Comparison of biopreservatives obtained from a starter culture of *Pediococcus acidilactici* by different techniques

Ana Teresa Noguerol, Marta Igual, Mª Jesús Pagán*

Universitat Politècnica de València, Food Technology Department, Food Investigation and Innovation Group, Camino de Vera s/n, 46022 Valencia, Spain

**Corresponding author. Tel.:0034963877000 ext.73621; fax: 0034963877369. E-mail address: jpagan@tal.upv.es (M. J. Pagán).*

Abstract

Due to the increase by consumers of healthy products, the exploration of natural antimicrobial compounds has been promoted through the use of by-products of lactic acid bacteria. Therefore, the aim of this research was to obtain an antimicrobial powder (cell free) by freeze-drying (FD) and spray-drying (SD) from the microbial stabilisation (filtration or partial purification) of a *Pediococcus acidilactici* fermentation broths. The antimicrobial activity of these powders was quantified, *in vitro*, against *Listeria innocua* CECT 4032 as a target microorganism. The physicochemical properties tested on these powders were water content, hygroscopicity, water activity, porosity, colour and solubility. As results, microbiological stabilisation is potentially better to perform a partial purification since the antimicrobial capacity against *L. innocua* CECT 4032 is higher than with filtration. On the other hand, SD is the best technique to obtain the powder, since it obtains a better productivity with a lower cost and also a more stable powder during storage.

Keywords: biopreservative; lactic acid bacteria; microbial stabilisation; freeze-drying; spray-drying; physicochemical properties; antimicrobial activity

1. INTRODUCTION

For many years, there has been a growing trend towards the consumption of food without additives or chemical preservatives. In recent years there has been an increase in the interest in biological preservation methods, owing to the consumer demand for minimally processed and fresher food (O'Sullivan et al., 2002; Gálvez et al., 2007). In this context, biopreservation represents an alternative to the use of chemical preservatives, because it is based on the application of antimicrobial metabolites are produced by microorganisms (Reis et al., 2012), which are naturally present in fermented products. In this kind of food, the antimicrobial metabolites are consumed with the microorganisms, which are commonly lactic acid bacteria (LAB). Besides, these microorganisms are capable of producing a variety of antimicrobial substances, like organic acids, hydrogen peroxide, antifungal peptides, and bacteriocins (Du et al., 2017). In general, lactic acid bacteria are recognized as safe for its use in food so due to this most of them have been granted GRAS (Generally Recognized as Safe) status by the US Food and Drug Administration or Qualified Presumption of Safety (Talon & Leroy, 2011) and by with a Qualified Presumption of Safety (QPS, in the EU) (La Storia et al., 2020). So, biopreservation using LAB and/or their antimicrobial metabolites represents an alternative for improving food safety.

In this context, bacteriocin proteins are bacterial metabolites with antimicrobial properties against other species of bacteria (Singh et al., 2015). But, pediococcal bacteriocins are generally have large variations among the bacteriocin peptides and antimicrobial activity, alone or in combination with other peptides (Dey et al., 2019). The incorporation of this metabolites as an ingredient is the commonly used approach. Metabolite preparations used can be any forms ranging from cell free supernatant, partially purified, and purified ones (Woraprayote et al., 2016). In order to take

advantage of all the antimicrobial compounds of the LAB fermentation broth, a stabilisation treatment must be undergone, which stops the fermentation process, and, at the same time, maintains the antimicrobial properties of the bioactive substances unaltered. But, for commercialization and effective use in food preservation, the product should have unique properties and also the purification strategies should be less cost and time demanding (Dey et al., 2019).

Commonly, the MRS (de Man, Rogosa and Sharpe) medium has been considered as suitable for promoting the growth of LAB, but it is expensive for its industrial production (Pandey et al., 2019; Musatti et al., 2020). For this reason, an attractive approach is to use by-products of the agro-food industry in the formulation of culture media.

Nowadays, techniques like freeze-drying (FD) or spray-drying (SD) are a good alternative to obtain powder products. FD is considered as a reference process. The sublimation of ice, coupled with a low process temperature, minimize thermal damage to heat sensitive nutrients (Mastrocola et al., 1997), making it more suitable than SD for some cultures (Gardiner et al., 2000). On the other hand, SD is a well-established and widely used method for transforming a liquid food product into powder form. Besides, SD has been widely used for production of starter cultures and dehydrated probiotic bacteria (Riveros et al., 2009), since the powder obtained can be transported at a low cost and can be stored in a stable form for prolonged periods (Gardiner et al., 2000).

