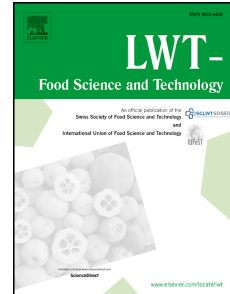


Accepted Manuscript

Stabilization of sourdough starter by spray drying technique: New breadmaking perspective

Anna Reale, Tiziana Di Renzo, Marco Preziuso, Gianfranco Panfili, Luigi Cipriano, Maria Cristina Messia



PII: S0023-6438(18)30852-1

DOI: [10.1016/j.lwt.2018.10.016](https://doi.org/10.1016/j.lwt.2018.10.016)

Reference: YFSTL 7482

To appear in: *LWT - Food Science and Technology*

Received Date: 25 May 2018

Revised Date: 12 September 2018

Accepted Date: 5 October 2018

Please cite this article as: Reale, A., Di Renzo, T., Preziuso, M., Panfili, G., Cipriano, L., Messia, M.C., Stabilization of sourdough starter by spray drying technique: New breadmaking perspective, *LWT - Food Science and Technology* (2018), doi: <https://doi.org/10.1016/j.lwt.2018.10.016>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **STABILIZATION OF SOURDOUGH STARTER BY SPRAY DRYING TECHNIQUE:**
2 **NEW BREADMAKING PERSPECTIVE**

3

4 **Anna Reale¹, Tiziana Di Renzo¹, Marco Prezioso², Gianfranco Panfili², Luigi Cipriano¹,**
5 **Maria Cristina Messia^{2*}**

6 ¹Istituto di Scienze dell'Alimentazione, Consiglio Nazionale delle Ricerche, Via Roma 64
7 83100, Avellino, Italy.

8 ²Dipartimento Agricoltura, Ambiente e Alimenti, Università degli Studi del Molise, Via De
9 Sanctis snc, 86100, Campobasso, Italy.

10

11

12

13

14

15

16 ***Corresponding author:**

17 Maria Cristina Messia

18 Dipartimento Agricoltura, Ambiente e Alimenti, Università degli Studi del Molise

19 Via De Sanctis, 86100 Campobasso, Italy

20 Phone + 39 0874 404624

21 Fax + 39 0874 404855

22 E-mail: messia@unimol.it

23

24

25 **Abstract**

26 Aim of the study was to produce traditional and typical breads using stabilized sourdough starter
27 without the addition of baker's yeast activator. To this end, the spray drying technique was
28 investigated as an alternative tool to freezing, freeze drying and drying techniques to preserve
29 sourdough starter. The stabilized sourdoughs were used in breadmaking to assess the ability to
30 leaven and ferment the doughs and to evaluate the effect of their use on the sensory
31 characteristics of the obtained breads. Positive and encouraging results were obtained mainly
32 with the spray-dried sourdoughs that better preserved the vitality of both yeasts and lactic acid
33 bacteria when compared to the traditional techniques. Furthermore, the results suggested that
34 spray-dried sourdough, opportunely refreshed, can be successfully used for breadmaking without
35 the use of baker's yeast activator, producing breads with sensory characteristics highly
36 comparable to those produced using fresh sourdoughs.

37

38 **Keywords:** *sourdough; lactic acid bacteria; yeasts; bread; spray drying.*

39

40

41

42

43

44

45

46

47

48

49

50

51 **1. Introduction**

52 Spontaneous sourdough fermentation is one of the oldest approaches used in the baking process
53 for the production of numerous homemade, typical and traditional breads and several
54 representative sweet products of the Italian tradition such as Panettone, Pandoro and Colomba
55 (Italian Ministerial Decree, 22 July 2005). Sourdough, on the basis of the technology applied, is
56 classified into 3 types (Types I, II, and III) (Decock & Cappelle, 2005). Type I, is a fresh
57 sourdough that is restarted using a part of the previous fermentation. It is largely used in the
58 modern biotechnology of baked goods as a natural leavening agent since it provides many
59 technological advantages compared to baker's yeast resulting in final products with higher
60 overall quality (Chavan & Chavan, 2011; Corsetti & Settanni, 2007). This kind of sourdough is
61 quite resilient, but to alive it may require extra cost and care as continuous daily or weekly
62 propagations using the mother sponge taken from the preceding fermentation, feeding with
63 proper ingredients and appropriate temperature to maintain the microorganisms in an active state
64 (De Vuyst et al., 2014). Furthermore, the microbial ecosystem of type I sourdough can easily
65 undergo modification due to factors affecting the management and preservation of the dough
66 itself, such as modification of the ingredients used and the type of flour, the change in the storage
67 temperature, the number of refreshments made, the hygienic conditions of the processing
68 environment and the operator (Lattanzi, Minervini & Gobbetti, 2014).

69 Type II is a sourdough using adapted strains to start fermentation. It is industrially produced and
70 it can be liquid, so it is easily pumpable in an industrial bakery.

71 Sourdough of type III is the most convenient way to introduce authentic bread taste into
72 nowadays high-tech bakery industry (Chavan & Chavan, 2011). It consists mainly in different
73 preparations of dried or lyophilized sourdoughs that, to enhance leavening ability, require the
74 addition of "*Saccharomyces cerevisiae*" yeast, which is presented as an "activator" but which
75 actually is responsible only for the leaven, often causing flattening of the aromatic characteristics
76 of the finished products.

77 A proper stabilization of the sourdough of type I over time could represent not only an important
78 milestone in economic terms in the bakery industry but also a prerequisite for the protection of
79 typical and traditional bakery products, without the use of baker's yeast activator.

80 Among traditional technologies used to store sourdough, freezing and freeze drying, despite
81 being efficient methods, have high manufacturing costs and energy consumption. For this reason,
82 in the last years increasing attention has been put on alternative drying processes as spray drying,
83 fluidized bed drying and vacuum drying (Peighambardoust, Golshan Tafti & Hesar, 2011).

84 Spray drying is considered a good long-term preservation method not only for liquid food
85 products (Barbosa, Brandão & Teixeira, 2017; Murugesan & Orsat, 2012; Caboni et al., 2005)
86 but also for starter cultures (Peighambardoust, Golshan Tafti & Hesari, 2011). Different
87 researches have been reported on the spray drying of bacteria and yeasts, as pure culture or
88 incorporated in food liquids, without loss of cell activity in order to overcome the difficulties
89 involved in their handling and maintaining (Huang et al., 2017; Lavari et al., 2015; Atalar &
90 Dervisoglu, 2015; Luna-Solano, Salgado-Cervantes, García-Alvarado, & Rodríguez-Imenes,
91 2015; Behboudi-Jobbehdar, Soukoulis, Yonekura & Fisk, 2013; Paez et al., 2013; Marquez-
92 Montes, Robles-Olvera & Luna-Solano, 2007). Spray drying could be a good alternative method
93 to obtain stabilized sourdough powders. In current literature, few studies related to spray dried
94 sourdough are available and they regard mainly the effect of incorporating spray-dried
95 sourdough, as convenient bakery ingredient, to enhance sensory features of breads obtained with
96 baker's yeast (Golshan Tafti et al., 2013a; Golshan Tafti, Peighambardoust, Hesari, Bahrami &
97 Bonab, 2013b).

