Research, Society and Development, v. 9, n. 10, e7369108942, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.8942

Probiotic gummy candy with xylitol: development and potential inhibition of *Streptococcus mutans* UA 159

Bala de goma probiótica com xilitol: desenvolvimento e inibição potencial de *Streptococcus mutans* UA 159

Caramelos de goma probióticos con xilitol: desarrollo y posible inhibición de *Streptococcus mutans* UA 159

Received: 10/01/2020 | Reviewed: 10/10/2020 | Accept: 10/14/2020 | Published: 10/17/2020

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Abstract

Gummy candies are nutritionally poor foods; however, they have a large consumer market. Enhancing these products with nourishing and/or functional matrices is a challenge. This study aimed to develop the features of a gummy candy probiotic, containing *Bifidobacterium*

lactis HN019, and evaluate its effectiveness in inhibiting *Streptococcus mutans* UA159. The candy was suitable for probiotic development, whose viable bacterial count was 8.54 Log CFU/g in the ready-to-eat product; high viability was maintained during the 28-day shelf life. Additionally, the rate of probiotic survival under in vitro gastrointestinal simulation was 98% and 93% in the ready-to-eat product and after shelf life, respectively. The sensory analysis revealed scores over 8.0 in all attributes evaluated. Lastly, the inhibition test of *S. mutans* UA159 showed 25% and 98% inhibition, with 10% and 20% xylitol respectively. Probiotic and xylitol gummy candy is a potential functional food and can target a niche food market, such as teenagers.

Keywords: *Bifidobacterium lactis*; Functional food; Ready-to-eat; Acceptability.

Resumo

Balas de gomas são alimentos nutricionalmente pobres; no entanto, eles têm um grande mercado consumidor. Melhorar esses produtos com matrizes nutritivas e / ou funcionais é um desafio. Este estudo teve como objetivo desenvolver uma bala de goma com caracteristicas probióticas contendo *Bifidobacterium lactis* HN019, e avaliar sua eficácia na inibição de *Streptococcus mutans* UA159. A goma foi adequada para o desenvolvimento do probiótico, cuja contagem bacteriana viável foi de 8,54 Log UFC/g no produto pronto para consumo; a alta viabilidade foi mantida durante a vida útil de 28 dias. Além disso, a taxa de sobrevivência do probiótico sob simulação gastrointestinal in vitro foi de 98 % e 93 % no produto pronto e após o prazo de validade, respectivamente. A análise sensorial revelou pontuações superiores a 8,0 em todos os atributos avaliados. Por fim, o teste de inibição de *S. mutans* UA159 mostrou 25 % e 98 % de inibição, com 10 % e 20 % de xilitol, respectivamente. A bala de goma probiótica adicionada de xilitol é um alimento funcional em potencial e pode atingir um nicho de mercado de alimentos, como adolescentes.

Palavras-chave: *Bifidobacterium lactis*; Alimento funcional; Pronto para consumo; Aceitabilidade.

Resumen

Los caramelos de goma son alimentos nutricionalmente pobres; sin embargo, tienen un gran mercado de consumidores. Mejorar estos productos con matrices nutritivas y / o funcionales es un desafío. Este estudio tuvo como objetivo desarrollar las características de un probiótico de caramelo gomoso, que contiene *Bifidobacterium lactis* HN019, y evaluar su eficacia para inhibir *Streptococcus mutans* UA159. El caramelo era apto para el desarrollo de probióticos,

cuyo recuento de bacterias viables era de 8,54 Log UFC/g en el producto listo para consumir; se mantuvo una alta viabilidad durante la vida útil de 28 días. Además, la tasa de supervivencia de los probióticos bajo simulación gastrointestinal in vitro fue del 98% y 93% en el producto listo para comer y después de la vida útil, respectivamente. El análisis sensorial reveló puntuaciones superiores a 8.0 en todos los atributos evaluados. Por último, la prueba de inhibición de *S. mutans* UA159 mostró un 25% y un 98% de inhibición, con un 10% y un 20% de xilitol respectivamente. Los caramelos gomosos de probióticos y xilitol son un alimento funcional potencial y pueden apuntar a un mercado de alimentos de nicho, como los adolescentes.