The aim of this research was to obtain an antimicrobial powder (cell free) by FD and SD from the microbial stabilisation (filtration or partial purification) as a by-product of a *Pediococcus acidilactici* fermentation simplified and economical food-grade medium that can be used in meat industry. The antimicrobial activity of these powders was quantified, *in vitro*, against *Listeria innocua* CECT 4032 as a target microorganism.

2. MATERIAL AND METHODS

2.1. Raw materials

The starter microorganisms *Pediococcus acidilactici* was specifically isolated by the company Josefa Estellés Mayor S.L., Lliria, Spain from raw-cured products for use in its meat products. Besides, the fermented broth made with food-grade ingredients (29 g/L of dextrose and 31 g/L of yeast extract) and the dextrin used as support were supplied by the company Josefa Estellés Mayor S.L., Lliria, Spain.

2.2. Microbial stabilisation

With the aim of obtaining a broth enriched in metabolites and without microbial cells a microbial stabilisation was carried out.

In order to obtain the cell free extracts (CFEs), the food grade broth was inoculated with *P. acidilactici* (10^3 CFU/mL) and was fermented for 8 h at 37 ºC. Then, a microbiological stabilisation of this fermented broths (10^8 CFU/mL) was carried out, by two techniques. Filtration (F) was described by De Jesús (2016) with modifications. Firstly, a centrifugation of the fermentation broths was carried out for 20 min at 3,000 x g and at 4 ºC (Eppendorf AG 5804R, Hamburg, Germany). The supernatant was filtered under vacuum conditions using a polyethersulfone (PES) membrane of 0.22 μm (UltraStep membrane, GVS, USA), which, due to its low protein retention, would allow the pass of peptide substances, not retaining any inhibitory activity (Ananou et al., 2010).

Partial purification (PP) was put into practise by adapting the procedures described by Cabo et al. (1999), Guerra & Pastrana (2001) and Ünlü et al. (2016). Thus, the pH of the fermentation broths was adjusted to 3.5 with 5 N HCl (Panreac, Barcelona, Spain) and

allowed to stand for 10 min. Subsequently, a pasteurization was carried out in a thermal bath (Julabo TW20, Seelbach, Germany) at 80 ºC for 10 min. After this time, the samples were cooled in an ice bath for 15 min. Then, they were centrifuged under the same conditions and time as the filtrated samples. Finally, the pH of the supernatant was adjusted to 6.5 with 10 N NaOH (Panreac), to neutralise acids and test the antimicrobial activity of the other different metabolites.

To check the effectiveness of the microbial stabilisation methods, lactic acid bacteria (LAB) count of fermented broths were performed using De Man, Rogosa y Sharpe (MRS) (Scharlau, Barcelona, Spain) incubated at 37 ºC for 48 h.

2.3. Freeze-drying and spray drying conditions

Two processes were used to obtain the powder biopreservative, FD and SD, both were described by De Jesús (2016). For the preparation of the feed mixture of both processes, 10% (w/v) of dextrin was added to CFEs stabilised by both F and PP, and mixed until a total dissolution.

For FD, the samples were placed in sterile glass Petri dishes (diameter 20 cm) (approximately 200 g per Petri dish), and was immediately frozen at -45 ºC for 48 h. Then, the Petri dishes were introduced into a Telstar LyoAlfa-6 freeze dryer (Barcelona, Spain) at 0.051 ± 0.013 mbar pressure and a temperature of -55 ± 3 ºC, for 48 h. The obtained cakes were ground (Minimoka GR-020, Coffemotion S.L., Lleida, Spain).

For SD, the mixture was fed into Büchi B-290 (Switzerland) mini spray dryer with the following operating conditions: inlet temperature 157 ºC; aspirator rate 85% (33 m^3/h); atomization air 414 L/h with a co-current flow; 35% pump rate (10 mL/min). Once the experiment was finished, and when the air inlet temperature fell below 50 ºC, the samples were collected from the product collection vessel.

In both cases, the powder was packed in impermeable plastic bags supplied by Josefa Estellés Mayor S.L., Lliria, Spain and these bags were stored in a cooled incubator FOC 225I (VELP Scientifica, Italy) at 30 ºC, to simulate the normal weather condition in the industry. Two batches were performed, one for physicochemical tests and other for antimicrobial activity analysis.

