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Stevia-based sweeteners as a promising alternative to table sugar: the effect on *Listeria monocytogenes* and *Salmonella* Typhimurium growth dynamics.

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Abstract

Sugar is commonly substituted with stevia-based products in food industry and in our daily-life. This substitution results in a change in food product characteristic formula and properties that may affect the growth dynamics of food pathogenic and spoilage bacteria. This work studies the effect of table sugar (TS), laboratory sucrose (LS), commercial stevia (St) and steviol glycosides (SG) ¹ on the growth dynamics of *Salmonella* Typhimurium and *Listeria monocytogenes*. Experiments were carried out in general and minimal culture media at 3 equivalent concentration levels in terms of sweetness intensity (TS and LS at 3, 9 and 15% (w/v); St at 0.3, 0.9 and 1.5% (w/v); and SG at 0.01, 0.03 and 0.05% (w/v)). Incubation temperatures were: 4, 8 and 20 °C for general media, and for minimal media 20 °C. To decipher the role of these sweeteners, their concentration evolution in minimal media was determined via HPLC analysis. The results revealed slow maximum specific growth rates (μ_{max}) of *S. Typhimurium* in general media with increasing concentrations of TS and LS at 20 °C; and reduced maximum cell population (N_{max}) at 8 °C. The growth of *L. monocytogenes* in general culture media remains invariable independently of the sweetener added, except at 4 °C. At this critical temperature, the presence of TS, LS and St seems to facilitate the growth of *L. monocytogenes*, presenting higher μ_{max} values in

¹ Abbreviations: table sugar, TS; laboratory sugar, LS; commercial stevia, St; steviol glycosides, SG.

comparison to SG and the control. Varying bacterial response to changes in media formulation suggests that further research is required, focusing on revealing the microbial dynamics in structured media, as well as in real food products.

Keywords: steviol glycosides, novel-sweeteners, HPLC, food pathogens, minimal media

1. Introduction

Human predisposition for sweet food products in combination with a sedentary life style is leading to a situation in which obesity and diabetes are reaching alarming levels (Hracek et al., 2010). Overweight and obesity prevalence has worldwide increased from 29% of adults being obese or overweight in 1980, to 38% in 2013 (Ng et al., 2014). Under this situation, consumer trend towards healthier nutritional habits is challenging food industry to develop novel product formulations characterised by, e.g., the substitution of added-sugars with no-caloric sweeteners. *Stevia rebaudiana* (Bert.) Bertoni, commonly known as Stevia, is a perineal shrub found in Paraguay and south of Brazil from the leaves of which steviol glycosides (SG), its sweet principle, are obtained. These SG have a sweetness intensity of about 200-300 times stronger than table sugar and are non-caloric, making them an ideal candidate for sugar replacement in caloric-reduced products (Geuns, 2010). The plant of *Stevia* has been used for centuries by the indigenous Guarany natives as a traditional sweetener (Puri et al., 2011); nevertheless, in the last years research studies and food industry have focused on its potential use as a food additive for sugar substitution. SG are the sum of mainly stevioside (Stev) and rebaudioside A (Reb A) and in a lower amount, Reb B, C, D, E and F; dulcoside A (Dulc A), rubusoside (Rub) and steviolbioside (SV). The use of SG (95% purity) was Generally Recognised as Safe (GRAS) by the US Food and Drug Administration (USFDA) in 2008 (Pasricha, 2009), and by the European Food Safety Authority (EFSA) in 2010 (EFSA and Food Ingredients and Packaging (FIP), 2010). Stevia-based sweeteners have been proposed as a suitable alternative to table sugar for direct consumption, or as an additive in industrial beverages, milk products such as flavoured milk (chocolate, vanilla, etc.) that include between 3 and 15% of added sugars. Several beneficial properties have been attributed to *Stevia rebaudiana* extracts and/or specific SG, such as anti-hyperglycaemic effects from intravenously infused Stev (2.0 g/kg BW) (Jeppesen et al., 2002), antioxidant activity of *Stevia rebaudiana* extracts (Xi et al., 1998) and anti-hypersensitive effect from 250 mg of Stev oral intake (Chan et al., 2000). Adequate

organoleptic properties, high stability under thermal treatment (Jookan et al., 2012; Kroyer, 1999), weight loss promotion, no glycaemic effect, etc., are other properties that made stevia-based products appear as a potential alternative to traditional sweeteners. However, little is known about their effect on foodborne bacteria of concern and their relation with sucrose substitution. It has been reported that sugar replacement may affect functionality and microbial safety of foods, since its partial or total elimination increases the water activity (a_w) in a range from 0.985 to 1.00, leading to a reduction in the food-intrinsic biopreservative ability (Hracek et al., 2010). Al-Dabbas and Al-Qudsi, (2012) described that table sugar can act as a preservative as well as enhancing and bringing out the characteristics of other favouring ingredients, providing bulk, colloidal osmotic pressure and influencing the physical properties of food.

Salmonella Typhimurium and *Listeria monocytogenes* are foodborne pathogens that can easily contaminate food products such as poultry, meat, unpasteurised milk, peanut-butter, raw cookie-dough, etc. (Braden and Tauxe, 2013). These bacteria are responsible for salmonellosis and listeriosis. Salmonellosis is a foodborne disease that causes enteritis or, the more serious, Typhoid fever. In the last report from the EFSA (EFSA and European Centre for Disease Prevention and Control (ECDC), 2015), *Salmonella* remained the most frequently detected causative agent in the foodborne outbreaks reported (22.5 % of total outbreaks), with 82,000 human cases of salmonellosis. Although the number of reported listeriosis cases is lower, 1763 in 2013, its high fatality rate (15.6% of the reported cases) in combination with the increasing prevalence, makes *L. monocytogenes* a target microorganism for the food industry (EFSA and ECDC, 2015). Moreover, the EFSA (2014) has estimated that the overall economic burden of human salmonellosis and listeriosis could be as high as EUR 3 and EUR 2.6 billion a year respectively, arising an economical issue on top of the health concern. Therefore, unravelling the influence of these novel low-calorie sweeteners on the behaviour of foodborne microorganisms of concern is required to guarantee optimal food safety and quality. This work assesses the effect of different levels of St, SG and TS (equivalent among them in terms of sweetness intensity) on the growth dynamics of *S. Typhimurium* and *L. monocytogenes* in general and minimal culture media, at room and refrigeration temperatures of 4 and 8 °C.

2. Materials and Methods

2.1. Bacterial strains and Inoculum preparation

Selected pathogenic strains of *Salmonella enterica* serovar Typhimurium SL1344, kindly provided by the Institute of Food Research (IFR, Norwich, UK), and *Listeria monocytogenes* LMG 13305 isolated from soft cheese and obtained from the Belgian Co-ordinated Collection of Microorganisms (BCCM, Ghent, Belgium), were stored in Luria Bertani broth (LB) and Tryptone Soya Broth (TSB; Oxoid Ltd., Basingstoke, UK) supplemented with 0.6% (w/v) Yeast Extract (YE; Merk, Germany) (TSBYE), respectively, at -80 °C. In both cases, stock cultures were cryoprotected with 25% (v/v) glycerol. Cells were recovered by streaking onto a Tryptone Soy Agar (TSA; Oxoid Ltd., Basingstoke, UK) plate supplemented with 0.6% (w/v) YE (TSAYE) in the case of *L. monocytogenes*. These plates were incubated for 24 hours at optimal temperatures, being 37 °C for *S. Typhimurium* and 30 °C for *L. monocytogenes*. To prepare the precultures, a colony was transferred from the agar plate to a 50 mL Erlenmeyer flask containing 20 mL of sterile TSB/TSBYE. These precultures were incubated at optimal temperatures under static conditions. After 9 h, 20 µL of stationary cell cultures were transferred into fresh 20 mL of TSB/TSBYE and incubated at the same temperatures for 15 h. After incubation, cells were harvested by centrifugation at 5974 g for 10 min at 4 °C and washed twice in sterile TSB/TSBYE-dextrose free [TSB/TSBYEdf], the media in which cells are going to be inoculated, and in order to remove the TSB/TSBYE. Serial decimal dilutions were prepared in TSB/TSBYEdf to obtain an initial concentration in the culture media of approximately 10³ cfu/mL. For the experiments in chemically defined minimal media, cells were washed and diluted with sterile saline solution NaCl 0.9% (w/v).

2.2. Sweeteners solutions: preparation and growth experiments

Growth experiments were conducted in general culture media at 4, 8 and 20 °C to simulate refrigeration conditions and room temperature, respectively. These media were enriched with 4 different sweeteners at 3 concentration levels. The selected sweeteners for the study were: commercial stevia (St; Pure Via®) and table sugar (TS; Tiense Suikerraffinaderij®; Belgium), both purchased at the local market; a mixture of steviol glycosides (SG), kindly donated by Stevia One (Peru) and laboratory sucrose (LS, 99.7% purity) (Sc, Acros, Applichem, Thermo Scientific, USA). In TS the main component is sucrose (dimers of glucose and fructose), while in St the SG are mixed with a bulking agent, in this case maltodextrin. Sweeteners were added at 3 different concentration levels that, although different in terms of weight, they all have the same sweetness, i.e., SG and St are 300 and 10 times, respectively, sweeter than TS.

The concentration range was based on the levels at which TS is commonly used in food products, such as flavoured milk. These concentrations were 3, 9 and 15% (w/v) for TS and LS; 0.3, 0.9 and 1.5% (w/v) for St and 0.01, 0.03 and 0.05% (w/v) for SG. In the case of the experiments with TS in chemically defined minimal medium, an additional control with glucose (Glu) was performed. Based on the intermediate concentration value of TS used in this work, 9% (w/v), 2 Glu concentrations were established: 9% (w/v), which coincides with this intermediate concentration of TS, and 4.7% (w/v), the equivalent of Glu found in 9% (w/v) of sucrose. Since every molecule of sucrose contains one molecule of Glu, the amount of Glu present in sucrose was calculated by establishing a simple relation between both molecular weights (sucrose 342.2965 g/mol, and Glu 180.1559 g/mol). For experiments with *L. monocytogenes*, the general and chemically defined minimal media were TSBYEdf, and Modified Welshimer's Broth (MWB) without glucose, respectively. In order to obtain MWB-glucose free, the medium was elaborated in the lab as described in Premaratne et al. (1991), but leaving glucose aside. Experiments with *S. Typhimurium* were performed in TSBdf as general culture medium, and in M9-glucose free (Gibco®, USA) as chemically defined minimal medium. TSBdf/TSBYEdf were sterilised by autoclaving at 121 °C for 20 min. M9-glucose free and MWB-glucose free were filter-sterilised through a 0.2 µm-pore-size filter (Filtropur S 0.2; Sarstedt, Germany). Afterwards, all media were separately enriched with the sweeteners under study and subsequently filter-sterilised through a 0.2 µm-pore-size filter with the aid of a syringe (10 mL of Norm-Ject; Henke Sass Wolf, Germany). Once sterilised, the total volume of each sweetener solution was inoculated with an adjusted cell population of about 10³ cfu/mL, and afterwards, individual samples of 1 mL were aseptically distributed in eppendorfs and incubated at the above specified temperatures. At regular time intervals, duplicate samples were aseptically taken to estimate the cell density with viable plate count (VPC). From each eppendorf, a 100 µL aliquot was taken, serially decimal diluted in saline solution 0.9% (w/v) and plated following the Drop Plate Method (Hoben and Somasegaran, 1982) on TSA/TSAYE. Plates were enumerated after 48 h incubation at 37 °C for *S. Typhimurium* and 30 °C for *L. monocytogenes*, and the obtained results were ln-transformed and plotted as a function of time.

