"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"

Synopsis of Ph.D. Thesis

Submitted by:
Kankona Dey

Guide:
Prof. Mini Sheth

Department of Foods and Nutrition
Faculty of Family and Community Sciences
The Maharaja Sayajirao University of Baroda
Vadodara

2023

Table of Contents

INTRODUCTION

Functional constipation (FC) is not an uncommon problem in Indian communities and in clinical practice. Constipation is a functional GI disorder that may significantly affect the quality of life and healthcare cost. It is not only restricted to elderly, but happens in middle age and young adults as well, and sedentary lifestyle and physical inactivity is one of the major causes (Lacy B.E. et al, 2016).

Functional Constipation is defined as the elimination of hard stools associated with one of the following characteristics: Pain or straining while passing stools, scybalous stools (hard, inspissated stool), unproductive calls ("want to but cannot"), infrequent stools, or incomplete evacuation; < 3 bowel movements per week, daily stool weight < 35 g/day, or straining > 25% of the time. Cylindrical and cracked or cylindrical and thick stools, stool frequency less than three times per week. Acute constipation may cause closure of the intestine, which may even require surgery (Hyams J., et al, 2002).

Global prevalence of constipation varies substantially because of differences among ethnic groups in how constipation is perceived. According to a study in Japan, 9.2% was reported as the prevalence for constipation (Katsuhisa Omagari et al, 2020). According to a Lancet study in 2021, a meta-analysis conducted with 45 studies for 80 population types (275260) reported a prevalence of 10% (Brigida et al, 2021). In spite of the scarcity of data, available studies indicate to be a common health problem in India, contradicting the popular belief of its infrequency due to vegetarianism with high fiber intake, and higher frequency of bowel movement, suggesting that Constipation might be, in fact, under-reported. According to the National Health Portal, prevalence of constipation ranges between 24-37%.

An improved understanding of this hidden organ will reveal secrets that are relevant to human health (Shin A et al, 2019). Our gut microbial community become more diverse and more stable as we become adults. How changes in lifestyle, illness, puberty, and so on affect the microbiota and its stability is still a matter of speculation (Spor, A., Koren, O., & Ley, R. 2011). Our dietary habits have a strong effect on our gut microbiome and metabolism. Low consumption of dietary fibers leads to low production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate involved in the modulation of host immune and inflammatory health status (Maslowski KM, Mackay CR**,** 2011).

Imbalance in gut microbiome is Dysbiosis

Dysbiosis is often defined as an "imbalance" in the gut microbial community that is associated with disease. This imbalance could be due to the gain or loss of community members or changes in relative abundance of microbes. Unsolicited perturbations in gut microbiota diversity and composition (gut dysbiosis) are key elements underlying chronic diseases including several low-grade inflammatory disorders of human gastrointestinal tract & associated complications. It is now well established that the gastrointestinal (GI) microbiota has a fundamental impact on metabolic, immunological, and endocrine functions of the host (Nagpal R, Shively CA, Register TC et al. 2019).

Dysbiosis is linked to Constipation

The host-microbe interrelationship is viewed as mutualistic, contributing to overall host health However, aberrations in both microbiota composition and function can result in the development of several chronic disease (Maslowski KM, Mackay CR**.** 2011). Dysfunctions in these mechanisms are linked to a range of conditions in the gastrointestinal tract, including functional gastrointestinal disorders, like functional constipation.

Functional Constipation is linked with Depression

It is well established that mood and anxiety disorders in Functional Gastrointestinal Disorder (FGD) patients is higher than in the general population up to 50% or even more (Henningsen P et al, 2003 & Van Oudenhove L, 2004). Studies on constipation in adults have confirmed the important role of psychological factors (Williams M etal, 2005*)*. The most prevalent disorder in constipated patients is major depression (or Mood Depressive Disorder). The second prevalent disorder was Generalized Anxiety Disorder (GAD) (31.5%) (Hosseinzadeh ST et al, 2011).

Functional Constipation and Chrononutrition

Maladjusted circadian rhythms in the bowel have been linked to digestive pathologies, including constipation (Duboc et al, 2020). Time Restricted eating (TRE) is a simple and well-tolerated diet that generates many beneficial health effects based on chrono nutrition principles. More rigorous studies are needed, however, to confirm those effects, to understand their mechanisms and to assess their applicability to human health. (Aadafer et al, 2020). Circadian rhythms regulate much of gastrointestinal physiology including cell proliferation, motility, digestion, absorption, and electrolyte balance (Voigt et al, 2019).

Modes of treatment for Functional Constipation:

According to a 2019 report from the Bowel Interest Group, constipation affects one in seven adults and one in three children and the treatment costs approximately £162 million (Lancet Editorial, 2019). The initial approach of management involves-

(1) *Diet:* Empirical treatment of constipation comprising an increase in dietary fiber content to approximately 25–30 g per day and increased hydration (2–2.5 L per day) is an inexpensive and effective method to increase evacuatory frequency and reduce laxative use (F. Mearin, et al, 2016).

(2) *Laxatives:* The second step in the management of intestinal constipation involves the use of osmotic laxatives, such as polyethyleneglycol (PEG) (1A) and lactulose (1C) and laxatives associated with the formation of fecal matter (psyllium, methylcellulose and polycarbophil (A.C. Ford and N.C. Suares, 2011). However, unwanted effects such as abdominal cramps and, in chronic treatment, the development of gastrointestinal tolerance may result from the use of stimulant laxatives (Potter J, Wagg A, 2005).