2.4. Efficiency, drying ratio and productivity.

Efficiency was defined as the ratio of the mass of solutes present in the powder obtained at the end of each SD or FD period, to the mass of solutes present in the mixture prior to SD or FD respectively (Vardin & Yasar, 2012). Drying ratio and productivity for SD and FD were calculated according to Cai & Corke (2000), slightly modified. The drying ratio was calculated by equation (1) (powder solid content / feed solid content).

$$\text{Drying ratio} = \frac{(X_w^i + 1)}{(X_w^f + 1)} \quad (1)$$

where X_w^i is the mixture feed moisture (dry basis), and X_w^f is the powder moisture (dry basis). Productivity (g/h) = feed rate (g/h) / drying ratio. Feed rate was calculated from the mass of mixture feed (g) and the process time (h).

2.5. Physicochemical analysis

The water content (x_w) of the biopreservative powders (g water/100 g sample) was performed by vacuum oven drying (Vaciotem, J.P. Selecta, Spain) at 60 ºC until constant weight (AOAC, 2000).

Water activity (a_w) of the samples was determined using a dew point hygrometer (AquaLab PRE LabFerrer, Pullman, USA).

ºBrix of initial fermentation broths was measured using a refractometer (PAL-BX/RI, Atago, Japan).

154 The hygroscopicity (Hg) was determined using the method described by Cai & Corke
155 (2000). Each sample was weighed seven days later. The hygroscopicity was expressed
156 as g of water gained by the powders.
157 Solubility measurement was realised following the methodology suggested by
158 Benlloch-Tinoco et al. (2013).
159 The porosity determination was described by Agudelo et al. (2016) with some
160 modification. The porosity (ε) was determined from the true and bulk densities
161 (equation 2). The true density (ρ) was calculated from its individual components, water
162 and carbohydrates, own and aggregates (equation 3). For bulk density (ρ_b)
163 determination, about 2 g of the powder were put into a 10 mL graduated test tube and
164 hit 20 times on a firm surface. The bulk density was calculated by dividing the mass of
165 the powder by the volume occupied after hitting.

166 $$\varepsilon = \frac{\rho - \rho_b}{\rho} \quad (2)$$

167 $$\frac{1}{\rho} = \frac{x_w^p}{\rho_w} + \frac{x_{CH}^p}{\rho_{CH}} \quad (3)$$

168 where ε is the porosity; ρ and ρ_b are the true and bulk densities, respectively; χ and ρ
169 are the mass fraction and density, respectively, of water ($_w$) and carbohydrates ($_{CH}$) of
170 the mixture, with ρ (20 ºC) 0.9976 g/mL and ρ_{CH} (20 ºC) 1.4246 g/mL (Okos, 1986).
171 The colour of the powder samples was measured using a Konica Minolta CM-700d
172 colorimeter (Konica Minolta CM-700d/600d series, Tokyo, Japan) with standard D65
173 illuminate and 10º visual angle. The powder was placed in a circular aluminium sample
174 holder of 17.7 mm in diameter and 9.53 mm in height. A reflectance glass (CR-A51,
175 Minolta Camera, Japan) was placed between the sample and colorimeter lens. The
176 measurement window was 6 mm in diameter. The results were express in CIELab
177 system (CIE, 1986). Chroma, C^*_{ab} (saturation), hue angle, h^o_{ab}, and the total colour

178 difference (ΔE) were also calculated. In order to observe the colour differences
179 produced by the stabilisation method used (F or PP), ΔE_1 was determined, and ΔE_2 was
180 performed for the differences produced by the powder extraction process (FD or SD).
181 The samples were analysed by quadruplicate on days 0 and 28, to know the effect of the
182 storage.
183
184 **2.6. Antimicrobial activity**
185 The evaluation of the antimicrobial capacity of the samples was carried out by agar
186 diffusion method (Guerra & Pastrana, 2001; Turcotte et al., 2004). *Listeria innocua*
187 CECT 4032 was used as a target microorganism. After the addition of a concentration
188 of 10^5 CFU/mL of *Listeria innocua* CECT 4032 in plates, 15 mL of Tryptona Soya
189 Agar (TSA) (Sharlau, Barcelona, Spain) were added. Once solidified, 5 wells of 9 mm
190 diameter were made. The wells were inoculated with 100 µL of a dilution obtained from
191 the regeneration of the biopreservative powders in peptone water (1:9, w/v). On each
192 plate, a negative control was performed by adding 100 µL of sterile water to a well. The
193 plates were incubated in an oven at 37 ºC for 18-24 h. After this time, the diameters of
194 the inhibition halos were measured. The results were expressed by calculating the area
195 of inhibition (mm^2). Samples were analysed on days 0, 1, 2, 7, 14 and 28, to know the
196 effect of storage.
197
198 **2.7. Statistical analysis**
199 Analysis of variance (ANOVA), with a confidence level of 95% ($p<0.05$), using
200 Statgraphics Plus 5.1 Software (Statistical Graphics Corporation, USA) was applied to
201 evaluate the differences among powder samples. A factor analysis was applied to
202 physicochemical values of studied samples, using SPSS program version 16.0.

