level and presence of FC among the teaching staff. The results of the study are not in line with the above findings where 20% of the subjects who were urban residents with higher level of education were found to have the presence of FC.

A study by Gamze et al (2020) revealed a negative correlation between low bowel movement and BMI. The findings of the present study that obesity is linked to FC is corroborated with the literature review that suggests that adults with obesity classes II and III category have a higher prevalence of FC ($p<0.05$). Hence assessing constipation in people who are obese by health professionals is crucial for tracking FC (Silveira et al, 2021; Papatheodoridis GV et al, 2010). Obesity has been associated to increased levels of inflammatory cytokines and gastrointestinal symptoms (Pourhoseingholi et al, 2009). Other explanation for this connection could be that obesity and constipation are caused by insufficient physical activity, inadequate dietary fibre consumption, and poor nutritional habits including fast food, insufficient fibre consumption, and insufficient water consumption) (Verkulij et al, 2020).

Studies indicate that bowel function can be improved with regular exercise (Silveira et al, 2021). A study conducted by Hamaguchi et al (2020) explored the connection between GI symptoms and daily step counts and the findings showed a definite relationship between locomotor activity and GI discomfort and symptoms. Similar results were observed in the current study where subjects with locomotor problems due to knee and joint pains were found to experience FC comparatively more than the others ($p<0.05$). A study conducted on adults found no association between physical activity and constipation (Markland et al, 2013). A study by Gamze et al (2020) revealed that when compared to inactive participants, those who were moderately active (OR: 0.77, 95% CI: 0.62-0.91) and active (OR: 0.74, 95% CI: 0.59-0.90) had a reduced chance of constipation ($p<0.05$).

A study by Jajam et al (2017) revealed that although oral symptoms vary in frequency across gastrointestinal disorders, these changes might indicate an underlying medical condition and can help with an early diagnosis. A case-control study by Tosello et al (2001) demonstrated that poor oral functional features increases your likelihood of developing gastrointestinal pathology which was also seen in the current study where having dental problems including dry mouth and cavities showed a positive association with the presence of FC ($p<0.05$).

Genetic predisposition has been one of the strong etiological factors in FC (Forootan et al, 2018; Rajindrajith S, 2011). A study conducted by Olaru et al (2016) indicated that a positive family history of the participants with FC (38.49%) and in 61 of these cases (46.56%) the FC status of the mother was mentioned. The findings of the current study are at par with the literature, where 20% of the mothers of the study participants were suffering from FC ($p<0.05$).

Constipation was found to be more common with higher coffee and tea consumption, and some dietary factors, such as less fruit and vegetable consumption, according to data from the first National Health and Nutrition Examination Survey (Verkulij et al, 2020; Sandler RS et al, 1990). In the current study, regular tea intake showed a significant association with the presence of FC ($p<0.05$). Theophylline, which promotes extracellular dehydration and constipation, may be the cause of the association between tea and constipation (Gamze et al, 2020).

According to a study conducted by Lacy et al (2016), general practices including having a daily schedule for defecation, elevating the feet with a foot stool or using an Indian style toilet which is lower to the ground can serve as part of approaches of treatment for FC. The current study also recorded a significant negative association between having a fixed schedule for defecation and presence of FC ($p<0.05$). Subjects who were using an Indian style of toilet also recorded a negative association with the presence of FC ($p<0.05$). A study found that using a western toilet increased the risk of constipation where in subjects were assessed both before and after switching from sitting to squatting for defecation. These researchers came to the conclusion that the best posture modification for minimizing gastro intestinal issues was sitting with feet elevated (Suri et al, 2020).

According to the Rome IV criteria for FC, which included manual defecation maneuvers, straining, lumpy or hard stools (Bristol stool form type 1 or type 2), incomplete evacuation, anorectal blockage, and decreased stool frequency (WHO criteria of fewer than three bowel movements per week), FC was diagnosed. The respondents required to have at least two of the aforementioned problems as well as occasionally passing loose stools without first using laxatives in order to meet the criterion for constipation. Additional constipation symptoms like failure to pass stool, prolonged straining, stomach bloating, anal pain, and abdominal pain were also asked (Mearin F et al, 2016).