2.3. Data analysis and mathematical modelling

Obtained growth curves of *S. Typhimurium* and *L. monocytogenes* were fitted with the primary growth model of Baranyi and Roberts (1994). Growth parameters were estimated from the set of experimental data corresponding to each experimental condition via the minimization of the sum of squared errors (SSE), using the lsqnonlin outline with the Levenberg-Marquardt optimization algorithm of the Optimization Toolbox of Matlab version R2010b (The MathWorks, Inc., Natick, MA). Standard errors of parameter estimates were calculated from a Jacobian Matrix (Pronzalo and Walter, 1997) and graphical illustrations were also generated.

2.4. Analysis of sweetener concentration

The water activity and pH of sweeteners solutions in general culture media were determined with the aid of an a_w -Kryometer (AWK-40; Nagy, Germany) set on the “liquid” modus and a pH meter (DocuMeter, Sartorius, Germany), respectively. Measurements were performed on media without bacterial cells.

HPLC analyses were carried out to determine the concentration of SG in St 0.3, 0.9 and 1.5% (w/v), SG in the 3 SG-mix concentrations used (0.01, 0.03 and 0.05% (w/v)), and sucrose in TS 3, 9 and 15% (w/v), at different time points of the growth experiments in minimal medium. Sucrose was analysed with an HPLC apparatus (Thermo Scientific, Waltman, USA) consisting of an SCM1000 vacuum degasser, a P1000 XR pump, an AS1000 autosampler with a fixed injection volume of 20 μ L and a Shodex RI101 refractive index detector (RID). Separations were performed on a Phenomenex Luna 5u NH2 100A, 250x4.6 mm ID column with appropriate guard column. The column and RID temperature were kept at 40 °C. An isocratic eluent of 75 % (v/v) acetonitrile 25 % (v/v) ultrapure water with a flow rate of 1.5 mL/min was used. The sugar standard was dried overnight at 60 °C, dissolved and diluted in ultrapure water. A calibration line was generated between 2 and 50 g/L of sucrose. St and SG-mix samples were analysed with an HPLC apparatus (Thermo Scientific, Waltman, USA) consisting of a SCM1000 vacuum degasser, a P4000 pump, an AS1000 auto sampler with a fixed injection volume of 20 μ L, and an UV6000LP diode array detector with a flow cell of 10 μ L and a path length of 5 cm. The analysis was performed according to the method described earlier in Jookan et al. (2012), where the specific SG analysed were Stev, SB, SV, Reb A, Reb B, Reb C, Reb F, Reb G, Rub, Dulc A and Dulc B. Separations were done on KINETEX- Columns (C18; 2.6 μ m; 150 x 4.6 mm) of Phenomenex. All

samples were eluted by a linear gradient using 25 mM H₃PO₄ (solvent A) and acetonitrile (ACN: solvent B) as the eluent, as follows: 0 min, 30% B; 2 min, 30% B; 6 min, 40% B; 10 min, 48% B; 14 min, 82% B. (Flow: 1 mL/min.). UV spectra were recorded between 195 and 360 nm for identification purposes, and the compounds were quantified at 200 nm. The Limit Of Detection (LOD) of the used method is 0.0025 mM, which is equivalent to 2.4 mg/kg RebA, and obtained results present a Relative Standard Deviation (RSD) of 2%. All samples were filtered through a 0.20 µm Chromafil syringe filter (Macherey-Nagel, Germany) and eventually diluted with ultrapure water prior injection; each sample was analysed twice.

2.5. Statistical analysis

An analysis of variance (ANOVA) test was performed to determine whether there are any significant differences among means of parameter estimates for the tested sweetener concentrations, at a 95.0% confidence level ($P = 0.05$). If the ANOVA indicated significant differences between the parameters for the different mixtures, a Fisher's least significant-difference (LSD) test was used to identify which means were significantly different. Standardised skewness and standardised kurtosis were used to assess whether the data sets came from normal distributions. These analyses were performed using the Statgraphics Centurion XVI.I Package (Statpoint Technologies, Warrenton, VA). Test statistics were regarded as significant when $P \leq 0.05$.

3. Results and discussion

3.1. General culture media at room and refrigerated temperatures

3.1.1. *L. monocytogenes*

The growth of *L. monocytogenes* in TSBYEdf enriched with 3 different concentrations of the selected sweeteners (TS, LS, SG and St) and in TSBYEdf with no sweetener added (control), was individually monitored at 4, 8 and 20 °C. Experimental data on the evolution of cell concentration vs. time, and growth kinetics for every tested condition are respectively detailed in Fig. 1 and Table 1. Due to the similar behaviour of *L. monocytogenes* obtained among sweeteners and their concentrations, results description and discussion are here grouped by temperatures: a) 20 and 8 °C, and b) 4 °C.

Under these conditions, all results present typical sigmoid curves with maximum cell density values of about 20 ln cfu/mL in all the cases. Although the statistical analysis reveals significant

differences for certain conditions, results for maximum cell density always remained in the same order of magnitude. *L. monocytogenes*, as a psychrotrophic microorganism, is able to grow at very low temperatures, with a limiting value of -1.5 °C (Couvert et al., 2010). Indeed, the stationary phase of growth was reached after less than 200 h at 8 °C and between 200 and 400 h at 4 °C. As generally acknowledged in literature, *L. monocytogenes* maximum specific growth rates decreased for the three temperatures tested as a consequence of the decreasing temperature (Adams and Moss, 1995; Baka et al., 2014; International Commission on Microbiological Specifications for Foods, 1996; Magalhães et al., 2016).

a) **At 20 and 8 °C**, no significant differences are observed among concentrations of the same sweetener including the control, and neither among sweeteners ($P > 0.05$). To date, most of the studies focus on the potential antimicrobial properties of SG or dried leaf extracts from the *Stevia* plant using water, acetone, chloroform, etc., as solvents (Debnath, 2007; Ghosh et al., 2008; Jayaraman et al., 2008; Tadhani and Subhash, 2007), but less on the commercial product directly added to the medium. Belda-Galbis et al. (2014) studied the growth dynamics of *L. innocua* in TSB at 37 °C with 3 different *Stevia* extracts (SG purified extract with >95% of SG, a crude extract containing $\geq 20\%$ of SG, and leaf infusion of 2.5% (w/v)). Growth parameters were estimated by fitting the growth curves to the modified Gompertz equation. Among the three extracts, the leaf infusion and the crude extract presented antimicrobial activity against *L. innocua*, being both able to halve the μ_{max} , and to multiply the lag phase twice and 10 times, respectively, in comparison to a control with no *Stevia* extracts added. Based on these results, the antimicrobial effect of the most active, i.e., the leaf infusion, was afterwards assessed at 3 concentration levels (0.5, 1.5 and 2.5% (w/v)) and three temperatures: 10, 22 and 37 °C. These selected conditions for the leaf infusion are, overall, comparable to those assayed in this paper in terms of temperature and SG concentrations. Obtained results by Belda-Galbis and co-authors at 10 °C are similar to those here presented at 8 °C, since there were no statistically significant differences ($P > 0.05$) for any of the leaf concentrations assessed. Nevertheless, an inhibitory effect was obtained at 22 °C with 1.5 and 0.5% (w/v); and also at 37 °C with a concentration of 2.5% (w/v). In our work no inhibition was observed at 20 °C, and these differences might be attributed to a higher purity of our SG-mix. As discussed in the work of Belda-Galbis et al. (2014), the antimicrobial properties of *Stevia* can be linked

to other plant compounds than the SG, which can be reduced by further purification. The different species used, *L. innocua* vs. *L. monocytogenes*, can also be a reason for the different effect.

b) Results at 4 °C reveal a faster growth in the media with TS, LS and St, compared to SG and the control. These results reveal that the presence of these sweeteners at the studied concentrations favours the growth of *L. monocytogenes* at 4 °C, the most strict refrigeration temperature. The fact that these differences appear at 4 °C, and not at 8 or 20 °C, can be due to a higher vulnerability of this microorganism from this temperature and below. It has been described that in spite of the ability of this bacterium to grow at very low temperatures, 4 °C seems to be a critical point regarding growth behaviour (Adams and Moss, 1995; Chan and Wiedmann, 2008; Magalhães et al., 2016). While the growth of *L. monocytogenes* is very slow below this temperature (International Commission on Microbiological Specifications for Foods, 1996; Yousef and Lou, 1999), a slight rise is enough to considerably facilitate the growth rate and shorten the lag phase (Lianou and Koutsoumanis, 2013; Magalhães et al., 2016). Moreover, it has been widely reported that besides temperature, other factors such as pH (Seeliger and Jones, 1986; Tienungoon et al., 2000), a_w (ICMSF, 1996) or certain components present in food (Angelidis and Smith, 2003; Bayles et al., 1996; Omac et al., 2015; Sleator et al., 2003), can influence the growth of *L. monocytogenes* at suboptimal temperatures. Therefore, an enrichment of the media with sweeteners, which can act as a nutrient source, could promote the growth of *L. monocytogenes* at this critical temperature in comparison to the control in TSBYEdf. On the other hand, the addition of SG to the media had a negligible effect on the growth of *L. monocytogenes*, as compared to the control. An explanation for the slower growth of this microorganism in the media with SG can be the low concentrations added, from 0.01% to 0.05% (w/v), as compared to the other sweeteners whose concentration values are between 0.3% and 15% (w/v). Although statistical analysis indicates significant differences for the obtained maximum specific growth rates, values are almost equal (Table 1). Comparable results to those obtained here were reported by Li et al. (2014). They studied the effect of Reb A, the main component of the SG mix, on the microbial diversity of the mouse gut. As part of the study, experiments in vitro were performed with *L. monocytogenes* and *S. Typhimurium* among others. The growth of these bacteria was assessed in general culture media with Reb A in a concentration range

between 0.01 and 1.00 % (w/v) and monitored with optical density (OD), posing little differences in the growth of *L. monocytogenes* and *S. Typhimurium*; however temperature conditions are not mentioned.

3.1.2. *S. Typhimurium*

In Fig. 2 the growth curves of *S. Typhimurium* are depicted. This growth was monitored in TSBdf enriched with all the sweeteners at three concentration levels at 20 and 8 °C, and curves were fitted with the model of Baranyi and Roberts (1994). Estimated parameters together with the statistical analysis are detailed in Table 2. At a first glance, while *S. Typhimurium* presents typical growth curves at 20 °C independently of the added sweetener, significant differences appeared for decreasing temperatures. As expected, at 4 °C none of the media supported the growth of *S. Typhimurium* since this value is below the minimum temperature (Adams and Moss, 1995; ICMSF, 1996) (data not shown). For an easier understanding, results are hereunder grouped and described by sweetener and temperature.