3. RESULTS AND DISCUSSION

In previous studies (data not shown), *P. acidilactici* was fermented in this food grade broth during 24 h at 37 ºC and the antimicrobial activity was tested in stabilised broths by filtration and partial purification. The maximum values of antimicrobial capacity were found at 8 h of fermentation in both stabilised broths (F and PP) (data not shown). So that, in this work the broth was fermented 24 h at 37 ºC and the mean values (with standard deviation in brackets) of ºBrix, LAB count and antimicrobial capacity of this broth stabilised by filtration and partial purification were 13.93 (0.13), 0 CFU/mL, 396 (5) mm^2, and 13.97 (0.06), 0 CFU/mL, 556 mm^2, respectively. Those are the results which were obtained from the broth and will now be compared to the same results once the broth is turned into powder, giving us the data.

There are important differences between the methods of obtaining powders studied in yield term. FD is considered as a benchmark for powders of high quality (Mastrocola et al., 1997), however, the main disadvantage of this technique is its high cost, both in term of time and energy. SD is the most commonly used encapsulation method in the food industry (Rajam & Anandharamakrishnan, 2015). In this work, it can be observed that the SD efficiency was ≈72%, and FD efficiency was significantly ($p<0.05$) higher (≈98%), in both cases without significant differences ($p>0.05$) between the microbial stabilisation methods. The SD efficiency is satisfied because a successful spray-drying must have efficiency higher than 50% according to Tontul & Topuz (2017). On the other hand, the values of drying ratio and productivity in SD were significantly ($p<0.05$) higher than FD values. Whilst FD showed 1.85 and 1.88 g/h of productivity for FDF and FDPP respectively, SDF and SDPP showed 75 and 48 g/h. The microbial stabilisation by filtration in SD technique presented significantly higher productivity

than partial purification probably as a consequence of the higher fluency of SDF due to low protein retention such as peptide substances. The properties of spray-dried powders and its yield are mainly affected by the process conditions and one of the important factors is the feed properties (Igual el at., 2014). Once the powders were obtained, they were characterised in terms of their physicochemical properties and their stability during the storage.

Table 1 shows the mean values of physicochemical parameters studied. The values of water activity presented significant ($p<0.05$) differences between the samples. As it is shown that the sample FDF showed more a_w in comparison with the remaining samples. The water content of FD powders varied between 2.51-3.02 g water/100 g, which are normal values for a freeze-dried product according to Benlloch-Tinoco et al., (2013). The water content of SD powders varied between 0.4-0.52 g water/100 g. As indicated by Tontul & Topuz (2017), the water contents of the powder produced by spray-drying were generally lower than 5% and could be classified as microbiologically safe and can be stored for long-term. Significant ($p<0.05$) differences can be seen between FD or SD powders. The hygroscopicity (Hg) of powders was significantly ($p<0.05$) higher in those obtained by FD in comparison with SD. Generally, food powders with lower hygroscopicity and water content are considered a good powdered product (Igual et al., 2014). The bulk density was higher in the samples obtained by FD. Because of this, these samples showed a lower porosity. Significant ($p<0.05$) differences in bulk density and porosity were observed between the powders obtain methods (FD or SD). In both cases without significant differences between the microbial stabilisations. A greater porosity corresponds to a more free-flowing powder (Agudelo et al., 2016). For the solubility (DS) values, no differences were observed in the stabilisation method nor in the obtaining of the powders, so all the samples being equal, showing an excellent