98 Aim of this paper was, therefore, to produce breads using a stabilized sourdough starter, avoiding
99 the use of bakery' yeast activator. For this reason, different techniques such as spray drying,
100 freezing, drying and freeze drying were examined for the sourdough starter stabilization.
101 Breadmaking experiments, using the stabilized sourdoughs, were carried out and the leavening
102 dough ability and the sensory features of breads were evaluated.

103

104 **2. Materials and Methods**

105 **2.1 Sourdough sample**

106 A traditional type I sourdough, daily reactivated at 25-28°C with refined wheat flour, was
107 obtained by a local bakery. It presented the following characteristics: pH = 4.1 ± 0.15 , total
108 titratable acidity = 15.9 ± 0.16 (mL), lactic acid bacteria 9.17 ± 0.17 log cfu/g and yeasts 7.53 ± 0.12
109 log cfu/g. Sourdough was divided into individual fractions containing 500g each that were
110 treated using four preservation techniques: freezing, drying, freeze drying and spray drying. To
111 evaluate the effect of the different storage treatments, lactic acid bacteria and yeasts were
112 counted before and after treatments and after 6 months of storage.

113

114 **2.2 Preservation techniques**

115 **2.2.1 Freezing (F).** Freezing was performed at -18°C for 24 h in sterile air-tight-capped
116 containers (Sarstedt, Germany) and stored at -18°C for 6 months.

117

118 **2.2.2 Drying (D).** Fresh sourdoughs were delivered into sterile Petri dishes and dried in a
119 thermostated oven at 40°C for 48h under vacuum condition (-87 kPa). The dried samples were
120 packed in sterile air-tight-capped containers and stored at room temperature for 6 months.

121

122 **2.2.3 Freeze drying (FD).** Fresh sourdoughs were frozen at -40°C and then freeze-dried under
123 vacuum at 15 Pa in a freeze-drier Genesis 25ES (VirTis, NY, USA) for 48 h (maximum shelves'
124 temperature $+20^{\circ}\text{C}$). The resulting freeze-dried powders were packed in sterile air-tight-capped
125 containers and stored at room temperatures for 6 months.

126

127 **2.2.4 Spray drying (SD) procedure and optimization of spray drying condition**

128 Sourdoughs were spray-dried in a Büchi B-290 mini spray dryer (Büchi Labortechnik AG,
129 Switzerland) equipped with a two-fluid nozzle. The drying medium was air. The drying
130 proceeded until the desired moisture content was reached in the sprayed particles and the product
131 was then separated from the air. The material was sprayed in the same direction as the flow of
132 hot air through the apparatus. To optimize spray drying conditions, batches of sourdough
133 diversely resuspended (distilled sterile water or physiological sterile solution) and diluted (ratio
134 sourdough solution of 1:1, 1:2, 1:5) were spray-dried.

135 By regulating the aspirator speed, the amount of heated drying air can be increased or decreased.
136 For this reason, spray drying experiments were performed at varying inlet air temperatures
137 (130/140°C), different % aspiration (95/100%) and feed flow rate (4/6/8 mL/min) (Table 1).
138 Each experiment was carried out in duplicate. The spray-dried sourdough was sealed in plastic
139 bag and stored at room temperatures for 6 months. After spray drying, the drying yield (%) was
140 determined as the % of the powder weight collected from the receiver to the amount initial of
141 solids contained in the solution feed.

142 The spray-dried sourdough obtained with the optimal spray drying condition was compared with
143 sourdoughs obtained with the other techniques above described.

144

145 **2.3 Optimization of rehydration procedures**

146 Sourdough powders were suspended in different rehydration media: a) physiological solution,
147 PHI; b) skim milk (10%) in physiological solution, SM; c) skim milk (10%) plus sucrose (1%) in
148 physiological solution, SMSUC; d) flour plus skim milk (10%) in physiological solution, FSM;
149 e) flour plus sucrose (1%) in physiological solution, FSUC. Sourdough powders were dissolved
150 in the media described above, mixed gently to form a slurry and rested for about 1 h at 28°C.
151 Lactic acid bacteria and yeast counts were determined at time zero and after 1h of incubation.

152

153 **2.4 pH, Total titratable acidity (TTA), water activity (a_w), moisture, ash and protein**
154 **assessment**

155 pH and TTA were measured on 10g of sample (fresh sourdough or freeze/dried/freeze-
156 dried/spray-dried powders), homogenized with 90 mL of deionized water at room temperature
157 for 1 min in a Stomacher laboratory blender (Bag Mizer 400, Interscience, France). The pH
158 measurements were done using a pHmeter Medidor PH Basic 20 (CRISON, Spain). TTA was
159 expressed as the amount (mL) of 0.1 N NaOH necessary to achieve pH 8.3.

160 The water activity of the powders was measured using a water activity meter (Aqualab, Decagon
161 Devices, USA) at a constant temperature of 23 ± 1 °C.

162 Ash, moisture and protein content on wheat flour and sourdoughs were determined according to
163 AOAC official method 923.03, 925.09 and 920.87 respectively (AOAC International, 2000).

164

165 **2.5 Lactic acid bacteria and yeast viability**

166 For LAB and yeast counts, samples were prepared according to the following procedure: 10 g of
167 each sample was aseptically transferred into a sterile stomacher bag and diluted with 90 mL of
168 physiological solution (9 g/L NaCl). After 1 min of agitation in Stomacher, the samples were
169 serially diluted and plated in duplicate. Lactic acid bacteria were counted using MRS (Oxoid,
170 Milan, Italy) agar medium supplemented with 4 mg/100 mL cycloheximide (SIGMA Aldrich,
171 Germany) after incubation at 30°C for 72 h in anaerobic conditions (Gas Pack AnaeroGen™,
172 Oxoid). Yeasts were counted on YPD Agar (20.0 g/L dextrose, 20.0 g/L bacteriological peptone,
173 20.0 g/L agar, 10.0 g/L yeast extract) using spread plate technique at 28°C for 72h. Plates with
174 30-300 colonies were selected and the colonies were counted and the cfu/mL calculated. For
175 microbial survival, counts were transformed to logarithmic reduction using the equation: \log
176 (N/N_0) , where N was the microbial cell count in the fresh sourdough and N_0 was the microbial
177 cell count immediately after treatments.

178

179 **2.6 Bread production by stabilized sourdoughs**

180 Six different breads were produced using fresh sourdough (C); freeze sourdough (FS) and
181 refreshed freeze sourdough (rFS), refreshed dried sourdough (rDS), refreshed freeze dried
182 sourdough (rFDS), refreshed spray-dried sourdough (rSDS).

183 Refreshment was made mixing sourdough with equivalent part of flour, 10% sucrose and 60%
184 physiological solution, and incubated at 28°C for about 20h. After refreshment bread doughs
185 were prepared. The basic ingredients were: wheat flour (ash= 0.61% d.w., protein= 12.5% d.w.),
186 sourdough (20%), salt (1.5%), and water (60%). The dough was kneaded in a mixer for 20 min
187 and rested for about 10 min in a proofer, after that it was kneaded for others 10 min. Doughs
188 were cut, formed and placed in the molds (250 gr). Then, followed leavening for about 4-5h at
189 28°C. In the production laboratory leavened doughs were baked, cooled and assessed. Baking
190 was carried out at $225 \pm 5^\circ\text{C}$ for 30 min in a deck oven. Loaves were allowed to cool for 120 min
191 at room temperature before further analysis.