This diagnostic criteria showed a presence of 20% of FC among the study population in the current study. However, if only Rome IV criteria was used, more number of subjects could have been diagnosed upto 94% (Rome IV). According to Dimidi et al (2019), there is little agreement between public perceptions of constipation and Rome IV criteria, and the opinions of general public on constipation varies noticeably from those of professional clinicians. These findings highlight the importance of educating medical professionals and the general public about constipation symptoms. The study suggest 94% of individuals who self-reported constipation satisfied the official diagnostic criteria (Rome IV). Twenty nine of the 1,623 participants who did not self-report constipation also met these requirements. Therefore, nearly one in three "healthy" people were clinically constipated but were unaware of it. This data suggests that some individuals might still meet the formal diagnostic criteria for chronic constipation even though they do not think they have the condition. The discrepancy between symptoms thought to be crucial for a diagnosis of constipation and those included in Rome IV criteria could be one explanation.

Chrono nutrition profile evaluates a person's distinct dietary habits and behavioral traits, which might give insight into their present health status. When a study was conducted on many countries to find out which meal was the biggest, France and Switzerland claimed lunch was the biggest meal of the day which is in corroboration with our study findings, whereas North America stated evening was the biggest meal. A study by Almoosawi et al (2016) found that eating the largest meal earlier in the day or having a larger breakfast was more beneficial for improved body functions (Almoosawi et al, 2016). A study by Yamada et al (2021) showed that during the three-year period of research, a lack of fruit consumption and declining habits, such as missing breakfast and becoming sedentary, were linked to an increase in constipation status.

An important requirement to consider when examining the eating habits and sleep schedule of constipation sufferers is their chrononutrition profile. Constipation and other digestive disorders have been linked to improper circadian rhythms in the gut (Duboc H et al, 2020). The circadian rhythm is an endogenous time system with a 24-hour cycle that regulates an individual's behavioral and physiological activities, such as metabolism, energy balance, sleeping and eating patterns throughout the day (Lee et al, 2021). A significant association ($p<0.05$) was recorded for subjects for their sleeping pattern on a free day with constipation profile. A network of circadian clocks that interact constantly controls the circadian rhythm

(Wehrens et al, 2017). As per Murakami and Tognini (2020), delaying or skipping meals causes circadian misalignment which is observed among 10% subjects in case of breakfast as observed in the current study. The "circadian misalignment" refers to improper sleep-wake schedules and improper feeding patterns (Mirghani, 2021).

Dietary intake has a direct impact on chronotype of individuals. Individuals with an evening chronotype are less likely to consume a healthy diet (Mazri et al, 2019). Compared to morning chronotypes, individuals with an evening chronotype prefer to consume more items that are high in calories, sugar, fat, and saturated fatty acids. The study's findings indicated that there is no significant association between the presence of FC with the diet consumed which has been in debate in recent years. A common cause of FC is lack of fiber and water intake (Corsetti et al, 2021; Forootan et al, 2018). Similar results were obtained in the study which indicated that intake of insufficient drinking water, total fiber and soluble fiber was negatively associated with the presence of FC.

Another important newly developed phenomenon for improved digestion and reduced risk of FC is mindful eating. One of the major criteria of mindful eating involves chewing of food which promotes the breakdown of food into absorbable components through enzymatic and mechanical processes. The limit is set at a minimum of 30 times/morsel (Cherpak C E, 2019). Chewing insufficiency is associated with FC which was proved by Khayyatzadeh et al (2018). The current study shows that 33% of study participants weren’t aware of the chewing time and 50% participants were chewing their food <20 times and this result was strongly significant with the presence of FC.