However, according to a 2017 US survey of patients with chronic idiopathic constipation, 59% of patients were not satisfied with the treatment they received (Lancet Editorial, 2019).

Prebiotics as a line of treatment:

The definition of prebiotics was given by Gibson and Roberfroid (1995). It is already known that all prebiotics are dietary fibers, but all fibers cannot be called as a "Prebiotic". There are certain specifications laid down which confirms that a Prebiotic should be:

(1) A food ingredient should resist gastric acidity, hydrolysis by mammalian enzymes and absorption in the upper intestinal tract
(2) Fermented by the intestinal microflora
(3) Selectively stimulating the growth and or activity of intestinal bacteria potentially associated with health and well being

Studies suggest that prebiotics even at a low dose of 5-8g/day significantly amplifies fecal bacteria like *Bifidobacteria* and *Lactobacillus*. An alternative among nutritional therapies for constipation is the use of prebiotics, including galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) (Davis LMG, Martínez I, Walter J, Hutkins R.2010). Various studies have proved that feeding of FOS in adults have increased the colonization of *Bfidobacteria* and *Lactobacillus* and reduced the colonization of pathogenic bacteria like *E.coli* and *Clostridum* (Sheth & Jain & Assudani, 2017; Mahendra & Sheth 2015).

Galactooligosaccharide (GOS)

Enzymatic conversion of lactose present in whey fraction of cow's milk, by use of b-galactosidases. It contains galactose chains, starting with glucose, with degree of polymerization between 2-10. GOS is approved by USA Food and Drug Administration (FDA) with GRAS status; European Food Safety Authority (EFSA) novel foods; Foods for particular nutritional uses (PARNUTs) status within EU; Food for Specified Health Uses (FOSHU) in Japan.

Gummies as a medium for Clinical trial:

According to Sessler, Weiss, and Vodovotz (2013), confectionery such as chewing gum, candy and 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. These factors influence the confectionery market, which has improved its products with the incorporation of ingredients and technologies that meet the needs of the most demanding consumers (Li & Srigley, 2017). The laxative effect of GOS is believed to be caused by its action as a soluble fibre. Oligosaccharides pass undigested into the large intestine and stimulate bacterial fermentation in the colon. The bacterial fermentation of oligosaccharides increases bacterial mass, which in turn increases faecal bulk. Undigested oligosaccharides and fermentation products may also produce an osmotic effect in the gut, which increases the water content of faeces (Topping DL, Clifton PM, Physiol Rev. 2001)

RATIONALE

Dysbiosis is common in constipated population and determining the role of prebiotics in improving gut health is vital. Constipation also leads to stress and vice versa due to low fiber and water intake, physical inactivity and increased westernized diet and lifestyle. There are only a handful of studies that describes the treatment efficacy for chronic constipation using dietary fibers. Several Prebiotics are considered as effective modes of treatment and may prove to be beneficial in treatment for functional constipation. However, there are no randomized, double-blind, placebo-controlled clinical trials undertaken using GOS in treating adults in the teaching profession suffering from functional constipation. Gummies are popular "sugary" confectionary amongst all age groups. Moreover, FSSAI has mandated to reduce sugar intake. There is a need to study the feasibility & acceptability of sugar substituted GOS gummies. There are a few studies on GOS and bowel movement for the elderly population and infants. However, there are limited studies which focus on the relationship of GOS intake and

functional constipation in the adult population. Hence the present study was aimed to focus on a potential novel line of treatment for functional constipation with improved gut health of the adult population with a teaching profession suffering from functional constipation.

OBJECTIVES

Broad Objective:

To study the 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 Constipation Profile and Gut Health

Specific Objectives:

1. To select and train the sensory panel members
2. To make standardized gummies with respect to various levels of sugar, water, gelling agent, GOS, acid.
3. To conduct shelf life analysis for the GOS substituted gummies for a period of 6 months
4. To develop, pretest and validate the tool (Questionnaire) to determine the presence of constipation among the teaching staff of The M. S. University of Baroda.
5. To screen the teaching staff of The M. S. University of Baroda for the presence of functional constipation with respect to their general information, family history, medical history, physical activity pattern, defecation profile, chrono nutrition profile and food frequency with the help of pre tested and validated questionnaires.
6. To standardize the methods for laboratory analysis.
7. To investigate whether Galacto-oligo-saccharides (GOS) relieves constipation status in teaching staff in The M. S. University of Baroda
8. To investigate whether Galacto-oligo-saccharides (GOS) relieve depression status in teaching staff in The M. S. University of Baroda.
9. To study the effectiveness of GOS Gummies on the defecation profile teaching staff in The M. S. University of Baroda.
10. To study the effectiveness of GOS Gummies on the quality of life in the teaching staff in The M. S. University of Baroda.
11. To investigate whether Galactooligosaccharide (GOS) improve the gut microflora in teaching staff in The M. S. University of Baroda.

HYPOTHESIS

Null Hypothesis:

Supplementation of GOS gummies will not bring about any improvement in the functional constipation status, depression, quality of life and gut health in terms of gut microflora (*Bifidobacteria, Lactobacillus*, *E.coli*) of the constipated adults.

Alternate Hypothesis:

Supplementation of GOS gummies will bring about an improvement in the functional constipation status, depression, quality of life and gut health in terms of gut microflora (*Bifidobacteria, Lactobacillus*, *E.coli*) of the constipated adults.

