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Development of Galactooligosaccharide (GOS) added gummies: sensory, characterization and shelf quality

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Abstract

Sugar loaded confectioneries have a rapidly growing market globally. Consumption of such products may lead to multiple health risks. Products like gummies can be substituted with healthy sugar alternatives such as Galactooligo-saccharide (GOS), which can be consumed by all age groups.

The aim of the study was to develop standard gummies and sugar substituted gummies, conduct sensory analysis, shelf life studies and characterize it for its physico chemical properties.

The standard gummies were made using agar, sugar, citric acid, water, and FSSAI (Food Safety Standards Authority of India) certified natural colours and flavours, while GOS supplemented gummies were made by replacing sugar in varied amounts, upto 100%. A trained panel (n = 8) evaluated the gummies using a composite score card in triplicates for a variety of sensory attributes. GOS recovery analysis, physicochemical variables such as colour, moisture, pH, and texture were assessed. Shelf life Studies of 100 percent GOS supplemented gummies were carried out at accelerated temperatures (37 °C) over a period of 6 months.

The results revealed that gummies with varying levels of GOS were acceptable to the panelists, with no significant differences in the keeping quality. However, F test revealed a significant improvement (p < 0.05) in the texture of the gummies with a slight reduction in colour and flavour at the end of 6 months. The moisture content and pH values were 24.8% and 3.37 respectively. HPLC analysis revealed a recovery of 95% GOS in the prepared gummies.

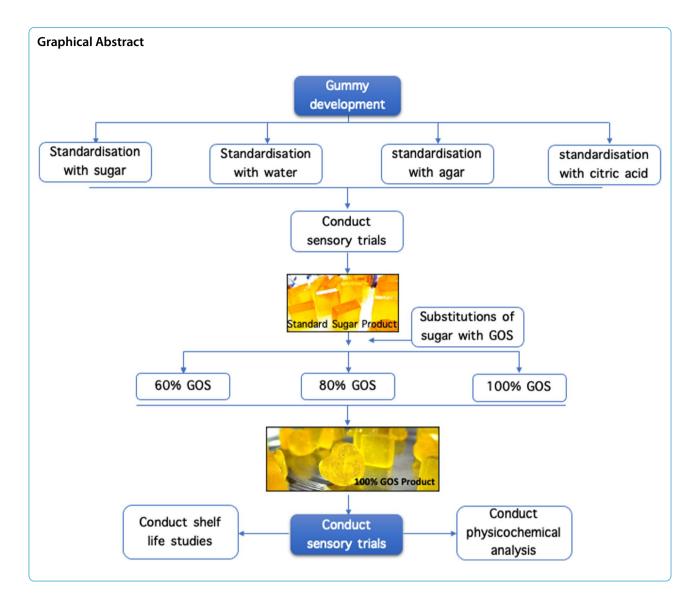
Hence, sugar can be substituted with 100% GOS to fulfil the increasing demand for healthy confectioneries without any change in organoleptic qualities and shelf life for 6 months.

Keywords: Gummy, Galactooligosaccharide, Shelf life, Sensory, Prebiotics, Physico-chemical

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Introduction

The size of the global gummy market, which was estimated at USD 16.28 billion in 2020, is anticipated to increase at a CAGR (Compound annual growth rate) of 12.6% from 2020 to 2028. Due to the growing number of health-conscious consumers who desire convenient dosage forms of supplements, the market is expanding significantly. Due to unhealthy and stressful lives brought on by the quick rate of social and technological change, cardiovascular illnesses (CVDs) and cardiac issues are more common today. Preventive health practises are greatly influenced by the desire and apparent ability to live longer and healthier lives. Compared to hard pills and tablets, gummies are easier to chew, have a gel-like consistency. Additionally, the product comes in a range of tastes. All of these elements are increasing product demand globally and fostering market expansion (Gummy Market size 2022).