In addition to other factors, dietary factors like eating habits, food preferences, and intake of macro- and micronutrients have most frequently been shown to affect the activity of the gastrointestinal system (Schneeman, 2004). Daily consumption of fibrous foods has been positive associated with the presence of constipation which was similar to our study with respect to consumption of pulses. As per NIN-ICMR recommendations (2020), a total of 40g of dietary fiber is recommended for a 2000 kcal diet. A ratio of 1:3 of soluble fiber to insoluble fiber for daily wellbeing is suggested as a dietary reference for Indians. The current study revealed that the ratio of fiber consumed by the respondents was not at par with the guidelines and hence a significant negative association was recorded between fiber intake and the presence of FC ($p<0.05$).

Lower constipation levels were linked to grains, lipid-rich foods, total fats, and starch, while higher constipation levels were linked to sugary foods, sodium, and a greater calorie consumption (Rollet et al, 2022). As per the current study findings, University staff was not consuming more of processed foods, on either monthly or fortnightly basis, hence no positive correlated with recorded with FC.

Presence of functional constipation in the teaching staff of The M.S. University of Baroda was recorded to the tune of 19%.

PHASE II

Prebiotics are defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as "a substrate that is utilized by host microorganisms selectively to provide a health advantage". Prebiotics like GOS, are dietary components that have the ability to alter the intestinal microbiota of the host without causing any adverse effects, such as abdominal discomfort, bloating, or the development of pathogenic intestinal microbiota.

Food products have been able to develop with varying levels of prebiotics. In the currentstudy, it was feasible to replace and substitute sugar with prebiotics. Similar studies have reported the potential prebiotic effects of Fructooligosaccharide (FOS) and Xylooligosachharide (XOS) to substitute sugar when incorporated into food products such as cakes, ice creams, and other sweetmeat products (Sheth and Heena, 2017; Sheth and Viral 2017; Thakuria & Sheth, 2019).

According to FDA, sucralose is permitted for use as a sweetener in foods. No adverse events have been recorded with the use of sucralose in foods. Using non-nutritive sweeteners (NNS) can potentially lower the energy value of a diet by replacing sugar intake while keeping its sweet flavour. Sucralose is one of the NNS that is frequently used to replace table sugar (Wilk et al, 2022). Hence in the current study sucralose (6.5%) was added to increase the sweetness of the 100% GOS gummies.

It is advised to keep colour depths at no higher than what 20 Lovibond units can match when used with Lovibond RYBN (Red Yellow Blue and Neutral) units. The accuracy depends on the path length selection. The more intense the colour, the shorter the path length, unless working to a certain specification (Lovibond.com; Gupta and Sheth, 2015). Consumer perception of color additives has always been a matter of concern hence natural color was used in the study. Foods with too bright or strange coloration are seen as unnatural by consumers, creating a sense of suspicion that anything so strange is probably harmful (Abu Khader et al, 2021).

Similar findings of moisture and pH in sugary candies were reported previously (Mutlu C et al, 2018). The gummies had a low moisture level even in the absence of sugar in them, which is a positive indication for better shelf life and transportation (Saleh et al, 2016). Gummy's textural characteristics are mostly influenced by the amount of water and type of gelling agent

present. Gummy with a higher water content is substantially softer than candy with a lower water content, regardless of the gelling agent used (Minifie, 1971). The surface may become excessively hard and trap moisture if skin development happens too quickly. In turn, this may cause the candy's surface to "sweat" while being stored (Sudarshan et al, 2004). Therefore controlling the rate of drying of gummies and candies is necessary. Optimum acidity is a desirable characteristic in gummies. A very low pH is not preferred because the product won't be stable and a gel might not form. Gelation is likely to happen if a hydrocolloid is kept at its isoelectric point, pH for which the net charge is zero (Edwards, 2000).