REVIEW OF LITERATURE

This chapter will focus on the available literature under the following heads:

1. Incidence and prevalence of functional constipation
- Global prevalence
- Indian prevalence
2. Criteria to diagnose constipation
3. Types of constipation
4. Etiology of constipation
- Chrono nutrition profile as one of the factors
- Dietary intake as of the factors
5. Approaches to manage and treat constipation
- Dietary approaches
- Alternative systems to treat constipation including ayurveda, homeopathy, yoga etc
- Nutraceuticals
- Galactooligosaccharide as a prebiotic to combat constipation
6. Health complications as a result of constipation
7. Gut dysbiosis and constipation
- Specific microorganisms of concern in gut dysbiosis
- Alterations in short chain fatty acids profile in constipation
8. Gummies as a confectionery and medium of supplementation
9. Physicochemical properties of gummies

METHODOLOGY

This chapter focusses on the various tools and techniques used for arriving at the results of the stated objectives. The experimental design for the methods of study is presented in Table 1.The detailed methods for the various phases of the study are presented as under the following heads:

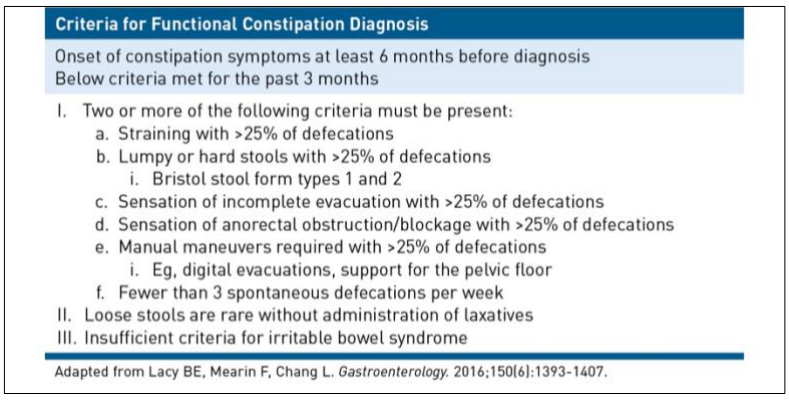
Phase 1: *Screening for the presence of constipation in the teaching staff of The M. S. University of Baroda*

Phase 2: *Acceptability Trials of GOS addition to standard gummies at varying levels and its shelf life studies*

Phase 3: *Impact evaluation of GOS added gummies on Constipation, Depression, Gut health and Quality of Life of constipated subjects.*

Phase 1: Screening for the presence of constipation in the teaching staff of The M. S. University of Baroda

University teaching staff (n=364) were screened using a cross-sectional study design and purposive sampling technique, for the presence of functional constipation. A pre-tested and validated structured questionnaire was administered to them bearing questions related to their gastrointestinal tract functionality, constipation profile, BMI, medical history, physical activity, personal habits, perceptions, practises, chrono nutrition profile, food frequency and dietary habits (Fig 1). Participants were scored on a scale of 20 based on the criteria laid down by World Health Organisation (WHO), The Rome Foundation (Fig 2A) and Bristol stool chart (Fig 2B) to assess the presence and degree of constipation.



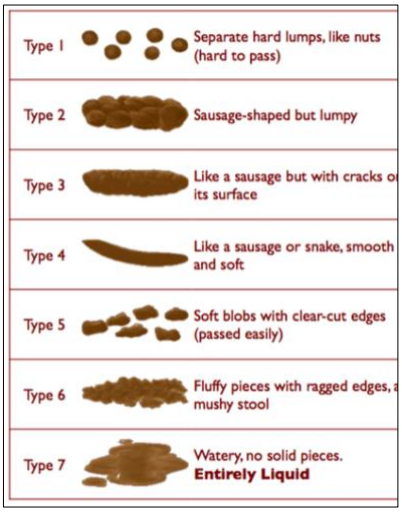


Fig 2: (A) Rome IV criteria for Constipation (B) Bristol Stool Chart

Table 1: Experimental Design for the study

Phase 1 *Screening for the presence of constipation in the teaching staff of The M. S. University of Baroda*	Phase 2 *Acceptability Trials of GOS addition to standard gummies at varying levels and its shelf life studies*	Phase 3 *Impact evaluation of GOS added gummies on Constipation, Depression, Gut health and Quality of Life of constipated subjects*
Snapshotting the presence of constipation (n=364) - General information - Medical history - Family History - Personal habits/ Addictions - Physical activity - Perceptions and practices - Constipation Profile ▪ WHO criteria ▪ Rome IV criteria ▪ Bristol stool chart - Chrono nutrition profile (Alison 2018) - Frequency of consumption of selected food groups (Krause 2016) - Dietary intake (Krause 2018)	Trained panel (n=8) was recruited for sensory evaluation - Standardisation of gummies ▪ Water (65ml, 75ml, 85ml) ▪ Sugar (50g, 60g, 70g, 80g) ▪ Agar (2g, 2.5g, 3g, 3.5g) ▪ Citric acid (0.48g, 1.9g, 3.8g, 5g) ▪ Sugar substitution with GOS (60%, 80%, 100%) ▪ Addition of sucralose (5g, 5.5g, 6g) - Physico-chemical Properties of GOS gummies ▪ Color intensity ▪ pH/ Acidity ▪ Texture profile ▪ Moisture analysis - Recovery of GOS in 100% GOS gummies - Shelf Life studies of GOS gummies on Day 0, 30, 60, 90 days at 37^{o}C ▪ Sensory properties ▪ Microbial properties (Refai and FAO, 1979). o TPC o Yeast and moulds o *E.coli*	Studying the impact evaluation of GOS added gummies (n=48) ▪ Randomised Double blind placebo control technique - Constipation Profile - Depression profile (Becks Depression Inventory, 1996) - Quality of life profile (PACQOL, 2004) - Gut Microbiota profile for the following ge & phyla (Krumbeck et al, 2018) ▪ Bifidobacteria ▪ Lactobacillus ▪ Clostridium ▪ Bacteroides ▪ Firmicutes phyla ▪ Bacteroidetes phyla - Short chain fatty acid (SCFA) profile ▪ Acetic acid ▪ Butyric acid ▪ Propionic acid

