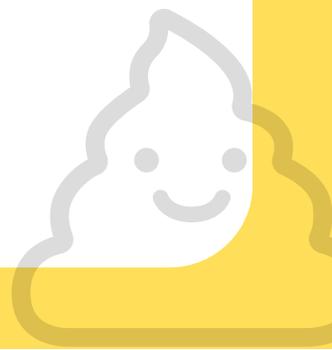
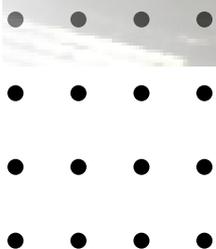


ABSTRACT



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Functional constipation (FC) is a gastrointestinal disorder which may significantly affect one's quality of life and depression status. It is not only restricted to elderly, but happens in middle age and young adults as well, and sedentary lifestyle is one of the major causes. The global prevalence of FC varied from 10-15% when different diagnostic criteria were used. As per literature review, among the Indian population, the prevalence of constipation ranges from 10-58% however, limited information is available regarding the prevalence of FC in the teaching fraternity. Use of dietary fibres is a common approach to manage constipation. Few oligosaccharides such as FOS and inulin have shown their potential to alter the composition of gastrointestinal microbiota and/or activity in specific ways that are beneficial to the host's health. Galactooligosaccharide (GOS) is a less explored oligosaccharide which undergoes selective fermentation and known to have a bifidogenic effect on the gut. Given the market's high demand for sweet candies, gummies in this category may be a potential vehicle for the introduction of GOS that may confer beneficial effects on the defecation profile of the individuals.

The specific objective of this study included screening the University teaching staff for the presence of FC along with exploring the association with underlying factors including medical history, family history of constipation, personal habits, chrono nutrition profile and dietary pattern of constipated subjects; use of GOS as a nutraceutical to develop a sugar-free vegetarian healthy gummy; and impact evaluation of GOS gummies supplementation on their constipation profile, selected gut microflora, short chain fatty acids, depression score and quality of life (QOL) of constipated subjects.

University teaching staff (n=364) were screened using a cross-sectional study design for the presence of FC. A pre-tested and validated structured questionnaire was administered to them bearing questions related to their gastrointestinal tract functionality, constipation profile, BMI, physical activity, perceptions and practises, medical history, family history of constipation, personal habits, chrono nutrition profile and dietary pattern of the subjects. Participants were scored on a scale of 20 based on the criteria laid down by WHO, The Rome Foundation and Bristol stool chart to assess the presence and degree of constipation. Agar, sugar, citric acid, water, and naturally flavoured, FSSAI-certified colours and flavours were used to make the standard gummies. GOS was used in the gummies to replace sugar in varied amounts, up to

100%. A trained panel (n = 8) evaluated the gummies using a composite score card in triplicates for a variety of sensory attributes. In addition to GOS recovery analysis, physicochemical variables such as colour, moisture, pH, and texture were assessed. Shelf life Studies of 100 percent GOS added gummies were done at accelerated temperatures of 37°C.

A double-blind, placebo-controlled clinical trial was used for the impact evaluation phase for the study. After obtaining the consent, the teaching staff (n=35) at The M.S. University of Baroda who suffered from functional constipation was selected for the study. The participants were divided into two groups; the experimental group (n=17) received 10g gummies enriched with 100% GOS for a period of 4 weeks, while the placebo group (n=18) received sugar-based gummies. Data from pre- and post-administration of validated questionnaires, such as PAC-QOL and Beck's depression inventory, was collected. QOL was studied for physical discomfort, appetite/ food intake, psychological disturbances and degree of satisfaction related to defecation profile. Fecal samples were collected on day 0 and day 30 to assess the gut health in terms of short chain fatty acid (SCFA) profile with respect to acetic acid, propionic acid, butyric acid and relative abundance of genera including Bifidobacterium, Lactobacillus, Clostridium, Bacteroides, and two prominent phyla Bacteroidetes, and Firmicutes. DNA was extracted for the microbial analysis and subjected to RTPCR. Normalization of gene expression was done using 16s rRNA. The data obtained was subjected to statistical analysis using JASP software (MAC version).

General information of the subjects 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. Obesity in various degrees was observed among 43.9% subjects (n=160). Statistically significant inverse correlation ($r = -0.126^*$) was observed between obesity and constipation at $p < 0.05$. 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 ($r = -0.113^*$) at $p < 0.05$. Most of the subjects fell in the fair category (n=209) with respect to their chrononutrition profile and no significant correlation was observed with presence of FC. No significant correlation was observed with presence of FC with chrono nutrition profile in terms of the working day profile, whereas in terms of a free day profile, chrono nutrition profile

showed a significant association ($p < 0.05$). Type of diet consumed by the subjects did not show any significant association with the constipation profile. Female subjects ($n=50$) were more constipated than the male respondents and the results were statistically significant ($r = -0.120^*$) at $p < 0.05$. Many subjects (12%) felt constipation is a social taboo and 60% respondents felt constipation is a minor health issue.

The results of the product development phase revealed that most acceptable standard gummies were prepared with 75ml water, 60g sugar, $1/4^{\text{th}}$ tsp citric acid and 2g agar ($n=24$). Sugar could be substituted with GOS to produce most acceptable gummies upto 60%. However, in order to prepare 100% GOS gummies, 5.5g of sucralose was added per batch of gummies ($n=24$). The physico chemical properties of GOS gummies revealed presence of 24% moisture, acidic pH, 7 lovibond units indicating yellow color with optimum springiness and adhesiveness. HPLC analysis revealed a recovery of 95% GOS in the prepared gummies. The GOS gummies were shelf stable with respect to its sensory attributes and keeping quality for 6 months at accelerated temperatures.

The results of the intervention phase of the study demonstrated GOS gummy supplementation increased Bifidobacterium and Lactobacillus by 1230% and 322% respectively ($p < 0.001$; $p < 0.01$) with reduced Clostridium by 63%, phylum Firmicutes by 73%, and Bacteroidetes by 85% ($p < 0.01$). The GOS-supplemented group demonstrated a higher F/B ratio (4.2) indicating improved gut health ($p < 0.01$) with reduced gut dysbiosis and constipation severity. GOS gummies enhanced acetic acid and butyric acid levels compared to the control group ($p < 0.01$; $p < 0.001$). Post supplementation, there was 40% reduction in depression (40%) ($p < 0.01$) and 22% improvement in quality of life ($p < 0.05$).

To summarize, FC was observed in $1/5^{\text{th}}$ of the study population which was inversely correlated with obesity and physical activity and positively correlated with female gender. It also confirms that GOS can be successfully used to substitute sugar up to a hundred percent in candies to fulfil the increasing demand for healthy confectioneries by consumers without any change in organoleptic qualities and with increased shelf life for 6 months. This study confirms the predicted beneficial effects of short-term GOS consumption on the constipation profile, gut health profile, quality of life and depression status of constipated subjects and was associated with significantly higher colonization of beneficial bacteria including *Lactobacillus* sp. and *Bifidobacterium* sp. and an improved F/B ratio which is a common indicator of gut health.