Using texture profile analysis, it is possible to test for hardness, chewiness and stickiness (Nowakowski, 2000). The results obtained in the present study are in coordination with previous studies reported in terms of cohesiveness (Teixeira et al, 2021). Agar used as a gelling agent in the present study may have contributed to the textural characteristics of the gummies. Prebiotics exhibit a property of moisture retention which increases the softness and stickiness of the product (Jain et al, 2013). Similar observations have been observed in a study which reported that oligofructose contributes humectancy to soft food items (Kaur and Gupta, 2002).

Studies conducted on 14 Indian fried and non-fried desserts prepared with Fructooligosaccharide (FOS) as the prebiotic revealed a recovery of FOS in the range of 87–100% (Sheth and Viral, 2017; Sheth and Shah, 2017) and a loss of 4% inulin was reported in roasted chapati (Parnami and Sheth, 2010) using HPLC analysis This corroborates with the present study where GOS was used as the prebiotic in making gummies and exhibited a similar retention of GOS of 95 per cent which indicated a lower loss of the prebiotic even when subjected to higher temperatures.

The most prevalent microorganisms that cause deterioration in confectioneries are yeast and molds. The results are in corroboration with Yadav et al. (2021) where no mold growth was observed at the end of 2 months. The gummies were packed in airtight HDPE bottles and contained citric acid as a flavouring and salivating agent, which may have contributed to their longer shelf life at accelerated temperatures. Several factors are known to have contributed to the increased shelf life of the gummies. The findings are consistent with those reported by Čižauskaitė, U et al (2019) and Sabeera Muzzaffar et al. (2016) who also noted a moisture loss after a period of 3 months of shelf life studies. Lower acidity in foods can result in longer shelf life in terms of pH (Scott, 1957; Devi et al, 2016).

Analytical or sensory approaches, as well as a combination of tests, may be used in shelf life testing. Sensory testing is nearly always included in shelf life testing protocols because, in the end, the term "shelf life" refers to the period of time when the consumer no longer deems the confectionery acceptable (Ergun et al, 2014).

Previous studies have also reported a similar decrease in the color and flavor attributes of the candies prepared, at the end of their shelf life studies of 60 days and 180 days (Yadav et al, 2021; Kohinkar et al, 2014). This could be a result of how temperature affects the overall sensory perceptions of appearance and flavour.

The extensively performed sensory analysis of various compositions of gummies suggests that nutraceuticals like GOS can be incorporated in confectioneries making it easier to consume the vegetarian sugar free gummies. The GOS gummies were accepted by the trained panel even after 180 days of storage, which suggests that the gummies are exhibiting better shelf-life and keeping quality and can be of commercial interest. HPLC analysis revealed a recovery of 95% which suggests a high recovery of GOS. Hence, we conclude that the gummies supplemented with GOS can meet the increasing demand of consumers for healthy confectioneries without any change in organoleptic qualities and with an increased shelf life of 6 months at accelerated temperature.

PHASE III

According to the WHO/FAO and EFSA recommendations, dietary fiber supplementation with 25 to 30 g per day is recommended for patients with FC, but it's vital to understand how it works. Insoluble fibers, such wheat bran, raise the water level of the small intestine and speed up the passage of food through the small intestine and colon, increasing the frequency of stools. Bran has a sizable amount of fermentable fibre, which may make several related symptoms of FC worse, like bloating, flatulence, and pain in the abdomen (Corsetti et al, 2021).

With an increase in stool size and frequency, soluble fibre like psyllium raises small bowel and colonic water content but not colonic gas (Erdogan A et al, 2016). A study by Ford et al, (2014), suggested that tolerance and compliance may be impacted by dose-dependent bloating, distention, and flatulence. The current study findings also suggest an increased flatulence as a side effect of GOS supplementation due to the ingestion of soluble fiber.

