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Designing a functional rice muffin formulated with prebiotic oligosaccharides and sugar reduction

Cláudia Amorim [a,*], Beatriz B. Cardoso [a], Sara C. Silvério [a], Jessica C. Silva [a], Joana I. Alves [a], Maria Alcina Pereira [a], Ramón Moreira [b], Lígia R. Rodrigues [a]

[a] CEB-Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal
[b] Chemical Engineering Department, Universidade de Santiago de Compostela, Campus Vida, 15782, Santiago de Compostela, Spain

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ABSTRACT

Innovation of pastry products towards higher nutritional and commercial value remains a challenge to the growing field of healthy food. In this study, the prebiotic supplementation and sugar reduction were explored in a widely consumed pastry product with a low level of innovation. The prebiotic potential of commercial agave inulin and galacto-oligosaccharides (GOS) was evaluated and compared by an in vitro model using human fecal inocula. Rice muffins containing 100% of sugar or 75% of sugar supplemented with 0.8% GOS were produced and compared with commercial rice muffins regarding their physical and textural properties. GOS fermentation led to the highest production of lactate and short-chain fatty acids, besides the most significant reduction of the final pH value and of the ammonia and methane production. Inulin presented a higher selectivity towards Lactobacillaceae (51 ± 1% of all), while GOS are more efficient to stimulate Bifidobacteriaceae growth (65 ± 7% of all).

Both prebiotics were effective in reducing Methanobacteria. The reduction of sugar content modified the air bubbles characteristics (size and population with a greater number of tunnels) present in the muffin crumb, without relevant differences in apparent porosity. Textural results indicated that springiness and resilience of the muffin with low sugar content are acceptable, but hardness and chewiness were increased. The new-formula muffins presented very relevant textural parameters with comparable values to those reported in the literature for the commercial ones, thus anticipating a good consumer acceptance. This study is an important contribution towards more innovative and diversified healthy pastry products.

1. Introduction

The increasing prevalence of overweight and obesity is associated with many diet-related chronic diseases. In this sense, consumers today are pivoting their preferences towards healthier options, such as low-sugar, low-fat and functional food products. The latter are characterized for containing functional ingredients (e.g. prebiotics) that will confer extra health benefits above the original nutritional value of the food product (Mohanty et al., 2018; Szutowska, 2020). In this context, prebiotics have attracted global attention due to their beneficial effects over different physiological functions. These compounds have been reported as promoters of beneficial effects on both human and animal health, namely on the gastrointestinal (GI) tract, cardiometabolism, mental health and bones, among others (Devaraj et al., 2020; Gibson et al., 2017; Sreenivas & Lele, 2013). According to the updated definition by The International Scientific Association for Probiotics and Prebiotics (ISAPP), a prebiotic is "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017). In addition, these compounds can be fermented by intestinal bacteria leading to the production of short-chain fatty acids (SCFA) and gases that beneficially modulate the gut environment (Amorim et al., 2020a, 2020b).

The global market of the prebiotic ingredients is expanding with a forecasted compound annual growth rate (CAGR) of 9.8% (2019–2024) (Ahuja & Mamtani, 2019). On the other hand, pastry products are widely consumed by people throughout the world. For instance, the European market of bakery products, including muffins and pastries, is expected to grow at a CAGR of 3.12% (2020–2025) (Mordor Intelligence, 2020). However, there is a lack of innovation in the traditional pastry sector, namely regarding the delivery of healthier alternative

* Corresponding author.
E-mail address: claudia.oliveira.amorim@ceb.uminho.pt (C. Amorim).

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products.

Prebiotic oligosaccharides can be used as low-calorie sweeteners, presenting favorable organoleptic properties, temperature and acidic stability, which make them potential food ingredients. Inulin and galacto-oligosaccharides (GOS) are commercially well-established prebiotics, recognized as safe food ingredients by the European Food Safety Authority and have been used in the European Union over two decades (Amorim et al., 2019). Inulin is comprised by water-soluble oligo- and polysaccharides consisting of fructose moieties linked to each other through β-(2→1) linkages and to a terminal glucose residue by α-linkages (Ahmed & Rashid, 2019). GOS are composed of galactose (Gal) moieties linked to each other and terminally connected to a glucose (Glc) residue via β-linkages according to the formula Galn-Glc, with a degree of polymerization (DP) varying from 2 to 8 (Torres et al., 2010).

In this study, an in vitro model using human fecal inocula and high-throughput sequencing (16S rRNA gene) of the microbiota was used to evaluate and compare the prebiotic potential of commercial agave inulin and GOS. Unlike the in vivo studies, the in vitro approach using fecal inocula is a cheap and fast methodology to evaluate the prebiotic potential in a preliminary way (Date et al., 2014). The use of fecal inocula and high-throughput sequencing techniques to analyse the microbiota modulation is compatible and well-aligned with the guidelines stated for the in vitro studies by ISAPP (Gibson et al., 2017). The compounds presenting highest prebiotic potential were then incorporated in a rice muffin, in order to increase its commercial and nutritional value. Muffins containing 100% of sugar and 75% of sugar supplemented with prebiotics were produced and compared with a commercial rice muffin.

The main challenges of this work were (a) to confirm the in vitro prebiotic potential of marketed GOS and agave inulin and (b) to create a healthier and potentially functional pastry option for consumers who either follow a health-based lifestyle or have a special medical condition.

2. Materials and methods

2.1. Materials

Agave inulin was purchased from Entelees® (Guadalajara, Mexico) and GNC Prebiotic GOS from GNC® (Cleveland, Ohio, USA). All chemicals and media components were of analytical grade and obtained from Sigma - Aldrich Quimica S.L. (Lisboa, Portugal). Except for the prebiotics, the ingredients used for muffins preparation were purchased on a local market: Branca de Neve wheat flour from Fábricas Lusitana, S. A. (Alcains, Portugal), Ceifeira rice flour from Dacsa Atlantic, S.A. (Lisbon, Portugal), sugar from RAR, S.A. (Porto, Portugal), Vaqueiro butter from Upfield Inc. (Lisbon, Portugal), medium size eggs from Derovo, S.A. (Pombal, Portugal), Terra Nostra semi-skimmed milk from Bel Portugal, S.A. (Ribeira Grande, Açores, Portugal) and Royal baking soda from AMD Portugal, S.A. (Mora, Portugal).

