

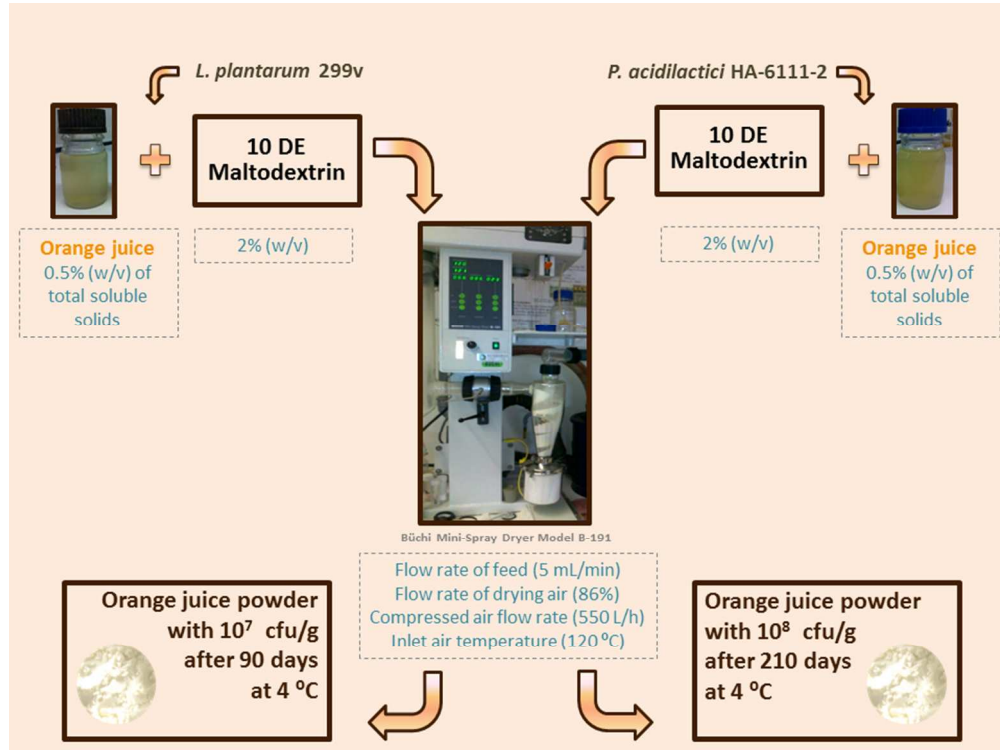


Spray drying conditions for orange juice incorporated with lactic acid bacteria

Journal:	<i>International Journal of Food Science and Technology</i>
Manuscript ID	IJFST-2016-21762.R1
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	n/a
Complete List of Authors:	Barbosa, Joana; Universidade Catolica Portuguesa Centro Regional do Porto, CBQF Brandão, Teresa; UCP, CBQF Teixeira, Paula; UCP, CBQF
Keywords:	Probiotics, Spray Drying, Fruit Juices

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Graphical abstract

254x190mm (96 x 96 DPI)

Review

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3 1 **Spray drying conditions for orange juice incorporated with lactic acid bacteria**
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8 3 Barbosa, J., Brandão, T.R.S. and *Teixeira, P.
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24 10 **Running title:** Probiotic orange juice powder
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3 22 **Abstract**
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5 23 This work aimed to develop an orange juice powder by spray drying with lactic acid bacteria
6
7 24 (*Lactobacillus plantarum* 299v and *Pediococcus acidilactici* HA-6111-2), testing their survival
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10 25 both during drying and storage (room temperature and 4 °C). Initially, the best conditions for
11
12 26 spray drying were chosen to allow the best survival of each LAB: i) inlet air temperature of 120
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15 27 °C and ii) 0.5:2 ratio of the orange juice soluble solids and drying agent added (prebiotics: 10DE
16
17 28 maltodextrin or gum Arabic). Survival of LAB was not affected by drying process and it was
18
19 29 higher when cultures were stored at 4 °C. A slightly higher protection was conferred by 10DE
20
21 30 maltodextrin, in the case of *L. plantarum* and at 4 °C. *Pediococcus acidilactici* was more resistant
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23 31 during storage at 4 °C, with logarithmic reductions lower than 1 log-unit. It was demonstrated
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25 32 that it is possible to produce a functional non-dairy product, orange juice powder supplemented
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27 33 with prebiotic compounds, containing viable LAB for at least 7 months, when stored at 4 °C.
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36 36 **Keywords:** Spray drying, *Lactobacillus plantarum* 299v, *Pediococcus acidilactici* HA-6111-2,
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38 37 maltodextrin, gum Arabic
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46 **Introduction**

47 The production of oranges in Portugal is more than 200,000 tons per year (OMAIAA, 2011) and
48 the possibility of producing natural orange juice powder would be an advantage at economic
49 level, not only by the reduction in volume and weight of the packages, easier transportation and
50 storage, but especially by increasing the shelf life of the product. At the same time, the use of
51 probiotics as food supplements is increasing, because of their health benefits, as well as the
52 increased diversity in food choices they provide. Presently, probiotics are defined as “live
53 microorganisms, which, when administered in adequate amounts, confer a health benefit on the
54 host” by the Food and Agriculture Organization of the United Nations and World Health
55 Organization (FAO/WHO, 2002). Producing an orange juice with probiotic bacteria can be an
56 innovative way to increase this diversity in food choices, especially among consumers who prefer
57 functional non-dairy based foods. Whilst dairy products are the priority for the development of
58 novel probiotic foods, an increase of vegetarianism, milk cholesterol content, and lactose
59 intolerance justify the need for non-dairy probiotic products (Granato *et al.*, 2010).