The sample size was calculated as per the following formula:

$$\frac{\mathbf{Z}_{\alpha/2}^{2} * \mathbf{N} * \mathbf{p} * \mathbf{q}}{\mathbf{e}^{2} * (\mathbf{N} - \mathbf{1}) + \mathbf{Z}_{\alpha/2}^{2} * \mathbf{pq}}$$

where,

p = Proportion of constipation = 0.092

q = Proportion of not constipation = 0.908

$Z_{\alpha/2}$= The value of the standard variate at confidence interval (99%) = 2.576

e = Margin of error = 0.05

By substituting all these values in the formula, sample size was 193.

Attrition rate=20%

Drop out = (1/1-0.2) *193 = (1/0.8)* 193 = 1.25* 193 =241.25 = **241**

The final sample size for Phase 1 was calculated to be 241

Phase 2: **Acceptability Trials of GOS addition to standard gummies at varying levels and its shelf life studies**

Threshold test was performed to select the panel members (20-35 years) to investigate the ability to recognize basic taste in solution (salt, sugar and acid). The selected panel members were trained for a period of 7 consecutive days to evaluate the standardised gummy for its sensory attributes like colour and appearance, mouthfeel, texture, taste, and aftertaste (Silva et al, 2014).

The gummies were standardized using varying levels of each ingredients including water, sugar, agar and citric acid (Fig 1). All the products were subjected to organoleptic testing in triplicates to select the standardized gummy. All ingredients used for preparation of gummies were FSSAI certified. Sugar content in the standardized gummies was substituted with varying levels of GOS and sucralose and was subjected to organoleptic testing (Fig 1).

The trained panel (n=8) evaluated the GOS substituted gummies with the help of a composite scoring test and the most acceptable GOS added gummy was taken further for physico chemical analysis and shelf life analysis. Difference test was conducted to choose the most acceptable gummy with 100% GOS and sucralose. The test was conducted in triplicates.

The 100% GOS substituted gummies was subjected to physico chemical analysis including colour intensity, moisture, pH, texture and recovery of GOS in triplicates. The analyses were carried out at the Food and Drug Laboratory, Vadodara, India.

The following formula was used to calculate the amount of GOS present in a gummy:

Concentration of GOS in one piece of gummy (3.5g) =

$$\frac{\text{Average area of the sample concerned}}{\text{Average area of the standard GOS}} \times 100$$

Shelf life tests was performed on the 100 percent GOS substituted gummies packaged in High density polyethylene (HDPE) bottles kept at accelerated temperatures with respect to sensory evaluation and microbial parameters (*E. coli*, total plate count, and yeast and mould count) on days 0, 30, 60, 90, and 180 (Refai and FAO, 1979).

Phase 3: Impact evaluation of GOS added gummies on the Constipation, Depression, Gut health Profile and Quality of Life

Using a Randomized double blind placebo controlled trial, the teaching staff of The M. S. University of Baroda aged 25-42 years suffering from functional constipation was selected for this phase of the study.

This phase of the study are presented as under the following heads:

Sample size calculation:

The sample size was calculated as per the following formula:

$$\mathbf{n = [(Z\ \alpha/2\ + Z\ \beta\ )\ 2\ \times \{(p\ 1\ (1\text{-}p\ 1\ ) + (p\ 2\ (1\text{-}p\ 2\ ))\}]/(p\ 1\ -\ p\ 2\ )\ 2}$$

where

p 1 = proportion of subject effectiveness by treatment A = 0.7,

p 2 = proportion of subject effectiveness by Placebo = 0.2,

p 1 -p 2 = clinically significant difference = 0.5

Z α/2 : This depends on level of significance, for 5% this is 1.96

Z β : This depends on power, for 80% this is 0.84

Level of significance = 5%, Power = 80%, Type of test = two-sided

Attrition rate=20%

Based on above formula the sample size required per group is 19.

Hence total sample size required is **48** (Ref: Danielda et al, 2018)

Since no Indian study on adult population to detect functional constipation using Rome IV criteria is available, hence a study conducted in Japan is used as the reference.

Inclusion criteria and Exclusion criteria

Subjects who were a teaching faculty in The M.S. University of Baroda and were having symptoms related to functional constipation for >6 months and were in between the age group of 25-62 years were selected for the study. Subjects who underwent intestinal resection or had a history of GI diseases like hiatal hernia, fissures, haemorrhoids, IBS/IBD, chronic metabolic disease such as symptomatic cardiovascular disease, insulin-requiring or uncontrolled diabetes, current active treatment of cancer, or were on antibiotic/any medication for any chronic illness use within the last 12 weeks prior to enrolment, or on regular use of NSAIDs (Eg, aspirin), or on excessive alcohol intake (more than two drinks for men and one drink for women daily), or who were consuming probiotics, prebiotics, or synbiotic on a regular basis and pregnant and lactating women were excluded from the study.