Palabras clave: *Bifidobacterium lactis*; Comida funcional; Lista para el consume; Aceptabilidad.

1. Introduction

Probiotics show beneficial effects on human health and are associated with the microbial balance of the digestive tract and the immune system (Abuajah et al., 2014). Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al., 2014). To obtain the probiotic status, microorganisms must fulfill a number of criteria related to safety, functional effects, and technological properties (Foligné et al., 2013). In this sense, Bifidobacterium and Lactobacillus are the most popular probiotics employed in foods (Tufarelli & Laudadio, 2016) that can be added to nondairy products, including cereals, juices, chocolate, chewing gum, and dairy products such as yogurt, cheese, and ice cream (Ranadheera et al., 2010).

Xylitol, a natural sugar substitute, defined as a nutritive sweetener, has a low caloric value, thus helping consumers to reduce their energy intake and lose weight (EU legislation, 2008). Xylitol is widely known as an anticariogenic agent, is not fermented by *S. mutans*, on the other hand, exerts an inhibitory effect on the microbial group (Watthanasaen et al., 2017). Recently, studies have also demonstrated the effectiveness of both probiotics and xylitol as anticariogenic agents preventing caries and other oral diseases (Witzler et al., 2017; Kaur et al., 2018). Scientific evidence demonstrates that xylitol meets the safety and efficacy prerequisites for use in caries prevention programmes in all age groups. These characteristics make xylitol a good substitute for sugars in candies, the consumption of which is common, especially among children.

Candies that usually do not provide any nutritional value are considered versatile

matrixes and thus can act as vehicles for the supply of vitamins, fibers, and other compounds with health benefits (Archaina et al., 2019). Considering these factors, the scope of the current work was to develop a gummy candy with *Bifidobacterium lactis* HN019 and xylitol and study its physicochemical properties, acceptance, probiotic in vitro stability, scanning electron microscopy of probiotic gummy candy, and the effect of xylitol on the growth of *S. mutans* UA159.

2. Methodology

This is a quantitative study, as it used the collection of numerical data that had been analyzed applying statistical analysis relevant to the study (Pereira et al., 2018)

2.1 Probiotic

B. lactis HN019 (Dupont, Brazil) was added (0.01% w/v) to skimmed milk powder reconstituted at 10 % (w/v), sterilized at 115 °C/10 min and cooled. The culture was incubated at 37 °C/24 h. For the development of gummy candy, the probiotic was mixed to whey protein isolate (WPI) (Alpha Galvano Chemical) in the ratio 1:1(v/w) as protector agent. The homogenized and lyophilized (Liobras L101, Liotop) mix was used in the gummy candy formulation (Patent BR1020160307902).

2.2 Production of gummy candy

The candy was prepared using 12.3% unflavored gelatin powder (Royal, Brazil), 4.8% bidistilled glycerin (Arcolor, Brazil), 6.6% xylitol (LaboNathus, Brazil), 20.5% maltitol syrup (Lycasin 80/55, LaboNathus, Brazil), 12.7% of the mix WPI with probiotic, 12.7% toasted and ground peanut (Yoki, Brazil), 30.20% water and 0.2% potassium sorbate (Synth, Brazil). The candy was produced by agglutination of ingredients followed by molding, and stored in plastic bags (low density polyethylene) at room temperature (±25 °C).

2.3 Physico-chemical characterization and probiotic stability during storage

The pH, moisture, protein, lipids, and ash of the candy were determined by AOAC (2005) and carbohydrates according to Dubois et al. (1956). The probiotic stability on 1, 7,

14, 21 and 28 days of storage at ±25 °C was evaluated. The viability of the probiotic was evaluated by Orange Serum agar in anaerobic conditions at 37 °C/72 h (Champagne et al., 2011).

