LWT - Food Science and Technology 50 (2013) 17–24

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Optimization of microencapsulation of probiotics in raspberry juice by spray drying

Kartheek Anekella [a], Valérie Orsat [b,*]

[a] Department of Food, Bioprocessing and Nutrition Sciences, Schaub Hall, North Carolina State University, Raleigh, NC 27695, USA
[b] Bioresource Engineering Department, McGill University, 21111 Lakeshore Rd., Ste-Anne de Bellevue, QC, Canada H9X 3V9

ARTICLE INFO

Article history:
Received 27 April 2012
Received in revised form
31 July 2012
Accepted 2 August 2012

Keywords:
Sub-lethal thermal effect
Probiotic/prebiotic
Lactobacillus

ABSTRACT

Aims: Probiotics were microencapsulated in raspberry juice through spray drying.
Methods & Results: A combination of probiotics (*Lactobacillus acidophilus* NRRL B-4495 and *Lactobacillus rhamnosus* NRRL B-442) was chosen to offer high viability. Maltodextrin's role as a carbon source was also assessed for its prebiotic potential. Spray drying inlet temperature (°C), total solids: maltodextrin ratio, and inlet feed rate (mL/min) were fixed as independent variables while % recovery, % survival and color were the dependent outputs.
Conclusions & Significance: High temperatures during spray drying are detrimental to probiotics and can be circumvented by sub-lethal thermal shock (50 °C for *L. acidophilus* and 52.5 °C for *L. rhamnosus*). Increasing the microencapsulating material concentration increased the survival rate of the probiotics. Non-dairy probiotic foods are becoming popular as they do not pose problems of lactose intolerance while they offer an alternative.

1. Introduction

Probiotics are considered as "good buddies" to human health. Although historically, probiotics were products of the pharmaceutical industry, the current trend is moving toward the health food sector, making Hippocrates' statement *"Let food be your medicine"* true once again.

Probiotics are usually sold as capsules, powders and combinations of different species which may have multiple advantages (Timmerman, Koning, Mulder, Rombouts, & Beynen, 2004). It was proven in various animal studies that the use of one or more strains/species of probiotics can have beneficial effect and it is logical to assume that a mosaic of probiotics could help in exerting multiple benefits they possess (Famularo, de Simone, Matteuzzi, & Pirovano, 1999; Sanders, 1999). In many cases however, the functionality of probiotics is an issue due to the poor quality in the standards of preparation of probiotics foods and lack of sound and thorough clinical evidence (Azcarate-Peril, Tallon, & Klaenhammer, 2009; Hamilton-Miller & Shah, 2002; Klaenhammer, 2000; Timmerman et al., 2004). The main goal of any food industry is to increase the versatility in consumption of different forms of food without losing its basic properties of providing nutrition and ensuring health.

Formulating and enriching foods with probiotics would not only improve public health but also the diversity in food choices. Most probiotic foods in the current market are refrigerated dairy-based products (Burgain, Gaiani, Linder, & Scher, 2011) while preparations of non-dairy foods will attract a broader range of consumers with different preferences. The food matrix encapsulation must act as a buffer during storage as well as in the stomach transit until the probiotic is delivered to the intestinal tract along with offering a protection during thermal processing (Ranadheera, Baines, & Adams, 2010). The probiotic microorganisms present in food should survive in significant numbers of at least 10^6–10^8 CFU/g, although the numbers vary from strain to strain (Boylston, Vinderola, Ghoddusi, & Reinheimer, 2004; Chávez & Ledeboer, 2007; Ishibashi & Shimamura, 1993; Kailasapathy & Rybka, 1997). It is possible to induce a sub-lethal effect on microorganisms which adapts them to adverse conditions during drying, storage and other processes (Broadbent & Lin, 1999). Usually a temperature rise of 10 °C above the optimal growth temperature leads to shock (Teixeira, Castro, & Kirby, 1994). Thermal sub-lethal treatment can increase the survival rate of *Lactobacilli* remarkably (between 16 and 18 folds depending on the adaptation media) during and following spray drying (Desmond, Stanton, Fitzgerald, Collins, & Paul Ross, 2001; Gardiner et al., 2002). The stress resistance proteins are produced

* Corresponding author.
E-mail addresses: kanekel@ncsu.edu (K. Anekella), valerie.orsat@mcgill.ca (V. Orsat).

0023-6438/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2012.08.003

mainly during the sub-lethal exposure prior to drying (Dabbah, Moats, & Mattick, 1969; Teixeira et al., 1994).

Although spray drying of *Lactobacillus* cultures was first done in 1914 by Rogers, it was not adopted due to very low survival rate, difficulty in storage as well as poor rehydration capacity (Porubcan & Sellers, 1975; Teixeira, Castro, Malcata, & Kirby, 1995). Use of berries with probiotics has been tested for uropathogenic and urogenital disorders. The proanthocyanins present in berries, such as cranberries, can modulate the immune system in conjunction with probiotics (Reid, 2002). Anthocyanin rich raspberries also have a high amount of dietary fibers (6.5 g/100 g) with good absorption characteristics which could potentially serve as a carrier and microencapsulating agent as well as a prebiotic (Chiou & Langrish, 2007). This supports our premise of studying the combination of probiotics organisms with raspberry juice for spray drying. Additionally, thermoplasticity and hygroscopicity of fruit juice might pose problems during the spray drying causing them to adhere to the chamber wall due to their stickiness, clogging and caking (Chegini & Ghobadian, 2005). Adding suitable wall materials such as maltodextrin can reduce the caking and stickiness to the walls and increase the free flowing nature of the spray dried powder.