On the other hand, India is set to be a global leader in nutraceuticals with a market of USD 4–5 billion. By 2025, experts and estimates predict it will increase to over USD 18 billion. In India, the market for dietary supplements is estimated to be worth USD 3924.44 million in 2020, and by 2026, it's expected to be worth USD 10,198.57 million, according to reports. The industry is expanding at a rate of 22% annually (Naresh K 2021).

According to a report by Expert market research, Indian market accounts for one of the top vegan food markets in the Asia Pacific region. The expansion of the worldwide vegan food market, which has already reached a value of USD 15.4 billion in 2020, is helping to promote the growth of the vegan food market in India. The market for vegan food is anticipated to expand further from 2022 to 2027 at a CAGR of 26%. The general consensus is that vegan supplements are both healthier and safer than

products made with animals. Additionally, prospects for expansion are being created by ethical concerns about the usage of animal components.

Consumption of sugary confectioneries is on the rise, which could be considered a public health risk. Food additives, sugar, and glucose syrup supplemented at a high level (above 50%) are examples of ingredients that could be harmful to human health (Garcia 2000). Garcia (2000), also estimates that around half of the market is made up of the confectionery category, which includes gummies, nougat, and hard and soft sweets.

Functional foods provide basic nutrients and biologically active compounds, which have an overall positive impact on the host's health (Marchand and Vandenplas 2000). Nutraceuticals like probiotics and prebiotics can be used to make functional food products in levels that give consumers the most health benefits. Prebiotics are non-digestible dietary components that bypass digestion and may improve health by selectively focusing on the growth and/or activity of a limited number of good bacteria in the colon (Gibson et al. 2004). Consumption of prebiotics can have significant health benefits because of their anti-cancer properties, influence on mineral absorption, lipid metabolism, anti-inflammatory and immune modulating effects (Macfarlane et al. 2008). Prebiotics have been proved beneficial for reducing bloating, abdominal pain, constipation and abdominal cramps (Mezzasalma et al. 2016). Prebiotics have shown to improve blood sugar balance in the body and also regulate appetite (Dibase et al. 2008). Galactooligosaccharide is a naturally occurring prebiotic present in human milk. Bifidobacteria bifidum and Lactobacillus rhamnosus are the most researched probiotic strains that have shown to increase in great numbers upon consumption of GOS (Arnold et al. 2021; Bakker et al. 2005; Fischer and Kleinschmidt 2015). The B-galactosidase enzyme can also be used to manufacture GOS commercially from lactose (Ekhart and Timmermans 1996).

The present study of replacing sugar with GOS in the gummies is a pioneering work. The objective of the study was to create healthy vegan gummies with prebiotic properties using Galactooligosaccharide (GOS). The data presented in this study is part of the research to fulfil the doctoral research work of the researcher.

Materials and methods

The present study was conducted in the Department of food and nutrition, The Maharaja Sayajirao University of Baroda, India.

Procurement of equipments, reagents, galactooligosaccharide and other raw materials

Chemical analysis was carried out in Food and Drug Laboratory, Vadodara, India. Lovibond Tintometer Colorimeter

(Model F) was used for color estimation. Electronic pH meter (Cole-Parmer P100) was used for pH analysis. Karl Fischer Moisture Titrator (Model: MKV-710 M) was used for moisture analysis. For texture profile analysis, texture analyser (Brookfield CT3 version 3.0 Texture Analyser) was used and Recovery analysis was carried out using HPLC (Brand: Agilent Technologies, Model: LC Agilent 1260 Infinity). HPLC grade chemicals were used for HPLC analysis.

Galactooligosaccharide (95%) with the necessary certifications and toxicological testing was purchased from Tata Chemicals, Pune. All additional ingredients used for the preparation of gummies (agar, sugar, citric acid, sucralose, natural colours and flavours) were FSSAI certified and purchased from the local markets and e-commerce websites.

