Development and characterization of the gummy-supplements, enriched with probiotics and prebiotics

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ABSTRACT
The aim of this study was to develop gummy-supplements (G-S) based on probiotics (Lactobacillus plantarum LUHS135 and L. paracasei LUHS244), prebiotics (psyllium husk), and apple pomace as a pectin source, and to evaluate viable lactic acid bacteria (LAB) count, total phenolic compounds (TPC) content, antioxidant activity, colour coordinates, texture parameters, and overall acceptability of the developed G-S. Antimicrobial properties of the used LAB strains against Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, and Streptococcus mutans were investigated. Higher lightness, yellowness, and acceptability of the G-S with gelatin were found. G-S with agar showed harder texture, and agar/gelatin selection has a significant influence on TPC content in G-S. The antioxidant activity of G-S was depended on the strain of LAB and the use of psyllium husk. LUHS244 inhibited all the tested pathogenic strains. The developed G-S formula simply allowed to produce higher value products.

Desarrollo y caracterización de suplementos gomosos enriquecidos con probióticos y prebióticos

RESUMEN
El presente estudio tuvo como finalidad desarrollar suplementos gomosos (G-S) que incorporaran probióticos (Lactobacillus plantarum LUHS135 y Lactobacillus paracasei LUHS244), prebióticos (cáscara de psililo) y pulpa de manzana como fuente de pectina. Asimismo, pretendió evaluar el conteo de bacterias lácticas (LAB) viables, el contenido total de compuestos fenólicos (TPC), la actividad antioxidante, las coordenadas de color, los parámetros de textura y la aceptabilidad general de los G-S desarrollados. Además, se investigaron las propiedades antimicrobianas de las cepas LAB utilizadas contra Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis y Streptococcus mutans. Se comprobó que los G-S con gelatina mostraron mayor liviandad, amarillez y aceptabilidad, mientras que los G-S con agar exhibieron una textura más dura. Asimismo, se constató que la selección de agar/gelatina incide significativamente en el contenido de TPC de los G-S y que la actividad antioxidante de éstos depende de la cepa de LAB y del uso de cáscara de psililo. Todas las cepas patógenas examinadas fueron inhibidas por LUHS244. La fórmula G-S desarrollada permitió obtener productos de mayor valor.

1. Introduction
Supplements play an outstanding role in the food market, demonstrated by the demand derived from the increasing cost of healthcare and the steady increase of life expectancy (Wildman, 2016); therefore, research in this field is considered as a high priority (Doré et al., 2017). Often the bioactive ingredients in supplements are probiotics, prebiotics, and other substances having antioxidant and/or other positive activities for consumer health. Probiotic bacteria are living supplementary organisms that have been shown to provide beneficial health effects to the host by replenishing natural gastrointestinal microbiota (Das & Pathak, 2016). Among the probiotics, Lactobacillus spp. are well known as having many properties which make them beneficial to control pathogenic microorganisms (Garcia et al., 2016). These include the ability to adhere to cell, the reduction of pathogenic bacteria adherents, the co-aggregation, the production of organic acids, hydrogen peroxide, bacteriocins, and so on (Satpute et al., 2016). The predominant and most important bacteria species that are used as probiotics are the lactobacilli. Lactobacillus plantarum and L. paracasei are able to produce a biosurfactant which antimicrobial and anti-adhesive activities against several pathogens have been demonstrated, and the ability to co-aggregate with those pathogens and to adhere to epithelial cells enhance the probiotic properties and applications of these strains (Ivanovska, Pavlova, Mladenovska, & Petrushevska-Tozi, 2014). Combinations of probiotics and prebiotics act synergistically to confer health benefits to the host. As a therapeutic strategy, they provide a gentle yet powerful method for modulating the composition and metabolic output of the human gut microbiota (Timmis & Timmis, 2017). The resistant carbohydrates of psyllium husk (Plantago ovata) belong to soluble dietary fibres and are considered as important prebiotics that maintain biological functions, including glucose homeostasis, lipid regulation, colon disease prevention, and probiotics characteristics (Huang et al., 2016). Non-
targeted metabolomics implies that psyllium husk is recommended by the Food and Agriculture Organization of the United Nations for human health. Psyllium contains phenolics and flavonoids that possess reducing capacity and reactive oxygen species scavenging activities (Patel, Mishra, & Jha, 2016). Other compound, pectin, is one of the most common functional ingredients, naturally found in the walls of the plants (Pagiaro, Cirimina, Rodriguez, & Chavarria, 2015). Pectin composed of macromolecular compounds of plant origins consisting of galacturonic acid, galactose, arabinose, and other monosaccharides (Wang, Chen, & Lu, 2014). Pectin substances in foods are added due to their functional health (fibre and natural prebiotic) and technological functionality – pectin reduces the amount of fat and sugar, can alter the physical and chemical properties, and structure of foods that persist even after heat treatment (Tyagi, Kumar, & Malviya, 2015). The main technological feature used in the food industry is the ability of pectin to form gels with aqueous solutions, because of these properties, pectin is widely used in marmalade, jam, gel candy, and other confectionery bakery products (Prakash et al., 2014). The processing of apples generates large amounts of residues, which are commonly known as apple pomace, which could be utilized either for direct extraction of useful compounds, or for the production of value added products, or for lactic acid bacteria (LAB) immobilization, in case to enhance their viability (Bartkiene et al., 2017).