2.4 Texture profile of probiotic gummy candy with xylitol

The analysis of texture (hardness, adhesiveness, elasticity and chewing) was carried out on the Brooksfield CT3 Texture Analyzer. The analysis parameters assigned to the equipment for determination of the Texture Profile Analysis (TPA) were: TA4/1000 probe; target 2.0 mm; 0.05 N trigger load; pre-test speed: 2 mm/s; test speed: 1 mm/s. Texture parameters were evaluated on 1, 7, 14, 21 and 28 days of storage at ±25 °C.

2.5 Sensory Acceptance

Sensory analysis was carried out with a panel of 100 untrained individuals (25 children, 25 teenagers and 50 adults). Evaluation in children was conducted using a hedonic scale with facial expression in two categories (extremely like and extremely dislike). Teenagers and adults used an unstructured 9-point line scale ranging from 9 (extremely like) to 0 (extremely dislike) for each sensory characteristic: appearance, aroma, flavor, texture and overall acceptability. The product was analyzed for the presence of thermotolerant coliforms, coagulase-positive staphylococci, and Salmonella sp., as required by legislation (Brasil, 2005) prior to sensorial test, and were in accordance with this regulation. Approximately 10 g portions of the gummy candy were served in disposable transparent polyethylene plates. The test was carried out under controlled conditions, with mineral water and cream crackers available to the panelists. The Ethics Committee of the Universidade Pitágoras Unopar approved the study (Register 1.148.291), and consent forms indicating voluntary and fully informed participation were signed by all panelists. Children and teenagers were recruited from a school located in Londrina-PR, and parental consent to participate was obtained along with the children's assent. Students and staff from Unopar were recruited as adult panelists.

2.6 In vitro resistance of probiotic in simulated gastrointestinal conditions

The survival of *B. lactis* HN019 under gastrointestinal conditions was determined on 1, 7, 14, 21 and 28 days of storage (Buriti et al., 2010).

2.7 Scanning electron microscopy (SEM)

The WPI containing the probiotic *B. lactis* HN019 and the probiotic gummy candy were fixed onto aluminum stubs and made electrically conductive by gold coating before examination on the SEM apparatus (FEI Quanta 200, FEG, Netherlands). The microstructures were imaged at an accelerating voltage of 30 kV.

2.8 Xylitol effect in the *S. mutans* inhibition

The inhibition of *S. mutans* was evaluated in the Brain Hearth Infusion (BHI) broth with 0 (control), 1, 5, 10, 15 and 20% xylitol, sterilized at 121 °C/15 min and tested with three *S. mutans* UA159 inoculum (2, 3 and 4 Log CFU/mL) incubated at 37 °C/24 h. The percent inhibition of *S. mutans* was calculated by the equation:

% Inhibition = [(A Abs – B Abs)/A Abs] x 100

A = absorbance in the broth with 0% xylitol (control), after incubation;

B = absorbance in the broths with different concentrations of xylitol, after incubation.

2.9 Statistical analysis

The candy characterization data were analyzed using the analysis of variance (ANOVA) and Tukey procedure ($p < 0.05$) while the sensory data were analyzed using Student's t-test ($p < 0.05$) with the software Statistic 13.0.