192

193 **2.7 Sensory analysis**

194 A sensory evaluation test was performed for all breads. The samples were evaluated by 10
195 judges, aged 30-50 years, with food expertise. The samples were sliced into equally sized pieces
196 and served as coded randomized duplicates. Bread produced with fresh sourdough was used as a
197 standard. The comparative preferences were rated on a 7-point hedonic scale (7= excellent, 6=
198 very good, 5=good, 4=satisfactory, 3=unsatisfactory, 2=bad, 1= very bad) for appearance, flavor,
199 texture and overall quality.

200

201 **2.8 Image Acquisition and Digital Images Analysis**

202 The images acquisition of bread slices took place after 24h from baking. For each bread sample,
203 three loaves were sliced transversely using an electric slicer to obtain 15 mm thick slices. They
204 were scanned in colour and in black and white images using a flatbed scanner (HP ScanJet 8300,

205 Hewlett Packard Co., CA, USA) with 300 dpi of resolution and the following settings: highlight
206 70, shadows 58, and midtones 0.5. The images were saved in TIFF format.

207 Images were analyzed by Software Image-Pro Plus 4.5 (Media Cybernetics, Georgia, USA,
208 Windows 98), to evaluate the distribution of aveoli and determine the ratio between alveolar
209 cross section and slice areas. The number of alveoli and pore circularity values were categorized in
210 different classes. Ten different classes were arbitrarily defined. In detail, Table 2 shows the min
211 and max range of area (mm^2) of alveoli for each class and Figure 1 shows an example of data
212 obtained by Digital Image Analysis performed on sliced breads.

213

214 **2.9 Statistical analysis**

215 Two biological replicates of all experimentations were performed on separate days. All analyses
216 were carried out in triplicate. Mean values and standard deviation were calculated. Analysis of
217 variance was performed to determine significant differences (Tuckey's HSD test $*P \leq 0.05$)
218 between means. Data were analysed using SYSTAT 13.0 for Windows (Systat Software Inc.,
219 Richmond, CA, USA).

220

221 **3. Results and Discussion**

222 **3.1 Optimization of spray drying condition**

223 Before to compare the different techniques to stabilize sourdough, the spray drying conditions
224 were optimized. For this purpose, three different experiments were carried out, changing some
225 parameters as inlet air temperature, aspiration rate (%) and feed flow rate (mL/min) as reported
226 in Table 1.

227 The outlet air temperature varied proportionally with air inlet temperature and feed flow rate
228 conditions. Moisture, dry matter (DM), ash, a_w , pH, TTA, drying yield, LAB and yeast counts
229 were assessed to evaluate the optimal spray drying condition (Table 3).

230 Fresh sourdough was characterized by a moisture value (%) of 42.2 ± 1.06 (average) and a_w value
231 of 0.992 ± 0.003 (average). All the experiments allowed to produce a sourdough powder with low
232 moisture content (%) ranged from 7.56 ± 0.07 (I-SD) to 6.23 ± 0.08 (II-SD) and low a_w values
233 ranged from 0.202 ± 0.02 (I-SD) to 0.199 ± 0.04 (II-SD).

234 It has been proven that moisture content and water activity are important prerequisites for
235 storage stability of spray-dried powders with good handling characteristics such as high flow
236 ability, low stickiness and agglomeration, as well as maximum microbial viability (Behboudi-
237 Jobbehdar et al., 2013). Water activity (a_w) is a key marker for spray-dried powder because it can
238 strongly influence the shelf life of the powder produced. Generally, food with $a_w < 0.6$ is
239 considered as microbiologically stable and if any spoilage occurs, it is induced by chemical
240 reactions rather than by microorganisms (Queck, Chok, & Swedlund, 2007). From our results, a_w
241 values of the powders were minor than 0.2. This meant that the produced spray-dried powders
242 were microbiologically stable.

243 As reported by other authors (Chavez & Ledebøer, 2007), in food powders containing probiotics,
244 water activity should be below 0.25 and moisture content below 5% for safe storage. Zayed and
245 Roos (2004) also demonstrated that the amount of water remaining after drying affects not only
246 the viability of bacteria, as determined immediately after the process, but also the rate of loss of
247 viability during subsequent storage. Indeed, the optimum moisture content for storage of freeze-
248 dried *L. salivarius* subsp. *salivarius* was reported to range from 2.8% to 5.6% (Zayed & Roos,
249 2004).

250 Our spray-dried sourdoughs, despite were characterized by a moisture content slightly higher (of
251 about 1.5 percentual point) when compared to data of Zayed and Roos (2004) and Chavez and
252 Ledebøer (2007), were however acceptable for a good storage. In fact, also after 6 months of
253 storage, the spray-dried powders were characterized by low moisture content ($< 7.5\%$) and
254 noticeable viability of lactic acid bacteria and yeasts of 7.0 and 5.1 log cfu/g, respectively,
255 recording a load reduction of minus of 1 logarithmic cycle. Nevertheless, moisture values of our

256 spray-dried samples were very similar to those of numerous commercial Active Dry Baker's
257 Yeast that are characterized by 5-8% moisture (Grabowski, Mujumdar, Ramaswamy &
258 Strumillo, 1997; Reale et al. 2013).

259 The spray drying did not substantially affect pH and TTA values (Table 3). In fact, TTA values
260 of the spray-dried sourdoughs (16.5 mL, median value) was slightly higher compared to that of
261 the fresh sourdoughs (16.0 mL, median value). Independently by spray drying condition, the
262 spray-dried powders had pH values relatively similar to fresh samples, of about 3.9 (median
263 value).

264 Fresh sourdough was characterized by an average ash value of 0.721 ± 0.156 (% d.m.). After the
265 treatment samples II-SD (0.814% d.m.) and III-SD (0.866% d.m.) were characterized by ash
266 content similar to those recorded by other authors of 1.01% (Stefanello et al., 2018) and
267 $1.9\% \pm 0.02$ (Golshan Tafti, Peighamardoust, Hesari, Bahrami & Bonab, 2013b), whereas
268 sample I-SD (4.493% d.m.) had an unexpected very high ash value.

269 Sourdough powder I-SD resulted in the highest ash value since the sourdough was originally
270 diluted in physiological solution (1:2) containing NaCl salt (0.9%).

271 For this reason, the condition I was not considered as optimal because high amount of salt in the
272 sourdough powder could negatively compromise cell viability during storage. Therefore, in the
273 subsequent trials this condition (I) was discarded.

274 Furthermore, the spray drying conditions strongly influenced the stickiness of the powders.

275 During the trials, within the experiment I and II a higher powder adhesion on the walls of the
276 drying chamber was observed compared to the experiment III, resulting in a loss of the product.

277 In fact, condition I (sourdough diluted in sterile physiological solution in 1:2 ratio) and II
278 (sourdough diluted in sterile water in 1:2 ratio) produced a low drying yield, 36.3% and 35.3%,
279 respectively. Experiment III, instead, produced the highest drying yield (56%) compared to the
280 other conditions. This high value was probably due to the different spray drying conditions

281 (higher aspiration rate and feed flow rate value and higher sourdough/distilled water ratio) that
282 almost certainly facilitated the atomization, increasing the drying yield.