With respect to the constipation profile of the subjects, the frequency of stools was considerably enhanced by GOS consumption as compared to the placebo fed group. In a large number of patients with functional constipation, a rise of 1.01 bowel movements per week by prebiotic ingestion normalized stool frequency (Dimidi et al, 2014). GOS is crucial for avoiding constipation. According to a clinical trial investigation, using GOS for three weeks improved constipation symptoms in women significantly (Teuri and Korpela, 1998). Prebiotics improve the gut's ability to bind water. Stool weight, frequency, and softening are all increased by these movements, which shorten transit time. Stool frequency is increased by various GOS levels in newborn formula (Ben et al., 2008). Infants receiving prebiotic supplements had softer feces and stool consistency similar to that of breastfed infants when compared to infants getting normal formula (Fedorak and Masen, 2004).

The current study confirmed a definite effect of GOS consumption on Bifidobacterium growth over time in comparison to the placebo group, adding to the notion that Bifidobacterium bacteria may be a factor in enhancing bifidogenic effect. All literature review found an increase in Bifidobacterium at the genus level, while the majority of studies also found an increase in Lactobacillus.

Our study revealed that, in comparison to the other control groups, the GOS supplemented group exhibited a higher ratio of Firmicutes to Bacteroidetes (F/B ratio). These results were in

accordance with studies of a similar nature conducted in constipated women of reproductive age, which likewise revealed a higher F/B ratio in healthy women when compared to constipated women (Li et al, 2021). It is commonly acknowledged that the Firmicutes/Bacteroidetes (F/B) ratio plays a significant role in preserving healthy intestinal homeostasis (Stojanov et al, 2020).

Based on previously published studies in humans and animals, which reported a range of treatments from 7 days (Nayakama and Oishi, 2013) to 12 weeks, the length of the treatment was determined (Davis et al, 2011). There are several similar research that have used GOS as a bioactive compound and conducted their investigations for around 4 weeks with positive outcomes. Short-term dietary interventions in healthy subjects with potentially bioactive substances can act as early markers of their ability to positively modulate the gut flora (Johnstone et al, 2021; An et al, 2019). In this current study, the GOS was supplemented for 30 days, and this led to appreciable changes in the composition of the gut microbial composition.

In this clinical trial, we examined the fecal microbiota of sedentary teaching staff with constipation based on 16S rRNA-based genera/phyla specific DNA amplification using Realtime PCR to evaluate and compare the specific microbial differences. At baseline, there were significant differences, with phyla Firmicutes, Bacteroidetes, and genus Clostridium being more prevalent and the genera Bifidobacterium, Lactobacillus, and Bacteroides being significantly less. According to an RT PCR-based investigation, Bifidobacterium and Bacteroides species were reduced in the subjects with functional constipation (Kim SE et al, 2015).

According to the current research, consuming GOS for 30 days increased the relative abundance of the beneficial genera including Bifidobacterium, Lactobacillus, in the gut of the subjects. The definite effect of GOS consumption on the increased colonization of Bifidobacterium post supplementation in comparison to the placebo group, added to the fact that Bifidobacterium bacteria may be a factor in enhancing bifidogenic effect. This suggests that encouraging the growth of prebiotic bacteria may have adaptive consequences that limit the colonization of pathogenic microorganisms including Clostridium.

According to Gibson et al (2017), prebiotics should be digested by specialized microbes conferring beneficial effects on the host health rather than being processed generically. This is

pertinent to other human studies using prebiotics such as inulin, FOS, and GOS found that treatment with prebiotics significantly improved the relative abundance of gut bacteria belonging to the phylum Actinobacteria, with Bifidobacterium genus making a major contribution, whilst the majority of studies also concluded an increased colonization in Lactobacillus spp. (Liu et al, 2017). According to Macfarlane et al (2008), an increase in Bifidobacteria in the gut can inhibit the growth of spoilage bacteria like Clostridium spp. and prevent the production of harmful fermentation products.