The aim of this study was to develop gummy–supplements (G-S) based on probiotics (L. plantarum LUHS135 and L. paracasei LUHS244), prebiotics (psyllium husk), and apple pomace as an pectin source, and to evaluate viable LAB count, total phenolic compounds (TPC) content, antioxidant activity, colour coordinates, texture parameters, and overall acceptability of the developed G-S. In addition, antimicrobial properties of the used LAB strains and their composition against Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Salmonella enterica, Staphylococcus aureus, Enterococcus faecalis, and Streptococcus mutans were investigated.

2. Materials and methods

2.1. G-S formula and preparation

LAB strains of L. plantarum LUHS135 and L. paracasei LUHS244 (collection of Lithuanian University of Health Sciences, Kaunas, Lithuania) were selected for G-S preparation. Strains were stored at −80°C in a Microbank system (Pro-Lab Diagnostics, UK) and multiplied in a de Man Rogosa Sharpe (MRS) broth (CM 0359, Oxoid Ltd., Hampshire, UK) at 30°C for 48 h before use.

Apple pomace (the dry part after the juice production, moisture content 55 g 100 g⁻¹) was obtained from MV Group Production wine factory (Anyksciai, Lithuania). Psyllium husk powder (fat 0.6 g 100 g⁻¹, total carbohydrates 88.6 g 100 g⁻¹, from which dietary fibre 88.5 g 100 g⁻¹, protein 1.5 g 100 g⁻¹) was obtained from the local supermarket (producer Livin, India). The agar powder (Gelidium sesquipedale algae, Rapunzel, Germany) was used as a polymer with mucoadhesive properties for G-S forming. In addition, gelatin was also tested (Klingai, Lithuania).

The formula of control G-S group consisted of sugar (20 g), glucose syrup (10 g), water (20 g), citric acid (0.90 g). For G-S of control (C1) group gelatine (10 g) was used. Also agar (10 g) was used for control (C2) G-S group. G-S were prepared by including different functional ingredients (probiotics, prebiotics, and apple pomace as sustainable pectin source) to the main formula. Recipe formulations are given in Table 1.

First, agar and/or gelatin powder was soaked in a water for 30 min, after that samples with agar were melted by heating for 15 min at 90°C, with gelatin were melted by heating for 15 min at 60°C. Sugar following glucose syrup was added and dissolved in the mixture under heating. Obtained mixture with agar was further heated to 90°C under stirring. Citric acid and different functional ingredients (probiotics, prebiotics, and apple pomace or their compositions) were incorporated into G-S mass at the end of the process (mass temperature 30°C). Obtained mass after mixing was poured into cast, and G-S were dried at 22–24°C for 24 h to get a gel-hard form.