283 The outlet air temperature is a parameter that varied according to the inlet air temperature, air
284 flow rate, product feed rate, medium composition and atomized droplet size. Generally, high
285 inlet temperatures hold higher temperatures of the outlet air and of the output product. Increasing
286 the pump rate lowers the outlet temperature and thus increases the temperature difference
287 between the inlet temperature and the outlet temperature.

288 Experimental condition III had the lowest outlet air temperature (54°C) compared to condition I
289 and II that recorded 70°C and 60°C, respectively (Table 1).

290 As reported by Peighambardoust, Golshan Tafti and Hesar (2011) the outlet air temperature is
291 believed to be the major drying parameter affecting the viability of spray-dried starter cultures.

292 In our experiment outlet air temperature affected the survival of lactic acid bacteria and yeasts.

293 Sourdough powder obtained in the Experiment III, in fact, after spray drying, showed a reduction
294 of only 1 logarithmic cycle for both microbial groups respect to those obtained in the

295 Experimental I and II that showed a reduction of more than 2 logarithmic cycles both for yeasts
296 and lactic acid bacteria (Fig. 2A). Despite the load reduction, the yeast/LAB ratio 1:100 did not

297 changed, reflecting the typical proportion existing in mature sourdoughs (Reale et al. 2011,

298 Corsetti & Settanni, 2007). In a paper of Golshan Tafti, Peighambardoust, Hesari, Bahrami &

299 Bonab (2013b), whereas inlet and outlet temperature were 180°C and 90°C, respectively, spray

300 drying reduced enough the moisture content and water activity to provide longer shelf-life in the

301 product, but reduced lactic acid bacteria population (*L. paralimentarius* used as starter) of

302 sourdough of almost four logarithmic cycles, from 10^9 cfu/g to 2×10^5 cfu/g. In our best condition

303 (experiment III), using an inlet temperature of 130°C and a subsequent outlet temperature of

304 54°C, we obtained a microbial survival rate of about 50%. Similar results were obtained by

305 Romano et al. (2014) that spray-dried two strains of *L. rhamnosus* in chestnut extract using inlet

306 and outlet temperature analogous to those of our experiment (140-65°C, respectively).

307 The optimization of the spray drying conditions are important to guarantee not only a proper
308 moisture content and water activity but also the best survival of microbial population.
309 So, our experiments allowed setting the optimal spray drying condition for sourdough,
310 specifically sourdough dissolved in sterile distilled water in 1:5 ratio, an air inlet temperature of
311 130°C, 95% aspiration rate and a feed flow rate of 8 mL/ min.

312

313 **3.2 Comparison of freezing, drying, freeze drying and spray drying techniques**

314 The optimized spray drying condition was compared with the other traditional techniques,
315 freezing, drying and freeze drying. Fig. 2B shows the effect of different preservation techniques
316 on the survival of lactic acid bacteria and yeasts occurring in sourdoughs.

317 Freezing strongly affected the microbial survival reaching a reduction of about 4 logarithmic
318 cycles both for LAB and yeasts. These results are in agreement with other authors (Lattanzi,
319 Minervini & Gobbetti, 2014) that evidenced that three different freeze-dried sourdoughs after 7 days
320 of storage achieved a reduction in yeast density of more than 4 logarithmic cycles. As reported
321 by Lattanzi, Minervini and Gobbetti (2014), freezing and subsequent thawing may have caused
322 severe injury to yeast cells and may have lowered the leavening power of sourdoughs.
323 Differently from these authors, in our experiment we obtained also a great reduction in LAB
324 count of about 3.5 ± 0.09 log cfu/g.

325 Regarding the drying and freeze-drying techniques in our experiment a reduction of almost 3
326 logarithmic cycles both for yeasts and lactic acid bacteria was also recorded in the sourdoughs.

327 These results highlighted the injurious effects of these techniques on survival of microbial
328 consortium of sourdoughs. Otherwise, as reviewed by Santivarangkna, Kulozik and Foerst,
329 (2007), microbial inactivation is not only induced by dehydration inactivation but also by
330 thermal- and cryo-injuries depending on the drying processes employed. Lattanzi, Minervini and
331 Gobbetti (2014) also evidenced that the dried form of sourdough showed markedly lower
332 leavening power and yeast cell density.

333 Although freeze drying is considered one of the best practices for reducing thermal damage
334 (Behboudi-Jobbehdar et al., 2013), in our experimentation we gain the best results using
335 optimized spray drying conditions. As showed in Fig. 2B, the spray drying technique get the
336 highest survival both of yeasts and lactic acid bacteria when compared to the other techniques,
337 showing a reduction of just 1-2 log cfu/g. Definitely, spray drying allowed to obtain the more
338 satisfactory results relating to viability of both yeast and lactic acid bacteria.

339 Our study highlighted the importance to gain a correct optimization of the spray-dry parameters
340 (Fig. 2A The III spray drying condition, otherwise, had an outlet air temperature of 54°C that,
341 certainly, influenced the microbial survival. This fact, was also observed by Behboudi-Jobbehdar
342 et al. (2013) that obtained the most significant change in microbial survival when the outlet
343 temperature was reduced from 91.5 to 60°C; over this temperature range, the survival rate of
344 microbial cell increased from 2.5 to 84%. Another factor that probably enhanced the microbial
345 survival was the elevation of the feed flow rate from 4 (I experimentation) to 8 (III
346 experimentation). This factor, as also evidenced by other authors (Behboudi-Jobbehdar et al.,
347 2013) probably causes a reduction in the droplets' surface temperature, which determine changes
348 in both heat and water diffusivity (Barbosa-Canovas, Ortega-Rivas, Juliano & Yan, 2005),
349 consequently reducing the physical damage to the cell membranes.

350 Then, freeze-dried sourdough (opportunistically thawed) and all the sourdough powders were used as
351 direct inoculation in dough leavening experiments waiting from 4 to 8 h for leavening.
352 Unfortunately, all the stabilized sourdoughs, both freeze and dried/freeze-dried/spray-dried
353 sourdoughs, had a relatively long lag phase before onset of growth, determining a delay in
354 leavening of the dough. Also for the spray-dried sourdoughs, despite the high cell density of lab
355 and yeasts occurred respectively of 7.9 ± 0.1 and 5.7 ± 0.2 log/cfu/g, the leavening ability of
356 surviving microbial cells was compromised following rehydration and reactivation.

357 Our experiments highlighted, as also showed by other authors (Lattanzi, Minervini & Gobbetti,
358 2014), that the dried/freeze-dried/spray-dried starter sourdoughs cannot be used for direct

359 inoculation in breadmaking fermentations probably due to the reduced microbial counts as well
360 as to the injured and stressed metabolic activity. For this reason, in subsequent breadmaking
361 experiments a refreshment of the injured sourdoughs was indispensable and a microbial
362 rehydration procedure was optimized.

363

364 **3.3 Sourdough rehydration**

365 Rehydration is considered to be a critical step in the recovery of starter culture powder. The
366 solution used for rehydration and the rehydration conditions may affect the survival of dried
367 microbial cultures. As described in the section 2.3, five different rehydratation conditions were
368 assessed. The powders were left to absorb physiological solution slowly for 10 min, mixed
369 gently to form a slurry, and left undisturbed for a further 45 min. The best rehydration procedure
370 consisted in mixing the treated sourdoughs with equivalent part of wheat flour plus sucrose (1%)
371 in physiological solution (FSUC). In fact, yeast and lab loads obtained in this condition were
372 respectively of about 0.9 ± 0.21 and 1.1 ± 0.13 log cfu/g higher than those reported by other
373 conditions.