Standardisation of the recipe of gummies and substitution with galactooligosaccharide and sucralose

The basic ingredients required for the preparation of gummies include agar, sugar, and water. The ingredients (sugar and water) were cooked to a soft ball stage, followed by the addition of agar, citric acid, and natural colours and flavours. The gummies were poured into the mould and allowed to set in the refrigerator for 1 h (Fig. 1). The gummies were then dried for 48 h under the fan. The standardised process was conducted with varying levels of each ingredient such as water content (55 ml, 65 ml, 75 ml, 85 ml), sugar content (50 g, 60 g, 70 g, 80 g), agar content (2 g, 2.5 g, 3 g, 3.5 g) and citric acid content (0.48 g, 0.95 g, 1.9 g, 3.8 g). All the products were subjected to organoleptic testing in triplicates to select the standardised gummy.

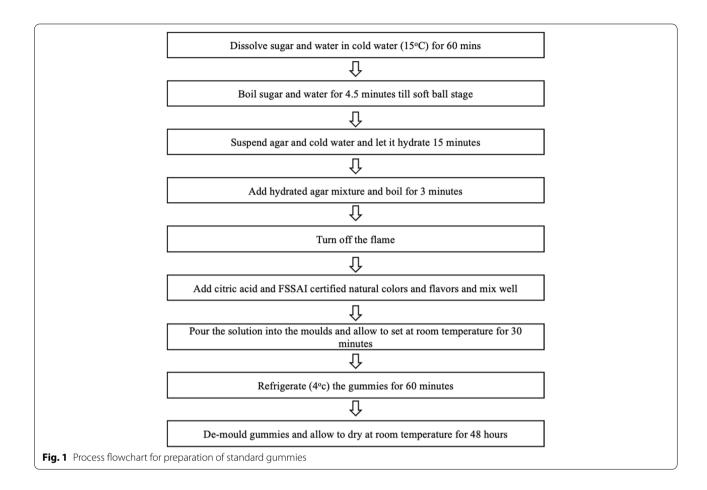
Sugar content in the standardised gummies was substituted with varying levels of galactooligosaccharide (60%, 80%, and 100%) and subjected to organoleptic testing. In an attempt to make the 100% substituted galactooligosaccharide gummies more acceptable, the gummies were prepared with varying levels of artificial sweetener (sucralose) (5 g, 5.5 g, 6 g) and subjected to organoleptic testing by the panel.

Physicochemical properties of the 100% Galactooligosaccharide substituted gummies

The 100% GOS substituted gummies were crushed using a mortar and pestle and were utilised for measuring colour intensity, moisture, and pH in triplicates. The analyses were carried out at the Food and Drug Laboratory, Vadodara, India.

Colour intensity

The crushed sample (2.5 g) was dissolved in 10 ml of methanol, placed in a glass cell inside the lighting cabinet, and



its colour intensity was assessed using a Lovibond Tintometer and compared with Lovibond colour racks of known colour characteristics. The racks are varied until a visual colour match is found for the light from the sample and its colour can then be expressed in Lovibond[®] units. The colour was matched by adjusting the yellow and red racks of the instrument in triplicates. (Gupta and Sheth 2015).

Moisture content

The moisture content was determined by the Karl Fischer Titrator method to obtain a direct analysis of the sample. The crushed sample of gummies (5 g) was solubilised in methanol and other reagents (Karl Fischer reagent) with a rotation speed of 5 RPM. The resulting moisture content was displayed digitally after 10 min (Robert A Martin 1977).

pH content

The sample (2.5 g) was crushed and dissolved in 50 ml of distilled water by incubation for 10 min at 80 °C in a water bath. The dissolved samples were cooled, filtered and checked for its pH using an electronic pH meter at ambient temperature. Results were obtained after calibration with a buffer solution of pH 4.0 and 9.2 (Yadav et al. 2021).

Texture profile

Texture profile analysis was carried out using a texture analyser to provide insight into how the samples behave when chewed. Using circular probes (TATX2 50 mm) moving at a speed of 1 mm/second, the gummy was placed on the base plate and compressed twice with a pause of 3 s (Cano-Lamadrid et al. 2020). The parameters assessed were hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness in triplicates.