60 Spray drying (SD) is the most common method used for converting liquid food products into dry
61 powder, because it is inexpensive and easy to operate. Briefly, the process involves the pumping
62 of liquid sample into the atomizer that transforms the liquid into small droplets, which rapidly
63 lose their moisture on contact with the hot and dry air (Silva *et al.*, 2011). It could be a good
64 method to get a natural orange juice powder, if the characteristics of the natural juice allowed the
65 powder production. Fruit juices are extremely sticky, due to the presence of low molecular
66 weight sugars and organic acids in their composition, their high hygroscopicity, water solubility
67 and low melting point (Bhandari *et al.*, 1997). The usual strategy to spray-dry sticky products is
68 the use of wall materials with high molecular weight. Several authors have been using drying aids
69 to a variety of fruit juices, being maltodextrins and gum Arabic the more common agents used

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3 70 (Martinelli *et al.*, 2007; Tonon *et al.*, 2010). Maltodextrins are low cost oligosaccharides, made
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6 71 from starch, that have dextrose equivalents (DE) and gum Arabic is a complex
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8 72 heteropolysaccharide and a natural exudate of Acacia tree (Bemiller & Whistler, 1996). Together
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10 73 with their ability as drying agents, several studies have provided evidence that both maltodextrin
11
12 74 and gum Arabic also have prebiotic effects (Anekella & Orsat, 2013; Slavin, 2013). Prebiotics are
13
14 75 defined as “nondigestible food ingredients that beneficially affect the host by selectively
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16 76 stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus
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18 77 improving host health” (Gibson & Roberfroid, 1995).
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20 78 Studies on SD of fruit juices with probiotic bacteria incorporated are rare (Anekella & Orsat,
21
22 79 2013; Pereira *et al.*, 2014), probably because there are many factors influencing the survival of
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24 80 probiotics before, during and after SD (reviewed by Barbosa *et al.*, 2016).
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26 81 This work aimed to develop an orange juice powder dried by SD and incorporating viable lactic
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28 82 acid bacteria (LAB), ensuring their survival both during drying and storage.
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36 84 **Materials and methods**

37 85 **Origin, growth and storage conditions of LAB isolates**

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39 86 Two LAB were used: *Lactobacillus plantarum* 299v (Probis Probiotika, Lund, Sweden) and
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41 87 *Pediococcus acidilactici* HA-6111-2 deposited in *Escola Superior de Biotecnologia* (ESB)
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43 88 culture collection (Barbosa *et al.*, 2015).
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46 89 Growth and storage of isolates were done according to Barbosa *et al.* (2015).
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53 91 **Conditions of the drying process of orange juice**

54 92 *Materials*

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3 93 Mature oranges exclusively originated in Portugal were randomly purchased from local
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5 94 commercial establishments (Porto, Portugal) and stored at room temperature until used (for no
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8 95 more than 24 h before experiments).

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10 96 The drying agents used were 10 DE maltodextrin (Sigma, Steinheim, Germany) and gum Arabic
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12 97 (Merck, Darmstadt, Germany).

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17 99 *Orange juice preparation*
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20 100 Oranges were squeezed using a domestic juicer and the juice was filtered in order to eliminate the
21
22 101 solids in suspension, preventing the obstruction of the atomizer of the spray dryer. The content of
23
24 102 the total soluble solids of the juice was measured using a digital refractometer (model PR-32 α
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26 103 (alpha), Brix 0–32%, Atago U.S.A., Inc., WA, U.S.A.) and adjusted to 0.5 or 1% (w/v). The
27
28 104 drying agents 10 DE maltodextrin and gum Arabic were added, both at the concentrations of 1 or
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30 105 2% (w/v), under magnetic stirring at 40 °C, until complete dissolution.

31 32 33 34 106 35 36 107 *Spray drying*

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38 108 The drying of orange juice was performed in a laboratory scale Büchi Mini Spray Dryer Model
39
40 109 B-191 (Büchi Laboratoriums-Technik, Flawil, Switzerland) with a two-fluid nozzle atomizer
41
42 110 with a 1 mm inside diameter and a concurrent drying chamber of 10.5 cm (Barbosa *et al.*, 2015).
43
44 111 The inlet air temperatures tested were 120 and 130 °C. The outlet air temperature cannot be
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46 112 regulated, resulting from a combination of the inlet air temperature, the feed rate, the drying gas
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48 113 flow rate and the solids content of the feed. A single cyclone air separator system was used and
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50 114 the dried powders were collected from the base of the cyclone.