2.2. Prebiotic potential

2.2.1. In vitro batch culture fermentations

Fecal samples were obtained from one healthy female donor, aged 23 years old. A voluntary informed consent was obtained from the fecal donor prior to this study. The samples were collected on site, diluted 1/10 (w/w) in anaerobic (100% N_2) phosphate-buffered saline solution (PBS, 0.1 M, pH 7.0). Static batch culture fermentations and samples analysis were performed as descrived in Amorim et al. (2020a; 2020b). The nucleotide sequences were deposited in the European Nucleotide Archive (ENA) under the BioProject accession number PRJEB37799.

2.3. Development of the traditional rice muffin

2.3.1. Preparation of the muffins

Control muffins (100S) were prepared according to the original recipe of the rice muffin, containing (% w/w): sugar (23.4), butter (9.4), eggs (19.1), milk (14.0), wheat flour (28.1), rice flour (5.6) and baking soda (0.5). The modified muffins were prepared based on the original recipe with a 25% (w/w) reduction of sugar and a 0.8% (w/w) addition of the most promising prebiotic (% w/w): sugar (18.4), butter (9.8), eggs (20.0), milk (14.7), wheat flour (29.3), rice flour (5.9), baking soda (0.5), GOS (0.8) and GOS excipients (0.7). Butter, sugar, baking soda and the lemon flavour were mixed using a hand mixer (BHM3133, Becken, Portugal) for 5 min. The eggs were added and mixed (3 min at speed 2) one by one. After incorporating the wheat and rice flour and when required GOS (1 min mixing at speed 2) into the batter, the milk was added, mixing 3 min at speed 2. The batter was divided in 80 g aliquots into paper molds, and baked at 180 °C during 30 min in a preheated electric oven (Balay 3HB569XC, BSH, Spain). The muffins were left to cool overnight at 25 °C and at controlled atmosphere (relative humidity of 53 ± 5%).

2.3.2. Physical, optical and textural properties

The height and diameter of the muffins were measured using a digital caliper. Moisture content, X kg water/kg dry solid (d.s.), of the muffins was determined by weighing after vacuum drying at 70 °C and 15 kPa (AOAC, 1995). Baking mass loss, BML, was determined as described by Heo et al. (2019), using Equation (1):

$$BML = \frac{\left(w_{batter} - w_{muffin}\right) 100}{w_{batter}} \quad (1)$$

where w_{batter} and w_{muffin} are the weights of the sample before and after baking (1 day). The color of the muffin crumbs was measured 1 day after baking, using a colorimeter (CR-400 Konica Minolta, Japan) previously calibrated, by means of six measurements on four different positions. The color parameters (L*, a* and b*) were accessed as described by Fariña et al. (2019). The total color difference (ΔE*) between samples was determined by:

$$\Delta E^* = \sqrt{\left(L^* - L_r^*\right)^2 + \left(a^* - a_r^*\right)^2 + \left(b^* - b_r^*\right)^2} \quad (2)$$

where the suffix r corresponds to the reference system (Hunter & Harold, 1987). The muffins were cut transversally and images of the cut surface of the crumb were captured. Images were analysed using the UTHSCSA ImageTool 3.0 program (ImageTool software copyright, University of Texas Health Science Center San Antonio, USA). The number of bubbles (with area above 0.008 cm^2) was counted along with their dimensions to evaluate the average pore diameter (after the determination of the length of major and minor axes by the irregular shape of bubbles) and area. Apparent density (g/cm^3) of the crumb was evaluated after cut a cylinder with a hollow punch and knife and measure its volume, V (cm^3), and weight, w (g). Apparent porosity, ϕ (%), (bubble volume x 100/sample volume) of muffin crumb was evaluated after the determination of the sample volume and the volume of the grounded sample. Specific density, ρ_s (g/cm^3), of the crumb was evaluated by Equation (3):

$$\rho_s = \frac{w}{V\left(1 - \frac{\varphi}{100}\right)} \quad (3)$$

The texture profile analysis (TPA) was performed using the texture analyzer (Stable Micro System Ltd, Model TA-XT2i) apparatus. Cylindric samples of 19.90 mm (d) x 20 mm (h) were cut from the bulk of the muffins. Muffin samples were double compressed to 50% of the original height, using a flat-ended cylindrical probe (P/25), a trigger force of 5 g, pre- and test-speed of 1 mm/s and a post-speed of 2 mm/s, 10 mm of distance and a rest period of 5 s between cycles. Textural measurements were performed during 4 consecutive days for muffins stored under controlled conditions (25 °C and 53 ± 5% of relative humidity). Hardness (g), springiness (cm), cohesiveness (-), chewiness, (g cm), and resilience (-) were recorded form curves because are considered the most relevant textural parameters for muffins (Fariña et al., 2019; Goswami