HPLC determination of GOS from 100% Galactooligosaccharide substituted gummies

High performance liquid chromatography analysis for determining the GOS content of the gummies was carried out as explained by shodex.com with some modifications using an Inertsil NH2 column (15 mm \times 4.6 mm, 32 °C) and a refractive index (RI) detector (32 °C). 1 g GOS gummy was weighed and dissolved in 50 ml distilled water by heating to 100 oC and subjected to vacuum filtration. Limit of quantification of GOS standards was 4 μ g/ μ l. 20 μ l of the sample was injected into the column, where GOS was eluted using a mobile phase of

Acetonitrile and water (ACN: H2O, 70:30 v/v) at a flow rate of 1.0 mL/min for 30 min. The analysis was carried out in triplicates.

The following formula was used in the present study to determine the concentration of GOS in the gummy:

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

Shelf life studies

Shelf quality tests were performed on the 100 percent GOS substituted gummies, which were packaged in High density polyethylene (HDPE) bottles and kept in an incubator at an accelerated temperature of 37 °C (Refai and

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

Selection of panel members using threshold test

A threshold test was conducted to select the panelists to perform sensory evaluation for the varying standardised recipes. Different solutions of sweet, salt, and sour with varying concentrations were given for tasting. Panelists who were able to identify the solutions correctly and gave consent were selected to be trained for sensory evaluation (Rangana 1986).

Training of the panel

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, mouth-feel, texture, taste, and aftertaste (Silva et al. 2014).

Development of score cards for sensory evaluation

Score cards were developed for numerical scoring tests and the Difference Test (IS 1971). A numerical score card was used to determine the quality attributes of the gummies, which included colour and appearance, flavour, mouthfeel, overall taste, aftertaste, and overall acceptability on a scale of 10 for each attribute for all the samples with varying concentrations. The difference test was used to measure the superiority, inferiority, or equal in terms of taste for the coded test samples against the standard gummy.

Sensory evaluation

Sensory evaluation of the gummies was performed using the numerical scoring test where the panellists were asked to evaluate the sensory attributes of the gummies such as colour and appearance, texture, mouth feel, taste, after taste, and overall acceptability on a scale of 10 (IS 1971). The test was conducted in triplicate.

The 100 percent galactooligosaccharide substituted gummy with varied quantities of artificial sweetness (sucralose) was evaluated using the difference test (IS 1971) to determine whether it was equal to, superior to, or inferior to the standard gummies. The panellists were asked to compare three coded samples to the standard gummy during the test, which was conducted in triplicate. Sample A, B, and C contained 5 g, 5.5 g, and 6 g of artificial sweetener, respectively.

FAO 1979). Sensory evaluation with similar parameters for standardisation and microbial testing in terms of *E. coli*, total plate count (TPC), and yeast and mould count on days 0, 30, 60, 90, and 180 was carried out. The results were expressed as CFU/g of sample.

Statistical analysis

Statistical analysis was performed using Microsoft Excel and the statistical package for IBM (SPSS version 34) software. 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 access 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 0.05. ANOVA- one way variance was used to determine any significant change in the shelf life of the gummies over the period of 6 months at a *p* value of 0.05.

Results and discussion

Sensory evaluation with respect to standardisation of galactooligosaccharide gummies

The mean scores, standard deviation, and F test results for organoleptic evaluation of gummies prepared with different concentrations of water, sugar, agar, citric acid, and GOS are presented in Tables 1, 2, 3, 4, 5 respectively. 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 difference in the gummies with varied quantities of citric acid.