253 solubility as the results showed by Benlloch-Tinoco et al., (2013) in FD kiwifruit.
254 Moreover, higher solubility is desirable especially when the obtained powder used as an
255 additive in the production of different products (Tontul & Topuz, 2017). Table 1 also
256 includes the physicochemical mean values of the samples at the end of storage. In
257 general, significant ($p<0.05$) changes were observed in water activity and water content
258 showed an increase. In case of the SDPP sample, water activity was stable during
259 storage. At the end of storage, the samples with the lowest water content were those
260 obtained by SD. As for hygroscopicity a significant ($p<0.05$) decrease was observed in
261 all samples tested at the end of storage. For bulk density and porosity only significant
262 ($p<0.05$) differences were observed during storage in the case of FDPP. No differences
263 were observed on values of solubility during storage, so it was stable. The water content
264 of the powdered products is related to drying efficiency, playing an important role in its
265 free-flowing behaviour and stability during storage (Santhalakshmy et al., 2015).
266 Therefore, powders with a low x_w, Hg and a high ε and productivity would be preferred,
267 so powders with these characteristics were obtained by SD.
268 Table 2 presents the mean values of colour parameters. In general, all studied samples
269 showed significant ($p<0.05$) differences among them in colour terms. As it can be
270 appreciated that powder obtained by FD showed less lightness (L*) and both, more
271 shades of red (a*) and yellow (b*) in comparison with samples obtained by SD. This
272 trend in lightness was observed in grapefruit powder when comparing these techniques
273 (Agudelo et al., 2016). The SD powder's tone was higher than FD powders whilst
274 chrome showed the contrary trend. Table 2 includes the total colour differences for each
275 stabilised method (PP or F) as a function of powder technique obtaining (ΔE_1) and total
276 colour differences too between PP and F for FD or SD (ΔE_2). Both PP and F showed
277 colour differences between studied drying techniques to obtain powders, being

278 significantly ($p<0.05$) higher in F stabilisation. However, for FD or SD, the total colour
279 differences between PP and F were below the perceptible sensory limit ($\Delta E>3$)
280 according Bodart et al. (2008) without significant ($p>0.05$) differences between them.
281 Figure 1 shows the colour changes of obtained powders along storage time. At the end
282 of storage, the colour of the samples slightly faded, above all FDPP (Figure 1.A).
283 However, FD powders suffered an increase of a* and b* due to storage time and, for SD
284 powders, a* and b* remained stables (Figures 1.B and 1.C). The total colour changes
285 (Figures 1.D) that took place throughout the storage in FD samples were significant
286 ($p<0.05$) higher than these in SD samples which were below the perceptible sensory
287 limit (Bodart et al., 2008). In case of FD samples, the use of F stabilisation showed
288 minor colour changes than the use of PP. According to colour results, SD allows for
289 obtaining powders more stably during storage time.
290 On applying a factor analysis (Figures 2 and 3) to the values of analysed
291 physicochemical properties (except solubility which not showed significant differences
292 among any sample) corresponding to all the powder samples at the initial and the end of
293 storage times, the first two factors showed eigenvalues of over 1. The consideration of
294 both factors accounted for 90.92% of the total variability (Figure 2). The first factor
295 (F1), explaining 73.12% of the variability, was associated with colour coordinates (L*: r
296 = 0.98; a*: r = 0.96; b*: r = 0.99; h: r = 0.97; C: r = 0.99), porosity (r = 0.97), bulk
297 density (r = 0.97) and water content (r = 0.80) values. The second factor (F2) accounted
298 for 17.8% of the variability and it was mainly associated with hygroscopicity (r = 0.90)
299 and water activity (r = 0.83) values. L*, h* and ε maintained a close relationship, whose
300 trend is unlike the rest of the parameters associated with F1. The relation between water
301 activity and hygroscopicity in the case of F2 is opposite. It can be observed in Figure 3,
302 F1 separate clearly powders obtained by FD and SD. FD samples show higher values of