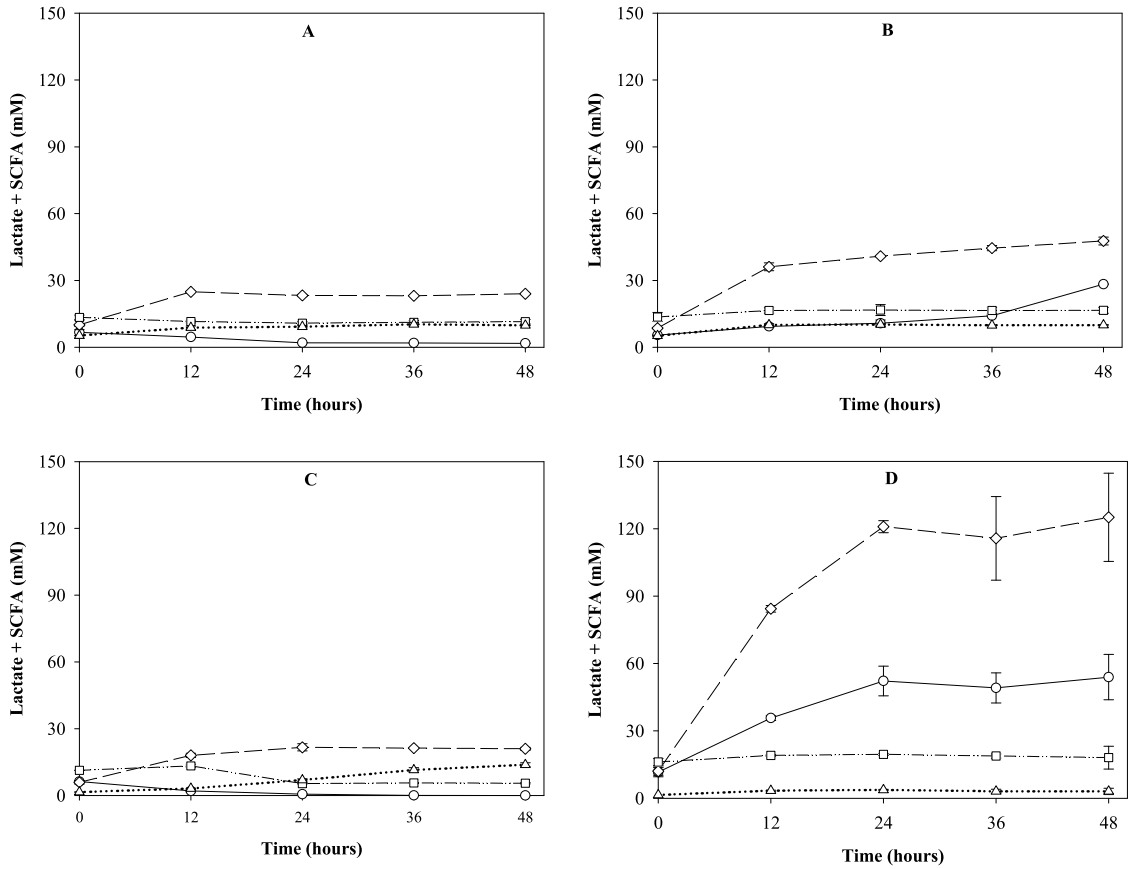

Fig. 1. Production of lactate and SCFA (lactate - circle; acetate - diamond; propionate - square; butyrate - triangle) during 48 h of in vitro fermentations using human fecal inocula of one donor: A - control 1, in the absence of prebiotics; B - culture medium enriched with inulin 10 g/L; C - control 2, in the absence of prebiotics; D - culture medium enriched with GOS 10 g/L. The results are the average of two independent fermentations ± standard deviation.

et al., 2015). Four different muffins from each recipe were measured in duplicate. Water adsorption isotherms were determined by a static gravimetric method employing saturated salt solutions to generate a range of water activities, a_w, from 0.11 to 0.92 at 25 °C. Triplicate samples (1.0 ± 0.1 g) of pre-dried muffins at 45 °C during 12 h were employed (X below 0.002 d.s.). At a_w higher than 0.5 thymol was employed in order to avoid microbial spoilage of samples. Around 14 weeks were necessary to achieve the equilibrium. Sorption isotherms were modelled using the Oswin model (Oswin, 1946), Equation (4):

$$X_{eq} = a\left(\frac{a_w}{1-a_w}\right)^b \quad (4)$$

where X_{eq} (d.s.) is the equilibrium moisture content and a and b are the fitting parameters.

2.4. Statistical analysis

Results are presented as mean values ± standard deviation. The statistical significance and differences were evaluated by one-way ANOVA using the software Prism version 7.05a (GraphPad Software Inc., California, USA). Tukey test was used for post hoc comparisons. Unpaired t-test was used when required. Significant differences were considered when $p < 0.05$.

3. Results and discussion

3.1. In vitro evaluation of the prebiotic potential

3.1.1. Production of lactate and short-chain fatty acids (SCFA)

The fermentation of prebiotic oligosaccharides by gut microbiota results in the production of SCFA and lactate, a precursor of SCFA, which play an important role on human health (Gill et al., 2018). The production of lactate (that in the gut can be a precursor of SCFA), acetate, propionate and butyrate during 48 h of fermentation with commercial inulin and GOS is presented in Fig. 1 together with the respective controls, conducted in the absence of prebiotics.

Similar results were obtained for the controls, although representing fecal samples from different days, which suggest inter-assay reproducibility of the technique (Fig. 1). In fact, the gut microbiota of each individual is relatively stable, although varying with different factors, including age, diet, and health condition (Rinninella et al., 2019). Regarding the experiments with inulin, after 48 h of fermentation a significant increase in the accumulation of acetate (24.00 ± 0.06 mM for control 1 and 48 ± 2 mM for inulin, $p < 0.0001$) and lactate (1.77 ± 0.01 mM for control 1 and 28.3 ± 0.2 mM for inulin, $p < 0.0001$) was observed when compared to the control fermentation (Fig. 1). While propionate showed a less statistically significant accumulation at the same time point (12 ± 1 mM for control 1 and 17 ± 1 mM for inulin at 48 h, $p < 0.05$), and butyrate revealed no significant variations relative to the control. When compared to inulin assays, the GOS fermentation led to a 2.9-fold higher relative production of lactate and SCFA at 48 h. Additionally, the fermentations supplemented with GOS showed an increased relative production of lactate and SCFA at an early time point (12 h) than inulin, having reached maximum values at 24 h (571 ± 29%