The most appropriate gummies formulation with the best evaluated parameters was prepared with 75 ml water, 60 g sugar, 2 g agar and 1.9 g citric acid with the help of the process (Fig. 1). The gummies with varying quantities of galactooligosaccharide showed a significant difference in F value in terms of mouthfeel, texture, flavour, general acceptability, and total score. Similar studies have reported the potential prebiotic effects of Fructooligosaccharide (FOS) and

Table 1 Standardization with varying levels of water addition

		SENSORY QUA	ALITIES							
Levels	of Water	Color & Appearance	Mouthfeel	Texture	Overall Taste	Flavor	After taste	Chew ability	Overall Acceptability	Total Score
Maxim	num Score	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(80)
55 ml	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
65 ml	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
75 ml	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
85 ml	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 2 Standardization with varying levels of sugar addition

		SENSORY QUA	LITIES							
Level	s of Sugar	Color & Appearance	Mouthfeel	Texture	Overall Taste	Flavor	After taste	Chew ability	Overall Acceptability	Total Score
Махі	mum Score	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(80)
50 g	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
60 g	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
70 g	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
80 g	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

Xylooligosachharide (XOS) to substitute sugar when incorporated into food products such as cakes, ice creams, and other sweetmeat products (Thakuria & Sheth 2019).

Scores of the panel with respect to different levels of GOS were presented in Table 5 and Fig. 2. The results

revealed a statistically significant reduction in mouth-feel, texture, overall acceptability, and total score as the GOS concentration exceeded 60%. To mitigate this, the gummies were supplemented with various levels of sucralose along with GOS, which improved the

Table 3 Standardization with varying levels of agar addition

		SENSORY QUA	ALITIES							
Levels	of Agar	Color & Appearance	Mouthfeel	Texture	Overall Taste	Flavor	After taste	Chew ability	Overall Acceptability	Total Score
Maxir	num Score	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(80)
 2 g	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
2.5 g	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
3 g	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
3.5 g	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 4 Standardization with varying levels of citric acid addition

		SENSORY QUA	LITIES							
Levels of citric acid		Color & Appearance		Chew ability	Overall Acceptability	Total Score				
Maxim	um Score	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(80)
0.48 g	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
0.95 g	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
1.9 g	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	5675
3.8 g	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	4870
	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*

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

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 5.5 g sucralose was found to be superior as compared to the other variations of sucralose (Table 6).

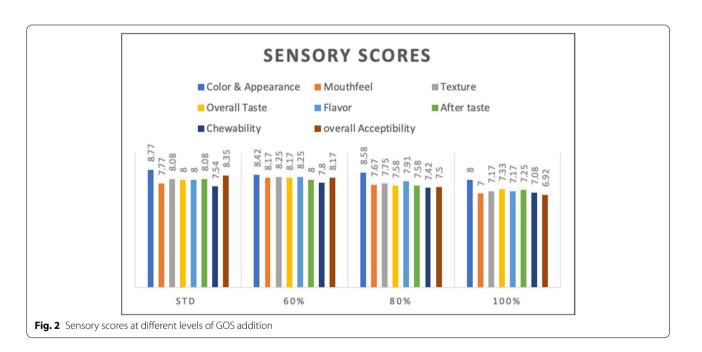
Table 5 Standardization with varying levels of GOS addition

		SENSORY QUA	ALITIES							
Levels	of GOS	Color & Appearance			Overall Taste	Flavor	After taste	Chew ability	Overall Acceptability	Total Score
Maxin	num Score	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(80)
STD	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*	0.14 ^{NS}	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)



Physico chemical properties

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 (Table 7) which was similar to the natural color

Curcumin. 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;

Table 6 Difference test for varying levels of sucralose addition in 100% GOS gummies

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

NS Not significant

Table 7 Physico chemical characteristics of the 100% GOS gummies

SI.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
5.	Recovery	95%, 2.24 g GOS/ gummy

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 moisture and pH content of the gummies were determined as 24.8% and pH at 3.37 respectively (Table 7). Similar findings were reported previously (Periche Á et al. 2016; Mutlu C et al. 2018; Yu-yong HE et al. 2014; Scott 1957). 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).

Texture profile analysis (Table 7) is an important parameter for candies in terms of acceptability and appeal. 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).