303 a*, b*, C*, bulk density and water content and lower values of L*, h* and porosity.
304 However, SD samples present the values of physicochemical properties associated to F1
305 contraries to FD. On the other hand, F2 increase during storage in all cases. At the end
306 of storage, powders showed higher values of water activity and lower values of
307 hygroscopicity. Only FD samples suffered changes in physicochemical properties
308 associated to F1 consequently to storage whilst SD samples remained stables.
309 While authors like Gardiner et al. (2000) indicated that FD is more suitable than SD for
310 some cultures, although other research had not found differences between these
311 methods. As human listeriosis is one of the most serious foodborne diseases under
312 European Union (EU) (Escolar et al., 2017) and some authors have described the use of
313 different metabolites producing by starter cultures could be used to inhibit the
314 outgrowth of *L. monocytogenes* in raw meat (Aymerich et al., 2000). So that, in this
315 study *L. innocua* CECT 4032 was selected as the target microorganism. Moreover,
316 Mauriello et al. (1999) and Silva et al. (2002) indicated that SD does not affect the
317 antagonist activity of some LABs, in this case *P. acidilactici* against *L. innocua* CECT
318 4032.
319 Results of the antimicrobial activity of samples tested against *L. innocua* CECT 4032
320 during the storage are shown in Table 3. For day 0, significant ($p<0.05$) differences
321 were observed between samples, the sample with the highest antimicrobial activity was
322 FDPP, with a value of inhibition of 581 mm^2. The sample with the lowest antimicrobial
323 activity (480 mm^2) was FDF. In addition, there was a significant ($p<0.05$) increase in
324 the antimicrobial activity of samples F regardless of the obtaining process of the
325 powder. Regarding to the antimicrobial activity evolution during storage, it can be
326 observed in Table 3 that samples FDPP and SDF experiment a decrease during storage,

327 remaining the latest day with less antimicrobial activity, 480 mm^2 and 476 mm^2,
328 respectively.
329 This may be due to the fact that these samples also presented higher water content and
330 water activity, so their antimicrobial activity may have been diminished. Moreover,
331 rehydration could also cause organisms to lose viability because they may have suffered
332 sublethal injury during drying and storage, and may not be able to repair the damage if
333 they are rehydrated under inappropriate conditions (Costa et al., 2000). The
334 antimicrobial activity of sample FDF remained stable for 28 days. Although significant
335 ($p<0.05$) differences were presented, these can be produced to the variability of the
336 inhibition areas, but no significant ($p>0.05$) differences were observed between the first
337 and the latest day of storage. Similar to some results indicated by Ananou et al. (2010)
338 in SD enterocin AS-48 at room temperature. As for the sample SDPP, it can be
339 observed an increase in the antimicrobial activity. As indicated by O'Bryan et al.
340 (2015), pediocins have a narrow spectrum of activity; all pediocins are active against
341 *Listeria*. The values of inhibition at days 0 and 28 were 543 mm^2 and 556 mm^2
342 respectively and significant ($p<0.05$) differences were shown between these days.
343 Besides, this sample (SDPP) presented the highest antimicrobial activity the day 28 of
344 storage. Thus, considerable research has been conducted on preservation by SD as a
345 means of preserving starter cultures and probiotic products, and also packaging, storage
346 conditions, and method of rehydration also affect viability and function (O'Bryan et al.,
347 2015). So, these results are an evidence of the antimicrobial capacity against *Listeria*
348 *innocua* CECT 4032, stabilising by PP and obtaining powders by SD, but investigating
349 the effect of these by-products against other pathogens should be tested in future
350 research.
351

4. CONCLUSIONS

Microbiological stabilisation is potentially better to perform a partial purification since the antimicrobial capacity against *L. innocua* CECT 4032 is higher than with filtration. However, it should be tested against other pathogens to verify its possible use as biopreservative in food. Spray-drying is the best technique to obtain the powder, since it obtains a better productivity with a lower cost and also a more stable powder during storage, both in its physicochemical properties and in antimicrobial capacity.

5. CONFLICT OF INTEREST

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

6. ACKNOWLEDGMENTS

No outside funding was received to perform this study, but we would like to thank Josefa Estellés Mayor S.L. (Lliria, Spain) company for supplying the necessary material and Helen Warburton for checking the English spelling.

7. REFERENCES

Agudelo, C., Igual, M., Camacho, M. M., & Martínez-Navarrete, N. (2016). Effect of process technology on the nutritional, functional, and physical quality of grapefruit powder. *Food Science and Technology International*, 23(1), 61-74.

Ananou, S., Muñoz, A., Martínez-Bueno, M., González-Tello, P., Gálvez, A., Maqueda, M., & E. (2010). Evaluation of an enterocin AS-48 enriched bioactive powder obtained by spray drying. *Food microbiology*, 27(1), 58-63.