Table sugar (TS). Results presented in Fig. 2a and 2b indicate an influence of the presence of TS in the media on the growth dynamics of *S. Typhimurium*. This influence varies with the concentration of TS and with the incubation temperature. **At 20 °C** (Fig. 2a) typical sigmoid curves were obtained for all the conditions tested, and only small differences were found between the three concentrations (3, 9 and 15% (w/v) in terms of maximum growth rate. While negligible differences are observed between the control and the media with 3 and 9% (w/v), a lower μ_{max} value characterises the growth of *S. Typhimurium* with 15% (w/v) TS. This slightly slower growth can be a consequence of the lower a_w of the media (Table 3). This effect has been previously described by Brocklehurst et al. (1995), who studied the growth of *S. Typhimurium* at 20 °C in TSB, with decreasing values of a_w from 0.992 to 0.919, by adding sucrose. The concentration range used was from 0 to 50% (w/v) sucrose, and the lowest sucrose concentration that presented a slower growth rate was 15% (w/v), supporting thus our results with TS. **At 8 °C** the effect of TS is more evident than that at 20 °C (see Fig. 2b). Under these conditions the maximum population of *S. Typhimurium* decreases drastically with increasing concentrations of TS. In the media with 3% (w/v) TS, the N_{max} is lower than the control although the μ_{max} is higher. Initially, this indicates that this concentration of TS promotes the growth of *S. Typhimurium*, but the faster growth also leads to a faster nutrient depletion or accumulation of metabolites and biomass which forces the end of the exponential growth with a lower concentration of cells (Wilson et al., 2002). The reduction

in the maximum cell population is more pronounced with higher concentrations of TS. While the reached N_{max} in the control is about 19 ln (cfu/mL), in the media with 9% (w/v) TS this parameter decreases about 10 ln, and finally no growth is detected in the media with 15% (w/v) of TS after 1000 h of incubation (see Fig. 2b and Table 2). Since the media with 9% (w/v) TS presents almost the same a_w values as the control and the media with 3% (w/v) of TS, this reduced growth can be attributed to a combination of the low temperature with certain antimicrobial properties attributed to commercial sugar itself (Chirife et al., 1983). For the media with the highest concentration of TS, 15% (w/v), the lack of growth indicates a hurdle effect (Leistner, 1978) as a result of combining a low temperature with the high concentration of TS, and the consequent lower a_w . Koutsoumanis et al. (2004) presented a study in which the growth boundaries of *S. Typhimurium* in TSBdf were modelled as a function of temperature, pH and a_w . This study concluded that small differences in any of these factors can drastically change the probability of *S. Typhimurium* to grow. They observed no growth at any pH value for a a_w value of 0.942, which is lower than those obtained in this paper, at 10 and 15 °C. Nevertheless, the lowest temperature tested was 10 °C, while experiments in the current work were performed at 8 °C. Considering that a small change in the environment can dramatically change the behaviour of *S. Typhimurium*, it seems possible that a 2 °C difference in temperature could increase the hurdle effect from the a_w . It can be concluded that although the addition of TS to the media barely affects the growth of *S. Typhimurium* at 20 °C, effective inhibition can occur under refrigeration conditions in terms of maximum cell population.

Laboratory sucrose (LS). The growth of *S. Typhimurium* in TSBdf enriched with LS is depicted in Fig. 2c and 2d, and it presents a similar behaviour to that in TS. **At 20 °C** (see Fig. 2c) typical growth curves were obtained for all the concentrations tested with no significant differences among them. These results are confirmed with the statistical analysis (Table 2). **At 8 °C** (see Fig. 2d) a dependency of the growth of *S. Typhimurium* on the concentration of LS in the media is observed again, with similar results to those obtained with TS. For all the concentrations tested, N_{max} values are lower than in the control (Table 2). In the medium with 3% (w/v) LS, the estimated N_{max} is about 1.5 ln lower than in the control; in the media with 9% (w/v) of LS no growth is detected after 900 h incubation, and in the media with 15% (w/v) LS a slight growth of 1.6 ln cfu/mL is observed. As previously described in this section, the

addition of (laboratory) sucrose to the culture media slightly decreases the a_w . Although this decrease does not represent a hurdle for *S. Typhimurium* at 20 °C, its combination with a low temperature triggers a bacteriostatic effect decreasing the growth. As summarised in Table 3, the addition of LS leads to lower a_w than TS for the same concentrations, which supports the absence of growth at 9% (w/v) LS.

Commercial stevia (St). To obtain a product with an equivalent sweetness taste to TS, the proportions specified in the packaging box were followed, i.e., 1 g of TS corresponds to 0.1 g of St. In this context, it should be considered that the total amount of total solutes in the solution with St is lower than that with TS. **At 20 °C** no differences are obtained in the growth of *S. Typhimurium* in the media with different concentrations of St (Fig. 2e), presenting almost equal N_{max} values and maximum specific growth rates among them (Table 2). Although the statistical analysis indicates small differences, obtained values are within the same order of magnitude. Results **at 8 °C** reveal different maximum specific growth rates depending on the St concentration in the media, being the control with no St added the slowest. The three concentrations of St yielded a faster growth of *S. Typhimurium* as compared to the control, and although statistical analysis results in significantly different values between them, no clear relation can be established for these three concentrations. This favoured growth can be attributed to a richer nutrient supply from the sweetener, compared to the TSBdf. It has been previously reported that certain bacteria are able to metabolise steviol glycosides. Jookan et al. (2013) demonstrated that soil bacteria from a Paraguayan *Stevia* plantation metabolised steviol glycosides to stevia after 1 week incubation at 37 °C. Considering that *S. Typhimurium* could also metabolise steviol glycosides, addition of a small amount of these compounds to the media under cold-stressing conditions could possibly lead to a faster growth. Li et al. (2014) presented a study in which the effect of Reb A on the growth of several bacteria, including *S. Typhimurium* was addressed; however little effect was reported on the growth of this bacterium. Nevertheless, in Li et al. (2014) a Reb A solution was used, while in this study a commercial product is being studied, that besides SG it also contains maltodextrin. Maltodextrin is a complex carbohydrate commonly used in food products as a bulking agent; moreover, the use of this carbohydrate as a source of energy has been reported for *S. Typhimurium* and other enterobacteria such as *E. coli* (Dippel and Boos, 2005; Morbach et al., 1993). Hence, the faster growth observed in this study can be a consequence of the maltodextrin present in the tested commercial product.

Steviol glycosides (SG). Fig. 2g and 2h illustrates the obtained growth curves fitted with the model of Baranyi and Roberts (1994), from *S. Typhimurium* in TSBdf containing a mix of SG at the three concentration levels assayed, equivalent in sweetness to those tested in TS. Considering that SG are 300 times sweeter than sugar, concentrations used were 0.01, 0.03 and 0.05% (w/v). At 20 °C very similar growth curves were obtained for all the studied conditions. At 8 °C, *S. Typhimurium* reveals differences in the estimated maximum specific growth rates. This parameter indicates a faster growth of about 0.008 1/h in all the SG-enriched media in comparison with the control, but no differences are observed among the 3 concentrations of SG. A potential metabolizing ability of SG by *S. Typhimurium* could explain this facilitated growth, as previously described in section 3.1.1., where the effect of SG on *L. monocytogenes* is reported (Li et al., 2014). In this case, the medium is enriched with a pure mix of SG, leaving aside an effect from the maltodextrin.

3.1.3. Conclusions from *L. monocytogenes* and *S. Typhimurium* in general media enriched with sweeteners

For a better understanding of the results, a brief summary of the consequences of substituting TS, a high-caloric sweetener, with St or SG, a non-calorie sweetener, on the growth dynamics of *L. monocytogenes* and *S. Typhimurium* is hereunder provided. The growth of *L. monocytogenes* at 8 and 20 °C remains invariable independently of the concentration and sweetener added; however, the addition of TS, LS and St under more stressful conditions, i.e., 4 °C, increases maximum specific growth rates of this bacterium. The media enriched with TS, LS and St result in more nutritious media as compared to the control and to the media with SG, which are added in very low concentration. This enrichment could only reveal its effect under critical temperature conditions, when *L. monocytogenes* seems to be more vulnerable to changes in the media. The growth of *S. Typhimurium* is affected in two opposite ways when the media is enriched with the high- and non-caloric sweeteners here studied, especially at low temperatures. On the one hand, high concentrations of TS and LS are able to reduce the growth of this microorganism, affecting the maximum cell population more significantly since this parameter was reduced for more than 10 ln with 9 and 15% (w/v) of TS and LS in the media. This reduction can be generally attributed to a hurdle effect between low temperature and the antibacterial properties of the product itself. Despite the favoured growth observed in μ_{max} values, λ values do not present any

significant difference when different sweetener concentrations and the control are compared. This lack in statistical difference can be due to very large standard errors. On the other hand, both St and SG seem to favour the growth of *S. Typhimurium*. This enhanced growth, observed in the maximum growth rates, is independent of the sweetener concentration. Based on literature, the metabolizing ability of SG by *S. Typhimurium*, that provides then a richer medium than TSBdf, could explain this favoured growth. As observed in the behaviour of *L. monocytogenes*, no significant differences are found for the duration of the lag phase of *S. Typhimurium* which, in the case of low temperatures can be caused by the large standard errors obtained.

3.2. Results in chemically defined minimal media

For a deeper insight in the role of the studied sweeteners on the growth of *L. monocytogenes* and *S. Typhimurium*, experiments were carried out in specific chemically defined minimal media at 20 °C. The experiments with these media aim to determine microbial nutritional requirements. Since results in TS and LS for both microorganisms in general culture media showed minor differences, it was decided to conduct the experiments with TS, which is more representative and realistic in food industry.

3.2.1. Growth dynamics of *L. monocytogenes* in Modified Welshimer's Broth-glucose free (MWB-glucose free)

L. monocytogenes has been reported to be a fastidious microorganism regarding nutritional requirements (Schneebeli and Egli, 2013). Thus, several minimal media have been developed for *L. monocytogenes*, e.g., Modified Welshimer's Broth (MWB) (Premaratne et al., 1991) and Hsiang-Ning Tsai medium (Tsai and Hodgson, 2003) among others. MWB-glucose free was the selected minimal medium for this work since it is the most commonly used in literature (Beumer et al., 1994; Marquis et al., 1993; Pérez-Conesa et al., 2011; Premaratne et al., 1991; Schauer et al., 2009; Tyrovouzis et al., 2014).