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57 116 *Analysis of powders*
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3 117 Immediately after the SD, drying yield and water activity (a_w) of the dried powders were
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5 118 determined (Barbosa *et al.*, 2015).
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10 120 **Spray drying of orange juice with LAB**

12 121 *Orange juice preparation*

13 122 The orange juice was prepared as described above. After the selected conditions, the total soluble
14 123 solids content of the juice was adjusted to 0.5% (w/v) and at this solution was added 2% (w/v) of
15 124 the drying agent (10 DE maltodextrin or gum Arabic), under magnetic stirring at 40 °C, until
16 125 complete dissolution.
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26 127 *Preparation of LAB cultures*

28 128 From Man, Rogosa and Sharpe (MRS) agar incubated at 37 °C for 24 h, one colony of each LAB
29 129 isolate was transferred to MRS broth and incubated at the same conditions. For the final
30 130 inoculum, the last culture was transferred to a new MRS broth (1:100) and incubated at 37 °C for
31 131 24 h to reach stationary phase. Each isolate was harvested by centrifugation (8877 x g, 10 min, 37
32 132 °C; Rotina 35R, Hettich, Germany), washed twice in sterile quarter strength Ringer's solution
33 133 (Lab M, Bury, United Kingdom) and re-suspended in the same volume of the final solution
34 134 prepared before (in Orange Juice Preparation).
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45 135 As control, 10% (w/v) of reconstituted skim milk (RSM) powder (Oxoid, Basingstoke, UK) was
46 136 used to re-suspend the LAB cultures.
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52 138 *Spray drying and powder analysis*

53 139 The drying of LAB cultures incorporated either in orange juice or in the RSM was achieved as
54 140 described above. The drying conditions chosen for both LAB cultures were: feed temperature of
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3 141 40 °C, feed flow rate of 5 mL/min; 86% of drying air flow rate; compressed air flow rate of 550
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5 142 L/h, inlet air temperature of 120 °C and outlet air temperature of about 65 °C.
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8 143 Drying yield and a_w of the dried powders were also determined.
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11 12 145 *Storage conditions*

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15 146 Dried samples were stored in plastic containers, hermetically sealed in glass flasks, in normal
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17 147 atmosphere (air), in the presence of daylight, at 4 °C and room temperature.
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21 22 149 *Enumeration of spray dried LAB cultures*

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24 150 The survival of each microorganism was assessed immediately after SD and at regular intervals
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26 151 throughout storage by rehydration of each dried sample to their initial solids concentration in
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28 152 sterile quarter strength Ringer's solution (Lab M). Each rehydrated sample was homogenized for
29
30 153 1 minute and kept at room temperature for 30 minutes followed by serial decimal dilutions and
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32 154 plated in duplicate for enumeration by the drop count technique (Miles and Misra, 1938) on MRS
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34 155 agar. The enumeration of each microorganism re-suspended in both orange juice or RSM before
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36 156 SD was also performed.
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38 157 The colonies were counted after incubation at 37 °C for 48 h and the CFU/mL calculated.
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46 159 **Data analysis**

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48 160 Each experiment was done in duplicate. All calculations were carried out using the software IBM
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50 161 SPSS Statistics (version 22.0, IBM Corporation, Armonk, NY, USA). A significance level of $P <$
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52 162 0.05 was applied to all statistical procedures.
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56 57 164 *Statistical analysis*

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3 165 Significant differences in microbial counts of each LAB before and after SD were analyzed using
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5 166 a paired-samples t-test.

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8 167 Microbial counts were transformed to logarithmic reduction using the equation: $\log (N/N_0)$,
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10 168 where N is the microbial cell count at a particular sampling time and N_0 is the microbial cell
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12 169 count after SD. To evaluate the values of the variable $\log N/N_0$ (between the first and the last
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14 170 storage time) among different isolates and used treatments (different drying agents) studied, we
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16 171 applied multivariate models of generalized estimating equations (GEE), with identity as binding
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18 172 function, i.e., it was assumed a linear time course over the months of storage. The GEE are a
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20 173 method that allow analyzing repeated or longitudinal measures, taking into account that the
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22 174 measurements in the same individual over time are correlated. The advantage of this method is
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24 175 that it provides consistent estimates of the parameters associated with covariances of the model,
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26 176 even if the assumed correlation structure would be wrong.
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33 34 178 *Logistic model*

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36 179 Data from logarithmic reductions of each LAB along storage were adjusted with Logistic model
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38 180 using the equation: $\log (N/N_0) = - C / (1 + Ae^{-B})$, where N is the microbial cell count at a
39
40 181 particular sampling time, N_0 is the microbial cell count after SD, C is the asymptotic value, which
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42 182 evaluates the tail tendency and B is related to the steepness of the curve (higher values are
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44 183 associated to higher inactivation rates) (Chen, 2007).
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50 185 **Results**