3. Results and Discussion

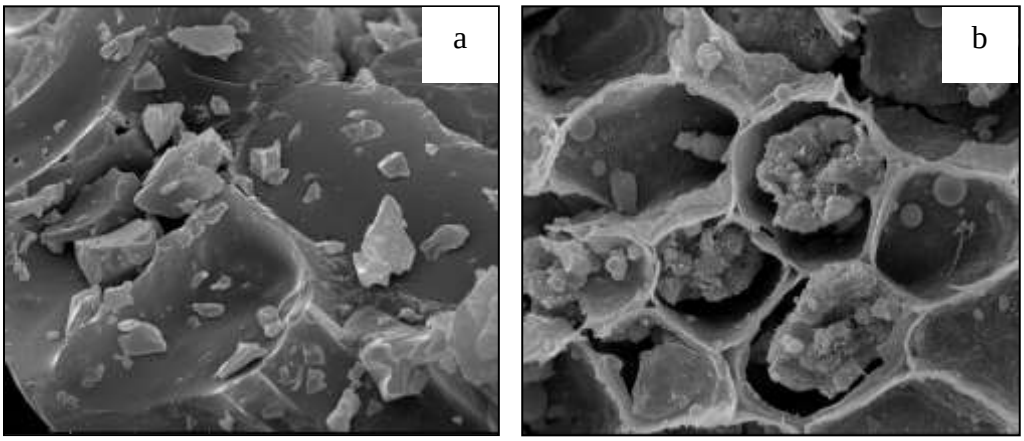
The protein content in the probiotic gummy candy was due to the addition of gelatin and, mainly, WPI (Table 1), which is the unique feature of this product, as candies are usually not protein sources. A significant decrease ($p > 0.05$) in the viability of *B. lactis* HN019 was not reported during the 28 days of storage whose counts were 8.5 and 8.0 Log CFU/g in fresh gummy and after 28 days of storage, respectively, indicating a suitable stability of the probiotic in this matrix. During the ingredient preparation, the probiotic was completely absorbed by the WPI, and the same condition was maintained after probiotic addition to the gummy candies, as shown in Figure 1A and 1B, which the WPI may have had a protective effect on the probiotic cells.

Table 1. Physicochemical characteristics of probiotic gummy candy with xylitol.

Physicochemical characteristics	**Probiotic gummy candy**
Moisture (%)	33.34 ± 0.07
Protein (%)	27.80 ± 0.16
Ash (%)	0.81 ± 0.04
Lipids (%)	1.33 ± 0.02
Carbohydrates (%)	36.72 ± 0.24
pH	5.89

Results are presented as means ± standard deviation. Fonte: Os autores.

Figure 1. Scanning electron microscopy (SEM) of whey protein isolate (WPI) containing the *Bifidobacterium lactis* HN019 probiotic (a) and probiotic gummy candy (b).



Fonte: Os autores.

During the 28 days of storage, an increase in hardness (from 1.65 to 6.94 N) and chewiness (from 2.65 to 13.77 mJ) of the gummy candies was observed ($p<0.05$) (Table 2). These results might indicate water loss during storage, which is a reflection of the storage conditions and kind of packing used.

Table 2. Texture of probiotic gummy candies with xylitol during storage.

Texture profile	**Storage time (days)**				
	1	7	14	21	28
Hardness (N)	1.65±0.02^{d}	3.53±0.14^{c}	5.30±0.42^{b}	5.44±0.06^{b}	6.94±0.40^{a}
Adhesiveness (mJ)	0.05±0.02^{b}	0.10±0.00^{b}	0.76±0.12^{a}	1.03±0.23^{a}	1.03±0.06^{a}
Elasticity (mm)	1.90±0.02^{b}	1.93±0.02^{ab}	1.98±0.01^{ab}	2.00±0.07^{ab}	2.01±0.04^{ab}
Chewiness (mJ)	2.65±0.04^{e}	4.73±0.12^{d}	5.73±0.12^{c}	7.03±0.25^{b}	13.77±0.29^{a}

Results are presented as mean ± standard deviation. Different superscript letters, on the same line, indicate significant difference by the Tukey test ($p < 0.05$). Fonte: Os autores.

Sensory analyses in previous studies demonstrated the effect of probiotic addition on the acceptance of gummy candies or similar products (Witzler et al., 2017; Bartkiene et al., 2018). Herein, we observed that 88% of the children liked the product (Table 3). The texture presented a difference when the results of teenagers and adults were compared, indicating greater acceptance by the adults. The presence of probiotic in food can affect the sensory characteristics due the production of metabolic components such as organic acids.

Table 3. Sensory acceptance of probiotic gummy candies with xylitol.