Aiming at determining if the studied sweeteners can act as carbon sources for the growth of *L. monocytogenes*, the originally defined glucose concentration in the media was substituted by the specific sweetener at the concentrations defined in section 2.2 and incubated at 20 °C to avoid further stress from low temperatures. A control in the minimal medium without any carbon source yielded a slight increase in cell concentration, as can be observed in Fig 3a, 3b and 3c in green. This growth in the

absence of glucose was not observed in previous studies, more specifically, Premaratne et al. (1991) described the strictly dependency of *L. monocytogenes* on a minimum glucose concentration of 0.5% (w/v) in the medium to grow. Nevertheless, it should be taken into account that the results in this work were obtained from VPC, while in previous works measurements were based on OD, whose specific detection limit is over the population reached in our media (Baka et al., 2015; Begot et al., 1996). Another influencing factor might be the strain of *L. monocytogenes* used. Several authors have reported the varying nutritional requirements depending on the strain of *L. monocytogenes* (Premaratne et al., 1991; Schneebeli and Egli, 2013).

Table sugar (TS). *L. monocytogenes* growth curves in MWB-glucose free are depicted in Fig. 3a. This growth is characterised by two different phases at the 3 concentrations tested. A biphasic growth was already described for *L. monocytogenes* in MWB at low temperatures by Tyrovouzis et al. (2014), who adapted the growth model of Baranyi and Roberts (1994) in order to fit it to the two sigmoid curves. This adapted model was applied to describe the results obtained in this work, and estimated parameters are detailed in Table 4.

The bi-phasic growth of *L. monocytogenes* shows two trends between the first and the second phase. The first phase is characterised by short lag phases ($\lambda_{(i)}$) and fast maximum specific growth rates ($\mu_{max(i)}$). The medium with the highest concentration of TS, 15% (w/v), presents the fastest maximum growth rate ($\mu_{max(i)}$), as well as the highest maximum cell population, estimated as $N_{max(i)}$ (Table 4). The second phase is characterised by lower $\mu_{max(ii)}$ values and longer lag phases ($\lambda_{(ii)}$), the latter showing a dependency on the TS concentration. In the medium enriched with 3% (w/v) of TS, *L. monocytogenes* presents the longest lag phase; and the shortest lag phase corresponds to the medium with 15% (w/v) of TS. Regardless these differences in lag phase, maximum growth rates and final cell populations present similar values for the three concentrations of TS (Table 4). This biphasic growth of *L. monocytogenes* indicates that the presence of TS in the media could promote the growth of this bacterium for the first 100 h of incubation. However, after this time in which cell population reaches a level between 15 and 16.5 ln (cfu/mL), the exponential growth ceases and cells enter in a secondary adaptation phase, whose duration is inversely proportional to the concentration of TS, i.e., cells in the medium with the highest TS concentration require the shortest adaptation time. The second exponential growth is slower than the

first one and independent of the TS concentration, since similar N_{max} values are reached with the three conditions. This biphasic growth is only observed in the media enriched with TS. This fact combined with the dependence on the TS concentration, brought up the assumption that this compound could be consumed in two phases. To confirm this assumption, TS concentration was determined by HPLC at the beginning of the experiment, end of the first exponential growth and end of the experiment. Obtained results are illustrated in Fig. 4a and, although the total amount of TS slightly decreases at the end of the experiments, measurements at the end of the first phase do not reveal a clear TS metabolism. Besides HPLC analysis, similar growth experiments with Glu 4.7 and 9% (w/v) were carried out to determine a potential consecutive metabolism of Glu and fructose (forming monomers of TS, i.e. sucrose), which could trigger then the biphasic growth. The corresponding results depicted in Fig. 3a in magenta (4.7% (w/v)) and purple (9% (w/v)), and the estimated parameters detailed in Table 4, present typical growth curves in both cases, with no lag phases ($\lambda = 0$), equivalent μ_{max} but higher N_{max} values, all as compared to the control. Overall, these growth values are lower than the ones obtained for the first growth-phases with TS, but higher than those obtained for the second phases, i.e., $\mu_{max(TSi)} > \mu_{max(Glu)} \equiv \mu_{max(Ctrl)} > \mu_{max(TSii)}$. Differences in specific growth rates and maximum cell population weaken the initial assumption of the two-steps TS metabolism. Complementary pH measurements at regular time intervals (see Fig. 4b) present a more pronounced drop that coincide with the end of the first growth phase, although, measured values remain between the growth limits of *L. monocytogenes*. A possible explanation for this phenomenon might be that the majority of a certain substrate is consumed during the first growth phase, provoking the cells to enter in a stationary phase. This substrate consumption leads to a decrease in the pH, however, the intense buffering capacity of WMB holds the pH of the media within optimal values (Premaratne et al., 1991). Nevertheless, deeper metabolic analyses to unravel the specific processes here occurring, as well as the biphasic growth, are out of the scope of this research.

Commercial stevia (St). Fig. 3b illustrates the growth curves fitted with the model of Baranyi and Roberts (1994) of *L. monocytogenes* in MWB-glucose free with St. The same concentrations as in TSBYEdf were here tested, 0.3, 0.9 and 1.5% (w/v), all equivalent to those used for TS based on sweetness intensity. In all cases *L. monocytogenes* presents typical growth curves, and no significant

differences occur between the three concentrations tested in terms of maximum specific growth rates or maximum population level. As mentioned in section 3.1.2, this commercial product contains SG and maltodextrin as bulking agent, which can also be used by *L. monocytogenes* to grow. In order to elucidate if the growth is due to the consumption of the SG, their concentration was measured at the beginning and at the end of the exponential growth via HPLC analysis. Obtained results for the three initial concentrations are graphically presented in Fig. 5. These analyses revealed that the main steviol glycoside present in the product is Reb A (98%) and that the total amount is much lower than expected. Based on the instructions in the product package, 10 times less of St must be added to achieve the same sweetness as with the addition of TS. Considering that SG, the sweet principle of St, is 300 times sweeter than TS, it was estimated that 1/30 of the St composition will be SG. Surprisingly, the HPLC analysis revealed that the amount of SG in St was not 1/30 but 1/100. Nevertheless, HPLC results do not show any reduction in the total amount of SG after the corresponding incubation time, but rather the opposite, as can be seen in Fig.5 for the three SG concentrations measured. Therefore, it can be assumed that the growth of *L. monocytogenes* in minimal media with St is mainly attributed to the present maltodextrin. The uptake of maltodextrin has been reported for *L. monocytogenes* (Gopal et al., 2010) as well as for other foodborne bacteria of concern such as *E. coli* (Dippel and Boos, 2005), *Staphylococcus xylosus* (Gopal et al., 2010) and *Lactobacillus lactus* (Monedero et al., 2008). The work of Gopal et al. (2010) revealed the presence of the specific genes evolved in the uptake of maltodextrin. They also performed growth experiments in TSB at optimal conditions, which confirmed the use of this complex carbohydrate.

Steviol glycosides (SG). The growth of *L. monocytogenes* in MWB-glucose free with SG is presented in Fig. 3c and the estimated parameters from the Baranyi and Roberts (1994) growth model are provided in Table 4. For the three concentrations of SG, *L. monocytogenes* is able to grow, proving the ability of this bacterium to grow on this substrate. In comparison to the control, obtained μ_{max} and N_{max} values are higher, and λ is shorter in the media with the three concentrations of SG than in the control. In fact, *L. monocytogenes* presents the fastest maximum specific growth rates with SG compared to the other sweeteners tested in minimal media. Although the statistical analysis indicates significant differences between μ_{max} in the media with 0.03% (w/v) SG and the other 2 concentrations, they might

be considered as similar since they are all in the same order of magnitude. Independently of the maximum specific growth rate values, after 54 h cells reached the stationary phase, with N_{max} values below $15 \ln$ (cfu/mL) and directly proportional to the SG concentration in the media. For a deeper insight in the evolution of the SG during these experiments, their concentration was measured at the beginning and at the end of the exponential growth via HPLC analysis. Results for each concentration are depicted in Fig. 6. These analyses show that after 54 h of incubation with *L. monocytogenes*, the total amount of SG is significantly reduced compare to time 0 h, due to the consumption of RebA and Stev, the main components in the SG mix. To date, no studies have been found in literature related to the metabolizing ability of SG by *L. monocytogenes*. Further research in this field is required for a better comprehension.

3.2.2. *S. Typhimurium* in M9-glucose free

The reference chemically defined medium for *S. Typhimurium* is M9-glucose free, as widely reported in literature (Antunes et al., 2011; Davis et al., 1981; Hartman et al., 2014). Hereunder, the results obtained from the growth experiments are individually described for every sweetener. Since the main objective of these experiments is to detect if growth can occur when the sweetener under study is the only carbon source, experiments were performed substituting the glucose with each sweetener at the concentrations defined in section 2.2. A control without any glucose or sweetener was also assessed, and since no carbon source was available, *S. Typhimurium* was not able to grow. Resulting graphs are depicted in Fig. 7, estimated growth parameters are detailed in Table 5, and the results from the HPLC analysis are presented in Fig. 8 for TS, Fig. 9 for St and Fig. 10 for SG.

Table sugar (TS). The growth of *S. Typhimurium* in M9-glucose free with TS is presented in Fig.7a. Contrary to the results in TSBdf, increasing concentrations of TS facilitate the growth of *S. Typhimurium*. This effect is observed at the level of N_{max} and μ_{max} (Table 5). The growth of *S. Typhimurium* with 3% (w/v) of TS is almost negligible; but increasing the concentration of this sweetener significantly enhances its growth, being the fastest with 15% (w/v) of TS. The N_{max} also increases with higher TS concentrations, but the medium with the (slightly) highest N_{max} does not correspond to the highest concentration of TS, as could be expected but with 9% (w/v). The faster growth in the medium with 15% (w/v) TS can trigger a faster accumulation of metabolites and nutrient depletion, as described for the same microorganism in general media with TS at 8 °C, explaining thus

the lower N_{max} in comparison with the medium with 9% (w/v). These results indicate that *S. Typhimurium* is able to use TS as carbon source. Sucrose concentration in TS was measured at the beginning and at the end of the experiment, as well as at the end of the exponential phase of growth via HPLC (see Fig. 8); which revealed that there is no large consumption of sucrose. With the aim to identify the role of the two forming monomers of TS, complementary growth experiments were carried out with Glu 4.7 and 9% (w/v), the same two concentrations as those assessed in the experiments with *L. monocytogenes* MWB-glucose free. Growth curves are also presented in Fig. 7a, in purple and magenta. In this case, *S. Typhimurium* presents a higher growth than with any TS condition. Both concentrations present equal μ_{max} values, and N_{max} values are directly proportional to Glu concentration. In the study presented by Antunes et al. (2011), M9-glucose free medium was used to determine if *S. Typhimurium* is able to use lysophosphatidylcholine as a carbon source. This experiment was also compared to the growth with 10% (w/v) Glu; however, no growth was obtained in the media with Glu. Microbial growth was measured with OD, explaining therefore the absence of growth compared to the results presented in this work, due to their higher detection limit (Baka et al., 2015; Begot et al., 1996).