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53 186 Powdered orange juice was initially obtained in a Büchi Mini Spray Dryer at i) constant feed
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55 187 temperature (40 °C), flow rates of feed (5 mL/min), drying air (86%) and compressed air (550
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57 188 L/h) and ii) varying inlet air temperatures (120 and 130 °C) as well as the ratio of total soluble
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3 189 solids (orange juice; 0.5 or 1% w/v): drying aid (10DE maltodextrin or gum Arabic; 1 or 2%
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6 190 w/v). Powders with different drying yield and a_w values were obtained (data not shown). In table
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8 191 S1 are presented the drying yields and a_w values obtained only for the selected conditions: inlet
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10 192 and outlet air temperatures of 120 °C and 65 °C, respectively, and ratio of soluble solids: drying
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12 193 aid of 0.5: 2. For both drying agents, drying yield was close to 50% and values of a_w between 0.3
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14 194 and 0.4 were obtained for the orange juice powders.
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20 196 In table 1 are shown the log CFU/ml of *L. plantarum* 299v and *P. acidilactici* HA-6111-2 before
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22 197 and after being spray dried. For each LAB, besides the strong correlation coefficient (>0.9), no
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24 198 significant differences were obtained ($P > 0.05$) between before and after the drying process.
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26 199 Moreover, no logarithmic reductions were obtained after SD for both LAB.
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32 201 Kinetic parameters for logarithmic reductions of each LAB along 210 days of storage at room
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34 202 temperature and 4 °C are detailed in Table S2.

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36 203 The survival of each microorganism during storage at room temperature is presented in Figure 1.
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38 204 For both LAB, survival during storage at room temperature was higher when cells were spray
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40 205 dried in RSM. Although, the reduction of both microorganisms had been lower for RSM initially,
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42 206 both were reduced to values below the level of the detection limit of the enumeration technique
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44 207 after 150 (*L. plantarum* 299v; graph A1) or 210 days of storage (*P. acidilactici* HA-6111-2;
45
46 208 graph B1).

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50 209 In spray dried orange juice, significant differences were obtained between the drying agents used
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52 210 regarding to the survival of LAB during storage period studied ($P < 0.05$). After 11 days of
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54 211 storage in orange juice dried with 10 DE maltodextrin (graph A2) an accentuated reduction in the
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56 212 number of cells of *L. plantarum* 299v occurred, and after 60 days the logarithmic reduction was
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213 to values below the level of the detection limit of the enumeration technique. In orange juice
214 dried with gum Arabic (graph A3), even though the similar behavior, higher reduction in the
215 number of cells of *L. plantarum* 299v occurred initially, compared with orange juice dried with
216 10 DE maltodextrin.

217 Despite survival of *P. acidilactici* HA-6111-2 dried in orange juice with 10 DE maltodextrin
218 (graph B2) or gum Arabic (graph B3) had been different along the storage period ($P < 0.05$), in
219 both cases, the reduction in the number of cells became more pronounced after 30 days of storage
220 and values below the level of the detection limit were attained after 120 days of storage.

221
222 In Figures 2 and S1 are presented the survival of *L. plantarum* 299v and *P. acidilactici* HA-6111-
223 2, respectively, during storage at 4 °C. A higher survival was observed during storage at 4 °C than
224 at room temperature, for both LAB.

225 In the case of *L. plantarum* 299v, the drying with RSM (Fig 2, graph A4) also conferred a
226 protective effect during storage at 4 °C, comparing with the other drying agents ($P < 0.05$).
227 Significant differences were also found among the additives used ($P < 0.05$). In the orange juice
228 dried with 10 DE maltodextrin (Fig 2, graph A5), there was a 4 log-units reduction in the survival
229 of *L. plantarum* 299v up to 90 days of storage and of 8 log-units until the end of the storage
230 period (210 days). In the presence of gum Arabic (Fig 2, graph A6), after this period of 90 days
231 this reduction was higher than in the presence of 10 DE maltodextrin, reaching values below the
232 detection limit after 210 days (> 9.9 log reduction).

233 No significant differences were observed in the survival of *P. acidilactici* HA-6111-2 during
234 storage at 4 °C ($P > 0.05$) for all the SD media investigated, RSM (Fig S1, graph B4) and orange
235 juice supplemented with 10 DE maltodextrin (Fig S1, graph B5) or gum Arabic (Fig S1, graph

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3 236 B6). At this storage temperature, *P. acidilactici* HA-6111-2 demonstrated a higher survival than
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6 237 *L. plantarum* 299v ($P < 0.05$), showing less than 1 log-unit reduction after 210 days.
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8 238 Orange juice dried with 10 DE maltodextrin conferred a slightly higher protection on the survival
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10 239 of *L. plantarum* 299v during storage at 4 °C.
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14 15 241 **Discussion**