	Teenager's (14-17 years old)	**Adults (> 18 years old)**
Appearance	7.96^{a}	8.12^{a}
Aroma	7.88^{a}	7.82^{a}
Flavor	7.52^{a}	7.92^{a}
Texture	6.87^{b}	7.56^{a}
Overall Acceptance	7.48^{a}	7.88^{a}

Results are presented as means. Different superscript letters, in the same line, indicate significant difference by the Student's t-test ($p < 0.05$). Fonte: Os autores.

In all simulated phases (Table 4), *B. lactis* HN019 was observed to show a high survival rate. In the gastric phase, a difference in probiotic counts in the enteric phases I and II

was noticed ($p<0.05$). However, noticeable changes in the total count were not reported, since the changes occur within one logarithm cycle. In Phase 3, an increase in *B. lactis* population at all evaluated periods was observed, indicating that the probiotic was not affected by the drastic pH conditions in the previous phases. The results showed that the probiotic resistance in the gastric and enteric juice conditions was not significantly affected during the storage time, indicating that the consumption of probiotic gummy candy after production and 28 days of storage has the same effect. The probiotic presented a high rate of survival of 98% in the ready-to-eat product and 93% after the shelf life under in vitro gastrointestinal simulation (Guo et al., 2009). In the study of Medeiros et al. (2019), the authors added *B. lactis* to curd cheese, and the minimal counting was observed only in day 1 of storage.

Table 4. *Bifidobacterium lactis* HN019 (Log CFU/g) counting during probiotic gummy candy storage at 25 °C and in vitro simulation.

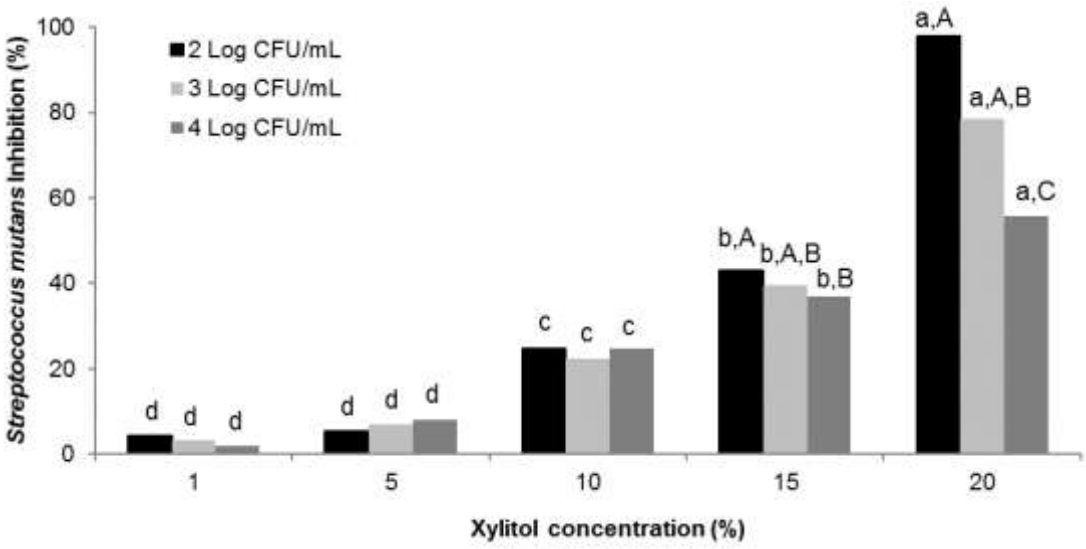
Gastrointestinal Phase	**Storage time (days)**				
	1	7	14	21	28
Gummy candy	8.53 ± 0.07^{Aa}	8.40 ± 0.11^{Aa}	8.36 ± 0.18^{Aa}	8.15 ± 0.18^{Aa}	7.97 ± 0.13^{Aa}
1	7.59 ± 0.22^{Ba}	7.59 ± 0.11^{Aa}	7.56 ± 0.05^{Ba}	7.56 ± 0.15^{Ba}	7.56 ± 0.06^{Aa}
3	7.80 ± 0.12^{Aa}	7.79 ± 0.13^{Aa}	7.79 ± 0.00^{ABa}	7.79 ± 0.11^{ABa}	7.77 ± 0.08^{Aa}
4	8.43 ± 0.09^{Aa}	8.40 ± 0.05^{Aa}	8.05 ± 0.14^{Aa}	8.05 ± 0.07^{Aa}	7.93 ± 0.12^{Aa}