374 So, new breadmaking experiment were carried out using rehydrated sourdough powders. In
375 detail, the sourdough powders were refreshed and left to ferment for 20-24h. Afterthat, the
376 mature doughs were kneaded and the doughs took about 4-5 h at 28°C to leaven. After that the
377 leavenend doughs were baked. Differently from other works, in this research, any commercial
378 baker's yeast was used for breadmaking manufacture using spray-dried sourdough.

379

380 **3.4 Sensory analysis of breads**

381 Results of sensory analysis of different breads are showed in Fig. 3.

382 Samples rSDS (refreshed spray-dried sourdough) and rFS (refreshed freeze sourdough) showed
383 good overall quality, very similar to Control sample (C), FS (freeze sourdough), rDS (refreshed
384 dried sourdough) and rFDS (refreshed freeze-dried sourdough) samples, instead, showed reduced

385 volume. The sensory scores showed that not all the bread samples were acceptable. In particular
386 FS, rDS and rFDS samples had the lowest scorers of texture (2.1 ± 0.56 and 4.5 ± 0.51) and
387 appearance (3.0 ± 0.52 and 5.0 ± 0.22) compared to the other samples, depending by a reduced
388 development of the dough and by a low leavening ability of the sourdough starter.

389 Sensory evaluation of the breads revealed significant differences regarding flavour, texture and
390 appearance between Control (C) and FS, rDS and rFDS breads ($*P < 0.05$). Instead, not
391 significant differences were observed between Control (6.0 ± 0.00), rSDS (5.6 ± 0.40) and rFS
392 (5.4 ± 0.65) samples respect to overall quality.

393

394 **3.5 Image acquisition and digital images analysis**

395 Digital images analysis was performed on the eighteen bread slices images of different bread
396 samples (three images for each sample) showed in Fig. 4. Samples C, rSD and rFS showed
397 homogeneous textures with sample rFS being slightly finer than other two samples. Sample FS
398 and rDS are an example of a non-uniform open crumb structure, evidencing a more reduced
399 ability of sourdough starter to leaven the dough.

400 To analyze the porosity and distribution of the alveolar structure of bread slices, different
401 characteristics of alveoli (number, size, dimension) were determined in the slice area. Digital
402 image analysis allowed to record parameters as number of objects, % Objects, Total Area, %
403 Area, Mean Area reported in Fig. 1.

404 Figure 5 reports the results of the % Area of alveoli of crumb of the most important classes
405 (from 1 to 5); Since FS and rDS breads were unleavened and compact (see Fig. 4), due to the
406 reduced leavening activity of the sourdough, only the digital images analysis data of Control,
407 rSDS, rFS and rFDS were considered for graphical representation. Graph (Fig. 5) highlighted
408 that Control and rSDS had the same profile showing that the spray-dried sourdough allowed to
409 obtain a similar porosity of the crumb structure. In fact, alveolature was represented by 80%
410 alveoli of Class 1 ($0.1-14 \text{ mm}^2$) and about 15% alveoli of Class 2 ($14.0-28.0 \text{ mm}^2$), whereas FS

411 and rFDS had alveolature more dissimilar having more percentage of alveoli of classes 3, 4 and
412 5.

413

414 **4. Conclusion**

415 Spray drying technique, highly used to storage pure culture starter, was used in this study to
416 preserve sourdough. This application is very promising and interesting, and its use allowed the
417 obtainment of sourdough characterized by a major vitality of both yeasts and LAB when
418 compared to the traditional techniques. Breadmaking experiments with the sourdough starter
419 treated in the different way, highlighted that spray-dried sourdough, opportunely refreshed, can
420 be successfully used for breadmaking purpose, avoiding use of baker's yeast activator, leading to
421 bread with sensory characteristics comparable to those produced using fresh sourdoughs. Digital
422 image analysis, able to attain and generate suitable data or elements beyond the scope of the
423 human perception, permitted to define objectively the internal appearance characteristics of the
424 slice of breads.

425

426 **References**

427 AOAC International. (2000). Official methods of analysis of AOAC International. 17th edition,
428 AOAC International, Gaithersburg, MD, USA.

429 Atalar, Y., & Dervisoglu, M. (2015). Optimization of spray drying process parameters for kefir
430 powder using response surface methodology. *LWT - Food Science and Technology*, 60, 751-757.

431 Barbosa, J., Brandao, T.R.S., & Teixeira, P. (2017). Spray drying conditions for orange juice
432 incorporated with lactic acid bacteria. *International Journal of Food Science & Technology*, 52,
433 1951-1958.

434 Barbosa-Canovas, G.V., Ortega-Rivas, E., Juliano, P. & Yan, H. (2005). Food Powders: Physical
435 Properties, Processing and Functionality; Kluwer Academic: New York.

- 436 Behboudi-Jobbehdar, S., Soukoulis, C., Yonekura, L., & Fisk, I. (2013). Optimization of spray-
437 drying process conditions for the production of maximally viable microencapsulated *L.*
438 *acidophilus* NCIMB 701748. *Drying Technology*, *31*, 1274-1283.
- 439 Caboni, M.F., Boselli, E., Messia, M.C., Velazco, V., Fratianni, A., Panfili, G. & Marconi E.
440 (2005). Effect of processing and storage on the chemical quality markers of spray-dried whole
441 egg. *Food Chemistry*, *92*, 293–303.
- 442 Chavan, R. S., & Chavan, S. R. (2011). Sourdough technology-A traditional way for wholesome
443 foods: a review. *Comprehensive Reviews in Food Science and Food Safety*, *10*, 170-183.
- 444 Chàvez, B. E., & Ledebøer, A. M. (2007). Drying of probiotics: optimization of formulation and
445 process to enhance storage survival. *Drying Technology*, *25*, 1193–1201.
- 446 Corsetti, A, & Settanni, L. (2007). Lactobacilli in sourdough fermentation. *Food Research*
447 *International*, *40*, 539-558.
- 448 Decock P., & Cappelle S. (2005) Bread technology and sourdough technology. *Trends in Food*
449 *Science & Technology*, *16*, 113–120.
- 450 De Vuyst, L., Van Kerrebroeck, S., Harth, H., Huys, G., Daniel, H.-M., & Weckx, S. (2014).
451 Microbial ecology of sourdough fermentations: Diverse or uniform? *Food Microbiology*, *37*, 11-
452 29.
- 453 Huang S., Vignolles M., Chen X.D., Le Loir Y., Jan G., Schuck P., & Jeantet R. (2017). Spray
454 drying of probiotics and other food-grade bacteria: A review. *Trends in Food Science &*
455 *Technology*, *63*, 1-17.
- 456 Golshan Tafti, A., Peighamardoust, S. H., Behnam, F., Bahrami, A., Aghagholizadeh, R.,
457 Ghamari, M., & Abbas Rafat, S. (2013a). Effects of spray-dried sourdough on flour
458 characteristics and rheological properties of dough. *Czech Journal of Food Science*, *31*, 361–
459 367.

