

Spray-Drying Encapsulation of Probiotics for Ice-Cream Application

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Ice-cream is generally considered as a nutritive food, representing an interesting vehicle for delivering beneficial microorganisms to consumers. Many ice-cream makers use industrial dry bases for its preparation. The aim of this study was to investigate the possibility of producing spray-dried probiotic formulations to be used for partial substitution of the commercial ice-cream bases.

A commercial cream base was selected for the study and, according to its composition and literature, two different formulations (FA and FB) were investigated. FA consisted in 46 % commercial skim milk powder, 24 % anhydrous glucose, 28 % maltodextrin (Maltrin® 40) and 2 % sodium alginate. FB contained prebiotic inulin fiber (Fibruline® instant) instead of Maltrin®. Powders were obtained with a lab-scale spray dryer dissolving each formulation in water at a 10 % w/v, with a 6 mL/min flow rate, 150 °C inlet temperature and increasing feed volume (100, 200, 300, 400 mL). The process gave a total dry solids yield ranging from 62.07 % (with 100 mL feed volume) to 58.14 % (with 400 mL) for FA, and from 65.55 % to 59.46 % for FB. Powder a_w was always < 0.3 .

Probiotic-enriched bases were obtained using FA and FB for encapsulation of a strain of *Lactobacillus paracasei*. Considering a recommended probiotics minimum daily intake of 10^9 CFU, a 100 g ice-cream serving, a 5 % substitution of the commercial base with the probiotic-enriched base and supposing no vital loss during the process, the cellular feed concentration for the spray-drying trials was calculated. However, in the presence of probiotics, FA could not be spray dried, while encapsulation with FB gave a 50.89 % yield, 0.42 a_w and 82 % cell mortality. Ice-cream was finally prepared in a domestic ice-cream maker using the probiotic-enriched FB after the pasteurization step and before the 12 h maturation step which caused limited additional mortality.

1. Introduction

Dairy products with incorporated probiotic bacteria are gaining popularity and the probiotics comprise approximately 65 % of the world functional food market (Akalin and Erişir, 2008). Although the application of probiotics in cheeses and especially in fermented milks has been widely explored in the literature, ice-cream is a relatively innovative and apparently suitable matrix for delivering probiotics in human diet because of its pleasant taste and attractive texture. Furthermore, ice-cream is highly accepted product by children, adolescents, and adults, as well as by the elderly public and even though it is more consumed in the summer due to its rather refreshing features, some people have the habit of consuming it throughout the year (Cruz et al., 2009).

Probiotics are live microorganisms that, if administered in adequate amounts, confer health benefits on the host. Therefore, probiotic food can be considered as functional food containing viable probiotic microorganisms which must be able to survive in the gastrointestinal tract. International guide lines, such as the Italian Health Ministry Guide lines on probiotics and prebiotics (2013), indicate a recommended daily intake of 10^9 vital cells (as colony forming units, CFU), even though the exact dosage depends on the selected probiotic, probiotic blend and desired clinical outcome.

Many “hand-made” ice-cream makers use industrial dry bases for product preparation. The aim of this study was, then, to investigate the possibility of producing dried probiotic formulations to be easily used together with commercial ice-cream bases.

Encapsulation is the envelopment of small solid particles, liquid droplets or gases in a coating. It can be successfully applied to entrap natural compounds, like essential oils or vegetal extracts containing antioxidant polyphenols (Spigno et al., 2013) or pigments (De Marco et al., 2013). Microencapsulation (final particle size 1–1000 nm) can also be used to preserve lactic acid bacteria, both starters and probiotics, in food and during the passage through the gastrointestinal tract, contributing to the development of new functional foods (Nazzaro et al., 2012). Probiotic cell concentrates often need to be stored over longer periods prior to food manufacture and ingestion, therefore sometimes they are dried after production. The most common procedure is to produce hydrogel-based microcapsules by extrusion or emulsification processes and, then, freeze dry them. An alternative method to achieve capsule-building and drying in a single step is spray-drying, which is a routine process in the food industry to convert liquids (Heidebach et al., 2012), including also particulate fluids (Oi et al., 2013), into dry powders. A variety of materials can be used as carrier for probiotics encapsulation, such as arabic gum, alginates, maltodextrins, pectins, milk proteins, starch and chitosan among the others. In particular, reconstituted skim milk (RSM) has shown a positive effect on the cellular survival after spray-drying in combination with prebiotics, such as inulin (Fritzen-Freire et al., 2012). Also glucose addition has reduced spray-drying mortality of probiotics (Ying et al., 2012).