Phase 1 counting after gastric simulation at pH 2.0; Phase 2 counting after enteral simulation of phase I at pH 5.0; Phase 3 counting after simulation of enteric phase II at pH 7.0. Results are presented as mean ± standard deviation. Different upper superscript case letters, in the same column, indicate significant difference by the Tukey test ($p<0.05$). Different lowercase superscript letters, on the same line, indicate significant difference by the Tukey test ($p<0.05$). Fonte: Os autores.

The inhibition of *S. mutans* at different concentrations of xylitol and inoculum of *S. mutans* (Figure 2) was evaluated. At xylitol concentrations of 10% and 20%, inhibition of *S. mutans* inoculum 2 Log CFU/mL was 24.64% and 97.96%, respectively. At a xylitol concentration of 15%, the inhibition percentage decreased as the inoculum increased. These results indicate that the inoculum of *S. mutans* directly affects the inhibition caused by xylitol and lowers the initial cellular concentration of the microorganism, implying greater inhibition. Kaur et al. (2018) evaluated the effect of chewing gums containing probiotics and xylitol on

the salivary *S. mutans* counts and concluded that both were equally effective in reducing *S. mutans* counts in children. The authors suggest that fluoride supplements, chewing gums containing probiotics and xylitol can be an alternative regimen for prevention of caries in children.

Figure 2. Effect of xylitol concentration in the inhibition (%) of different *Streptococcus mutans* UA159 inoculum (2, 3 and 4 Log CFU/mL).



Different lowercase letters indicate significant difference in *S. mutans* inhibition in the same inoculum among different xylitol concentration by the Tukey test ($p < 0.05$). Different upper case letters indicate significant difference in *S. mutans* inhibition in the same xylitol concentration among different inoculum by the Tukey test ($p < 0.05$). Fonte: Os autores.

4. Final Considerations

The gummy candy proved to be a stable and suitable matrix for the development and maintenance of the probiotic *B. lactis* HN019. The high protein content of the candy and the potential cariogenic effects are interesting characteristics of this type of product, especially with regard to the target audience, children and adolescents.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

Abuajah, C. I., Ogbonna, A. C., & Osuji, C. M. (2014). Functional components and medicinal properties of food: a review. *J Food Sci Technol.*, 52, 2522-2529. https://doi.org/10.1007/s13197-014-1396-5

AOAC (2005). Official Methods of Analysis of AOAC International. (18th ed.). Gaithersburg, MD: Association of Official Analytical Chemist.

Archaina, D., Sosa, N., Rivero, R., & Schebor, C. (2019). Freeze-dried candies from blackcurrant (*Ribes nigrum* L.) and yoghurt. Physicochemical and sensorial characterization. *LWT - Food Science and Technology*, 100, 444-449. https://doi.org/10.1016/j.lwt.2018.10.049

Bartkiene, E., Ruzauskas, M., Lele, V., Zavistanaviciute, P., Bernatoniene, J., Jakstas, V., Ivanauskas, L., Zadeike, D., Klupsaite, D., Viskelis, P., Bendoraitiene, J., Navikaite-Snipaitiene, V., & Juodeikiene, G. (2018). Development of antimicrobial gummy candies with addition of bovine colostrum, essential oils and probiotics. *International Journal of Food Science and Technology*, 53, 1227–1235. https://doi.org/10.1111/ijfs.13701

BRASIL. (2005). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução RDC n° 265, de 22 de setembro de 2005. *Regulamento Técnico para Balas, Bombons e Gomas de Mascar*. Diário Oficial da União – Brasília, 22 de Setembro de 2005.