Randomisation, allocation and Blinding

Constipated adults (n=48) were randomly assigned to 1 of the groups— control group (n=24) receiving placebo, experimental group (n=24) receiving 100% GOS gummies—through a randomization list prepared using computer generated random numbers. The allocation ratio followed was 1:1. The list was prepared by a senior professional in the Department of Foods and Nutrition who was not otherwise involved with the study. Appropriate coding was generated and allotted to the subjects to maintain blinding between the groups.

Study intervention process

The 2 intervention groups received kits with the gummy bottle, compliance sheet, uricols (stool collection container), a black polythene bag and a thank you card for participating in the trial. The kits for control subjects contained standard sugar gummies and kits for experimental group contained the GOS gummies. Subjects were provided with gummies for 4-week intervention period and were instructed to consume 4 gummies daily containing 10g GOS. At the end of the 4-week intervention period, subjects provided stool samples along with the post data on their constipation profile, depression profile and quality of life with the help of validated questionnaires.

Monitoring and Compliance

Compliance was monitored with appropriate follow ups and side effects experienced if any, using daily SMS reminders and phone calls weekly. A calendar was especially designed and

distributed to all the subjects to document daily consumption of gummies and any unusual symptoms or side effects if observed. Calendars were collected back after the completion of intervention period. They were asked to record for any pain, straining, difficulty passing stools and adverse effects (excessive pain, regurgitation or vomiting) in the compliance sheet during the intervention period.

Collection of fecal sample, storage and analysis:

Stool was collected on day 0 along with baseline information on their depression profile and quality of life. Fecal samples were collected in sterile uricols and stored right away at -80°C until DNA extraction was done. The samples were subjected to Fecal microflora quantification using Real time PCR technique with respect to *Bifidobacteri*a sp., *Lactobacillus sp.*, *Clostridium sp.*, *Bacteroides sp.* and major phyla Firmicutes and Bacteriodetes which constitutes for 90% of the human gut microbiome. Fecal samples were also subjected to short chain fatty acid (SCFA) analysis using Gas Chromatography technique with respect to acetic acid, propionic acid and butyric acid.

DNA extraction from fecal samples

DNA from the stool samples was extracted using the QIAamp DNA stool mini kit (Cat. No. 51504, Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA was quantified using Thermo Scientific NanoDrop Spectrophotomter with high accuracy and reproducibility.

Real-Time PCR analysis

To determine the relative abundance of bacterial genomes, real-time qPCR was used. DNA concentrations was determined by µDrop plate, Multiskan sky spectrophotometer (Thermo scientific, MA, USA). The DNA concentration for the PCR was established by serially diluting the DNA from 5 ng to 0.25 ng, and it was found that 1 ng/reaction was appropriate for all amplifications. All of the Real-Time qPCR experiments were performed using iTaq Universal SYBR Green Super mix (Bio-Rad, Cat. No. 1725124, Hercules, CA). QuantStudio3 (Life Technologies, Carlsbad, CA) was used for the Real-time PCR amplification, and the procedure was as follows: initial denaturation at 95 ºC for 10 minutes, denaturation at 95 ºC for 15 seconds, annealing for 1 minute at a temperature as calculated for 40 cycles, and melt curve analysis was performed at the end of the reaction.

Short chain fatty acid (SCFA) Analysis (Willian et al, 2018)

SCFA analysis was performed on a sub sample, with respect to acetic acid, propionic acid, butyric acid at baseline and after supplementation with gummies (experimental and placebo).

Statistical Analysis:

Statistical analysis was performed using Microsoft Excel and JASP software, MAC version. Results were expressed in terms of mean values and standard deviation for the prepared gummies. ANOVA- one way variance was performed to determine any significant difference among the samples with varying concentrations. Chi square test was used to assess the extent of differences among the scores obtained for the difference test which was used to study the superiority or inferiority between the standard gummy and the 100% galactooligosaccharide and sucralose added gummy at a p value of 0.5. ANOVA- one way variance was used to determine any significant change in shelf life of the gummies over the period of 6 months at a p value of 0.5. Mean and standard deviations will be calculated for various parameters to arrive at the appropriate results. Paired *t*-test will be used to study the significant differences after the supplementation when compared to baseline values. Chi-square test will be used for studying the associations between various parameters for the baseline data. Pearson correlation co-efficient will be used to arrive at significant association amongst the various parameters. ANOVA test shall be applied to study the significant differences in the mean values of sensory attributes of varying levels of GOS added gummies as well as shelf life studies. Students t test will be used to check the differences between the placebo and experimental group before and after intervention.

Ethical Committee Approval and Study Registration

The study protocol was approved by the Institutional Ethics Committee for Human Research (IECHR), Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda. The ethical approval number of the study is IECHR/ FCSc/PhD/2021/3. The intervention trial has been registered in CTRI, ICMR with the registration number CTRI/2021/10/037474. Written informed consent was obtained from the participants.

RESULTS

Based on the objectives of the study, the findings are presented under the following heads:

Phase I: **Screening for the Presence of Constipation in The teaching staff of The M. S. University of Baroda**

This phase of the study was planned to snapshot the presence of constipation among the teaching staff of The M.S. University of Baroda. 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 income of >INR 123,322.00 (42.3%) and their age ranged between 35-64 years.

Functional constipation was observed in 19.2% subjects (n=70), with 15.4%. 3.3% and 0.5% in mild, moderate and severe categories respectively. Twenty five percent subjects suffering from functional constipation experienced <3 stools/week and 23% subjects were having a regular bowel movement. Obesity in various degrees was observed among 43.9% subjects (n=160). Statistically significant inverse correlation was observed between obesity and constipation ($p<0.05$).