Considering the above reviewed literature and having in mind a concept of tailor made encapsulation process, according to which the encapsulating materials should be selected based on the composition of the target food application, this work investigated the possibility of encapsulating probiotic cultures in a formulation with a composition the closest possible to that of the ice-cream base. First, the influence of the use of maltodextrins rather than inulin in combination with RSM, glucose and alginate, and of the feeding volume on the process solids yield and final powder water activity was evaluated. Then, the two tested formulations (with maltodextrins or inulin) were applied for probiotics encapsulation to assess process feasibility and cellular survival. Finally, spray-dried cultures were employed for ice-cream preparation.

2. Materials and Methods

2.1 Preparation of modified ice-cream base formulations

A commercial base for full cream ice-cream preparation (Base Tuttapanna 100, Pernigotti, Italy) was used as reference encapsulating material and for ice-cream preparation. Base ingredients, in decreasing order, are: powder skim milk, powder full cream, dextrose, emulsifiers (E472, E471, E473), anhydrous glucose syrup, maltodextrins, concentrated milk proteins, stabilizers (E401, E410, E412), vanillin, natural flavour.

The commercial base and two different formulations were tested for spray-drying:

1. Formulation A (FA): 46 % commercial skim milk powder (Regilait), 24 % anhydrous glucose (Carlo Erba, Italy), 28 % maltodextrin (Maltrin® 40, Grain Processing Corporation GPC, kindly supplied by LEHVOSS Italia S.R.L.) and 2 % sodium alginate (Carlo Erba, Italy).
2. Formulation B (FB): as FA but with 28 % of prebiotic inulin fiber (Fibruline® instant, kindly provided by COSUCRA, VICTA Food & Trade, Italy) instead of Maltrin®.

All the used materials were characterised for moisture content (by drying at 105 °C until constant weight).

Powders were obtained with a lab-scale spray dryer (Büchi Mini Spray dryer B-290) dissolving each formulation at a 10 % w/v in water, with a 6 mL/min flow rate, 150 °C inlet temperature and increasing feed volume (100, 200, 300, 400 mL). Trials were carried out in triplicate. For each test, outlet temperature was recorded and the collected powder was weighted and analysed for moisture content (by drying at 105 °C until constant weight) and a_w (AquaLab Dew Point Water Activity Meter 4TE).

The total percent dry solids yield was calculated as the ratio of outlet dry matter to the inlet dry matter content.

2.2 Preparation of probiotics enriched formulations

For the preparation of the probiotic functionalised ice-cream the commercial base was substituted with a 5 % of a modified base (as FA or FB) enriched with probiotics (Figure 1). The required cellular concentration for the solutions to be spray-dried was calculated based on the following data or hypotheses:

- No vitality loss occurs during spray-drying;
- A final ice-cream with a 10^8 CFU/g is desired so that a 100 g serving would guarantee the recommended minimum 10^9 CFU daily intake;
- The ice-cream yield is 1350 g / 100 g of base (as evaluated in preliminary trials);
- Spray-drying feeding solution has a 10 % w/v solids of formulation ingredients.

Considering the process yields obtained with FA and FB and the required powder amounts for analysis and ice-cream preparation, it was established to spray-dry 600 mL of solution (Figure 1). Trials were carried out in duplicate and process evaluated as reported in section 2.1.

Probiotic strain *Lactobacillus paracasei* LMG S-27487 (o CBA-L66) was cultured in broth of De Man, Rogosa & Sharp (MRS) medium (Difco, US) at 37 °C in microaerophilic conditions for 18 h. A concentrated culture pellet from 1500 mL MRS was harvested by centrifugation at 8.000 rpm for 15 min, washed twice with sterile distilled saline and finally resuspended in 200 mL of water containing the base. The CFU concentration was evaluated and the suspension was stored at 4 °C until being diluted to 600 mL for spray-drying..

Viable probiotic cells in the suspension and powder were enumerated by decimal dilutions into maximum recovery diluent (MRD) (Difco, US) and plated onto agar MRS medium. Plates were anaerobically incubated at 37 °C for 72 h. The number of colonies for two parallel plates was counted from a dilution yielding 30 to 300 CFU/plate and the average was recorded.