16
17 242 To develop a new product such as an orange juice powder with functional properties, two LAB
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19 243 were selected to be incorporated: *L. plantarum* 299v - a commercial probiotic and *P. acidilactici*
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21 244 HA-6111-2 an isolated strain from a food matrix and with probiotic characteristics found after
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23 245 preliminary characterization (Barbosa *et al.*, 2015).
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29 247 Optimization of a drying process is the initial step to gather the best conditions for obtaining a
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31 248 powdered product of good quality. The different parameters evaluated to optimize the drying
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33 249 process of orange juice by SD were i) the content of soluble solids in orange juice, as well as the
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35 250 optimal ratio of drying agents tested, allowing the juice drying with the lowest loss of powder,
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37 251 and ii) the inlet air temperature of the SD. With the different tests carried out, conditions leading
38
39 252 to the highest drying yield and product with low a_w were selected: 0.5:2 ratio of the orange juice
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41 253 soluble solids and drying agent added.
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45 254 Another important parameter in SD is the outlet air temperature. As previously mentioned, in the
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47 255 Büchi Mini Spray Dryer used this is not an adjustable parameter, resulting from the combination
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49 256 of the various parameters such as inlet air temperature, pump and aspirator settings and feed
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51 257 concentration. Several researchers reported that outlet air temperatures above 85 °C were lethal
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53 258 for probiotic cultures (Gardiner *et al.*, 2000; Corcoran *et al.*, 2004). Since we intended to
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55 259 incorporate probiotics into orange juice and the outlet air temperatures are such an important
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3 260 parameter in the survival of bacteria during drying, all the conditions selected (Table S1) resulted
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5 261 in low outlet air temperatures (close to 65 °C) and, simultaneously, high drying yields and low a_w
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8 262 values.

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10 263 According to Bhandari *et al.* (1997) a total powder recovery of 50% in a laboratory scale spray
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12 264 dryer is considered to be the reference point for a marginally successful drying. From both
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15 265 concentrations of the drying agents tested, the addition of 2% (w/v) to the orange juice allowed a
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17 266 better drying yield than 1% (w/v). This is in agreement with some studies which stated that the
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20 267 increasing of drying agents concentration in fruit juices, also increased the powder yield (Quek *et al.*
21
22 268 *al.*, 2007; Fazaeli *et al.*, 2012). With the subsequent addition of probiotic cultures to orange juice,
23
24 269 it was important that the selected conditions allowed to obtain low a_w values, as water remaining
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26
27 270 after drying affects the viability of cultures, after the drying process and also during storage
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29 271 (Zayed & Roos, 2004). Moreover, dried products with a_w values below 0.6 are considered
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31 272 microbiologically stable (Quek *et al.*, 2007).

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36 274 After the selection of SD conditions for orange juice powder production, we proceeded to the
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38 275 incorporation of each LAB to the juice with each drying agent - 10 DE maltodextrin and gum
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40 276 Arabic - and subsequent drying. The survival of LAB was not affected during the drying process
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43 277 ($P > 0.05$). Good survival of probiotics after SD in fruit juices had already been reported
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45 278 (Anekella & Orsat, 2013).

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50 280 Drying of the selected LAB in RSM was used as a control since it has been demonstrated that it
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52 281 is as an efficient protector, both during drying and also during subsequent storage (Teixeira *et al.*,
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55 282 1995a; Gardiner *et al.*, 2000; Ananta *et al.*, 2005).