Commercial stevia (St). In Fig. 7b the growth of *S. Typhimurium* in M9-glucose free enriched with the three studied concentrations of St, is represented. At a first glance St has a similar effect to TS, i.e., increasing sweetener concentration facilitates the growth of the microorganism. In this case, *S. Typhimurium* is not able to grow with the lowest concentration tested (0.3% (w/v)); however it can with 0.9 and 1.5% (w/v) St. Increasing the concentration promotes a faster growth although the final population remains constant (Table 5). In this situation, the observed growth can be attributed to the components of St, either the SG or the maltodextrin. The HPLC analysis reveals that the SG concentration remains constant over time and, as in the experiments with *L. monocytogenes*, the total amount of SG present in the samples (see Fig. 9) is much lower than expected. Considering the ability of *S. Typhimurium* to metabolise maltodextrin (Morbach et al., 1993), it can be assumed that this growth is a consequence of, at least, the bulking agent and not only of the SG. Besides these experiments with St, the following results in M9-glucose free with SG will also help to understand the role of this sweetener in the growth of *S. Typhimurium*.

Steviol glycosides (SG). From the illustrated results in Fig. 7c, it can be seen that no growth is detected with any of the tested concentrations of SG. More specifically, cell population decreases and after about 160 h, cell concentration is below the detection limit. In Fig. 10 a, b and c, the SG concentrations determined by HPLC, at the beginning and after 72 h of incubation, are represented. Although the concentration of SG is reduced in the media with 0.01% (w/v) after the incubation period, this difference does not appear with the other two conditions. Results reveal that *S. Typhimurium* is not able to metabolise SG to obtain sufficient energy to grow. Nevertheless, to date no study has reported any effect of SG on *S. Typhimurium*.

4. Conclusions

Considering the high social and health actual impact of the use of stevia-based sweeteners as sugar substitutes, it has become of utmost importance to also investigate the effect of these additives on foodborne bacteria of concern. This work has demonstrated that while these ingredients have a mild effect on the growth of the Gram positive *L. monocytogenes*, a higher impact occurs on *S. Typhimurium*, especially at refrigeration conditions. It should be considered that refrigeration at 8 °C can promote the growth of *S. Typhimurium* if TS at high concentration values like 9 and 15% (w/v) has been replaced with a stevia-based sweetener at the equivalent concentrations of 0.9 and 1.5% (w/v). In the case of *S. Typhimurium*, the bulking agent is the main responsible for this growth promotion. However, this is not the case in the growth of *L. monocytogenes*, whose growth is invariable when pure SG-mix is added in the media. This presents SG as a suitable alternative for food products that can get easily contaminated with this microorganism, if combined with a different bulking agent than maltodextrin. Differences in the results between commercial stevia and steviol glycosides demonstrate the key role played by the additives included, like the bulking agent. Varying bacterial response to changes in media formulation, suggests further research is required, focusing on revealing the microbial dynamics in structured media, as well as in real food products. Information provided in this work regarding the effect of sweeteners and their concentrations, bulking agent used and storage temperature, should be considered for potential future studies. In this sense, high sweetener concentrations and low temperatures should be generally selected due to their stronger effect on microbial growth; as well as a lower initial concentration in accordance to the prevalence of pathogenic bacteria in food products.

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References

- Adams, M.R., Moss, M.O., 1995. Food microbiology, 1995, The Royal Society of Chemistry. Cambridge, UK.
- Al-Dabbas, M.M., Al-Qudsi, J.M., 2012. Effect of partial replacement of sucrose with the artificial sweetener sucralose on the physico-chemical, sensory, microbial characteristics, and final cost saving of orange nectar. *Int. Food Res. J.* 19, 679–683.
- Angelidis, A.S., Smith, G.M., 2003. Role of the glycine betaine and carnitine transporters in adaptation of *Listeria monocytogenes* to chill stress in defined medium. *Appl. Environ. Microbiol.* 69, 7492–7498.
- Antunes, L.C.M., Andersen, S.K., Menendez, A., Arena, E.T., Han, J., Ferreira, R.B.R., Borchers, C.H., Finlay, B.B., 2011. Metabolomics reveals phospholipids as important nutrient sources during *Salmonella* growth in bile in vitro and in vivo. *J. Bacteriol.* 4719–4725. doi:10.1128/JB.05132-11
- Baka, M., Noriega, E., Mertens, L., Van Derlinden, E., Van Impe, J.F.M., 2014. Protective role of indigenous *Leuconostoc carnosum* against *Listeria monocytogenes* on vacuum packed Frankfurter sausages at suboptimal temperatures. *Food Res. Int.* 66, 197–206. doi:10.1016/j.foodres.2014.08.011
- Baka, M., Noriega, E., Stamati, I., Logist, F., Van Impe, J.F.M., 2015. Critical assessment of the Time-to-Detection method for accurate estimation of microbial growth parameters. *J. Food Saf.* 35, 179–192.
- Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.* 23, 277–294. doi:10.1016/0168-1605(94)90157-0
- Bayles, D.O., Annous, B.A., Wilkinson, B.J., 1996. Cold stress proteins induced in *Listeria monocytogenes* in response to temperature downshock and growth at low temperatures. *Appl. Environ. Microbiol.* 62, 1116–1119.
- Begot, C., Desnier, I., Daudin, J.D., Labadie, J.C., Lebert, A., 1996. Recommendations for calculating growth parameters by optical density measurements. *J. Microbiol. Methods* 25, 225–232.
- Belda-Galbis, C.M., Pina-Pérez, M.C., Espinosa, J., Marco-Celdrán, A., Martínez, A., Rodrigo, D., 2014. Use of the modified Gompertz equation to assess the *Stevia rebaudiana* Bertoni antisterial kinetics. *Food Microbiol.* 38, 56–61.

- Beumer, R.R., Te Giffel, M.C., Cox, L.J., Rombouts, F.M., Abee, T., 1994. Effect of exogenous proline, betaine, and carnitine on growth of *Listeria monocytogenes* in a minimal medium. *Appl. Environ. Microbiol.* 60, 1359–1363.
- Braden, C.R., Tauxe, R. V, 2013. Emerging trends in foodborne diseases. *Infect. Dis. Clin. North Am.* 27, 517–533.
- Brocklehurst, T.F., Mitchell, G.A., Pleass, W., Smith, A.C., 1995. The effect of step changes in sucrose concentration on the growth of *Salmonella typhimurium* LT2. *J. Appl. Bacteriol.* 78, 495–500.
- Chan, P., Tomlinson, B., Chen, Y.-J., Liu, J.-C., Hsieh, M.-H., Cheng, J.-T., 2000. A double-blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. *Br. J. Clin. Pharmacol.* 50, 215–220.
- Chan, Y.C., Wiedmann, M., 2008. Physiology and genetics of *Listeria monocytogenes* survival and growth at cold temperatures. *Crit. Rev. Food Sci. Nutr.* 49, 237–253.
- Chirife, J., Herszage, L., Joseph, A., Kohn, E.S., 1983. In vitro study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in treating infected wounds. *Antimicrob. Agents Chemother.* 23, 766–773. doi:10.1128/AAC.23.5.766
- Couvert, O., Pinon, A., Bergis, H., Bourdichon, F., Carlin, F., Cornu, M., Denis, C., Gnanou Besse, N., Guillier, L., Jamet, E., Mettler, E., Stahl, V., Thuault, D., Zuliani, V., Augustin, J.-C., 2010. Validation of a stochastic modelling approach for *Listeria monocytogenes* growth in refrigerated foods. *Int. J. Food Microbiol.* 144, 236–42. doi:10.1016/j.ijfoodmicro.2010.09.024
- Davis, R.W., Roth, J.R., Botstein, D., 1981. Advanced bacterial genetics: a manual for genetic engineering. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, New York.
- Debnath, M., 2007. Clonal propagation and antimicrobial activity of an endemic medicinal plant *Stevia rebaudiana*. *J. Med. Plants Res.* 2, 45–51.
- Dippel, R., Boos, W., 2005. The maltodextrin system of *Escherichia coli*: metabolism and transport. *J. Bacteriol.* 187, 8322–8331.
- EFSA, n.d. Salmonella | European Food Safety Authority. URL <http://www.efsa.europa.eu/en/topics/topic/salmonella> (accessed 4.18.16).
- EFSA FIP (Food Ingredients and Packaging), 2010. Scientific Opinion on the safety of steviol glycosides for the proposed uses as a food additive | European Food Safety Authority. *EFSA J.* 8, 1537. doi:10.2903/j.efsa.2010.1537
- European Food Safety Authority and (EFSA) European Centre for Disease Prevention and Control (ECDC), 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSA J.* 13.
- Geuns, J.M.C., 2010. Stevia and steviol glycosides. Euprint, Heverlee.
- Ghosh, S., Subudhi, E., Nayak, S., 2008. Antimicrobial assay of *Stevia rebaudiana* Bertoni leaf extracts against 10 pathogens. *Int. J. Integr. Biol.* 2, 27–31.
- Gopal, S., Berg, D., Hagen, N., Schriefer, E.-M., Stoll, R., Goebel, W., Kreft, J., 2010. Maltose and maltodextrin utilization by *Listeria monocytogenes* depend on an inducible ABC transporter which is repressed by glucose. *PLoS One* 5, e10349.
- Hartman, H.B., Fell, D.A., Rossell, S., Jensen, P.R., Woodward, M.J., Thorndahl, L., Jelsbak, L., Olsen, J.E., Raghunathan, A., Daefler, S., Poolman, M.G., 2014. Identification of potential drug targets in *Salmonella enterica* sv. Typhimurium using metabolic modelling and experimental validation. *Microbiology* 160, 1252–1266. doi:10.1099/mic.0.076091-0
- Hoben, H.J., Somasegaran, P., 1982. Comparison of the pour, spread, and drop plate methods for