2.2. The evaluation of LAB count in G-S

For the evaluation of LAB count, 10 g sample was homogenized with 90 mL of saline (9 g L⁻¹ NaCl solution). Serial dilutions of 10⁻³ to 10⁻⁶ with saline were used for sample preparation. Sterile MRS agar (CM0361, Oxoid) of 5 mm thickness was used for bacterial growth (Man, Rogosa, Sharpe) on Petri plates. The plates were separately seeded with the sample suspension using sowing in surface and were incubated under anaerobic conditions at 30°C for 72 h. The number of bacterial colonies was calculated and expressed as a decimal log of colony forming units per gram of sample (CFU g⁻¹).

2.3. The evaluation of G-S colour characteristics, texture parameters, and overall acceptability

The colour characteristics were evaluated using a CIE L*a*b* system (CromaMeter CR-400, Konica Minolta, Japan; McGuire, 1992). Acceptability of G-S was carried out according to the ISO 8586–1 method (ISO, 2000) by 20 judges for preliminary sensory acceptability using a 150 mm hedonic scale ranging from 150 (extremely like) to 0 (extremely dislike). Texture parameter–hardness was evaluated using texture analyser Brookfield (Middleboro, USA).

2.4. The evaluation of TPC content in G-S and their antioxidant activity

The total content of phenolic compounds (TPC) in G-S was determined by spectrophotometric method, as reported elsewhere (Vaher, Matso, Levandi, Helmja, & Kaljurand, 2010). The absorbance of samples was measured at 765 nm using spectrophotometer J.P. SELECTA S.A. V-1100D (Barcelona, Spain). Antioxidant activity of G-S was evaluated according to the method reported by Zhu, Xiong, Yu, & Wu (2005).

2.5. The evaluation of LAB antimicrobial activity

Pure probiotic (LUHS135 and LUHS244) strains and their mix (LUHS135 × LUHS244) were used for the determination of their antimicrobial activities against variety of pathogenic and opportunistic bacterial strains (P. aeruginosa, P. mirabilis, E. coli, S. enterica, S. aureus, E. faecalis, and S. mutans).

LUHS135 and LUHS244 were grown in MRS medium (Biolife, Italy) at 30°C. Two percent of the pure LAB strain
2.6. Statistical analysis

All analytical experiments were carried out in triplicate. In order to evaluate the influence of three different formula compounds (probiotics, prebiotics, apple pomace, agar and/or gelatin) and their interaction on gummy candies parameters, data were subjected to analysis of the analysis of variance (ANOVA) and the Tukey’s HSD test as post-hoc test. The results were referred to statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Viable LAB count in G-S

Viable LAB count in G-S is given in Table 2. In all the G-S, which were prepared with LAB and gelatin, higher than $6.0 \log_{10}$ CFU g$^{-1}$ LAB count was established. However, samples prepared with agar, in most of the cases, showed lower LAB count, except samples produced with agar, LAB, and psyllium husk. In general, $6.0 \log_{10} – 7.0 \log_{10}$ of probiotic bacteria per milliliter or gram of food has been recommended for exhibition of health benefit (Angmo, Kumari, & Bhalla, 2016). Beneficial effects of probiotics include their antimicrobial properties, improving lactose intolerance, lowering serum cholesterol level, increasing utilization of nutrients, and decreasing use of antibiotics (Guo, Kim, Namb, Park, & Kim, 2010). It is very important that for supplements preparation used strains are capable to survive for longer periods in food matrices without reduction in their number (Fijakowski, Peitler, Rakocy, & Zywicka, 2016). Immobilized cells exhibit many advantages over free cells, including the maintenance protection of cells against damage and reduced susceptibility to contamination, however, immobilization of probiotic cells requires some specific processing steps which complicate the manufacture of the food product and increase its cost (Burgain, Gaiani, Linder, & Scher, 2011). In our study, the developed G-S formula allowed to produce higher value products with high content of the viable probiotic cells, without specific processing steps. Just, combination of (I) agar, apple pomace, psyllium husk, and probiotic bacteria, and combination of (II) gelatin, apple pomace, psyllium husk, and probiotic bacteria for the G-S production could be recommended.
Table 2. Viable lactic acid bacteria (LAB) count in G-S during the storage.