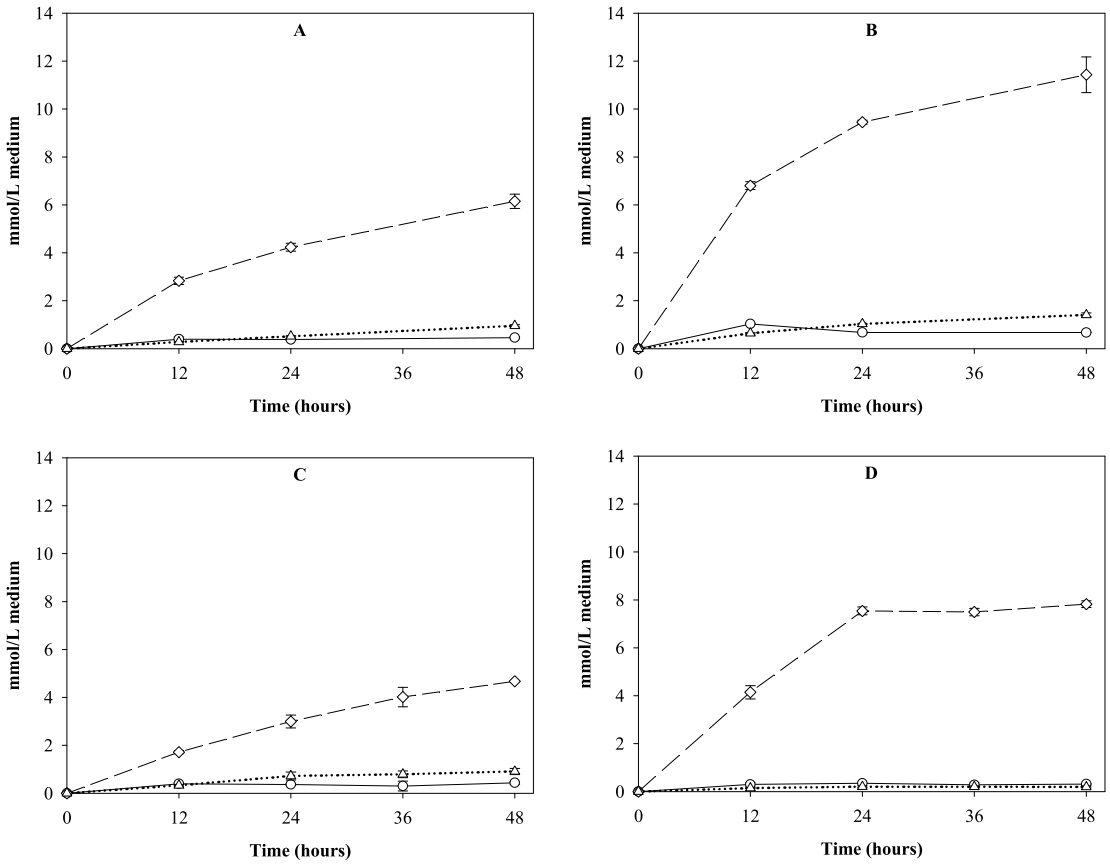

Fig. 2. Production of H_2 (circle), CO_2 (diamond) and CH_4 (triangle) during 48 h of in vitro fermentations using human fecal inocula of one donor: A - control 1, in the absence of prebiotics; B - culture medium enriched with inulin 10 g/L; C - control 2, in the absence of prebiotics; D - culture medium enriched with GOS 10 g/L. The results are the average of two independent fermentations and triplicate analysis of each sample ± standard deviation.

relative to the control). Contrariwise to inulin and GOS fermentations, the controls show a consumption of lactate leading to their absence after 48 h, suggesting that the growth of lactate-producing bacteria is being stimulated. Overall, acetate was the main SCFA produced, followed by the organic acid lactate, with propionate and butyrate being the least produced. Lactate and acetate production were significantly higher in experiments using GOS rather than inulin.

Rycroft et al. (2001) reported comparable results with increased production of acetate, propionate and lactate resulting from GOS supplementation when compared to inulin, both added at a concentration of 10 g/L. It is comprehensible that acetate was the most produced metabolite since its production pathways are present in many bacterial groups, while lactate, propionate and butyrate production pathways exhibit a more conserved distribution (Louis et al., 2007; Morrison & Preston, 2016). On the other hand, it is also known that inulin and GOS promote the growth of Bifidobacteria which are known to be non-butyrogenic bacteria (Gibson et al., 2017; Louis et al., 2007). The substrate concentration of 10 g/L is a widely used in in vitro models with supplementation of different prebiotics, including inulin and GOS (Amorim et al., 2020a, 2020b; Kanjan & Hongpattarakere, 2017; Pompei et al., 2008; Rodriguez-Colinas et al., 2013; Rycroft et al., 2001), therefore the results from this study could be easily compared with the literature. In addition, it is important to mention that this substrate concentration promoted significant changes on the metabolite production profiles when compared to the blank, thus avoiding technical limitations regarding sensitivity issues variations. However, after this first assessment of the prebiotic potential, the authors consider very relevant to determine the influence of the substrate concentration on the production profiles (i.e. study a wide range of concentrations) in a future work.

3.1.2. *Production of ammonia and final pH variation*

The accumulation of SCFA decreases the medium pH, promoting the reduction of pathogenic bacteria and allowing the increase of the population of beneficial bacteria. The change in the microbial population may lead to alterations in the metabolic pathways, such as the prevalence of saccharolytic fermentation over proteolytic fermentation, which in turn causes a reduction of ammonia production. Ammonia contributes to foul fecal odour and, being a toxic compound, can lead to colon carcinogenesis (Louis et al., 2007; Power et al., 2014). The final pH and ammonia concentration after 48 h of fermentation were determined in the control and the supplemented fermentations with prebiotics (Supplementary material, Table S1). A similar decrease in the pH value was observed due to inulin ($\Delta pH = 3.32$) and GOS fermentations ($\Delta pH = 3.26$). However, GOS resulted in a higher decrease in the ammonia concentration (398 ± 4 mg/L at 48 h), with a 5.4-fold reduction compared to the control fermentation (Supplementary material, Table S1). Pompei et al. (2008) reported similar results with a significant decrease of pH value in in vitro static batch fermentations of fecal microbiota supplemented with oligofructose, a well-established prebiotic, in comparison to the control fermentations. These authors also described a lower concentration of ammonia as a result of oligosaccharide supplementation.

The preference for saccharolytic fermentation and acidic pH are usual consequences associated to the in vitro models as used in this study, where only the distal colon conditions are mimicked and the medium pH is not controlled throughout the fermentation. Thus, the in vitro models are regarded as a semi-representation of the in vivo models due to their intrinsic limitations. Contrary to the in vivo models, the SCFA are not absorved and the different intestinal sections are not fully replicated. On the other hand, an in vitro model is a better approach to