- enumeration of *Rhizobium* spp. in inoculants made from presterilized peat. Appl. Environ. Microbiol. 44, 1246–1247.
- Hracek, V.M., Gliemmo, M.F., Campos, C.A., 2010. Effect of steviol glycosides and system composition on stability and antimicrobial action of sorbates in acidified model aqueous systems. Food Res. Int. 43, 2171–2175.
- International Commission on Microbiological Specifications for Foods, 1996. Microorganisms in Foods 5: Characteristics of Microbial Pathogens. Springer Science & Business Media.
- Jayaraman, S., Manoharan, M.S., Illanchezian, S., 2008. In-vitro antimicrobial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. Trop. J. Pharm. Res. 7, 1143–1149.
- Jeppesen, P.B., Gregersen, S., Alstrup, K.K., Hermansen, K., 2002. Steviol glycoside induces antihyperglycaemic, insulinotropic and glucagonostatic effects in vivo: studies in the diabetic Goto-Kakizaki (GK) rats. Phytomedicine 9, 9–14.
- Jooker, E., Amery, R., Monballiu, A., Moons, N., De Borggraeve, W., Dehaen, W., Meesschaert, B., 2013. Microbial Degradation of Steviol derived from steviol glycosides, in: Proceedings of the 7th Stevia Symposium 2013 Organised by EUSTAS. pp. 177–188.
- Jooker, E., Amery, R., Struyf, T., Duquenne, B., Geuns, J., Meesschaert, B., 2012. Stability of steviol glycosides in several food matrices. J. Agricultural Food Chem. 60, 10606–10612.
- Koutsoumanis, K.P., Kendall, P.A., Sofos, J.N., 2004. Modeling the boundaries of growth of *Salmonella Typhimurium* in broth as a function of temperature, water activity, and pH. J. Food Prot. 67, 53–59.
- Kroyer, G.T., 1999. The low calorie sweetener steviol glycoside: stability and interaction with food ingredients. LWT-Food Sci. Technol. 32, 509–512.
- Leistner, L., 1978. Hurdle effect and energy saving, Food quality and nutrition. Applied Sciences Publishers Ltd., London.
- Li, S., Chen, T., Dong, S., Xiong, Y., Wei, H., Xu, F., 2014. The effects of Rebaudioside A on microbial diversity in mouse intestine. Food Sci. Technol. Res. 20, 459–467.
- Lianou, A., Koutsoumanis, K.P., 2013. Strain variability of the behavior of foodborne bacterial pathogens: a review. Int. J. Food Microbiol. 167, 310–21. doi:10.1016/j.ijfoodmicro.2013.09.016
- Magalhães, R., Ferreira, V., Brandão, T.R.S., Palencia, R.C., Almeida, G., Teixeira, P., 2016. Persistent and non-persistent strains of *Listeria monocytogenes*: a focus on growth kinetics under different temperature, salt, and pH conditions and their sensitivity to sanitizers. Food Microbiol. 57, 103–108. doi:10.1016/j.fm.2016.02.005
- Marquis, H., Bouwer, H.G., Hinrichs, D.J., Portnoy, D.A., 1993. Intracytoplasmic growth and virulence of *Listeria monocytogenes* auxotrophic mutants. Infect. Immun. 61, 3756–3760.
- Monedero, V., Yebra, M.J., Poncet, S., Deutscher, J., 2008. Maltose transport in *Lactobacillus casei* and its regulation by inducer exclusion. Res. Microbiol. 159, 94–102.
- Morbach, S., Tebbe, S., Schneider, E., 1993. The ATP-binding cassette (ABC) transporter for maltose/maltodextrins of *Salmonella typhimurium*. Characterization of the ATPase activity associated with the purified MalK subunit. J. Biol. Chem. 268, 18617–18621.
- Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E.C., Biryukov, S., Abbafati, C., Abera, S.F., others, 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 384, 766–781.
- Omac, B., Moreira, R.G., Castillo, A., Castell-Perez, E., 2015. Growth of *Listeria monocytogenes* and *Listeria innocua* on fresh baby spinach leaves: Effect of storage temperature and natural

- microflora. Postharvest Biol. Technol. 100, 41–51. doi:10.1016/j.postharvbio.2014.09.007
- Pasricha, S., 2009. Stevia beverages and replacing aspartame. Frost & Sullivan.
- Pérez-Conesa, D., Cao, J., Chen, L., McLandsborough, L., Weiss, J., 2011. Inactivation of *Listeria monocytogenes* and *Escherichia coli* O157: H7 biofilms by micelle-encapsulated eugenol and carvacrol. J. Food Prot. 74, 55–62.
- Premaratne, R.J., Lin, W.-J., Johnson, E.A., 1991. Development of an improved chemically defined minimal medium for *Listeria monocytogenes*. Appl. Environ. Microbiol. 57, 3046–3048.
- Pronzalo, L., Walter, E., 1997. Identification of parametric models from experimental data, 1997th ed, Springer, Berlin Wentzell PD, Andrews DT, Hamilton. Springer.
- Puri, M., Sharma, D., Tiwari, A.K., 2011. Downstream processing of stevioside and its potential applications. Biotechnol. Adv. 29, 781–791.
- Schauer, K., Stolz, J., Scherer, S., Fuchs, T.M., 2009. Both thiamine uptake and biosynthesis of thiamine precursors are required for intracellular replication of *Listeria monocytogenes*. J. Bacteriol. 191, 2218–2227.
- Schneebeli, R., Egli, T., 2013. A defined, glucose-limited mineral medium for the cultivation of *Listeria*. Appl. Environ. Microbiol. AEM–03538.
- Seeliger, H.P.R., Jones, D., 1986. Genus *Listeria*, in: Bergey's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore, pp. 1235–1245.
- Sleator, R.D., Francis, G.A., O'Beirne, D., Gahan, C.G.M., Hill, C., 2003. Betaine and carnitine uptake systems in *Listeria monocytogenes* affect growth and survival in foods and during infection. J. Appl. Microbiol. 95, 839–846.
- Tadhani, M.B., Subhash, R., 2007. In vitro antimicrobial activity of *Stevia rebaudiana* Bertoni leaves. Trop. J. Pharm. Res. 5, 557–560.
- Tienungoon, S., Ratkowsky, D.A., McMeekin, T.A., Ross, T., 2000. Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. Appl. Environ. Microbiol. 66, 4979–4987. doi:10.1128/AEM.66.11.4979-4987.2000
- Tsai, H.-N., Hodgson, D.A., 2003. Development of a synthetic minimal medium for *Listeria monocytogenes*. Appl. Environ. Microbiol. 69, 6943–6945.
- Tyrovouzis, N.A., Angelidis, A.S., Stoforos, N.G., 2014. Bi-phasic growth of *Listeria monocytogenes* in chemically defined medium at low temperatures. Int. J. Food Microbiol. 186, 110–119.
- Wilson, P.D.G., Brocklehurst, T.F., Arino, S., Thuault, D., Jakobsen, M., Lange, M., Farkas, J., Wimpenny, J.W.T., Van Impe, J.F., 2002. Modelling microbial growth in structured foods: towards a unified approach. Int. J. Food Microbiol. 73, 275–289.
- Xi, Y., Yamaguchi, T., Sato, M., Takeuchi, M., 1998. Antioxidant activity of *Stevia rebaudiana*. J. Japanese Soc. Food Sci. Technol. 5, 310–316.
- Yousef, A.E., Lou, Y., 1999. Characteristics of *Listeria monocytogenes* important to food processors, 2nd ed, *Listeria: Listeriosis, and Food Safety*. CRC Press.

Figure captions

Figure 1. Growth curves at 20, 8 and 4 °C, of *Listeria monocytogenes* in TSBYEdf enriched with (a, b, c) table sugar (TS), (d, e, f) laboratory sucrose (LS), (g, h, i) commercial stevia (St) and (j, k, l) steviol glycosides (SG) at 3 concentration values presented with **blue** (TS/LS 3% (w/v), St 0.3% (w/v) and SG 0.01% (w/v)), **red** (TS/LS 9% (w/v), St 0.9% (w/v) and SG 0.03% (w/v)) and **black** (TS/LS 15% (w/v), St 1.5% (w/v) and SG 0.05% (w/v)). A control in TSBYEdf is presented with **green**. Symbols correspond to experimental data and lines to the fitting of Baranyi and Roberts model (1994).

Figure 2. Growth curves at 20 and 8 °C of *Salmonella* Typhimurium in TSBdf enriched with (a, b) table sugar (TS), (c, d) laboratory sucrose (LS), (e, f) commercial stevia (St) and (g, h) steviol glycosides (SG) at 3 concentration values presented with **blue** (TS/LS 3% (w/v), St 0.3% (w/v) and SG 0.01% (w/v)), **red** (TS/LS 9% (w/v), St 0.9% (w/v) and SG 0.03% (w/v)) and **black** (TS/LS 15% (w/v), St 1.5% (w/v) and SG 0.05% (w/v)). A control in TSBdf is presented with **green**. Symbols correspond to experimental data and lines to the fitting of Baranyi and Roberts model (1994).

Figure 3. Growth curves at 20 °C of *Listeria monocytogenes* in chemically defined minimal medium, WMB-glucose free, enriched with the sweeteners (a) table sugar (TS), (b) commercial stevia (St) and (c) steviol glycosides (SG) at 3 concentration values presented with **blue** (TS 3% (w/v), St 0.3% (w/v) and SG 0.01% (w/v)), **red** (TS 9% (w/v), St 0.9% (w/v) and SG 0.03% (w/v)) and **black** (TS 15% (w/v), St 1.5% (w/v) and SG 0.05% (w/v)). A control in WMB-glucose free is presented with **green**. In a) glucose controls are presented with **magenta** (◇, 4.7% (w/v)) and **purple** (◇, 9% (w/v)). Symbols correspond to experimental data and lines to the fitting of Baranyi and Roberts model (1994) and in a) to the adapted Baranyi and Roberts model of Tyrovouzis et al. (2014).

Figure 4. Concentration (a) in g/L and pH evolution (b) of table sugar (TS) with symbols and trend lines corresponding to (◇, -) 3% (w/v), 9% (○, --) and (□, ···) 15% (w/v) in WMB-glucose free with *Listeria monocytogenes* at 20 °C.

Figure 5. Concentration evolution in mg/L of steviol glycosides in commercial stevia (St) corresponding to the assessed concentrations of St: a) 0.3% (w/v), b) 0.9% (w/v) and c) 1.5% (w/v) in MWB-glucose free with *Listeria monocytogenes* at 20 °C. Graphs correspond to the HPLC analysis at 20 °C of samples taken at the beginning of the growth experiments and at the end of exponential phase.

Figure 6. Concentration evolution in mg/L of steviol glycosides (SG) corresponding to the assessed concentrations of SG: a) 0.01% (w/v), b) 0.03% (w/v) and c) 0.05% (w/v) in MWB-glucose free with *Listeria monocytogenes*. Graphs correspond to the HPLC analysis at 20 °C of samples taken at the beginning of the growth experiments and at the end of exponential phase.

Figure 7. Growth curves at 20 °C of *Salmonella* Typhimurium in chemically defined minimal medium, M9-glucose free, enriched with (a) table sugar (TS), (b) commercial stevia (St) and (c) steviol glycosides (SG) at 3 concentration values presented with **blue** (TS 3% (w/v), St 0.3% (w/v) and SG 0.01% (w/v)), **red** (TS 9% (w/v), St 0.9% (w/v) and SG 0.03% (w/v)) and **black** (TS 15% (w/v), St 1.5% (w/v) and SG 0.05% (w/v)). In a) glucose controls are presented with **magenta** (4.7% (w/v)) and **purple** (9% (w/v)). A control in M9-glucose free is presented with **green**. Symbols correspond to experimental data and lines to the fitting of Baranyi and Roberts model (1994).

Figure 8. Determined TS concentration in g/L of table sugar (TS) in M9-glucose free with *Salmonella* Typhimurium by HPLC analysis at 20 °C from the assessed TS concentrations 3, 9 and 15% (w/v).

Figure 9. Concentration evolution in mg/L of steviol glycosides in commercial stevia (St) corresponding to the assessed concentrations of St: a) 0.3% (w/v), b) 0.9% (w/v) and c) 1.5% (w/v) in M9-glucose free with *Salmonella* Typhimurium. Graphs correspond to the HPLC analysis at 20 °C of samples taken at the beginning of the growth experiments and at the end of exponential phase in case of growth.