2.3 Ice-cream preparation

Ice-cream was prepared with a domestic ice-cream maker (Il Gelataio ICK 5000, De Longhi, Italy), according to the process of Figure 1. Commercial fresh whole milk and sugars were used. The commercial base has to be used with a “warm process”, which means that it has to be mixed with the other ingredients and pasteurized. Literature (BahramParvar et al., 2012) indicates a flash treatment at 80 °C for 25 sec, followed by high pressure homogenization and maturation at 4 °C for a few hours. The process had to be adapted to laboratory facilities. Milk was pre-heated to 40-45 °C in a water bath. Sugar was added and mixed with a domestic beater (Ariete, Mixy210, 210 W). The mixture was left in the water bath until reaching a 80 °C temperature for 25 sec, rapidly cooled in water-ice bath until 10-12 °C and kept at 4 °C overnight (12 h). The final mixture was used for ice-cream making with a 30 min beating/freezing time, after which it was frozen, stored for 2 days at -18 °C and then analysed for CFU count as reported in section 2.2.

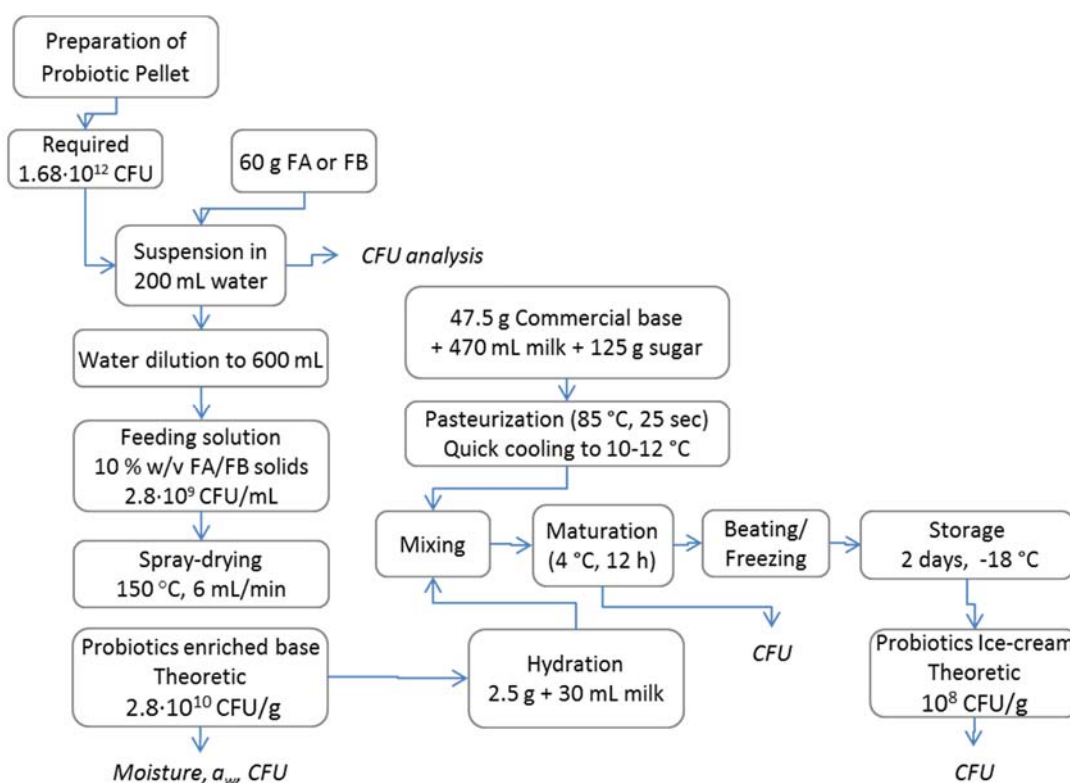


Figure 1: Process scheme for the preparation of probiotics enriched ice-cream

2.4 Statistics

The values are reported as means \pm SD, except for a_w which was measured on one sample for each spray-drying trial set. IBM SPSS® 20.0 (SPSS, Chicago, IL, USA) software for Windows was used to perform

statistical analysis of variance (ANOVA) followed by Tukey's post hoc test (for means discrimination) to assess the significance of variation among the different spray-drying trials. Variance homogeneity was confirmed according to Levene's test. All significance tests were conducted at $P \leq 0.01$.