| Gummy candies | After 0 days | After 10 days | After 20 days | After 30 days |
|---------------|--------------|--------------|--------------|--------------|
| CA            | 1.2 ± 0.1    | 1.3 ± 0.1    | 1.2 ± 0.2    | 1.3 ± 0.3    |
| CG            | 2.4 ± 0.2    | 2.5 ± 0.2    | 1.8 ± 0.2    | 1.9 ± 0.2    |
| ApA           | 3.3 ± 0.2    | 3.3 ± 0.1    | 2.8 ± 0.1    | 2.6 ± 0.2    |
| ApA135        | 4.4 ± 0.1    | 3.6 ± 0.2    | 3.4 ± 0.2    | 2.6 ± 0.2    |
| ApA244        | 4.7 ± 0.2    | 4.5 ± 0.2    | 3.2 ± 0.2    | 3.1 ± 0.2    |
| ApA135Psy     | 6.6 ± 0.1    | 6.7 ± 0.2    | 6.4 ± 0.2    | 6.4 ± 0.2    |
| ApA244Psy     | 7.3 ± 0.2    | 7.4 ± 0.1    | 7.2 ± 0.1    | 7.1 ± 0.1    |
| ApG           | 2.3 ± 0.2    | 2.5 ± 0.3    | 2.1 ± 0.3    | 2.1 ± 0.1    |
| ApG135        | 6.2 ± 0.2    | 6.4 ± 0.3    | 6.2 ± 0.3    | 6.1 ± 0.3    |
| ApG244        | 6.5 ± 0.2    | 6.3 ± 0.2    | 6.1 ± 0.2    | 6.3 ± 0.2    |
| ApG135Psy     | 6.4 ± 0.3    | 6.3 ± 0.2    | 6.0 ± 0.2    | 6.1 ± 0.2    |
| ApG244Psy     | 6.5 ± 0.3    | 6.5 ± 0.2    | 6.3 ± 0.2    | 6.4 ± 0.2    |

Gummy candies formula abbreviations: C – control; A – agar; G – gelatin; Ap – apple pomace; 135 – L. plantarum LUHS135; 244 – L. paracasei LUHS244; Psy – psyllium husk dietary fibre.

Data expressed as means (n = 3) ± SD; SD – standard deviation.

Abreviaturas de las fórmulas de dulces gomosos: C – control; A – agar; G – gelatina; Ap – pulpa de manzana; 135 – Lactobacillus plantarum LUHS135; 244 – Lactobacillus paracasei LUHS244; Psy – fibra dietética de cáscara de psilio.

Los datos figuran como medias (n = 3) ± DE; DE – desviación estándar.

3.2. Colour characteristics, texture parameters, and overall acceptability of the developed G-S

Colour coordinates, texture, and overall acceptability of the G-S are given in Table 3. In all the cases, higher lightness (L*) of the G-S prepared with gelatin, compare to samples prepared with agar, was found. Lightness coordinates of the G-S were ranged from 24.39 ± 1.56 NBS to 81.02 ± 1.56 NBS (of ApA – G-S produced with agar and apple pomace and of ApG135 – G-S produced with gelatin, apple pomace, and L. paracasei LUHS135, respectively). The highest coordinates of the redness (a*) of the ApA244 – G-S produced with agar, apple pomace, and L. paracasei LUHS244 were established (4.84 ± 0.29 NBS), and the lowest coordinates of the redness of the CA – control G-S were found (0.05 ± 0.01 NBS). In all cases, higher yellowness (b*) of the G-S prepared with gelatin was found, and the highest yellowness coordinates of the ApA244 – G-S produced with gelatin, apple pomace, and L. paracasei LUHS244, ApG135Psy – G-S produced with gelatin, apple pomace, L. plantarum LUHS135, and psyllium husk dietary fibre, and ApA244Psy – G-S produced with gelatin, apple pomace, L. paracasei LUHS244, and psyllium husk dietary fibre were established (19.42 ± 0.28 NBS, 19.43 ± 0.32 NBS, and 19.45 ± 0.08 NBS, respectively).