2. Materials and methods

Following a review of the literature on the health benefits of probiotics, two species widely used in commercial probiotic foods were chosen. *Lactobacillus rhamnosus* NRRL B-4495 and *Lactobacillus acidophilus* NRRL B-442 were obtained from the USDA's Agricultural Research Services Culture Collection. Dried pellets were reconstituted in 50 mL MRS broth (deMan, Rogosa and Sharpe medium, Difco) and grown overnight (14–16 h) in an incubator shaker (Model G24, New Brunswick Scientific, USA) at 110 rpm and 37 °C. This was subcultured and grown overnight again. The culture thus obtained after the second sub-culture was used for further experiments. A small part of the culture was stored in sterile 30mL/100 mL of glycerol at −80 °C (−86C ULT Freezer Model 5698, Thermo Forma, USA) for later usage. A similar recipe was adapted to prepare MD-MRS medium where the dextrose was replaced by maltodextrin (MD, referred as MD-MRS) with Dextrose Equivalent value 5–8 (Oxoid, USA).

2.1. Growth curve and dry biomass estimation

The increase in biomass over the growth cycle period provides information on the kinetics of the growth in relation to substrate utilization. A 5 mL sample of this culture was taken every 3 h and centrifuged in a pre-weighed centrifuge at 1130× *g* for 6–7 min. The supernatant was decanted and stored for substrate estimation. The weight of dry biomass was measured as the weight difference between the tube and the pellet after drying at 103 ± 2 °C in hot air oven (Thermoelectron, USA) for over 12 h (Eq. (1)).

$$K = [\log(X_{t1}) - \log(X_{t2})]/\ 0.301^{*}(t2 - t1) \quad (1)$$

K = number of populations doubling in an hour (growth rate constant)
X_{t1} = number of cells/mL at time t1; X_{t2} = number of cells/mL at time t2

During any phase, the doubling time (Td) or generation time is the time required for the cell biomass to double in number as represented in Eq. (2).

$$Td = 1/K \quad (2)$$

2.2. Determination of substrate concentration (anthrone method)

Understanding the substrate utilization pattern of a microorganism is also helpful to assess the growth behavior and survival in the gut which is the final destination (Verdenelli et al., 2009). A procedure similar to the one described by Sadasivam and Manickam (2005) was followed. Anthrone reagent (Sigma Aldrich, USA) was prepared by dissolving 200 mg of anthrone in 100 mL of 95 g/100 g sulfuric acid. A 1 mL volume of the supernatant obtained from the biomass estimation was diluted 10 times and 4 mL anthrone reagent was added. The mixture was heated in a boiling water bath (100 ± 2 °C) for 6–7 min until a stable green color was obtained after which the mixture was cooled immediately in an ice bath for 2 min. The maximum absorbance at OD_{620} was recorded. The concentration of pure dextrose/maltodextrin was estimated from the measured OD_{620} using the standard curve obtained using a known concentration (0.1 g/L) of dextrose/maltodextrin. OD_{620} was plotted on the *X*-axis and concentration of substrate on the *Y*-axis.

2.3. Raspberry juice

The extracted raspberry pulp had a solid content of 13–14 °Brix measured by a portable refractometer (Fischer Scientific, USA). The seeds and skin were sieved out using a fine metal sieve filter. The °Brix unit was adjusted to 11 (total solid concentration 0.1 g/L) as otherwise the pure extract was too viscous to be spray dried.

2.4. Sub-lethal temperature (T_{sl}) treatment

A sub-lethal temperature treatment was selected to subject the microbes to thermal stress before spray drying. Since the sub-lethal temperature of any given microorganism is strain-specific, each strain was tested individually. Five mL of late log phase *Lactobacillus* cultures (incubated at 37 °C, on a rotary shaker at 110 rpm for 12–14 h) was subjected to different sub-lethal temperatures (45 °C, 50 °C, 52 °C, 55 °C) in a hot water bath. Test tubes containing either MRS or raspberry juice (with inserted thermocouples) were used to regulate the temperature. One mL of sample was collected every 5 min and transferred into 9 ml of sterile MRD (maximum recovery diluents; 1 g/L- peptone, 8.5 g/L NaCl) up to 15 min. CFU analysis was performed from each of these samples at regular intervals (0, 5, 10, 15 min). T_{sl} was determined from the curves in the graph where the temperature was retaining the highest number of cells after 15 min of thermal stress exposure. Prepared graphs represent the average of triplicate Petri plates for each of three trials.

2.5. Spray drying

Spray drying of raspberry juice with maltodextrin as an additive (wall material) at different ratios and mixture of lactobacilli was performed using a Buchi B-290 mini spray dryer. A five mL volume of each probiotic cultures (at late exponential phase, grown overnight) were subjected to sub-lethal treatment and centrifuged at 1130× *g* for 1–2 min in order to extract the pure cells. These pellets were diluted in 1 mL of sterile distilled water each and mixed with 50 mL of raspberry pulp and maltodextrin thoroughly by magnetic stirring just before spray drying. Total number of cells just before spray drying was approximately 9.5 log CFU/mL. The spray dryer was allowed to reach uniform process temperature for 15–20 min prior to the spray drying. The aspiration was maintained at 100% and cyclone air flow rate at 30 m^3/h. The temperatures used to optimize the model were 100 °C, 115 °C and 130 °C. Feed rates used