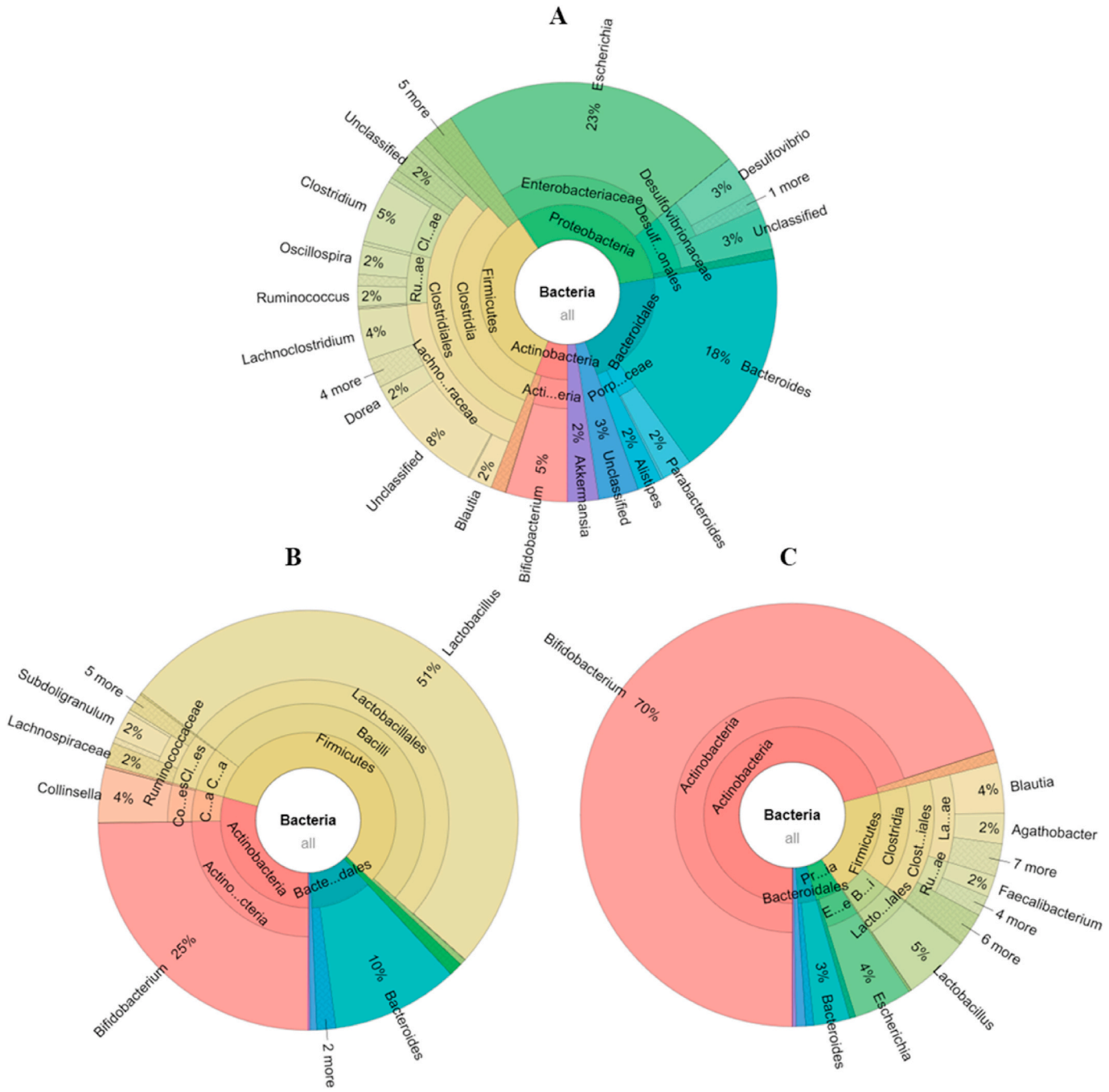

Fig. 3. Relative abundance of different bacteria after 48 h of in vitro fermentation by fecal human inocula in the absence of prebiotics (A) or enriched with a prebiotic solution of commercial agave inulin (B) or GOS (C) at 10 g/L.

evaluate the kinetics of colonic fermentation and to screen the prebiotic potential of different compounds (Payne et al., 2012).

3.1.3. Gas production

The fermentation of oligosaccharides by fecal human microbiota affects the production of gases such as carbon dioxide (CO_2), hydrogen (H_2) and methane (CH_4). CH_4 is only produced in a small portion of the population, who harbour methanogenic bacteria in their gut microbiota (Ghoddusi et al., 2007). Fig. 2 presents the gas production profiles during the 48 h of fermentation supplemented with inulin or GOS, and respective controls. As it occurred for the SCFA, the controls exhibited similar production profiles although carried out in different occasions, with different fecal samples from the same donor. For both compounds, higher concentrations of CO_2 were obtained when comparing to the control ($p < 0.0001$), presenting maximum concentrations at 48 h for inulin (11 ± 1 mmol/L) and at 24 h for GOS (7.5 ± 0.1 mmol/L), corresponding to a respective 1.9- and 2.5-fold increase relative to the control.

Regarding the production of CH_4, no significant differences were observed between control and inulin fermentation, contrary to the GOS fermentation. Interestingly, the GOS supplementation led to a decrease of 4.7-fold in the production of CH_4 when compared to the control. The composition (%) of the gaseous phase, in terms of CO_2, H_2 and CH_4, after 48 h of fermentation highlights the evidence that GOS addition led to the lower percentage of CH_4. (Supplementary material, Fig. S1). Furthermore, the supplementation with GOS resulted in no significant

alterations in CH_4 concentration from 12 h to 48 h (0.14 ± 0.02 mmol/L and 0.19 ± 0.04 mmol/L, respectively), while in the control increased amounts of CH_4 were produced (0.33 ± 0.04 mmol/L at 12 h and 0.91 ± 0.13 mmol/L at 48 h, $p < 0.0001$) (Fig. 2). Elevated CH_4 production has been associated with obesity, colorectal cancer, irritable bowel syndrome and chronic constipation. In fact, said gas has been shown to reduce intestinal transit time by 59% (Gaci et al., 2014; Lurie-Weinberger & Gophna, 2015). Therefore, further studies on the ability of GOS to reduce methane production should be carried out. The highest total gas production occurred as a result of inulin supplementation, while GOS supplementation led to a lower overall gas production. Similar results were reported by Hernot et al. (2009) and Rycroft et al. (2001) who compared the effect of different oligosaccharides. Hernot et al. (2009) after evaluating the gas production profile of fructans with different chain lengths and DP and other oligosaccharides, such as GOS, concluded that due to its branched structure, agave inulin was more rapidly fermented yielding the highest amounts of gas, while GOS yielded more moderate gas production. Similarly, in the results reported by Rycroft et al. (2001), the gas production profiles obtained revealed that fructooligosaccharides (FOS) and inulin produced the highest amounts of total gas, while GOS resulted in the lowest production. GOS are considered more prone to increase the number of Bifidobacteria than inulin, which could explain the differences herein found in the gas production, since these bacteria usually generate lower gas volumes.