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3 284 As expected, significant differences were obtained between the temperatures of storage used ($P <$
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5 285 0.05). Many authors reported the higher survival of spray dried bacteria during storage at low
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8 286 temperatures (Teixeira *et al.*, 1995b; Gardiner *et al.*, 2000; Silva *et al.*, 2002).
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10 287 Of the drying agents used in this study, 10 DE maltodextrin allowed better results on the survival
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12 288 of *L. plantarum* 299v in comparison with gum Arabic. Other studies have demonstrated the
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15 289 importance of maltodextrin as a drying agent in fruit juices, as well as its protective ability of
16
17 290 probiotic cultures during drying and subsequent storage (Anekella & Orsat, 2013; Pereira *et al.*,
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19 291 2014).
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21
22 292 Despite the scarce literature regarding the drying of *L. plantarum* and *P. acidilactici* strains
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24 293 incorporated in fruit juices by SD, many authors have studied the behavior of different strains
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26 294 after SD using maltodextrin as carrier. Lapsiri *et al.* (2012) found high survival rate of *L.*
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28 295 *plantarum* TISTR 2075 after SD. During storage at different temperatures, survival of spray dried
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30 296 cells was affected by elevated temperatures; while at 25 °C no cells have survived up to 90 days
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32 297 of storage, at 4 °C this strain had a decrease of only 1.62 log CFU/g after 12 months of storage in
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34 298 the absence of light. In the study of Reddy *et al.* (2009), using maltodextrin and nonfat skimmed
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36 299 as carriers, during 60 days of storage, the high temperatures also affected the survival rate of *L.*
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38 300 *plantarum* and *P. acidilactici* strains tested. At 4 °C a survival rate of 60% was obtained for both
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40 301 strains and carriers. At 30 °C, using maltodextrin as carrier, the survival rate decreased to 50% for
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42 302 both strains and using nonfat skimmed, the survival rate decreased to 67% for *L. plantarum* and
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44 303 to 53% for *P. acidilactici*. The authors concluded that maltodextrin is a good substitute of nonfat
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46 304 skimmed.
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49 305 Although maltodextrin could be a good encapsulating agent during SD, it also acts as a prebiotic,
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51 306 allowing the survival of the cultures (Reddy *et al.*, 2009; Lapsiri *et al.*, 2012; Anekella & Orsat,
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53 307 2013; Pereira *et al.*, 2014).
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3 308 In this study, and only focusing the results obtained during storage at 4 °C, it was possible to
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5 309 obtain spray dried cultures in orange juice, which could survive and remain viable in amounts of
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7 310 10^7 CFU/mL, over a certain period of time. As expected, different microorganisms had different
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9 311 behaviors: while the orange juice powder contained 10^7 CFU/mL of *L. plantarum* 299v only up
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11 312 to 90 days of storage at 4 °C, the orange juice powder with *P. acidilactici* HA-6111-2 contained
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13 313 10^8 CFU/mL, at least up to 210 days of storage. This means that the conditions are fulfilled for
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15 314 extra and important experiments being performed, especially in terms of i) storage conditions
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17 315 improvement, ii) validation of results in a larger industrial drier and iii) validation of the ability
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19 316 of the dried probiotic cultures to retain its functional properties. Nonetheless, the present data is
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21 317 promising and allowed to prove that it is possible to produce a functional non-dairy based food,
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23 318 such as an orange juice powder that incorporates probiotic ingredients.
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320 **Conclusions**

321 The conditions to obtain orange juice powder by SD were pooled. Another challenge was the
322 incorporation of bacteria with probiotic characteristics and that they were able to survive during
323 drying process and storage. In this study it was demonstrated that it is possible to produce a
324 healthy product, not only for the advantage of using a powder made from natural fruit juice, as
325 well as the beneficial characteristics conferred by a prebiotic and probiotic incorporated.
326 Within various additives which can be used in food industry, the prebiotic maltodextrin turned
327 possible not only to obtain a powder of good quality, but also a higher survival of one of the
328 bacteria incorporated into orange juice, along its storage at 4 °C. If this product is stored and sold
329 at refrigerated conditions it can have a long shelf life, depending on the probiotic used. The
330 potential probiotic *P. acidilactici* HA-6111-2 studied was more resistant than the probiotic *L.*
331 *plantarum* 299v. The challenge continues to be the preservation of these products at room

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3 332 temperature, so it is necessary to improve the storage conditions in order to increase their shelf
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5 333 life at the lowest possible cost.
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10 335 **Acknowledgments**

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12 336 This work was supported by funding from the National Funds from the *Fundação para a Ciência*
13 337 *e a Tecnologia* (FCT) through project UID/Multi/50016/2013. Financial support for author J.
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15 338 Barbosa was provided by a post-doctoral fellowship, SFRH/BPD/113303/2015 (FCT).
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426 **Table 1.** Survival of *L. plantarum* 299v and *P. acidilactici* HA-6111-2 before and after SD in
 427 RSM and orange juice supplemented with 10DE maltodextrin or gum Arabic

	log CFU/ml					
	<i>L. plantarum</i> 299v			<i>P. acidilactici</i> HA-6111-2		
	RSM	10 DE maltodextrin	gum Arabic	RSM	10 DE maltodextrin	gum Arabic
Before SD	10.0±0.09	10.0±0.53	9.4±0.44	10.7±0.29	9.0±0.07	9.1±0.04
After SD	11.3±0.10	11.2±0.05	9.9±0.70	11.0±0.11	9.1±0.23	9.9±0.15

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3 431 **Figure 1.** Logarithmic reduction (●) and Logistic model (—) of *L. plantarum* 299v (A) and *P.*
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5 432 *acidilactici* HA-6111-2 (B) incorporated in orange juice or RSM after SD and during 210 days of
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8 433 storage at room temperature: (—●) control (inoculum in 10% (w/v) of RSM); (—●) orange
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10 434 juice with 2% of 10 DE maltodextrin and (—●) orange juice with 2% of gum Arabic. The
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12 435 dotted lines mean that the isolate was reduced to values below the detection limited of the
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14 436 enumeration technique.

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22 439 **Figure 2.** Logarithmic reduction (●) and Logistic model (—) of *L. plantarum* 299v (A)
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24 440 incorporated in orange juice or RSM after SD and during 210 days of storage at temperature of 4
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26 441 °C: (—●) control (inoculum in 10% (w/v) of RSM); (—●) orange juice with 2% of 10 DE
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28 442 maltodextrin and (—●) orange juice with 2% of gum Arabic.