Figure 10. Concentration evolution in mg/L of steviol glycosides (SG) corresponding to the assessed concentrations of SG: a) 0.01% (w/v), b) 0.03% (w/v) and c) 0.05% (w/v) in M9-glucose free with

Salmonella Typhimurium. Graphs correspond to the HPLC analysis of samples taken at the beginning of the growth experiments and at the end of exponential phase in case of growth.

Figure 1

Figure 2

Figure 3

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Figure 7

Figure 8

Figure 9

Figure 10

Table 1. Kinetic parameters of Baranyi and Roberts model (1994) for growth of *Listeria monocytogenes* in TSBYEdf enriched with sweeteners at different concentrations and at various temperatures.

	T (°C)	% (w/v)	Kinetic parameters ¹				RMSE
			$\ln(N_0) \pm \text{SE}$ $\ln(\text{cfu/mL})$	$\ln(N_{\max}) \pm \text{SE}$ $\ln(\text{cfu/mL})$	$\mu_{\max} \pm \text{SE (1/h)}$	$\lambda \pm \text{SE (h)}$	
Table sugar	20	0	6.84 ± 0.34	20.80 ± 0.18 ^c	0.442 ± 0.016 ^a	1.57 ± 1.17 ^a	0.09
		3	6.46 ± 0.12	20.61 ± 0.09 ^{bc}	0.506 ± 0.008 ^b	3.67 ± 0.43 ^b	0.16
		9	6.45 ± 0.19	20.44 ± 0.14 ^b	0.493 ± 0.012 ^b	3.66 ± 0.68 ^b	0.24
		15	6.51 ± 0.22	20.00 ± 0.16 ^a	0.494 ± 0.016 ^b	4.10 ± 0.81 ^b	0.09
	8	0	6.74 ± 0.48	20.41 ± 0.20 ^b	0.091 ± 0.007 ^a	10.73 ± 8.06 ^a	0.48
		3	6.31 ± 0.25	20.44 ± 0.013 ^b	0.089 ± 0.003 ^a	6.91 ± 5.11 ^a	0.30
		9	6.32 ± 0.37	19.98 ± 0.19 ^a	0.083 ± 0.005 ^a	3.97 ± 7.70 ^a	0.44
		15	6.48 ± 0.24	19.93 ± 0.11 ^a	0.086 ± 0.003 ^a	8.07 ± 4.65 ^a	0.25
	4	0	6.51 ± 0.27	20.67 ± 0.16 ^c	0.034 ± 0.001 ^a	4.78 ± 11.02 ^a	0.40
		3	6.25 ± 0.23	20.17 ± 0.12 ^b	0.065 ± 0.002 ^{bc}	8.22 ± 5.94 ^a	0.23
		9	6.56 ± 0.41	19.98 ± 0.18 ^b	0.070 ± 0.005 ^c	18.41 ± 9.65 ^a	0.41
		15	6.61 ± 0.41	19.56 ± 0.21 ^a	0.063 ± 0.004 ^b	14.55 ± 10.72 ^a	0.41
Lab sucrose	20	0	6.84 ± 0.34	20.80 ± 0.18 ^{bc}	0.442 ± 0.016 ^{ab}	1.57 ± 1.17 ^a	0.09
		3	6.94 ± 0.33	20.59 ± 0.18 ^c	0.466 ± 0.019 ^a	3.36 ± 1.20 ^{ab}	0.41
		9	6.91 ± 0.27	20.35 ± 0.15 ^{ab}	0.479 ± 0.017 ^b	3.65 ± 0.98 ^b	0.34
		15	6.76 ± 0.14	20.07 ± 0.08 ^a	0.452 ± 0.008 ^{ab}	3.22 ± 0.53 ^b	0.17
	8	0	6.74 ± 0.48	20.41 ± 0.20 ^a	0.091 ± 0.007 ^a	10.73 ± 8.06 ^a	0.48
		3	6.45 ± 0.29	20.44 ± 0.16 ^a	0.090 ± 0.004 ^a	12.08 ± 6.16 ^a	0.37
		9	6.59 ± 0.21	20.23 ± 0.12 ^a	0.090 ± 0.003 ^a	14.49 ± 4.61 ^a	0.28
		15	6.51 ± 0.18	20.07 ± 0.11 ^a	0.087 ± 0.003 ^a	15.26 ± 4.12 ^a	0.24
	4	0	6.51 ± 0.27	20.67 ± 0.16 ^b	0.034 ± 0.001 ^a	4.78 ± 11.02 ^a	0.40
		3	6.05 ± 0.91	20.33 ± 0.44 ^{ab}	0.061 ± 0.008 ^b	14.52 ± 21.95 ^a	0.93
		9	6.63 ± 0.26	19.85 ± 0.13 ^a	0.062 ± 0.003 ^b	22.97 ± 7.57 ^a	0.29
		15	6.34 ± 0.35	19.96 ± 0.17 ^a	0.051 ± 0.003 ^b	0.00 ± 10.98 ^a	0.35
Stevia	20	0.0	6.84 ± 0.34	20.80 ± 0.18 ^{ab}	0.442 ± 0.016 ^a	1.57 ± 1.17 ^a	0.09
		0.3	6.83 ± 0.33	21.07 ± 0.20 ^b	0.465 ± 0.018 ^{ab}	2.97 ± 1.18 ^a	0.40
		0.9	6.84 ± 0.17	20.97 ± 0.11 ^b	0.476 ± 0.010 ^{ab}	3.05 ± 0.61 ^a	0.21
		1.5	6.86 ± 0.30	20.61 ± 0.17 ^a	0.467 ± 0.018 ^b	2.75 ± 1.05 ^a	0.35
	8	0	6.74 ± 0.48	20.41 ± 0.20 ^a	0.091 ± 0.007 ^a	10.73 ± 8.06 ^a	0.48
		0.3	6.76 ± 0.24	20.60 ± 0.13 ^a	0.089 ± 0.003 ^a	10.71 ± 5.10 ^a	0.30
		0.9	6.51 ± 0.35	20.37 ± 0.19 ^a	0.084 ± 0.005 ^a	7.28 ± 7.55 ^a	0.42
		1.5	6.26 ± 0.29	20.22 ± 0.15 ^a	0.087 ± 0.004 ^a	6.99 ± 6.14 ^a	0.35
	4	0.0	6.51 ± 0.27	20.67 ± 0.16 ^b	0.034 ± 0.001 ^a	4.78 ± 11.02 ^a	0.40
		0.3	6.56 ± 0.55	20.58 ± 0.28 ^a	0.073 ± 0.006 ^b	24.98 ± 12.47 ^{ab}	0.62
		0.9	6.72 ± 0.31	20.61 ± 0.15 ^a	0.066 ± 0.003 ^b	15.58 ± 7.95 ^{ab}	0.32
		1.5	6.56 ± 0.41	20.20 ± 0.20 ^a	0.067 ± 0.004 ^b	20.00 ± 9.72 ^{ab}	0.44

Steviol Glycosides	20	0.00	6.84 ± 0.34	20.80 ± 0.18 ^a	0.442 ± 0.016 ^a	1.57 ± 1.17 ^a	0.09
		0.01	7.22 ± 0.47	21.15 ± 0.17 ^b	0.432 ± 0.021 ^a	0.00 ± 1.62 ^a	0.49
		0.03	7.35 ± 0.55	21.16 ± 0.20 ^b	0.425 ± 0.024 ^a	0.11 ± 1.94 ^a	0.58
		0.05	7.36 ± 0.32	21.15 ± 0.12 ^b	0.414 ± 0.014 ^a	0.00 ± 1.16 ^a	0.34
	8	0.00	6.74 ± 0.48	20.41 ± 0.20 ^a	0.091 ± 0.007 ^a	10.73 ± 8.06 ^a	0.48
		0.01	7.13 ± 0.25	21.03 ± 0.13 ^b	0.098 ± 0.003 ^a	5.26 ± 3.87 ^a	0.25
		0.03	7.39 ± 0.43	21.04 ± 0.23 ^b	0.094 ± 0.005 ^a	7.06 ± 1.67 ^a	0.49
		0.05	7.34 ± 0.48	21.10 ± 0.26 ^b	0.092 ± 0.006 ^a	1.67 ± 8.05 ^a	0.49
	4	0.00	6.51 ± 0.27	20.67 ± 0.16 ^a	0.034 ± 0.001 ^a	4.78 ± 11.02 ^a	0.40
		0.01	7.22 ± 0.25	20.80 ± 0.14 ^{ab}	0.035 ± 0.001 ^{ab}	37.59 ± 11.49 ^c	0.32
		0.03	7.25 ± 0.25	21.01 ± 0.12 ^b	0.036 ± 0.001 ^b	14.67 ± 10.83 ^{ab}	0.28
		0.05	7.23 ± 0.21	20.93 ± 0.11 ^b	0.035 ± 0.001 ^{ab}	26.57 ± 9.89 ^{bc}	0.26

¹Estimated parameters from Baranyi and Roberts: initial cell population, N_0 ; maximal cell population, N_{max} ; specific maximum growth phase, μ_{max} ; lag phase, λ ; Root Mean Square Error, RMSE.

²For each sweetener, parameters of the Baranyi and Roberts model (1994) bearing different superscripts (no letters in common) are significantly different ($p \leq 0.05$).

Table 2: Kinetic parameters of Baranyi and Roberts model (1994) for growth of *Salmonella* Typhimurium in TSBdf enriched with sweeteners at different concentrations and temperatures. No detectable growth is represented by two lines (--).