Physical activity profile of the study subjects indicated 83% and 17% were in the sedentary and mildly active categories respectively. Statistically significant inverse correlation was observed between physical activity and constipation ($p<0.05$). Female subjects (n=50) were more constipated than the male respondents and the results were statistically significant ($p<0.05$). Many subjects (12%) felt constipation is a social taboo and 60% respondents felt constipation is a minor health issue.

No significant correlation was observed with constipation in terms of the working day profile, whereas in terms of a free day profile, chrono nutrition profile showed a significant association ($p<0.05$). The chrono nutrition profile of the respondents (n=364) also depicted that 10% subjects skipped their breakfast daily and 20% skipped their snacks daily. According to 50% subjects, lunch is their largest meal of the day. A significant association ($p<0.05$) was observed between duration of last meal and sleep onset on free day and time range for last meal of the day with the constipation status of the subjects.

62% of the respondents were vegetarians and 26% were non vegetarians. No significant association was seen among the different types of diet consumed and the presence of constipation. However, the type of diet consumed by the subjects did not show any significant association with the constipation profile.

With respect to frequency of consumption of various food groups, significant positive association was recorded with the consumption of whole and split pulses ($p<0.01$), natural sweeteners (jaggery) ($p<0.01$) and vegetables namely carrot, onion ($p<0.05$) with the constipation profile. Significant positive associations were observed between consumption of processed foods ($p<0.01$) with the presence of functional constipation. Intake of salt (< 1tsp) ($p<0.01$) and sugar (>2 tsp) ($p<0.05$) were significantly associated with the presence of constipation among the respondents.

Phase II: **Acceptability Trials of GOS addition to standard gummies at varying levels and its shelf life studies**

The trained panel did not find any significant differences in the organoleptic 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 differences ($p<0.05$) in the gummies with varied quantities of citric acid. The most accepted gummies' formulation were prepared with 75 ml water, 60 g sugar, 2 g agar and 1.9 g citric acid. The gummies with 60% sugar substituted with GOS was preferred in terms of mouthfeel, texture, flavour, general acceptability, and total score.

The physico-chemical parameters included determination of colour, moisture, acidity, and texture profile analysis. The colour intensity was measured using a tintometer (Lovibond) and the result depicted 7 lovibond units which was similar to the natural color Curcumin. The moisture and pH content of the gummies were determined as 24.8 % and pH at 3.37 respectively. Recovery of GOS in the most acceptable gummies exhibited a retention of GOS of 95%.

Shelf life studies conducted over a six-month period revealed no de-novo growth of *E. coli*, with no significant difference in TPC and yeast and mould count. Yeast and mould 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 mould count) were observed.

Acceptability trials demonstrated a considerable improvement in chewiness and a reduction in colour and flavour during shelf life testing. F test also showed no significant differences in the other organoleptic characteristics of gummies. The panellists accepted the gummies after 180 days of storage at accelerated temperatures (37^{o}c), demonstrating the successful shelf stability of the gummies even after six months on the shelf.

Phase 3: Impact evaluation of GOS added gummies on the Constipation, Depression, Gut health Profile and Quality of Life.

Constipated subjects (n=35) who gave informed written consent for participation and followed the inclusion and exclusion criteria were enrolled for the study. A double blind randomised technique was followed to divide the participants into control group and experimental group. The demographic profile of the participants chosen for the intervention trial demonstrates that the majority of respondents were females (74.36%), married (71.5%), and lived in nuclear families (62.92%), had a normal BMI, and were between the ages of 20 and 64 with a family income of >INR 123,322.00 (40.04%). During the intervention period, the experimental group experienced a significant reduction ($p<0.01$) in constipation symptoms, whereas the control group experienced an increase in symptoms and an associated reduction in quality of life by 22%.

At baseline, the placebo and GOS groups exhibited a similar pattern in the colonization of specific microbes with respect to harmful pathogens *Clostridium* (12.41±9.41 and 11.30±10.22), *Bacteroides(3.04*±4.04 and 4.26±6.03) and beneficial bacteria including *Bifidobacteria (5.05*± 6.45 and 10.14±9.87) and *Lactobacillus* (0.75±2.19 and 1.91±2.78) respectively. Two prominent phyla *Bacteroidetes* (44.70±21.07% and 35.63±21.92), and Firmicutes (34.04±14.50% and 32.18±14.02) were also studied in the placebo and experimental groups along with the F/B ratio (1.23± 1.14 and 2.66±6.1) respectively which is a measure for gut dysbiosis.

The experimental group post intervention with GOS gummies exhibited an increased colonisation of *Bifidobacterium* and *Lactobacillus* by 322% and 1230% respectively with

decreased colonisation of harmful pathogens including *Bacteroides* and *Clostridium* by 29% and 63% along with an improved F/B ratio (3.02 ± 0.73).

Depression was observed among 31% subjects in various categories ranging from mild mood disturbance and clinical depression to moderate depression. Post supplementation there was a significant reduction (40%) in various categories of depression in the experimental group ($p<0.01$). 67% subjects moved to normal category whereas 33% subjects moved from borderline clinical depression to mild mood disturbance category.

During the intervention period, the experimental group experienced a significant reduction ($p<0.01$) in constipation symptoms, whereas the control group experienced an increase in symptoms and an associated reduction in quality of life by 22%.