When comparing texture of the G-S prepared with gelatin and agar, higher hardness of the samples prepared with agar was found (ranged from 1.2 mJ to 7.6 mJ, of ApA244Psy – G-S produced with agar, apple pomace, L. paracasei LUHS244 and CA – control G-S produced with agar, respectively). Hardness of the samples group prepared with gelatin was ranging from 0.4 mJ to 0.8 mJ (of ApG135 – G-S produced with gelatin, apple pomace, and L. plantarum LUHS135 and of CG – control G-S produced with gelatin, respectively). According to results obtained, all of the used ingredients (probiotics, prebiotics, and apple pomace) reduced hardness of the G-S texture.

Analysis of the overall acceptability showed that higher acceptability of the G-S could be obtained using gelatin, compare to agar. The overall acceptability of the samples produced with agar was ranging from 48.2 ± 4.7 to 66.3 ± 4.9 (of ApA – G-S produced with agar and apple pomace and CA – control G-S produced with agar, respectively). According to the results obtained, all of the functional ingredients that were used in experiment reduced overall acceptability of the G-S, prepared with agar. However, all of the G-S prepared with gelatin were acceptable for consumers, and their acceptability was ranged from 91.0 ± 2.7 to 127.1 ± 4.3 (of ApG244Psy – G-S produced with gelatin, apple pomace, L. paracasei LUHS244, and psyllium husk dietary fibre and of ApG135 – G-S produced with gelatin, apple pomace, and L. plantarum LUHS135, respectively). Results of the ANOVA test indicated that there is a significant effect (p < 0.05) due to the use of probiotics, prebiotics, apple pomace, agar and/or gelatin on the G-S lightness (L*), yellowness (b*), texture, and overall acceptability; however, significant influence of the used ingredients on gummies redness (a*) was not found. It was some phenomena established that consumers primary selecting food in traditional form, and any modifications in form are unwelcome (Madukwe & Eme, 2012). In this case, it is very important to offer for consumers higher value foods and/or supplements in traditional form, and gummies could be alternative to improve consumers health using traditional product.