Sweetener	T (°C)	% (w/v)	Kinetic parameters ¹				RMSE
			$\ln(N_0) \pm \text{SE}$ $\ln(\text{cfu/mL})$	$\ln(N_{\max}) \pm \text{SE}$ $\ln(\text{cfu/mL})$	$\mu_{\max} \pm \text{SE (1/h)}$	$\lambda \pm \text{SE (h)}$	
Table sugar	20	0	6.42 ± 0.33	20.17 ± 0.20 ^a	0.519 ± 0.020 ^{bc}	1.97 ± 1.02 ^a	0.37
		3	6.50 ± 0.43	19.83 ± 0.19 ^a	0.551 ± 0.024 ^c	1.93 ± 1.10 ^a	0.43
		9	6.95 ± 0.44	19.75 ± 0.24 ^a	0.490 ± 0.028 ^b	1.86 ± 1.45 ^a	0.51
		15	6.88 ± 0.49	19.93 ± 0.27 ^a	0.438 ± 0.026 ^a	1.31 ± 1.76 ^a	0.56
	8	0	6.87 ± 0.35	19.26 ± 0.21 ^c	0.013 ± 0.001 ^a	36.01 ± 39.89 ^a	0.44
		3	6.69 ± 0.47	17.97 ± 0.94 ^b	0.020 ± 0.001 ^b	46.10 ± 34.97 ^a	0.55
		9	6.54 ± 0.09	9.37 ± 0.06 ^a	0.035 ± 0.005 ^c	52.07 ± 9.93 ^a	0.14
		15	6.97 ± 0.30	6.97 ± 0.30	--	--	--
Lab sucrose	20	0	6.42 ± 0.33	20.17 ± 0.20 ^b	0.519 ± 0.020 ^a	1.97 ± 1.02 ^a	0.37
		3	6.69 ± 0.41	19.61 ± 0.25 ^a	0.538 ± 0.037 ^a	3.35 ± 1.55 ^a	0.53
		9	6.63 ± 0.31	19.58 ± 0.20 ^a	0.529 ± 0.024 ^a	3.58 ± 1.10 ^a	0.41
		15	6.66 ± 0.46	19.68 ± 0.31 ^a	0.506 ± 0.035 ^a	3.93 ± 1.73 ^a	0.62
	8	0.0	6.87 ± 0.35	19.26 ± 0.21 ^a	0.013 ± 0.001 ^a	36.01 ± 39.89 ^a	0.44
		3	6.24 ± 0.47	17.79 ± 0.21 ^b	0.029 ± 0.002 ^a	58.30 ± 28.02 ^a	0.55
		9	6.52 ± 0.54	6.52 ± 0.54	--	--	--
		15	6.66 ± 0.20	8.26 ± 0.11 ^c	0.074 ± 0.089 ^a	60.00 ± 15.88 ^a	0.34
Stevia	20	0.0	6.42 ± 0.33	20.17 ± 0.20 ^{ab}	0.519 ± 0.020 ^a	1.97 ± 1.02 ^a	0.37
		0.3	6.44 ± 0.28	20.46 ± 0.16 ^b	0.570 ± 0.018 ^b	2.37 ± 0.74 ^a	0.32
		0.9	6.39 ± 0.36	20.52 ± 0.22 ^b	0.566 ± 0.023 ^b	2.47 ± 0.99 ^a	0.43
		1.5	6.58 ± 0.29	20.07 ± 0.19 ^a	0.590 ± 0.022 ^b	3.18 ± 0.79 ^a	0.36
	8	0.0	6.87 ± 0.35	19.26 ± 0.21 ^a	0.013 ± 0.001 ^b	36.01 ± 39.89 ^a	0.44
		0.3	6.82 ± 0.45	18.72 ± 0.25 ^b	0.017 ± 0.001 ^a	10.32 ± 36.59 ^a	0.49
		0.9	7.15 ± 0.45	19.65 ± 0.23 ^{bc}	0.015 ± 0.001 ^b	0.97 ± 44.85 ^a	0.53
		1.5	7.18 ± 0.43	19.36 ± 0.28 ^c	0.016 ± 0.001 ^b	54.47 ± 44.46 ^a	0.71
Steviol Glycosides	20	0.00	6.42 ± 0.33	20.17 ± 0.20 ^a	0.519 ± 0.020 ^a	1.97 ± 1.02 ^a	0.37
		0.01	7.14 ± 0.22	20.75 ± 0.09 ^b	0.534 ± 0.015 ^a	2.28 ± 0.73 ^a	0.25
		0.03	7.39 ± 0.13	20.80 ± 0.06 ^b	0.532 ± 0.010 ^a	3.02 ± 0.47 ^a	0.16
		0.05	7.25 ± 0.20	20.82 ± 0.08 ^b	0.526 ± 0.013 ^a	2.47 ± 0.67 ^a	0.23
	8	0.00	6.87 ± 0.35	19.26 ± 0.21 ^a	0.013 ± 0.001 ^a	36.01 ± 39.89 ^a	0.44
		0.01	7.42 ± 0.43	19.20 ± 0.27 ^b	0.017 ± 0.001 ^b	6.51 ± 38.77 ^a	0.72
		0.03	7.28 ± 0.31	19.26 ± 0.18 ^b	0.017 ± 0.001 ^b	2.35 ± 27.72 ^a	0.51
		0.05	7.28 ± 0.40	19.01 ± 0.21 ^b	0.018 ± 0.001 ^b	36.01 ± 39.89 ^a	0.66

¹Estimated parameters from Baranyi and Roberts: initial cell population, N_0 ; maximal cell population, $N_{\max}$; specific maximum growth phase $\mu_{\max}$; lag phase, λ ; Root Mean Square Error, RMSE.

²For each sweetener, parameters of the Baranyi and Roberts model (1994) bearing different superscripts (no letters in common) are significantly different ($P \leq 0.05$).

Table 3. pH and a_w values from the general media, TSBdf/TSBYE with the different sweeteners used.

Measurements were taken at 20 °C.

Sweetener	% (w/v)	TSBdf		TSBYEdf	
		pH	a_w	pH	a_w
Control	<i>0</i>	7.20	0.9921	7.20	0.9920
Table sugar	<i>3</i>	7.42	0.9925	7.13	0.9902
	<i>9</i>	7.38	0.9920	7.10	0.9869
	<i>15</i>	7.35	0.9837	7.09	0.9823
Lab Sucrose	<i>3</i>	7.32	0.9901	7.11	0.9905
	<i>9</i>	7.24	0.9861	7.09	0.9868
	<i>15</i>	7.18	0.9831	7.07	0.9819
Stevia	<i>0.3</i>	7.31	0.9925	7.13	0.9918
	<i>0.9</i>	7.32	0.9923	7.12	0.9906
	<i>1.5</i>	7.34	0.9920	7.12	0.9905
Steviol glycosides	<i>0.01</i>	7.20	0.9920	7.13	0.9922
	<i>0.03</i>	7.20	0.9920	7.13	0.9917
	<i>0.05</i>	7.20	0.9916	7.13	0.9904

Table 4. Kinetic parameters of Baranyi and Roberts model (1994) for growth of *Listeria monocytogenes* in MWB-glucose free enriched with sweeteners at different concentrations at 20 °C.

Sweetener	% (w/v)	Kinetic parameters ¹				
		$\ln(N_0) \pm \text{SE}$ $\ln(\text{cfu/mL})$	$\ln(N_{\max}) \pm \text{SE}$ $\ln(\text{cfu/mL})$	$\mu_{\max} \pm \text{SE (1/h)}$	$\lambda \pm \text{SE (h)}$	RMSE
Control	0.00	6.09 ± 0.18	10.50 ± 0.17	0.032 ± 0.005	45.00 ± 16.32	0.42
Table sugar²	3_i	6.64 ± 0.29	15.07 ± 0.27	0.163 ± 0.018	11.77 ± 4.17	0.92
	3_{ii}	15.07 ± 0.27	21.31 ± 0.48	0.042 ± 0.014	260.71 ± 32.71	
	9_i	6.94 ± 0.33	15.56 ± 0.27	0.168 ± 0.024	3.71 ± 5.19	0.92
	9_{ii}	15.56 ± 0.27	20.59 ± 0.27	0.064 ± 0.022	137.53 ± 15.98	
	15_i	6.84 ± 0.25	16.40 ± 0.42	0.190 ± 0.016	4.32 ± 4.17	0.52
	15_{ii}	16.40 ± 0.42	20.49 ± 0.30	0.056 ± 0.032	115.56 ± 31.60	
Glucose	0.00	6.09 ± 0.18	10.50 ± 0.17 ^a	0.032 ± 0.005 ^{ab}	45.00 ± 16.32	0.42
	4.7	6.45 ± 0.55	17.17 ± 0.48 ^b	0.037 ± 0.003 ^b	0.00 ± 24.48	0.58
	9.0	6.55 ± 0.86	16.60 ± 0.38 ^b	0.027 ± 0.004 ^b	0.00 ± 52.27	0.94
Stevia	0.3	6.56 ± 0.32	20.74 ± 0.21	0.153 ± 0.007	1.42 ± 3.86	0.74
	0.9	7.16 ± 0.42	20.62 ± 0.30	0.167 ± 0.012	0.00 ± 4.58	0.85
	1.5	6.96 ± 0.43	20.42 ± 0.30	0.165 ± 0.013	0.00 ± 5.12	0.98
Steviol Glycosides	0.01	7.26 ± 0.15	12.37 ± 0.10 ^a	0.182 ± 0.021 ^a	15.04 ± 2.18	0.38
	0.03	7.34 ± 0.20	13.76 ± 0.12 ^b	0.306 ± 0.046 ^b	13.16 ± 1.89	0.51
	0.05	7.20 ± 0.15	14.33 ± 0.09 ^c	0.220 ± 0.016 ^a	11.72 ± 1.56	0.36

¹Estimated parameters from Baranyi and Roberts: initial cell population, N_0 ; maximal cell population, $N_{\max}$; specific maximum growth phase $\mu_{\max}$; lag phase, λ ; Root Mean Square Error, RMSE.

²Estimated parameters from the adapted model of Baranyi and Roberts (Tyrovouzis et al., 2014) to the first (i) and second (ii) growth phases.

³For each sweetener, parameters of the Baranyi and Roberts model (1994) bearing different superscripts (no letters in common) are significantly different ($P \leq 0.05$).

Table 5. Kinetic parameters of Baranyi and Roberts model (1994) for growth of *Salmonella* Typhimurium in M9-glucose free enriched with sweeteners at different concentrations and temperatures.

Sweetener	% (w/v)	Kinetic parameters ¹				RMSE
		$\ln(N_0) \pm \text{SE}$ $\ln(\text{cfu/mL})$	$\ln(N_{\max}) \pm \text{SE}$ $\ln(\text{cfu/mL})$	$\mu_{\max} \pm \text{SE (1/h)}$	$\lambda \pm \text{SE (h)}$	
Control	0.00	7.08 ± 0.42	7.08 ± 0.42	--	--	--
Table sugar	3	6.70 ± 0.20	8.22 ± 0.18 ^a _A	0.041 ± 0.030 ^{aA}	15.00 ± 30.61	0.40
	9	5.74 ± 0.30	12.76 ± 0.32 ^c _C	0.059 ± 0.009 ^{abA}	10.00 ± 12.69	0.55
	15	6.52 ± 0.21	11.90 ± 0.14 ^b _B	0.085 ± 0.085 ^{bA}	2.49 ± 5.97	0.34
Glucose	4.7	7.26 ± 0.06	11.46 ± 0.04 ^a _D	0.151 ± 0.049 ^{aB}	7.48 ± 5.64	0.06
	9.0	7.33 ± 0.63	14.24 ± 0.52 ^b _E	0.088 ± 0.027 ^{aB}	0.00 ± 17.47	0.63
Stevia	0.3	7.02 ± 0.29	7.02 ± 0.29	--	--	--
	0.9	5.76 ± 0.28	11.58 ± 0.16 ^a	0.168 ± 0.039 ^a	5.00 ± 6.09	0.36
	1.5	6.84 ± 0.11	11.77 ± 0.05 ^a	0.350 ± 0.028 ^a	1.82 ± 0.89	0.19
Steviol Glycosides	0.01	7.26 ± 0.03	--	--	--	--
	0.03	7.27 ± 0.12	--	--	--	--
	0.05	7.05 ± 0.201	--	--	--	--

¹Estimated parameters from Baranyi and Roberts: initial cell population, N_0 ; maximal cell population, $N_{\max}$; specific maximum growth phase $\mu_{\max}$; lag phase, λ ; Root Mean Square Error, RMSE.

²For table sugar and glucose, parameters of the Baranyi and Roberts model (1994) bearing different underscripts (no capital letters in common) are significantly different ($P \leq 0.05$).