DISCUSSION

Constipation has a multifaceted pathogenesis, with particular emphasis on genetic predisposition, socioeconomic level, poor fibre intake, inadequate fluid intake, immobility, disruption of the hormone balance, drug side effects, or body structure.[3]An important requirement to consider when examining the eating habits and sleep schedule of constipation sufferers is their chrononutrition profile. Chrono nutrition is associated with constipation. Constipation and other digestive disorders have been linked to improper circadian rhythms in the gut[4].

The relationship between circadian rhythms, nutrition, and metabolism is explored by the emerging area of chrononutrition [14]. Chrononutrition encompasses both the concept of how our internal circadian system is impacted by nutritional composition as well as how food intake timing and biological rhythms affect our health, metabolism, and nutrition [15]. The effects of biologically active substances can be influenced by biological rhythms, and vice versa is also true when these substances are consumed.

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).

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).

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. HPLC method is the primary approach for identifying and quantifying oligosaccharides because of its high penetration rate and quick detection (Zhao et al, 2018). 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

The most prevalent microorganisms that cause deterioration in confectioneries are yeast and moulds. 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.

We 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 research 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. It is commonly acknowledged that the Firmicutes/Bacteroidetes (F/B) ratio plays a significant role in preserving healthy intestinal homeostasis (Stojanov et al, 2020)

CONCLUSIONS AND RECOMMENDATIONS

This study has been able to establish that functional constipation is a gastrointestinal issue of concern among the population with a prevalence rate of 19% among the teaching staff of the university. This research has brought into light the connection of gut dysbiosis with constipation and the need for improvement of both.

It is concluded from this study that gummies or sugary confectioneries can be successfully made by incorporation of prebiotic (GOS) with acceptable sensory properties, physico chemical properties and shelf life at accelerated temperatures (37^{o}c).

The teaching staff suffering from constipation was subjected to a double blind placebo control trial and was fed with 100% GOS gummies for a period of 30 days. Prebiotics such as GOS can be used as a potential remedy to alleviate the symptoms arising from constipation thereby modulating the gut bacteria upon its consumption. Quantification of selected gut microbes was carried out with the help of DNA isolation and RTPCR technique with respect to beneficial bacteria namely *Bifidobacteria* and *Lactobacillus* and harmful pathogens namely *Clostridium* and *Bacteroides* along with two prominent phyla Firmicutes and Bacteroidetes resulted in improved gut microbiome with increased significant relative abundance of the beneficial bacteria and reduced abundance of the pathogens. F/B ratio which is a measure for gut dysbiosis also improved upon supplementation with the resulted reduction in constipation profile.

Further studies needs to be undertaken to demonstrate the clinical efficiency of GOS incorporated in other products with respect to other gastrointestinal disorders. Further sequencing of the species could be carried out for more accurate information related to gut dysbiosis and functional constipation.

REFERENCES

1. Barile D, Rastall RA, Human Milk and related Oligosaccharides as prebiotics. Curr Opin Biotechnol 2013; 24 (2): 214-219
2. Becks Depression Inventory II, 1996, https://mdgcounseling.com/wp-content/uploads/2020/01/Asmnts-Initial-Part2-200106.pdf
3. Bomba, A. Nemcova,R. Mudronova,D. Guba,P. The possibilities of potentiating the efficacy of probiotics. Trends Food Sci. Technol. **2002**, 13, 121–126
4. Brigida et al, 2021, Global prevalence of functional constipation according to the Rome criteria: a systematic review and meta-analysis, The Lancet, vol 6 (8), 638-648
5. Danielda et al, 2018, Randomized, Double-Blind, Placebo-Controlled Parallel Clinical Trial Assessing the Effect of Fructooligosaccharides in Infants with Constipation, Nutrients, 10, 1602
6. Davis LMG, Martínez I, Walter J, Hutkins R. A dose dependent impact of prebiotic galactooligosaccharides on the intestinal microbiota of healthy adults. Int J Food Microbiol. 2010;144:285–92
7. Eline at al, 2013, Constipation – prevalence and incidence among medical patients acutely admitted to hospital with a medical condition, Journal of Clinical Nursing, 23, 2295–2302
8. Elvina & Namita, Dietary Intake of Tribal women of reproductive age group of Meghalaya, India, Int.J.Curr.Microbiol.App.Sci (2018) 7(10): 1847-1855
9. F. Mearin, B.E. Lacy, L. Chang, W.D. Chey, A.J. Lembo, M.Simren *et al*, 2016, Bowel disorders,. Gastroenterology, 150, pp. 407-1393 pp.
10. Fanaro S, Boehm G, Garssen J, Knol J, Mosca F, Stahl B, Vigi V Acta Paediatr Suppl. 2005 Oct; 94(449):22-6)
11. FAO 1979, Food and Nutrition paper manual of food quality control 4, Microbiology analysis FAO of UN (Rome)
12. Fatemeh et al (2013) Role of Fecal Calprotectin in Differentiating between Hirschsprung's Disease and Functional Constipation, Korean J Gastroenterol Vol. 62 (5), 288-291.
13. Ghoshal, Uday C et al. "Indian consensus on chronic constipation in adults: A joint position statement of the Indian Motility and Functional Diseases Association and the Indian Society of Gastroenterology." Indian journal of gastroenterology : official journal of the Indian Society of Gastroenterology vol. 37,6 (2018): 526-544.
14. Gibson, G.R., & Roberfroid, M.B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr.,125, 1401-1412