3.3. TPC content and antioxidant activity of the prepared G-S

TPC content and antioxidant activity of the prepared G-S are given in Table 3. The highest TPC content was found in CA – control G-S produced with agar (707.1 ± 23.1 mg 100 g−1 d.m.); however, in all G-S produced with gelatin (except control samples) TPC content was found higher and ranged from 642.0 ± 23.1 mg 100 g−1 d.m. (ApG244 – G-S produced with gelatin, apple pomace, and L. paracasei LUHS244) to 688.5 ± 13.5 mg 100 g−1 d.m. (ApG135Psy – G-S produced with gelatin, apple pomace, L. plantarum LUHS135, and psyllium husk dietary fibre), compare to samples produced with agar. Opposite tendencies of antioxidant activity of the prepared G-S were found, the lowest antioxidant activity of the CA – control G-S produced with agar (12.4 ± 1.6%) was established. Moderate negative correlation between the TPC and antioxidant activity of the prepared G-S (r = −0.4081) was found. Significant influence on TPC content had agar/gelatin selection (p = 0.0001), the use of apple pomace (p = 0.007), and...
| Gummy candies | L*       | a*       | b*       | Texture, mJ | TPC content, mg 100 g⁻¹ d.m. | Antioxidant activity, % | Overall acceptability |
|--------------|----------|----------|----------|-------------|-------------------------------|------------------------|-----------------------|
| CA           | 30.36 ± 2.31 | 0.05 ± 0.01 | 11.06 ± 0.41 | 7.6 ± 0.1 | 707.1 ± 23.1 | 12.4 ± 1.6 | 66.3 ± 4.9 |
| CG           | 40.47 ± 2.15 | 1.61 ± 0.12 | 11.34 ± 0.23 | 0.8 ± 0.1 | 477.9 ± 14.5 | 30.3 ± 2.8 | 106.6 ± 5.2 |
| ApA          | 24.39 ± 1.56 | 3.43 ± 0.09 | 8.73 ± 0.35 | 1.3 ± 0.1 | 475.6 ± 10.8 | 30.3 ± 2.6 | 48.2 ± 4.7 |
| ApA135       | 27.73 ± 1.38 | 4.23 ± 0.14 | 10.22 ± 0.09 | 1.4 ± 0.1 | 487.4 ± 11.4 | 51.8 ± 4.3 | 53.2 ± 4.9 |
| ApA244       | 25.19 ± 1.07 | 4.84 ± 0.29 | 10.52 ± 0.11 | 2.2 ± 0.1 | 447.5 ± 9.6 | 41.8 ± 2.5 | 56.1 ± 5.1 |
| ApA135Psy    | 27.10 ± 1.22 | 3.20 ± 0.17 | 9.99 ± 0.27  | 2.5 ± 0.1 | 415.9 ± 16.3 | 28.9 ± 3.4 | 57.0 ± 4.8 |
| ApA244Psy    | 28.63 ± 2.35 | 4.49 ± 0.16 | 10.98 ± 0.34 | 1.2 ± 0.1 | 472.2 ± 21.5 | 52.4 ± 2.6 | 61.8 ± 6.3 |
| ApG          | 62.92 ± 1.87 | 1.47 ± 0.09 | 15.73 ± 0.26 | 1.0 ± 0.1 | 655.5 ± 17.3 | 21.9 ± 1.9 | 100.4 ± 5.7 |
| ApG135       | 81.02 ± 3.01 | 1.81 ± 0.11 | 18.66 ± 0.17 | 0.4 ± 0.0 | 646.1 ± 13.4 | 20.9 ± 1.5 | 127.1 ± 4.3 |
| ApG244       | 77.79 ± 3.20 | 2.21 ± 0.24 | 19.42 ± 0.28 | 0.5 ± 0.0 | 642.0 ± 18.6 | 28.6 ± 2.3 | 121.5 ± 3.9 |
| ApG135Psy    | 69.19 ± 1.25 | 3.56 ± 0.21 | 19.43 ± 0.32 | 0.7 ± 0.0 | 688.5 ± 13.5 | 40.2 ± 1.8 | 91.0 ± 2.7 |
| ApG244Psy    | 74.46 ± 2.41 | 2.64 ± 0.13 | 19.45 ± 0.08 | 0.6 ± 0.0 | 655.8 ± 15.3 | 35.9 ± 2.1 | 96.2 ± 5.6 |

TPC – total phenolic compounds content.

Gummy candies formula abbreviations: C – control; A – agar; G – gelatin; Ap – apple pomace; 135 – L. plantarum LUHS135; 244 – L. paracasei LUHS244; Psy – psyllium husk dietary fibre.

Data expressed as means ($n = 3$) ± SD; SD – standard deviation.

TPC – contenido total de compuestos fenólicos.

Abreviaturas de las fórmulas de dulces gomosos: C – control; A – agar; G – gelatina; Ap – pulpa de manzana; 135 – Lactobacillus plantarum LUHS135; 244 – Lactobacillus paracasei LUHS244; Psy – fibra dietética de cáscara de psilio.

Los datos figuran como medias ($n = 3$) ± DE; DE – desviación estándar.
interaction of evaluated factors (agar/gelatin * psyllium husk dietary fibre; agar/gelatin * apple pomace; LAB (LUHS135/LUHS244) * psyllium husk dietary fibre) on TPC content in G-S was significant ($p < 0.001$; $p < 0.0001$; $p < 0.016$, respectively; Table 4). Antioxidant activity of G-S was dependent on the strain of LAB and the use of psyllium husk ($p < 0.0001$; $p < 0.006$, respectively), and interaction of evaluated factors (agar/gelatin * LAB (LUHS135/LUHS244); agar/gelatin * psyllium husk dietary fibre; agar/gelatin * apple pomace; LAB (LUHS135/ LUHS244) * psyllium husk dietary fibre) on G-S antioxidant activity was significant ($p < 0.002; p < 0.0001; p < 0.0001; p < 0.022$, respectively).