3.1.4. Microbiota analysis

Taking into account the results herein gathered, GOS presented the highest prebiotic potential and therefore, were selected to be incorporated in the rice muffins. The supplementation with inulin or GOS resulted in a distinct modulation of the gut microbiota after 48 h of in vitro fermentation (Fig. 3). Despite the fermentation of both prebiotics increased the relative abundance of members of Lactobacillaceae and Bifidobacteriaceae families, inulin presented a higher selectivity towards Lactobacillaceae strains (51 ± 1% of all), while GOS showed to be more efficient in stimulating the growth of Bifidobacteriaceae strains (65 ± 7% of all). Members of these families, such as Lactobacillus acidophilus, Limosilactobacillus fermentum, Lacticaseibacillus paracasei, Lacticaseibacillus rhamnosus, (formerly named Lactobacillus fermentum, Lactobacillus paracasei and Lactobacillus rhamnosus, respectively, emend. Zheng et al., 2020), Bifidobacterium longum and Bifidobacterium bifidum, are widely commercialized probiotic linked to several health benefits (Fenster et al., 2019; Silvi et al., 2003; Zheng et al., 2020). They are able to metabolize complex carbohydrates, producing mainly acetate and lactate and releasing CO_2 (Gibson et al., 2017), which corroborates the results previously presented. Additionally, the fermentation of inulin and GOS led to a significant reduction of the relative abundance of Proteobacteria, including Enterobacteriaceae strains, namely *Escherichia coli* (108 ± 51- and 38 ± 1- fold, respectively) (Supplementary material, Table S2). The reduction of the relative abundance of species belonging to Lachnospiraceae family (e.g. Blautia sp.) (6 ± 1- fold for inulin and 2.3 ± 0.3- fold for GOS) and the high reduction of Erysipelotrichaceae strains (51 ± 48- fold for inulin 23 ± 8-fold for GOS) was also shown for both prebiotics. The abundance of species from Enterobacteriaceae, Lachnospiraceae and Erysipelotrichaceae on gut microbiota is associated wtih gastrointestinal disorders, including inflammatory bowel disease (Rizzatti et al., 2017), visceral fat accumulation (Ozato et al., 2019) and colorectal cancer (Dinh et al., 2014). The presence of methanogenic archaea was also reduced by both prebiotics, in particular by GOS which led to the null values of its relative abundance, corroborating the decrease of CH_4 production previously observed (Supplementary material, Table S2). Taking into account the results herein gathered, GOS presented the highest prebiotic potential and therefore, were selected to be incorporated in the rice muffins. Further investigations should be performed to determine if the commercial GOS could be used as vehicle matrices to increase the survival rate of specific probiotic strains when tested on simulated gastrointestinal transit conditions (Pérez-Ramos et al., 2017). The most efficient combination of prebiotic and probiotic could be used afterwards to develop a synbiotic rice muffin.

Table 1
Physical properties and texture profile analysis of the studied muffins one day after their production: 100S (rice muffin with 100% of sugar); 75GOS (75% of sugar and 0.8% GOS; and Commercial (commercial rice muffin). A different superscript letter in the same row indicates statistical difference ($p < 0.05$).

	100S	75GOS	Commercial
Height (cm)	6.3 ± 0.4^a	5.6 ± 0.1^b	7.6 ± 0.5^c
Weight (g)	74 ± 1^a	75 ± 1^a	93 ± 8^b
Diameter (cm)	6.0 ± 0.1^a	6.0 ± 0.1^a	6.0 ± 0.2^a
Volume (cm^3)	178.9 ± 0.4^a	158.9 ± 0.2^a	212 ± 15^b
Baking loss (%)	9.0 ± 1.0^a	9.1 ± 0.3^a	–
Apparent density of crumb (g/cm^3)	0.28 ± 0.01^a	0.36 ± 0.01^b	0.33 ± 0.03^{ab}
Moisture content (d.s.)	0.35 ± 0.03^a	0.38 ± 0.02^a	0.49 ± 0.07^b
Apparent porosity of crumb (%)	74.3 ± 0.1^a	68 ± 1^b	68 ± 2^b
Number of bubbles[a]/cm^2	0.7 ± 0.1^a	2.2 ± 0.1^b	1.8 ± 0.2^b
Bubble area (cm^2)	0.07 ± 0.03^{ab}	0.04 ± 0.02^a	0.06 ± 0.06^b
Bubble diameter (cm)	0.29 ± 0.08^a	0.23 ± 0.06^a	0.2 ± 0.1^a
Specific density of crumb (g/cm^3)	1.60 ± 0.01^a	1.49 ± 0.01^b	1.35 ± 0.01^c
Color			
L*	66 ± 1^a	68 ± 2^a	57 ± 2^b
a*	-2.2 ± 0.1^a	-2.5 ± 0.1^a	-1.4 ± 0.3^b
b*	23.3 ± 0.3^a	27 ± 1^b	26 ± 1^b
ΔE*	9 ± 1^a	11 ± 2^a	–
Hardness (g)	205 ± 47^a	372 ± 49^b	209 ± 35^a
Springiness (cm)	0.89 ± 0.02^a	0.86 ± 0.01^b	0.82 ± 0.02^c
Cohesiveness (-)	0.70 ± 0.02^a	0.60 ± 0.04^b	0.39 ± 0.03^c
Resilience (-)	0.28 ± 0.01^a	0.23 ± 0.02^b	0.13 ± 0.01^c
Chewiness (g cm)	129 ± 29^a	190 ± 19^b	67 ± 13^c

[a] Pores with area higher than 0.008 cm^2.

3.2. Physical, structural and textural properties of rice muffins

Given the relevance of the GOS fermentation results, it would be interesting to transpose their potential beneficial effects to a highly consumed food product with a low level of innovation, such as the rice muffin, in order to increase its commercial and nutritional value. In this study, rice muffins were produced containing 100% of the sugar amount present in the original recipe (100S), 75% of sugar supplemented with 0.8% GOS (75GOS), and were then compared with a commercial muffin (Commercial). The concentration of 0.8% of GOS was established attending to the official recommended daily dose of the product (1.37 g/day) and assuming that a maximum of 2 cakes/day could be eaten by one person. Concentrations of GOS higher than the recommended daily dose could result in the occurrence of undesired secondary effects, including flatulence and diarrhoea. Thus, as a first approach, the authors selected a conservative concentration of GOS to ensure no repercussions for the consumers' well-being. On the other hand, the 25% sugar reduction was chosen based on the outputs of a preliminary sensory test with 50 subjects (data not shown). As expected, muffins with 25% sugar reduction were more well accepted in terms of their organoleptic properties than with 50% sugar reduction, since sugar plays an important role in the texture, flavour and moisture of the muffins. The physical characterization of the baked rice muffins are summarized in Table 1. Baking loss values are comparable to previous results corresponding to