The results of the present investigation showed that Bacteroidetes phylum levels in the constipated Day 0 samples were greater, and that these levels significantly decreased following GOS consumption in the constipated individuals after 30 days. Consistent with our findings Guo et al (2020) demonstrated that the presence of the phylum Bacteroidetes was almost two times higher in the gut of functionally constipated subjects as compared to the healthy group (Okhusa et al, 2019). In our investigation, it was found that the participants who had constipation had lower levels of total Firmicutes on Day 0 before intervention. The results also presented higher concentrations of Lactobacillus, a member of the Firmicutes phylum. The stool samples were examined for Firmicutes abundance using RT PCR post supplementation following GOS consumption, and the results indicated that the Firmicutes species had decreased in abundance while the Lactobacillus species had significantly increased. Previous studies have reported a possible reorganization of species within the phylum of firmicutes in which GOS supplementation fosters the growth of beneficial bacteria (Zhu et al, 2014).

Our study revealed that, in comparison to the other control groups, the GOS supplemented group exhibited a higher ratio of Firmicutes to Bacteroidetes (F/B ratio). These results were in accordance with studies conducted on constipated women of reproductive age, which likewise revealed a higher F/B ratio in healthy women when compared to constipated women (Li et al, 2021). It is commonly acknowledged that the Firmicutes/Bacteroidetes (F/B) ratio plays a significant role in preserving healthy intestinal homeostasis (Stojanov et al, 2020).

Short chain fatty acids are also called as postbiotics which are functional bioactive compounds formed during the fermentation of prebiotics in the gut (Thorakkatu et al, 2022). The study findings reported an significantly improved level of acetic acid ($p<0.05$) and butyric acid ($p<0.01$) which has been corroborated with previous studies which stated GOS supplementation is believed to improve short-chain fatty acid profile, in particular acetate and

butyrate (Grimaldi et al, 2016). The byproducts of fermentation by bacteria like Bifidobacterium and Lactobacillus are SCFAs. Fecal microbiota from breastfed children produced the same SCFAs in an in vitro experiment as those from supplemented GOS feeding (Knol et al., 2005).

The importance of mental health evaluations in all patients with functional gastrointestinal disorders, particularly among constipated individuals should be considered (Adibi et al, 2022). The most reliable and widely utilised tool for assessing the severity and behavioral aspects of depression is the Beck Depression Inventory (BDI). A strong relationship has been established between mood and specific bowel habits (Ballou et al, 2019; Palsson et al, 2020). Previous studies suggests a positive relationship between depression and functional constipation which is similar to our study results on baseline. Constipation is most likely caused by altered colonic transit and ano-rectal activities as a result of gut motility modification through the brain-gut axis (Devaranarayana and Rajindrajith, 2009).

According to literature review, FC significantly impacts the lives of individuals and exerts an unfavorable impact (Lim et al, 2016). Patient assessment of constipation quality of life (PACQoL) is a patient-centered concept that refers to questions regarding the severity/extent of symptoms, how constipation impacts their daily life, feelings related to constipation, life with constipation, and degree of treatment satisfaction. Constipation has a significant negative impact on patient's quality of life suffering from constipation (Palsson et al, 2020) as reported in our study findings. Similar results were observed in a study conducted by Baker et al (2016) wherein they had compared the PACQOL metrics for subjects suffering from FC. Another study conducted by Lewicky-Gaup et al, (2005) concluded that individually, the PAC-QOL components measuring physical discomfort, fears and anxieties, and satisfaction were all linked to reductions in stool symptoms. There is a perception among patients who report to be constipated that their general health, emotional health, social functioning and mental health are impaired (Hosseinzadeh et al, 2011). Management of symptoms may prove to be an effective way of improving QoL for a substantial number of patients (Belsey et al, 2010).

It is widely accepted that GOS is a natural substance having prebiotic potential. It has been demonstrated that consuming prebiotics will help you maintain an optimal equilibrium in your gut flora. In actuality, GOS is marketed as a blend of galactosyl oligosaccharides with various geometries and levels of polymerization. Researchers will likely pay close attention to screening for β-galactosidase with transgalactosylation due to the crucial role that GOS plays in the field of functional foods (Mei et al, 2022).