Recovery of GOS in the gummies

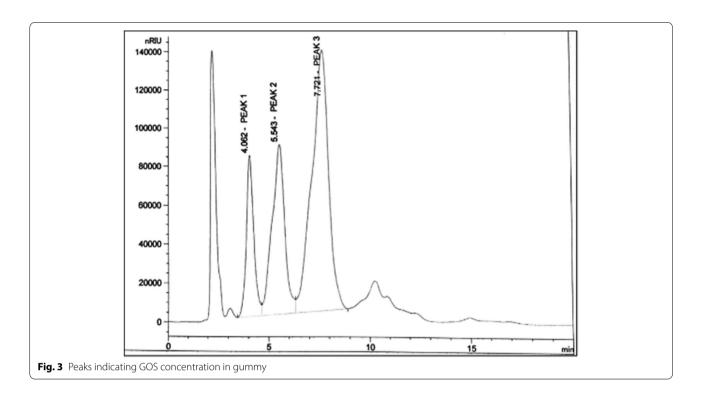
The present study of replacing sugar with GOS also focuses to study the retention of GOS in the final product. HPLC method is the primary approach for identifying and quantifying oligosaccharides because of its high penetration rate and quick detection (Zhao et al. 2018). Studies conducted on 14 Indian 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 making gummies and exhibited a similar retention of GOS of 95 per cent (Fig. 3) which indicates a lower loss of the prebiotic even when subjected to higher temperatures.

Shelf life studies

Effect of storage on microbial growth

The most prevalent microorganisms that cause deterioration in confectioneries are yeast and moulds. However, at the end of 6 months, no significant presence of yeast and mould was detected (Tables 8 and 9). 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.

Shelf life studies conducted over a six-month period (Table 8) revealed no de-novo growth of *E. coli*, a slight increase in TPC (log10cfu/g), and a slight decrease in 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. 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). Upon storage, however, no significant differences in microbiological parameters (E. coli, total plate count, yeast and mould count) were observed.

Effect of storage on sensory profile

Analytical or sensory approaches, as well as a combination of tests, may be used in shelf life testing. Sensory

Table 8 Microbial Count (Log_{10} CFU/g of sample) of GOS Gummies Stored for a period of 6 months (37 °C)

Duration	Total Plate Count	Yeast & Molds	E.coli	
0 Day	2.12	3.14	Nil	
15 Day	3.18	3.18	Nil	
30 Day	3.0	2.26	Nil	
60 Day	3.15	2.26	Nil	
180 Day	3.48	2.26	Nil	
F test	1.92	2.38	-	
P value	0.12 ^{NS}	0.06 ^{NS}	-	

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).

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 (Table 9). The panellists accepted the gummies after 180 days of storage at accelerated temperatures (37°c), demonstrating the successful shelf stability of the gummies even after six months on the shelf (Tables 8 and 9). 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.

Conclusion

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

Table 9 Sensory analysis of GOS gummies stored for a period of 6 months (37 °C)

Duration		Color/ Appearance	Mouth feel	Texture	Overall 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	± 1.07	± 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 Day	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	± 1.61	± 0.91	± 0.9	± 0.75	± 0.83	± 5.42
	Range	7–10	6-9	6-9	5–10	6-9	6-10	7–9	7–9	54-71
	Percent	86.3	77.5	79.4	79.4	81.9	82.5	81.9	81.8	81.3
30 Day	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	± 1.45	± 0.86	± 0.96	± 0.95	± 0.96	± 5.11
	Range	7–9	7–10	7–9	6–10	6-9	81.3	7–10	7–10	57-75
	Percent	86.9	84.4	82.5	83.1	82.5		83.1	84.4	83.5
60 Day	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 Day	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 Non significant, 0.05-*, 0.005-**

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.

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Authors' contributions

K.D- Experimental work carried out and Prepared the manuscript. M.S.- Conceptualization and Guiding the experiment and correcting the manuscript. Both authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during analysis will be available from the corresponding author on request.

Declarations

Ethics approval and consent to participate

The study 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. Written informed consent was obtained to participate in the study.

Consent for publication

Not applicable

Competing interests

None

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