- 460 Golshan Tafti, A., Peighamardoust, S. H., Hesari J., Bahrami A., & Bonab E.S. (2013b).
461 Physico-chemical and functional properties of spray-dried sourdough in breadmaking. *Food*
462 *Science and Technology International*, 19, 271-278.
- 463 Grabowski, S., Mujumdar, A. S., Ramaswamy, H. S., & Strumillo, C. (1997). Evaluation of
464 fluidized versus spouted bed drying of baker's yeast. *Drying Technology*, 15, 625-634.
- 465 Lavari, L., Ianniello, R., Páez, R., Zotta, T., Cuatrin, A., Reinheimer, J., Parente, E., &
466 Vinderola, G. (2015). Growth of *Lactobacillus rhamnosus* 64 in whey permeate and study of the
467 effect of mild stresses on survival to spray drying. *LWT - Food Science and Technology*, 63,
468 1322-330.
- 469 Lattanzi, A., Minervini, F., & Gobbetti, M. (2014). Assessment of comparative methods for
470 storing type-I wheat sourdough. *LWT - Food Science and Technology*, 59, 948-955.
- 471 Luna-Solano, G., Salgado-Cervantes, MA., García-Alvarado, MA., & Rodríguez-Imenes, G.
472 (2015). Improved viability of spray dried brewer's yeast by using starch (grits) and maltodextrin
473 as processing aids. *Journal of Food Process Engineering* 23, 453-462.
- 474 Marquez-Montes, R., Robles-Olvera V. & Luna-Solano, G. (2007). Metabolic Activity of the
475 Dehydrated Yeast by Spray Drying. *Drying Technology*, 25, 1281-1285.
- 476 Murugesan, R. & Orsat, V. (2012). Spray Drying for the Production of Nutraceutical Ingredients
477 - A Review. *Food Bioprocess Technology*, 5, 3-14.
- 478 Paez, R., Lavari, L., Audero, G., Cuatrin, A., Zaritzky, N., Reinheimer, J., & Vinderola, G.
479 (2013). Study of the effects of spray-drying on the functionality of probiotic lactobacilli.
480 *International Journal of Dairy Technology*, 66, 155-161.
- 481 Peighamardoust, S.H., Golshan Tafti, A., & Hesar, J. (2011). Application of spray drying for
482 preservation of lactic acid starter cultures: a review. *Trends in Food Science & Technology*, 22,
483 215-224.
- 484 Quek, S. Y., Chok, N.K., & Swedlund, P. (2007). The physicochemical properties of spray-dried
485 watermelon powders. *Chemical Engineering and Processing*, 46, 386-392.

- 486 Reale, A., Di Renzo, T., Succi, M., Tremonte, P., Coppola, R., & Sorrentino, E. (2011)
487 Identification of lactobacilli isolated in traditional ripe wheat sourdoughs by using molecular
488 methods. *World Journal Microbiology and Biotechnology*, 27, 237–244.
- 489 Reale, A., Di Renzo, T., Succi, M., Tremonte, P., Coppola, R., & Sorrentino, E. (2013).
490 Microbiological and fermentative properties of baker's yeast starter used in breadmaking.
491 *Journal of Food Science*, 78, M1224-M1231.
- 492 Romano, A., Blaiotta, G., Di Cerbo, A., Coppola, R., Masi, P., & Aponte, M. (2014). Spray-
493 dried chestnut extract containing *Lactobacillus rhamnosus* cells as novel ingredient for a
494 probiotic chestnut mousse. *Journal of Applied Microbiology*, 116, 1632-1641.
- 495 Santivarangkna, C., Kulozik, U., & Foerst, P. (2007) Alternative drying processes for the
496 industrial preservation of lactic acid starter cultures. *Biotechnology Progress*, 23, 302–315.
- 497 Stefanello, R., Machado, A., Cavalheiro, C., Santos, M., Nabeshima, E., Copetti, M., & Fries, L.
498 (2018). Trehalose as a cryoprotectant in freeze-dried wheat sourdough production. *LWT - Food*
499 *Science and Technology*, 89, 510–517.
- 500 Zayed, G., & Roos, Y. H. (2004). Influence of trehalose and moisture content on survival of
501 *Lactobacillus salivarius* subjected to freeze drying and storage. *Process Biochemistry*, 39: 1081–
502 1086.
- 503

504 **FIGURE CAPTIONS**

505 **Fig. 1.** Digital image analysis of a sliced bread.

506

507 **Fig.2.** Survival of LAB and yeasts in sourdoughs after different spray drying conditions (A) and
508 after different storage techniques (B). Survival was expressed as reduction of log (N/N₀) cycles,
509 where N₀ and N are the number of viable cells, respectively, before and after exposure to storage
510 treatment. Letters on plot bars indicate significant differences (Tukey's HSD, * P < 0.05) in
511 survival within lactic acid bacteria (a, b, c, d) and yeasts (A, B, C, D) grown in the different
512 conditions.

513

514 **Fig. 3.** Sensory analysis of breads obtained with sourdoughs stabilized with different techniques.
515 Bread produced with fresh sourdough (Control, C); bread produced with freeze sourdough (FS);
516 bread produced with refreshed freeze sourdough (rFS); bread produced with refreshed dried
517 sourdough (rDS); bread produced with refreshed freeze-dried sourdough (rFDS); f) bread
518 produced with refreshed spray-dried sourdough (rSDS) samples.

519

520 **Fig. 4.** Bread slices used for digital image analysis obtained by breads produced with fresh
521 sourdough (Control); freeze sourdough (FS); refreshed freeze sourdough (rFS); refreshed dried
522 sourdough (rDS); refreshed freeze-dried sourdough (rFDS); refreshed spray-dried sourdough
523 (rSDS) samples.

524

525 **Fig. 5.** Contribution of the different cells dimensional classes to the total alveolate area; *Class 1:*
526 $0.1 < \text{cell size} < 14 \text{ mm}^2$; *Class 2:* $14 < \text{cell size} < 28 \text{ mm}^2$; *Class 3:* $28 < \text{cell size} < 41.9 \text{ mm}^2$;
527 *Class 4:* $41.9 < \text{cell size} < 55.8 \text{ mm}^2$; *Class 5:* $55.8 < \text{cell size} < 69.7 \text{ mm}^2$. Breads produced with

528 fresh sourdough, *Control*; with freeze sourdough, *FS*; with refreshed freeze sourdough, *rFS*; with
529 refreshed spray-dried sourdough, *rSDS*.