Chronic and degenerative diseases such as cancer, diabetes, and cardiovascular diseases are the main causes of death worldwide (WHO, 2014). A strategy to prevent such diseases is through the consumption of supplements (Santana-Gálvez, Pérez-Carrillo, Velázquez-Reyes, Cisneros-Zevallos, & Jacobo-Velázquez, 2016). It was published that polysaccharides, as well as agar, has a moderate antioxidant activity (Souza et al., 2012). Psyllium is a potential source of dietary supplementation and possesses important biological, antioxidant, and anti-inflammatory properties (Berea et al., 2009). The extract contains terpenes (Patel et al., 2016), including saponins, and it was demonstrated that saponins might possess anticancer activity (Zhu et al., 2005). In psyllium, seeds have higher content of TPC than in the leaves and in the husk, therefore, seeds show a higher total antioxidant and DPPH scavenging activity, followed by the leaves and the husk (Patel et al., 2016). Antioxidant activities inhibit oxidation processes and play a key role in promoting health (Shahidi & Zhong, 2010). Scavenging and antioxidant activities, in most of the cases, depend on the content of polyphenolic compounds (Shahidi & Ambigaipalan, 2015). LAB also show antioxidant activity (Campanella et al., 2017). LAB are associated with a reduced risk of developing chronic diseases, and these abilities are mainly focused on viable LAB and cell-free extracts; furthermore, cell-free supernatant is a well-known good source of antioxidants (Xing et al., 2015). Finally, the combination of plants and probiotic bacteria could be a promising formula for G-S preparation.

### 3.4. Antimicrobial activities of the tested LAB strains and their combinations

Antimicrobial activities of the tested LAB strains and their combinations are given in Table 5. The pure LUHS135 strain showed a lower inhibition of the tested pathogenic strains, as compared to LUHS244 and LUHS135 and LUHS244 mix, which inhibited all the tested strains. The highest inhibition zones of the LUHS244 against *E. coli* (12.0 ± 0.4 mm) and of the LUHS135 and LUHS244 mix against *S. mutans* (12.0 ± 0.7 mm) were observed. Among target areas, the restoration of a healthy oral microbiota is generally based on the ability of probiotics to suppress growth of pathogens relevant to the oral cavity (Zoumpopoulou et al., 2013), such as *S. mutans*, which is widely known as the main causative microorganism in dental caries development. Today, many functional foods are available in the market, with probiotic microorganisms (i.e. fermented foods having probiotic microorganisms as starters or adjuncts) being the vast majority (Georgalaki et al., 2017). Generally, initial assessment of probiotic bacteria focuses on their tolerance to the hostile environment of the human gastrointestinal tract and their ability to colonize the host, as well as safety issues, and further, antimicrobial activity, immunomodulatory ability, and proteolytic activity leading to the release of biologically active peptides are considered major probiotic features (Papadimitriou et al., 2015). It was published that the *L. plantarum* represented an inhibitory activity against *S. aureus*, *S. epidermidis*, and *E. coli* (Jabbari et al., 2017), and that the *L. paracasei* inhibits the growth of the *E. coli* and *Shigella* sp. (Sharma, Mahajan, Attri, & Goe, 2017). However, both tested strains showed antimicrobial properties, but higher antimicrobial activity of the LUHS244 strain and LUHS135 and LUHS244 mix was established.