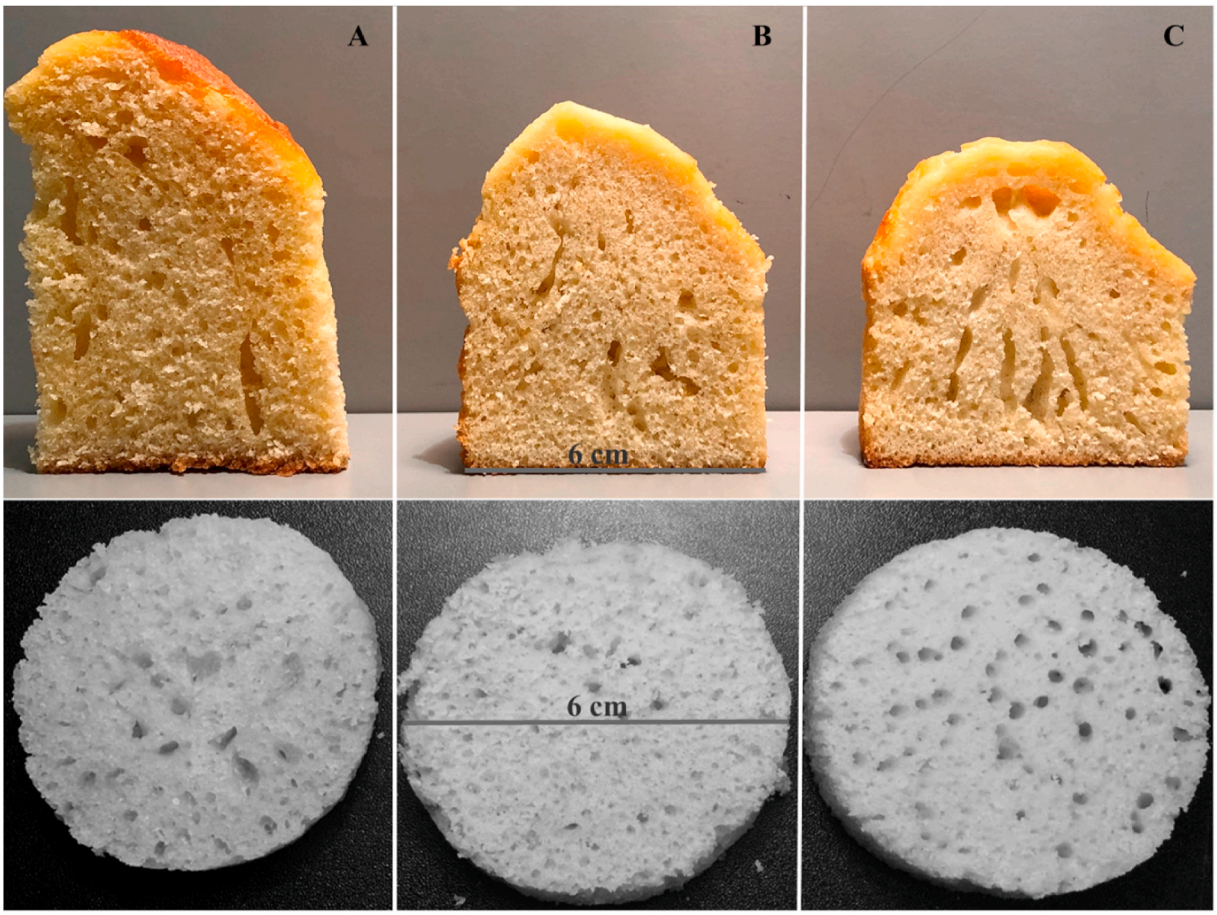

Fig. 4. Vertical and transversal sections of the studied muffins one day after their production: A) Commercial rice muffin (Commercial); B) 100% of sugar (100S) and C) 75% of sugar and 0.8% GOS (75GOS). Note that when "Lactobacillus" is referred in this figure, refers to the formerly Lacobacillus genus, before the emended description by Zheng et al. (2020).

other muffins (Goswami et al., 2015) and no significant differences between homemade samples were observed. Regarding color parameters of the muffin crumb, only relevant differences were found in L* parameter for the commercial sample. Possibly, the use of higher temperatures during baking promoting Maillard reactions could explain this difference (Shevkani & Singh, 2014). Minor colour differences between 100S and 75GOS ($\Delta E^* < 3$) can be considered (Goswami et al., 2015). The 75GOS sample showed a lower volume than the other samples. In fact, the sucrose content is relevant for the development of desired structural characteristics as it improves the starch gelatinization and protein denaturizing temperatures, thus increasing the samples volume (Hoseney, 1994). Muffins are porous food materials due to the presence of many air bubbles, Fig. 4.

The bubbles are produced during the mixing process and grow during baking. Starch gelatinization and protein denaturizing set the structure during baking and is completed with the formation of starch gel during cooling. The muffin with prebiotic exhibited a larger number of bubbles with smaller size than the other tested muffins. Nevertheless, the vertical section shows the existence of some tunnels at the centre of the muffin (Fig. 4C), which evidences that the capacity of starch and protein structure to retain the air bubbles adequately was reduced and some collapse took place (Heo et al., 2019). Despite these differences found for the porous structure, the apparent porosity of crumb (consequently, also the apparent density) of the tested muffins varied in a narrow range (from 68 to 74.3%). Finally, specific density values are in the range of other muffins prepared with amaranth and buckwheat flours (Dizlek, 2015). A linear relationship ($r^2 > 0.99$) between specific volume and moisture content was established.

Results are in the range of other muffins made from other flours and different additives (Alvarez et al., 2017; Chung et al., 2010). Commercial and 100S muffins showed similar hardness and springiness. The muffin recipe with higher starch and protein content in its formulation (75GOS) showed a higher hardness and thus, higher chewiness values (and higher apparent density). Also, the high moisture content of the 75GOS sample could be explained by the water retention capacity of starch and protein. Springiness is a desirable muffin property related to elastic characteristics (Shevkani & Singh, 2014). This property varied in a narrow range for the tested crumb muffins (from 0.82 up to 0.89 with significant differences between samples) and it is similar to the reported values for other muffins, suggesting an adequate acceptability of the product by the consumer (Sanz et al., 2009). No relationship was found between air bubbles characteristics or specific volume or hardness and, it seems that the developed protein network and starch gel jointly provide a muffin structure with similar elasticity for the tested samples. Despite the lower porosity of the 75GOS muffin comparing to the 100S muffin, the springiness value could be due to the formation of tunnels (Chung et al., 2010). Cohesiveness measures the strength of internal bonds in the muffin and, resilience measures how well a product fights to regain its original position (Trinh & Glasgow, 2012, pp. 23–26). Usually, linear correlations between both parameters can be established as in this case ($r^2 = 0.999$). In parallel, both parameters increased linearly ($r^2 > 0.98$) with increasing specific density of the tested muffins.