530

ACCEPTED MANUSCRIPT

Table 1 –Parameters of three different spray drying experiments

Parameter	Spray drying conditions		
	I	II	III
Inlet Air Temperature	140 °C	130°C	130°C
Outlet Air Temperature	75°C	60°C	54°C
Aspiration rate (maximum capacity 40 m ³ /h)	100%	95%	95%
Feed Flow Rate (mL/min)	4	6	8

Table 2 – Classification of min and max of area (mm²) of objects (alveoli) for each class

Class	Range of Area (mm ²)	
	min	max
1	0.1	14.0
2	14.0	28.0
3	28.0	41.9
4	41.9	55.8
5	55.8	69.7
6	69.7	83.7
7	83.7	97.6
8	97.6	111.5
9	111.5	125.4
10	125.4	139.3

Table 3 – Characteristics of sourdough powders obtained in three different spray drying conditions

Experiment		Signle	Moisture (%)	Ash (% d.m.)	a _w	pH	TTA	Cyclone recovery - Drying yield (%)
I	Fresh Sourdough	I-Control	41.00 ± 0.21 ^a	0.871±0.01 ^a	0.995±0.01 ^a	4.0±0.1 ^{abc}	16.0±0.1 ^{abc}	---
	Spray dried sourdough diluted in physiological 1:2	I-SD	7.56 ± 0.07 ^d	4.493±0.22 ^b	0.202±0.02 ^b	4.0±0.2 ^{abc}	16.2±0.0 ^{abc}	36.3±2.5 ^a
II	Fresh Sourdough	II-Control	42.6 ± 0.05 ^b	0.559±0.02 ^c	0.989± 0.03 ^a	4.1±0.1 ^{abc}	15.8±0.1 ^b	---
	Spray dried sourdough diluted in water 1:2	II-SD	6.23 ± 0.08 ^c	0.814±0.03 ^{ac}	0.199± 0.04 ^b	3.9 ±0.0 ^b	16.5±0.1 ^{abc}	35.5±1.8 ^a
III	Fresh Sourdough	III-Control	43.0 ± 0.14 ^c	0.732±0.04 ^{ac}	0.991± 0.02 ^a	4.3±0.0 ^c	16.1±0.7 ^{abc}	---
	Spray dried sourdough diluted in water 1:5	III-SD	7.09 ± 0.31 ^d	0.866±0.12 ^a	0.200± 0.01 ^b	3.9 ±0.1 ^b	16.7±0.2 ^c	56.0±4.1 ^b

Different superscript letters within a column indicate statistically significant differences (P<0.05) between values

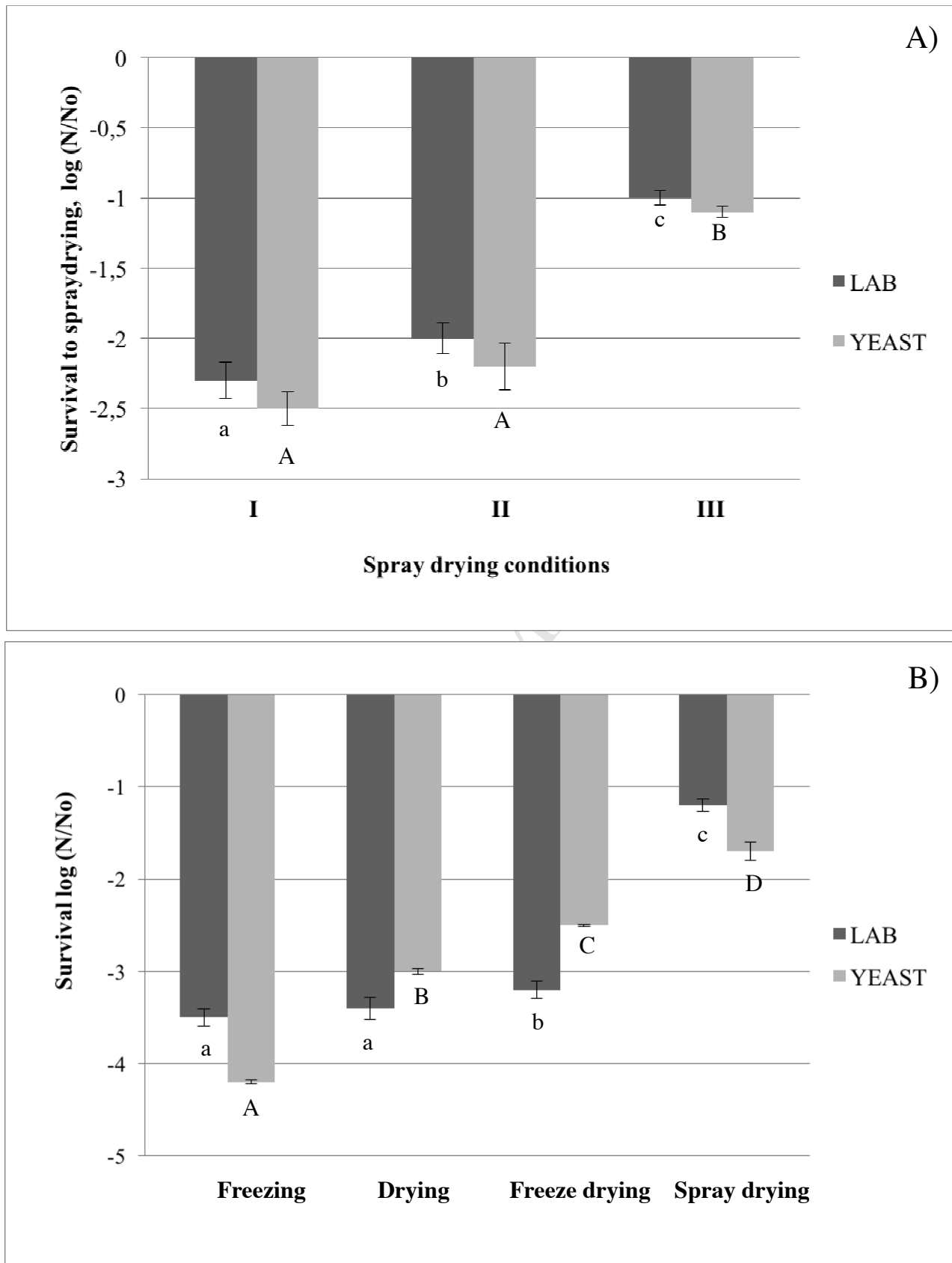


Figure 2

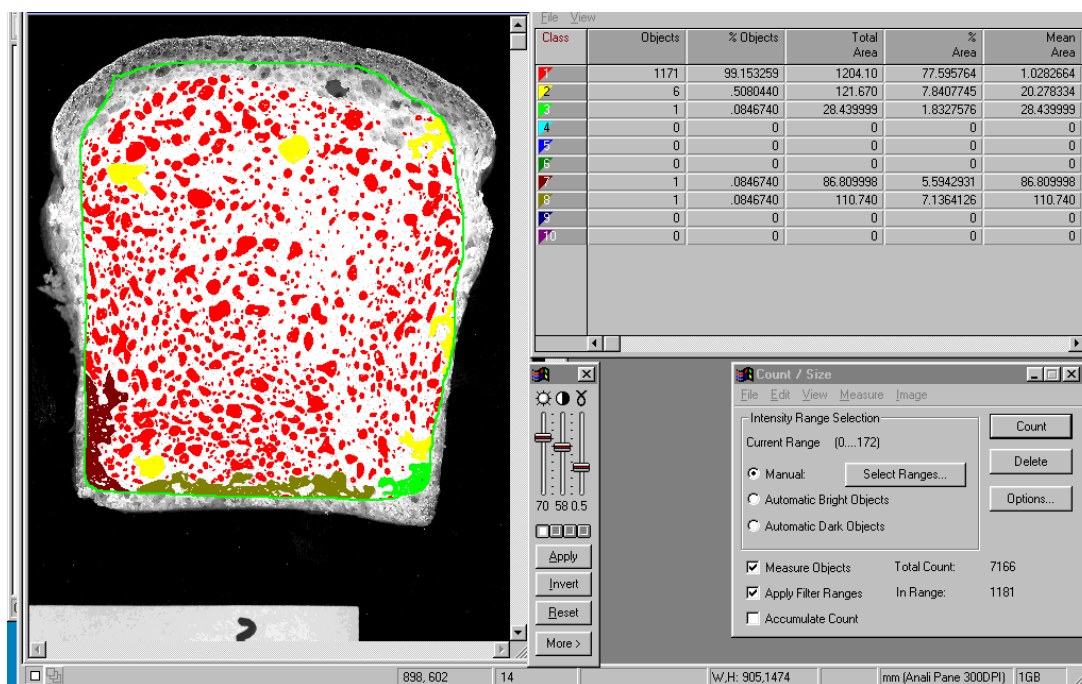


Figure 1.