3. Results and Discussion

3.1 Preparation of modified ice-cream base formulations

The idea behind this research is the development of encapsulation formulations for delivery of functional ingredients, such as probiotics, into food matrixes using materials that are already components of the final product. A commercial base for full cream ice-cream was selected for the study, since a milk-based ice-cream could represent a nutritional complete lunch substitute.

Direct spray-drying of the commercial base dissolved in water, even at a concentration below 10 % w/v, was not possible since no powder could be recovered, probably due to the excessive content of emulsifiers and stabilizers. It was then decided to select only some of the base components taking into account the literature about probiotics encapsulation, as reported in section 1. The specific types of maltodextrins and inulin (Maltrin® 40 and Fibruline®) were suggested by the relative suppliers as the most suitable for ice-cream application. The spray-drying results are reported in Table 1.

Table 1: Results of spray-drying trials. The values followed by different superscript letters in the same column were statistically different according to ANOVA and Tukey's post-hoc test. FA: formulation with Maltrin® 40; FB: formulation with Fibruline®; FBP: FB with probiotics

Feed volume (mL)	Formulation	Total Solids Yield (%)	Powder Dry matter (%)	a_w	T_{outlet}
100	FA	62.07 ± 4.42 ^a	96.23 ± 0.21 ^a	0.2774	65.00 ± 1.73 ^{ab}
200	FA	59.92 ± 2.43 ^{ab}	95.97 ± 0.07 ^a	0.2509	64.50 ± 1.50 ^{ab}
300	FA	60.80 ± 0.34 ^a	96.38 ± 0.54 ^a	0.2413	65.67 ± 0.58 ^a
400	FA	58.14 ± 0.46 ^{ab}	94.34 ± 0.13 ^b	0.2744	65.50 ± 1.50 ^a
100	FB	65.55 ± 5.71 ^a	96.12 ± 0.14 ^a	0.2609	62.00 ± 0.00 ^b
200	FB	60.16 ± 2.14 ^{ab}	96.22 ± 0.27 ^a	0.2482	67.50 ± 0.50 ^a
300	FB	59.15 ± 1.11 ^{ab}	96.04 ± 0.13 ^a	0.2425	65.50 ± 0.50 ^a
400	FB	59.46 ± 0.42 ^{ab}	93.56 ± 0.27 ^b	0.2952	64.50 ± 0.50 ^{ab}
600	FBP	50.89 ± 1.60 ^b	88.72 ± 0.63 ^c	0.4230	61.80 ± 1.50 ^b

Experimental data showed the possibility of obtaining spray-dried powders from both the investigated formulations. The carrier composition did not substantially influence the total solids yield, while a slight decrease with increasing feed volume was observed. This is due to a progressive fouling of the equipment atomizer, which will of course limit the operation time of an hypothetical industrial scale process. The yield values are in the range of literature results, even though higher yields are reported for different process conditions and carrier composition (Fontes et al., 2014). Outlet temperature was almost the same in all the trials and below 70 °C. Residual moisture was not influenced by the formulation, but it was statistically lower when 400 mL were spray-dried. Water activity of all the samples was below 0.3, which is very positive for powder stability since it represents less free water available for biochemical reactions and hence longer shelf-life (Fritzen-Freire et al., 2012).

Visually the two modified bases were similar but slightly different than the original base which is more yellow and fine (Figure 2). The latter difference can be easily overcome with a milling step, while the colour depends on the composition (the original base contains full cream and concentrated milk proteins).

Both the formulations were then tested for probiotics incorporation.

3.2 Preparation of probiotics enriched formulations

The microorganism used in this study is a strain of *L. paracasei*, a species widely employed for probiotic aims in the market of the nutritional integrators. The strain was isolated by AAT from faeces of healthy child and studied for both the safety of use in humans and for its probiotic *in vitro* and *in vivo* efficiency. In particular, according to the EFSA guidance (2012), the strain was tested for antibiotics sensitivity showing to be sensible to the 8 antibiotics indicated by EFSA, which assures its safety for human consumption. Furthermore, the strain revealed to be able to survive after the transit through the gastro-intestinal barrier, and to stimulate dendritic cells (which represents an anti-inflammatory profile).