15. Gibson, G.R.; Probert, H.M.; van Loo, J.; Rastall, R.A.; Roberfroid, M. Dietary modulation of the human colonic microbiota: Updating the concept of the prebiotics. Nutr. Res. Rev. 2004, 17, 259–275.
16. Gibson, R.G.; Roberfroid, M.B. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr. 1995, 125, 1401–1412.
17. Goyal et al, 2021, Prevalence, overlap, and risk factors for Rome IV functional gastrointestinal disorders among college students in northern India, Indian J Gastroenterogy,40(2):144-153.
18. Hill, P.; Muir, J.G.; Gibson, P.R. Controversies and recent developments of the low-FODMAP diet. *Gastroenterol. Hepatol.* 2017, *13*, 36–45.
19. http://www.ubic-consulting.com/template/fs/The-World-Prebiotic-Ingredient- Market.pdf
20. https://plantbio.okstate.edu/images/pdfs/Roche_RT-PCR_Manual.pdf
21. https://www.thehealthsite.com/news/22-of-indian-adults-suffer-from-constipation-abbott-gut-health-survey-ag0118-552169/
22. Hyams J., et al J. Pediatr. Gastroenterol. Nutr. 2002;3:110–117
23. ILSI (2011). ILSE Europe Concise monograph on Nutrition and Immunity in man,2nd ed, ILSI Europe Brussels, 1-30.
24. ILSI Europe Concise Monograph. 2013:1-32. Ninno Binns, Probiotics, Prebiotics and Gut Microbiota, Commissioned by the Prebiotics and Probiotics Task Forces.
25. Janice L Raymond, Kelly Morrow, Krause and Mahan's Food & the Nutrition Care Process, Saunders; 15th edition (2020), ISBN-13: 978-0323636551
26. Jeannette S. Messer, Eugene B. Change in Physiology of the Gastrointestinal Tract (Sixth Edition), 2018
27. Katsuhisa Omagari et al, 2020, Prevalence of Chronic Constipation and Irritable Bowel Syndrome, and their Overlap, among Female Undergraduate Students in Japan, OBM Hepatology and Gastroenterology, 4(2)
28. Krumbeck, J.A., Rasmussen, H.E., Hutkins, R.W. *et al.* Probiotic *Bifidobacterium* strains and galactooligosaccharides improve intestinal barrier function in obese adults but show no synergism when used together as synbiotics. *Microbiome* 6, 121 (2018).
29. Lewis, S. J., & Heaton, K. W. (1997). Stool form scale as a useful guide to intestinal transit time. Scandinavian journal of gastroenterology, *32*(9), 920–924.
30. Macfarlane S, Macfarlane GT, Cummings JHAliment PharmacolTher. 2006 Sep 1; 24(5):701-14
31. National Health portal. https://www.nhp.gov.in

32. Nikjooy A PhD, Jafari H PhD, Saba MA M-Phil, Ebrahimi N M-Phil, Mirzaei R MD. Patient Assessment of Constipation Quality of Life Questionnaire: Translation, Cultural Adaptation, Reliability, and Validity of the Persian Version. Iranian Journal of Medical Sciences. 2018 May;43(3):261-268.
33. Rajput M, Saini SK. Prevalence of constipation among the general population: a community-based survey from India. Gastroenterol Nurs. 2014 Nov-Dec;37(6):425-9.
34. Rijnierse A et al (2011), Food derived oligosachhharides exhibit pharmaceutical properties, Eur J Pharmacol 2011:668 Suppl 1:S117-23
35. Shin A, Preidis GA, Shulman R, Kashyap PC. The Gut Microbiome in Adult and Pediatric Functional Gastrointestinal Disorders. Clin Gastroenterol Hepatol. 2019 Jan;17(2):256-274.
36. Slavin, J. (2013). Fiber and Prebiotics: Mechanisms and Heath Benefits. Nutrients , 5(4), 1417-1435
37. Spor, A., Koren, O., & Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome, Nature Reviews Microbiology, 9(4), 279–290.
38. Stephan J. Ott (2004), Quantification of Intestinal Bacterial Populations by Real-Time PCR with a Universal Primer Set and Minor Groove Binder Probes: a Global Approach to the Enteric Flora.
39. The cost of constipation, (2019), Lancet Editorial, Lancet Gastroenterol Hepatol.; **4**: 811
40. WHO (2009), Global Recommendations on Physical Activity for Health.
41. Duboc H, Coffin B, Siproudhis L. Disruption of Circadian Rhythms and Gut Motility: An Overview of Underlying Mechanisms and Associated Pathologies. J Clin Gastroenterol. 2020 May/Jun;54(5):405-414.
42. Voigt, R. M., Forsyth, C. B., & Keshavarzian, A. (2019). Circadian rhythms: a regulator of gastrointestinal health and dysfunction. Expert review of gastroenterology & hepatology, 13(5), 411–424. https://doi.org/10.1080/17474124.2019.1595588
43. Ribeiro, W. R., Vinolo, M. A. R., Calixto, L. A., & Ferreira, C. M. (2018). Use of Gas Chromatography to Quantify Short Chain Fatty Acids in the Serum, Colonic Luminal Content and Feces of mice. Bio-protocol, 8(22), e3089. https://doi.org/10.21769/BioProtoc.3089
44. Stojanov, S., Berlec, A., & Štrukelj, B. (2020). The Influence of Probiotics on the Firmicutes/Bacteroidetes Ratio in the Treatment of Obesity and Inflammatory Bowel disease. *Microorganisms*, *8*(11), 1715. https://doi.org/10.3390/microorganisms8111715