Buriti, F. C. A., Castro, I. A., & Saad, S. M. I. (2010). Viability of Lactobacillus acidophilus in symbiotic guava mousses and its survival under in vitro simulated gastrointestinal conditions. *Int. J. F. Microb.*, 137, 121-129. https://doi.org/10.1016/j.ijfood micro.2009.11.030

Champagne, C. P., Ross, R. P., Saarela, M., Hansen, K. F., & Charalampopoulos, D. (2011). Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices. *Int. J. F. Microb.*, 149, 185-193. https://doi.org/10.1016/j.ijfood micro.2011.07.005

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem*., 28, 350-356.

Foligné, B., Daniel, C., & Pot, B. (2013). Probiotics from research to market: The possibilities, risks and challenges. *Curr. Opin. Microbiol*., 16, 284-292. https://doi.org/10.1016/j.mib.2013.06.008

Guo, Z., Wang, J., Yan, L., Chen, W., Liu, X. M., Zhang, H, P. (2009). In vitro comparison of probiotic properties of Lactobacillus casei Zhang, a potential new probiotic, with selected probiotic strains. *LWT-Food Science and Technology*, 42, 1640-1646. https://doi.org/10.1016/j.lwt.2009.05.025

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C. & Sanders, M. E. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol*., 11, 506–14. https://doi.org/10.1038/nrgastro.2014.66

Kaur, K., Nekkanti, S., & Madiyal, M. (2018). Effect of Chewing Gums Containing Probiotics and Xylitol on Oral Health in Children: A Randomized Controlled Trial. *J. Intern. Oral Health*, 10, 237-243. https://doi.org/10.4103/jioh.jioh_170_18

Medeiros Júnior, F. C. de, Pereira, I. C., Borba, K. K. S., Miranda Júnior, R. N. C., Moreira, R. T., & Santos, E. P. (2019). Viability of probiotic cultures Lactobacillus acidophilus and Bifidobacterium lactis added to the Curd Cheese. *Research, Society and Development*, 8(5), e2885986. https://doi.org/10.33448/rsd-v8i5.986

Pereira, A.S., Shitsuka, D.M., Parreira, F.J., Shitsuka, R. (2018). Metodologia da pesquisa científica. [e-book]. Santa Maria. Ed. UAB/NTE/UFSM. Retrieved from https://repositorio.ufsm.br/bitstream/handle/1/15824/Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf?sequence=1. Accessed in: 13 out. 2020.

Ranadheera, R. D. C. S., Baines, S. K., & Adams, M. C. (2010). Importance of food in probiotic efficacy. *Food Res. Int*., 43, 1-7. http://dx.doi.org/10.1016/j.foodres.2009.09.009

Regulation (EC) no 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. http:// eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:3 54:0016:0033:en:PDF.

Tufarelli, V., & Laudadio, V. (2016). An overview on the functional food concept: prospectives and applied researches in probiotics, prebiotics and synbiotics. *J. Exp. Biol. Agric. Sci*., 4(3S). https://doi.org/10.18006/2016. 4(3S).273.278

Watthanasaen, S., Merchant, A. T., Luengpailin, S., Chansamak, N., Pisek, A., Pitiphat, W. (2017). Xylitol-containing chewing gum for caries prevention in students with disabilities: A Randomised trial. *Oral Health Prev Dent*., 15, 519-527. https://doi.org/10.3290/j.ohpd.a39668

Witzler, J. J. P., Pinto, R. A., Valdez, G. Font de, Castro, A. D. de, &. Cavallini, D. C. U (2017). Development of a potential probiotic lozenge containing Enterococcus faecium CRL 183. *LWT- Food Science and Technology*, 77, 193–199. https://doi.org/10.1016/j.lwt.2016.11.011

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