AOAC. (2000). Official Methods of Analysis. Association of Official Analytical Chemist. *Volume I, agricultural chemicals, contaminants, drugs / edited by William Horwitz.* EUA.

Commission Internationale de l'Eclairage (CIE). (1986). Colorimetry. 2nd Edition, Publication CIE No. 15.2. Commission Internationale de l'Eclairage, Vienna.

Aymerich, T., Garriga, M., Ylla, J., Vallier, J., Monfort, J. M., & Hugas, M. (2000). Application of enterocins as biopreservatives against Listeria innocua in meat products. *Journal of food protection*, *63*(6), 721-726.

Benlloch-Tinoco, M., Moraga, G., Camacho, M. M., & Martínez-Navarrete, N. (2013). Combined drying technologies for high-quality kiwifruit powder production. *Food and Bioprocess Technology*, *6*(12), 3544-3553.

Bodart, M., De Peñaranda, R., Deneyer, A., & Flamant, G. (2008). Photometry and colorimetry characterisation of materials in daylighting evaluation tools. *Building and Environment*, *43*(12), 2046-2058.

Cabo, M., Murado, M., González, M., & Pastoriza, L. (1999). A method for bacteriocin quantification. *Journal of applied microbiology*, *87*, 907-914.

Cai, Y. Z., & Corke, H. (2000). Production and properties of spray-dried amaranthus betacyanin pigments. *Journal of food science*, *65*(7), 1248-1252.

Costa, E., Usall, J., Teixido, N., Garcia, N., & Vinas, I. (2000). Effect of protective agents, rehydration media and initial cell concentration on viability of Pantoea agglomerans strain CPA-2 subjected to freeze-drying. *Journal of Applied Microbiology*, *89*(5), 793-800.

De Jesús, C. (2016). Producción y recuperación de sustancias bioconservantes a partir de cultivos iniciadores de productos cárnicos curados. Ph.D. Thesis. Universitat Politècnica de València, Spain, 257pp.

Dey, B. C., Rai, N., Das, S., Mandal, S., & Mandal, V. (2019). Partial purification, characterization and mode of action of bacteriocins produced by three strains of Pediococcus sp. *Journal of food science and technology*, *56*(5), 2594-2604.

Du, L., Liu, F., Zhao, P., Zhao, T., & Doyle, M. P. (2017). Characterization of Enterococcus durans 152 bacteriocins and their inhibition of Listeria monocytogenes in ham. *Food microbiology*, *68*, 97-103.

Escolar, C., Gómez, D., del Carmen Rota García, M., Conchello, P., & Herrera, A. (2017). Antimicrobial resistance profiles of Listeria monocytogenes and Listeria innocua isolated from ready-to-eat products of animal origin in Spain. *Foodborne pathogens and disease*, *14*(6), 357-363.

Gálvez, A., Abriouel, H., López, R. L., & Ben Omar, N. (2007). Bacteriocin-based strategies for food biopreservation. *International journal of food microbiology*, *120*(1-2), 51-70.

Gardiner, G. E., O'sullivan, E., Kelly, J., Auty, M. A. E., Fitzgerald, G. F., Collins, J. K., Ross R. P. & Stanton, C. (2000). Comparative survival rates of human-derived probioticlactobacillus paracasei and l. salivarius strains during heat treatment and spray drying. *Applied and environmental microbiology*, *66*(6), 2605-2612.

Guerra, N., & Pastrana, L. (2001). Enhanced nisin and pediocin production on whey supplemented with different nitrogen sources. *Biotechnology Letters*, *23*, 609–612.

Igual, M., Ramires, S., Mosquera, L. H., & Martínez-Navarrete, N. (2014). Optimization of spray drying conditions for lulo (Solanum quitoense L.) pulp. *Powder Technology*, *256*, 233-238.

La Storia, A., Di Giuseppe, F. A., Volpe, S., Oliviero, V., Villani, F., & Torrieri, E. (2020). Physical properties and antimicrobial activity of bioactive film based on whey protein and Lactobacillus curvatus 54M16 producer of bacteriocins. *Food Hydrocolloids*, *108*, 105959.

Mastrocola, D., Dalla Rosa, M., & Massini, R. (1997). Freeze-dried strawberries rehydrated in sugar solution: mass transfers and characteristics of final products. *Food Research International*, *30* (5), 359–364.