**Table 4.** The influence of different formula compounds (probiotics, prebiotics, apple pomace, agar, and/or gelatin) and their interaction on G-S antioxidant activity and TPC content.

| Source | Type III sum of squares | Mean square | F   | p   |
|--------|-------------------------|-------------|-----|-----|
| Agar/gelatin | 46,605.170 | 46,605.170 | 184.744 | 0.0001 |
| LAB (LUHS135/LUHS244) | 542.040 | 271.020 | 1.074 | 0.357 |
| Psyllium husk dietary fibre | 36.015 | 36.015 | 0.143 | 0.709 |
| Apple pomace | 2178.908 | 2178.908 | 8.637 | 0.007 |
| Agar/gelatin * LAB (LUHS135/LUHS244) | 1086.970 | 1086.970 | 543.485 | 2.154 | 0.138 |
| Agar/gelatin * Psyllium husk dietary fibre | 3947.535 | 3947.535 | 15.648 | 0.001 |
| Agar/gelatin * Apple pomace | 125,522.108 | 125,522.108 | 497.572 | 0.0001 |
| LAB (LUHS135/LUHS244) * Psyllium husk dietary fibre | 1693.440 | 1693.440 | 6.713 | 0.016 |
| LAB (LUHS135/LUHS244) * Psyllium husk dietary fibre | 3.890 | 3.890 | 0.239 | 0.630 |
| Psyllium husk dietary fibre | 242.016 | 242.016 | 1.000 | 0.0001 |
| Apple pomace | 145.534 | 145.534 | 8.926 | 0.006 |
| Agar/gelatin * LAB (LUHS135/LUHS244) | 145.534 | 145.534 | 8.926 | 0.006 |
| Agar/gelatin * Psyllium husk dietary fibre | 276.416 | 276.416 | 138.208 | 4.876 | 0.002 |
| Agar/gelatin * Apple pomace | 1735.934 | 1735.934 | 735.934 | 45.135 | 0.0001 |

The results statistically significant at $p < 0.05$. LUHS135 = *L. paracasei* LUHS13; LUHS244 = *L. plantarum* LUHS244.

Los resultados son estadísticamente significativos en $p < 0.05$. LUHS135 = *Lactobacillus paracasei* LUHS13; LUHS244 = *Lactobacillus plantarum* LUHS244.
Table 5. Antimicrobial activity of the L. plantarum LUHS135, LUHS244 – L. paracasei, and their mix.

| Microorganisms       | LUHS135 | LUHS244 | LUHS135x LUHS244 |
|----------------------|---------|---------|------------------|
| Staphylococcus aureus| 8.0 ± 1.0| 11.0 ± 0.5| 9.0 ± 0.4        |
| Escherichia coli     | 9.0 ± 0.7| 12.0 ± 0.4| 11.0 ± 0.9      |
| Enterococcus faecalis| –       | 10.0 ± 0.5| 10.0 ± 0.4      |
| Proteus mirabilis    | –       | 11.0 ± 0.6| 11.0 ± 0.3      |
| Pseudomonas aeruginosa| 11.0 ± 0.4| 10.0 ± 0.5| 11.0 ± 0.7      |
| Salmonella enterica  | –       | 11.0 ± 0.5| 11.0 ± 0.5      |
| Streptococcus mutans | 11.0 ± 0.6| 11.0 ± 0.5| 12.0 ± 0.7      |

LUHS135 – L. plantarum LUHS135; LUHS244 – L. paracasei. (−) – not inhibited.

Data expressed as means (n = 5) ± SD; SD – standard deviation.

4. Conclusions

Different ingredients have different influence on G-S parameters, and there is a significant effect (p < 0.05) due to the use of probiotics, prebiotics, apple pomace, agar and/or gelatin on the G-S lightness (L*), yellowness (b*), texture, and overall acceptability. The most acceptable G-S group was prepared with gelatin. Agar/gelatin selection and the use of apple pomace have significant influence on TPC content in G-S, and the highest TPC content in G-S with gelatin (except control samples) was found, compare to samples produced with agar. Antioxidant activity of G-S was dependent on the strain of LAB and the use of psyllium husk. The pure LUHS135 strain showed a lower inhibition of the tested pathogenic strains, as compared to LUHS244 and LUHS135 and LUHS244 mix, which inhibited all the tested strains. Finally, the developed G-S formula allowed to produce higher value products with high content of the viable probiotic cells, without specific processing steps, and the best formulation for G-S, consisting of gelatin, apple pomace, L. paracasei LUHS244, and psyllium husk, can be recommended.

Disclosure statement

No potential conflict of interest was reported by the authors.

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