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36 445 **Figure S1.** Logarithmic reduction (●) of *P. acidilactici* HA-6111-2 (B) incorporated in orange
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38 446 juice or RSM after SD and during 210 days of storage at temperature of 4 °C: (—●) control
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40 447 (inoculum in 10% (w/v) of RSM); (—●) orange juice with 2% of 10 DE maltodextrin and
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42 448 (—●) orange juice with 2% of gum Arabic. Logistic model was not applied to this data.

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50 450 **Table S2.** Formal kinetic parameters for logarithmic reductions of each LAB along storage
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Table S1. Yield and a_w of orange juice powder at selected drying conditions

Drying agent	Orange juice (0.5% of total soluble solids)	
	Yield ^a	a_w ^b
2% 10DE Maltodextrin	40.90 ± 1.13	0.409 ± 0.004
2% gum Arabic	53.05 ± 1.48	0.321 ± 0.021

^aThe powder yield is represented as the media of percentages of powder obtained ± the standard error of the mean

^bThe a_w is represented as the media of a_w values obtained ± the standard error of the mean

Table S2. Formal kinetic parameters for logarithmic reductions of each LAB along storage

Condition	Parameters	<i>L. plantarum</i> 299v		<i>P. acidilactici</i> HA-6111-2	
		Room temperature	4 °C	Room temperature	4 °C
RSM (control)	A ± IC/2	7.035±2.903	4.056±3.630	10.895±4.057	n/a
	B ± IC/2	0.028±0.007	0.012±0.010	0.025±0.005	
	C ± IC/2	11.987±1.112	5.624±4.754	11.866±1.299	
	R ²	0.963	0.828	0.977	
Orange juice with 2% of 10DE MD	A ± IC/2	15.092±18.495	6.264±4.053	35.947±26.417	n/a
	B ± IC/2	0.357±0.151	0.015±0.011	0.070±0.014	
	C ± IC/2	10.232±0.556	10.344±6.223	9.051±0.322	
	R ²	0.939	0.842	0.990	
Orange juice with 2% of GA	A ± IC/2	25.223±18.131	19.137±13.238	83.619±107.016	n/a
	B ± IC/2	0.456±0.100	0.026±0.009	0.067±0.020	
	C ± IC/2	9.862±0.263	10.557±1.808	10.028±0.502	
	R ²	0.985	0.958	0.983	

Legend: B - related to the steepness of the curve; C - the asymptotic value
n/a – not applicable