Mauriello, G., Aponte, M., Andolfi, R., Moschetti, G., & Villani, F. (1999). Spray-drying of bacteriocin-producing lactic acid bacteria. *Journal of food protection*, *62*(7), 773-777.

Musatti, A., Cavicchioli, D., Mapelli, C., Bertoni, D., Hogenboom, J. A., Pellegrino, L., & Rollini, M. (2020). From Cheese Whey Permeate to Sakacin A: a circular economy approach for the food-grade biotechnological production of an anti-Listeria bacteriocin. *Biomolecules*, *10*(4), 597.

O'Bryan, C.A., Crandall, P.G., Ricke, S.C. & Ndahetuye, J.B. (2015). Lactic acid bacteria (LAB) as antimicrobials in food products: types and mechanisms of action. In Journals & Books, Elsevier Ltd., *Handbook of Natural Antimicrobials for Food Safety and Quality* (pp. 117-136). Woodhead Publishing.

O'Sullivan, L., Ross, R. P., & Hill, C. (2002). Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. *Biochimie*, *84*(5-6), 593-604.

Okos. (1986). Physical and chemical properties of food. Michigan: American Society of Agricultural Engineers, pp. 35–77.

Pandey, K. K., Sood, S. K., Verma, S. K., Kumar, S., Rani, S., & Ganguli, S. (2019). Bioutilization of paneer whey waste for production of paneer making powder containing pediocin PA-1 as a biopreservative to enhance shelf life of paneer. *LWT*, *113*, 108243.

Rajam, R. & Anandharamakrishnan, C. (2015). Microencapsulation of Lactobacillus plantarum (MTCC 5422) with fructooligosaccharide as wall material by spray drying. *LWT – Food Science and Technology 60*: 773–780.

Reis, J. A., Paula, A. T., Casarotti, S. N., & Penna, A. L. B. (2012). Lactic Acid Bacteria Antimicrobial Compounds: Characteristics and Applications. *Food Engineering Reviews*, *4*(2), 124-140.

Riveros, B., Ferrer, J., & Borquez, R. (2009). Spray drying of a vaginal probiotic strain of Lactobacillus acidophilus. *Drying Technology*, *27*(1), 123-132.

Santhalakshmy, S., Bosco, S. J. D., Francis, S., & Sabeena, M. (2015). Effect of inlet temperature on physicochemical properties of spray-dried jamun fruit juice powder. *Powder Technology*, *274*, 37-43.

Silva, J., Carvalho, A. S., Teixeira, P., & Gibbs, P. A. (2002). Bacteriocin production by spray-dried lactic acid bacteria. *Letters in Applied Microbiology*, *34*(2), 77-81.

Singh, M., Smith, J. & Bailey, M. (2015). Using natural antimicrobials to enhance the safety and quality of poultry. In Journals & Books, Elsevier Ltd., *Handbook of Natural Antimicrobials for Food Safety and Quality* (pp. 375-401). Woodhead Publishing.

Talon, R., & Leroy, S. (2011). Diversity and safety hazards of bacteria involved in meat fermentations. *Meat science*, *89*(3), 303-309.

Tontul, I., & Topuz, A. (2017). Spray-drying of fruit and vegetable juices: effect of drying conditions on the product yield and physical properties. *Trends in Food Science & Technology, 63,* 91-102.

Turcotte, C., Lacroix, C., Kheadr, E., Grignon, L., & Fliss, I. (2004). A rapid turbidometric microplate bioassay for accurate quantification of lactic acid bacteria bacteriocins. *International journal of Food Microbiology, 90,* 283-293.

Ünlü, G., Nielsen, B., & Ionita, C. (2016). Inhibition of Listeria monocytogenes in hot dogs by surface application of freeze-dried bacteriocin-containing powders from lactic acid bacteria. *Probiotics and antimicrobial proteins, 8*(2), 102-110.

Vardin, H., & Yasar, M. (2012). Optimisation of pomegranate (Punica Granatum L.) juice spray-drying as affected by temperature and maltodextrin content. *International journal of food science & technology*, *47*(1), 167-176.

Woraprayote, W., Malila, Y., Sorapukdee, S., Swetwiwathana, A., Benjakul, S., & Visessanguan, W. (2016). Bacteriocins from lactic acid bacteria and their applications in meat and meat products. *Meat science*, *120*, 118-132.