Figure 2: Original commercial ice-cream base (on the left); modified base with formulation A (in the middle); modified base with formulation B (on the right)

Interestingly, when FA was used, no powder could be collected, indicating interactions between the microbial cells and Maltrin® 40. This might be due to the type of maltodextrin, to the microorganism species or to the specific encapsulating material composition, since other authors have succeeded in spray-drying probiotic cultures with maltodextrins. Ying et al. (2012) encapsulated *L. rhamnosus* GG using whey protein isolates, inulin, glucose and our same Maltrin® 40. Sohail et al. (2012) spray-dried *L. rhamnosus* GG in co-culture with *L. acidophilus* NCFM using sodium alginate and maltodextrin (Fieldose 10C DE 9.8). Even though maltodextrins have been used as a carrier material in many studies, they have a potency to penetrate the cell membrane which is largely dependent on their molecular weight (Semyonov et al., 2010).

The total solids yield in the presence of probiotics was lower (Table 1). This might have been caused by the higher volume of feed solution, and by a slightly higher inlet solid concentration due to the pellet contribution. More probably, other components of the pellet, such as cell wall polysaccharides, may have increased stickiness of the powder during the process. The most negative result was the higher moisture content of the powder, and the water activity above 0.4 which can seriously compromise powder stability and cell vitality during storage.

Compared to the required cell concentration in the feed (Figure 1), the actual measured value was slightly higher ($5.7 \cdot 10^9$ CFU/mL). Considering the actual feed load, supposing that the product lost in the plant has the same composition of the recovered product and assuming no vitality loss during the process, a theoretical value of $5.2 \cdot 10^{10}$ CFU/g in the final powder was calculated. The actual measured value was $9.5 \cdot 10^9$ CFU/g, revealing a 82 % viability reduction during drying. The result is in agreement with literature, where Sohail et al. (2012) reported cell death ranging from 73 to 92 %. Even though spray drying is a well-established process characterized by high production rates and relatively low operational costs, the typical high working temperature can cause cell death due to simultaneous dehydration and thermal inactivation. The drying temperature of this study (150 °C) was selected based on literature works on spray-drying microencapsulation of probiotics (De Castro-Cislaghi et al., 2012; Fritzen-Freire et al., 2012). Lower temperature have also been used, such as 140 °C by Malmo et al. (2012) for *L. reuteri*, or 120 °C by Sohail et al. (2012) for *L. rhamnosus* and *L. acidophilus*, and could be tested to improve process survival.

3.3 Ice-cream preparation

Ice-cream was prepared substituting only a 5 % of the commercial base with the modified base (formulation B enriched with probiotics). This low substitution level should reduce the influence of base modification on technological performances of final product (such as color, melting rate, overrun and melting destabilization) but, especially, it allows the addition of the probiotic ingredient after the pasteurization. In spite of the low vital cells concentration in the spray-dried enriched base, ice-cream was produced to evaluate any further mortality. Considering the dosages reported in Figure 1, the probiotic concentration in the ice-cream mixture before beating and freezing was calculated in $3.5 \cdot 10^7$ CFU/g. The viable cells enumeration of the matured mix after 3 days storage at 4 °C gave $4.3 \cdot 10^7$ CFU/mL, corresponding to $3.79 \cdot 10^7$ CFU/g (taking into account the mixture density of 1.135 g/mL). This shows that the encapsulated cells remained vital after rehydration. Analysis of the final ice-cream after 2 days storage at -18 °C, gave $3.1 \cdot 10^7$ CFU/g count corresponding to a limited additional 18 % mortality. Stability during longer storage period has, anyway, to be further evaluated.

4. Conclusions

This study showed the possibility of producing spray-drying carrier materials suitable for functionalization of commercial ice-cream bases, since containing the same bases ingredients. Two formulations were developed, one with maltodextrins, widely used in the food industry and spray-drying processes, and one with inulin, a prebiotic fibre with both beneficial effects for humans and probiotics preservation. Process yield (58-66 %) and powder a_w (< 0.3) were not influenced by the formulation type. Probiotics encapsulation was possible only with inulin formulation, but with a lower process yield (51 %) and higher powder a_w (> 0.4) compared to the original formulation. A 82 % death cell occurred during drying, but the cells survived in the ice-cream making process. The results represent an important starting point for the development of encapsulated multiple bioactive ingredients containing probiotics, prebiotics and other functional compounds such as polyphenols.

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