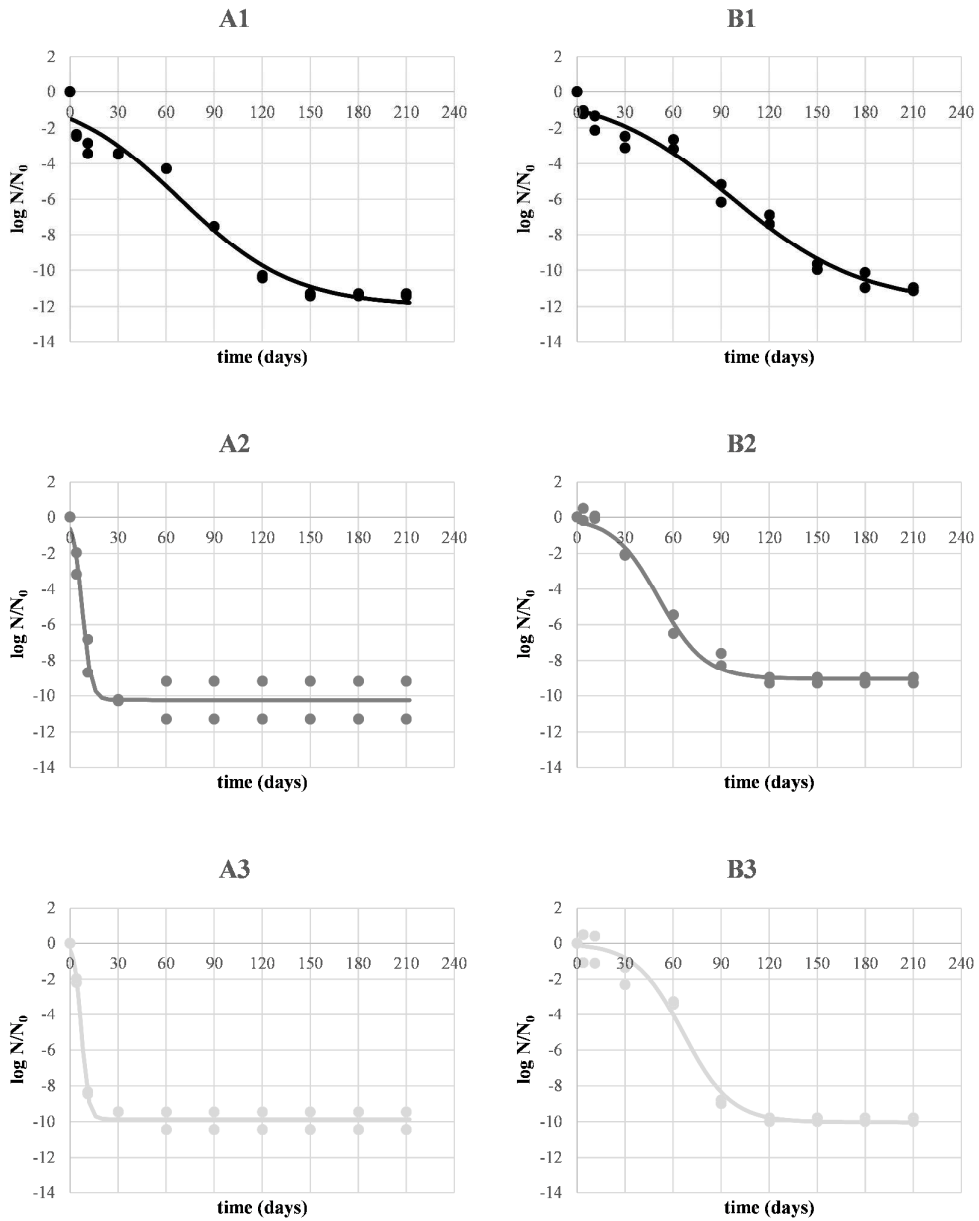


Figure 1.

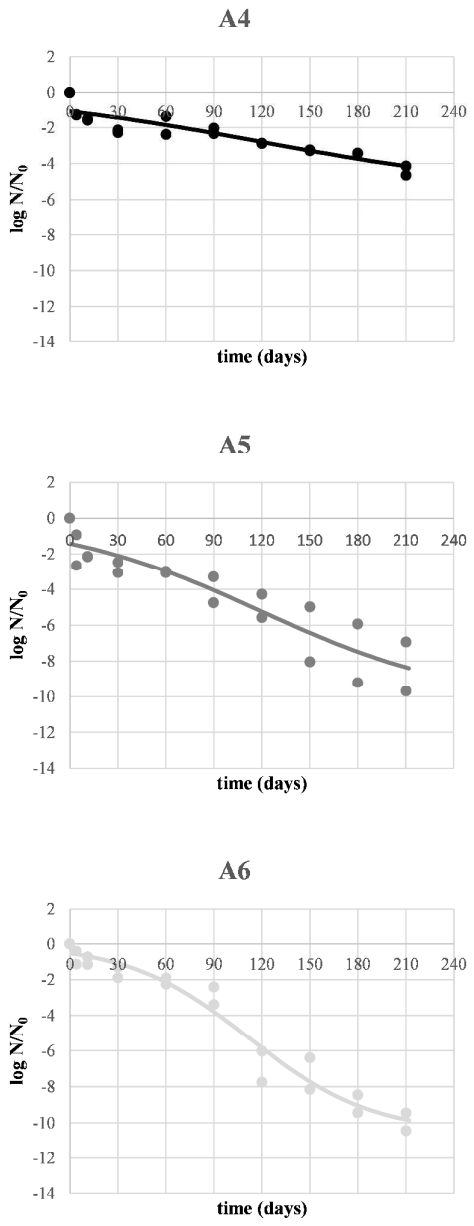


Figure 2.

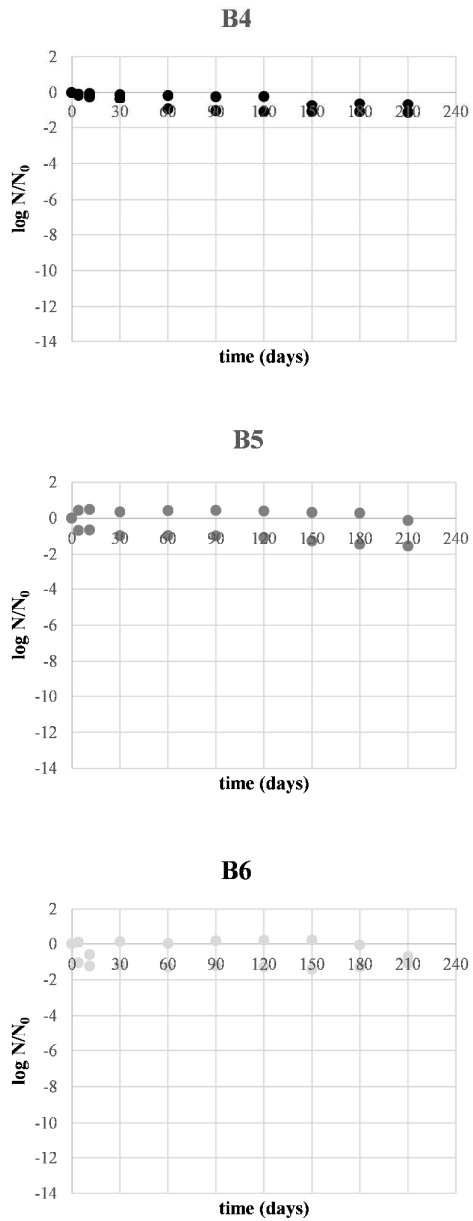


Figure S1.