Texture of the samples was determined during four days after preparation. Parameters determined at day 1 (Table 1) were used as reference and relative values of every parameter were calculated. Moisture content of samples varied slightly during these four days with

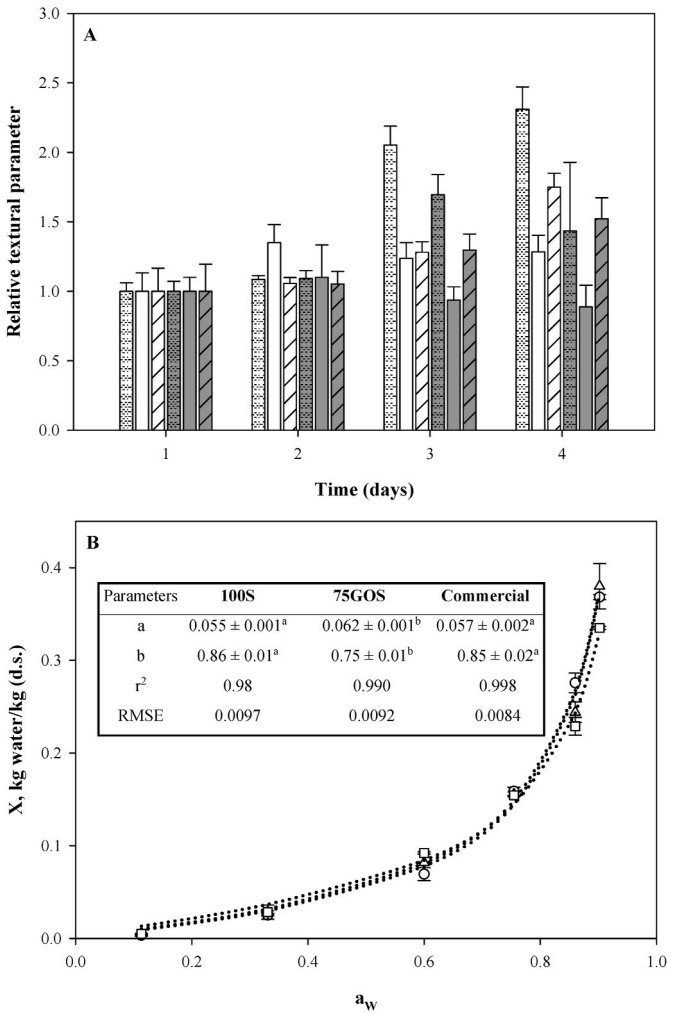

Fig. 5. Characterization of the rice cakes: A - Evolution of hardness (white) and chewiness (dark grey) during time for the rice cake with 100% of sugar (short dash), rice cake with 75% of sugar and 0.8% GOS (no fill) and commercial rice cake (long dash); B - Water adsorption isotherms obtained at 25 °C for the rice cake with 100% of sugar (triangle), rice cake with 75% of sugar and 0.8% GOS (square) and commercial rice cake (circle). Dashed lines corresponding to Oswin model (Equation (4)) fittings with the respective parameters presented in the insert (different superscript letter in the same row indicates statistical difference ($p < 0.05$)). RMSE stands for root mean square error.

an increase of 2 ± 1 d.s. Relative springiness continuously decreased over a narrow range (up to 0.95 ± 0.01 for 75GOS sample); relative cohesiveness also decreased but more markedly (up to 0.7 for 75GOS sample); and relative resilience remained constant for the Commercial sample and dramatically decreased for homemade muffins (up to 0.7 ± 0.1 for 75 GOS sample). Fig. 5A shows, as an example, the evolution of hardness and chewiness during storage time. As expected, the relative hardness increased in all samples. In fact, 100S muffin after 3 days and Commercial muffin after 4 days showed values above two. Relative chewiness showed for both muffins the same trend. Relative hardness of the 75GOS sample increased slightly (up to 1.3 ± 0.1) and relative chewiness decreased (up to 0.9 ± 0.1). These last trends are interesting because initial values for both parameters were high. Nevertheless, the texture profile analysis (TPA) results with time clearly suggest that the product should conveniently be consumed during the first two days after baking.

Fig. 5B shows the water adsorption isotherms at 25 °C of the tested muffins. In all cases, the equilibrium moisture content values increase (up to 0.37 d.s.) with the increase of water activity. Therefore, water adsorption isotherms can be classified as Type III according to Brunauer's classification (Brunauer et al., 1940). Slight differences can be observed between Commercial and 100S samples, thus confirming that these samples were prepared with a similar formulation. At high water activities (above 0.8), the 75GOS sample showed lower equilibrium moisture content than the other samples due to its low sugar content (Moreira et al., 2009). The Oswin model is one of the most used models for starchy products (Brett et al., 2009). Fig. 5B shows the fitting parameters of the Oswin model for the tested muffins and the statistical parameters are indicative of the goodness of the fittings (coefficient of determination, $r^2 > 0.98$ and root mean square error, RMSE < 0.0097). The water sorption isotherms indicate that the tested samples can be considered perishable food materials since they have high water activities (around 0.9). The storage of these products under a standard relative humidity (45%) would give as result, if no spoilage takes place, a very dried product (around 0.046 d.s.). Therefore, hygroscopic and textural results allow to further conclude that these products must be consumed in a short period of time (one or two days) if additional preservatives or packaging are not used.

4. Conclusions

When compared with inulin, the fermentation of GOS resulted in the highest production of short-chain fatty acids, bifidogenic effect and pH, ammonia and CH_4 reduction. The GOS fermentation results confirmed their high prebiotic potential, which was further exploited into a widely consumed food product with a low level of innovation, such as the rice muffin, in order to increase its commercial and nutritional value. A rice muffin was developed containing 25% less sugar than the original recipe and 0.8% of GOS. The reduction of sugar content modified the air bubbles characteristics (size and population with a greater number of tunnels) and hardness and chewiness increased. Nevertheless, very relevant textural parameters were obtained for the new-formula rice muffins, like springiness and resilience, and these were considered acceptable and comparable to those reported in the literature for commercial muffins, thus anticipating a good consumer acceptance. Overall, the results gathered in this study comprise an important contribution for the development of potential healthier and functional pastry options designed for consumers who either follow a health-based lifestyle or have a special medical condition.

Author contribution statements

Cláudia Amorim: Funding acquisition, Investigation, Methodology, Writing – original draft **Beatriz B. Cardoso:** Investigation, Methodology **Sara C. Silvério:** Investigation, Methodology, Writing – review & editing **Jessica C. Silva:** Investigation, Methodology **Joana I. Alves:** Investigation, Methodology, Writing – review & editing **Maria Alcina Pereira:** Funding acquisition, Resources, Validation, Writing – review & editing **Ramón Moreira:** Funding acquisition, Resources, Validation, Writing – review & editing **Lígia R. Rodrigues:** Funding acquisition, Resources, Validation, Supervision, Writing – review & editing, Project administration.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2020.100858.

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