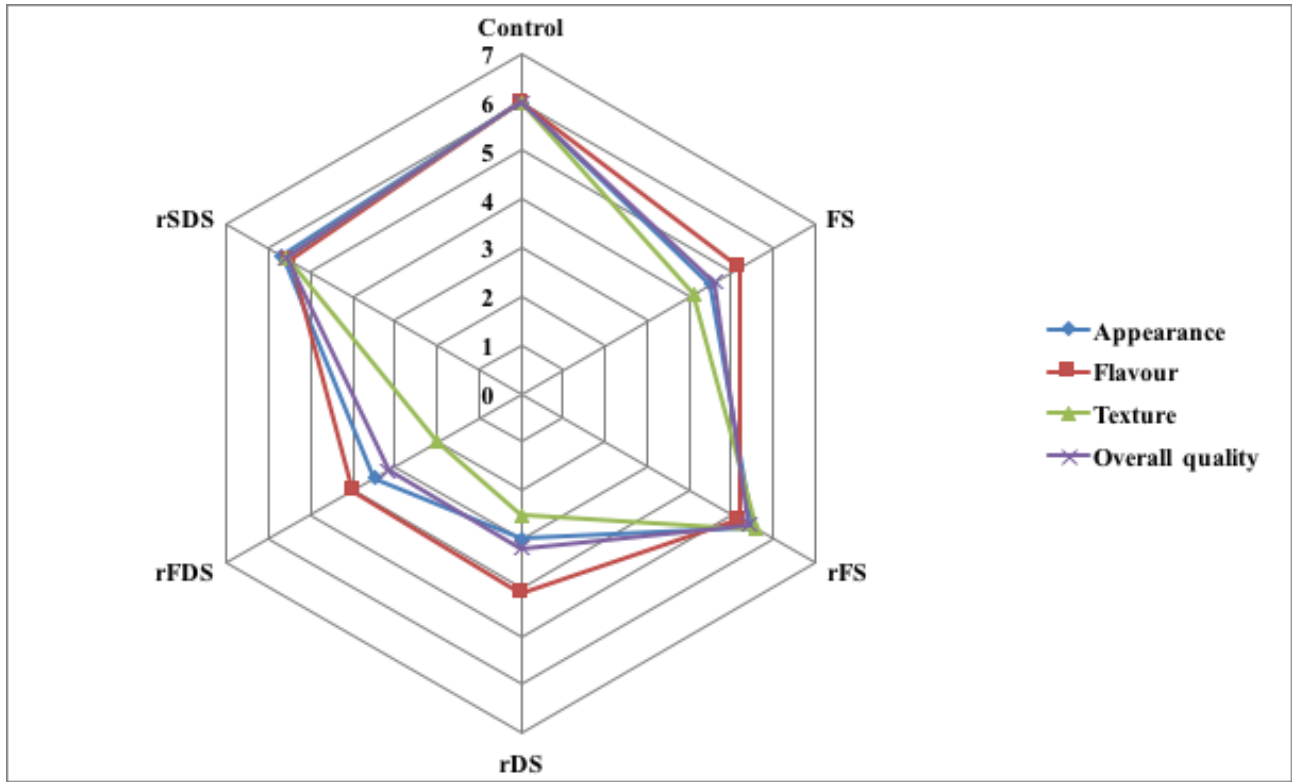


Figure 3



Figure 4

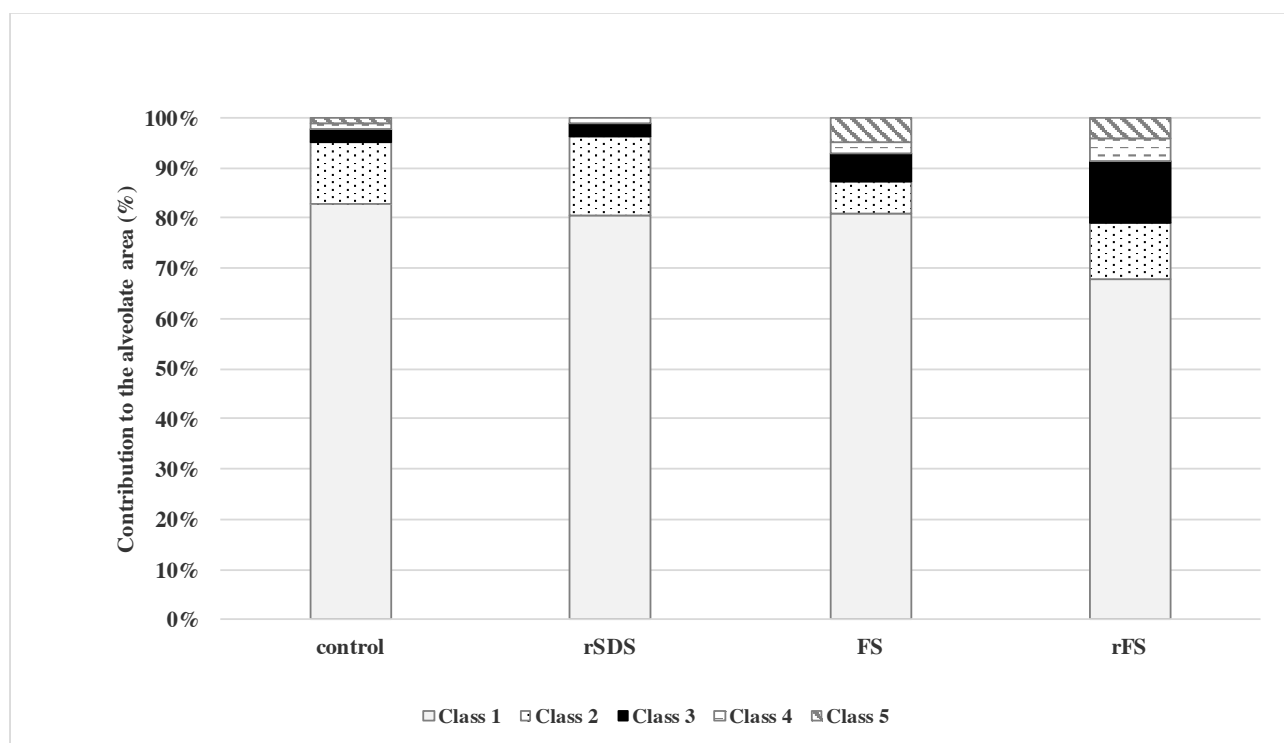


Figure 5

Highlights

1. Spray drying technique preserves good vitality of both yeast and LAB in sourdough
2. Spray drying sourdough can be successfully used in breadmaking, without any added yeast
3. Breads obtained with spray dried sourdough have high sensory characteristics

ACCEPTED MANUSCRIPT