Encapsulating probiotic bacteria by ultrasonic vacuum spray drying

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Introduction

While it is undoubted that clinical evidence supporting the health-promoting activity of probiotic cultures is of paramount importance, it is probably less well appreciated that the technological suitability of these strains is also critical to their exploitation (Ross et all. 2005). Probiotics are described as "live micro-organisms which when administered in adequate numbers confer a health benefit on the host" (FAO/WHO 2001). They are commonly included in fermented milks, yoghurts and cheese, but are also available in the form of dietary supplements where the probiotic is in the form of a dried product. Probiotic-containing foods can be categorized as functional foods, and along with prebiotics represent the largest segment of the functional foods market in Europe, Japan and Australia. The market for this food category continues to expand, in parallel with growing consumer awareness of the role of diet in health maintenance (Stanton et all. 2001), and represents an exciting market opportunity for the Food and Dairy Industries.

Lactobacillus is a genus of Gram-positive facultative bacteria. They are a major part of the Lactic acid bacteria group, named as such because most of its members convert lactose and other simple sugars to lactic acid. They are common and usually benign, even necessary, inhabitants of humans and other animals. In humans they are present in the vagina and the gastrointestinal tract, and are an important genus of the gut flora.

Lactobacillus and *Bifidobacterium* species are the most commonly used probiotics in foods for human consumption given the significant health benefits associated with ingestion of these micro-organisms. These micro-organisms share a number of common traits, such as generally regarded as safe (GRAS) status, acid and bile tolerance, and ability to adhere to intestinal cells (Dunne et all. 2001). It is recommended that the probiotic culture must be present in the product at minimum numbers of 10^7 CFU/ml and even higher numbers have been recommended (Ishibashi et all. 1993; Lee et all. 1995).

One of the major tasks is providing the functional bioactive ingredient intact, stable during processing and storage, and most important – bioavailable once being consumed. Probiotic cultures for food applications are frequently supplied in frozen or dried form, either as freeze-dried or spray-dried powders (Lievense et all. 1993; Holzapfel et all. 2001). Relatively successful drying of lactobacilli and bifidobacteria has previously been reported for a number of different strains, including *Lactobacillus paracasei* (Gardiner et all. 2000). However, most probiotic lactobacilli do not survive well, during the temperature and osmotic extremes to which they are exposed during the spray-drying process (Ross et all. 2005).

Spray-dried powder with high numbers of viable probiotics is a convenient means of storage and transport of probiotic cultures and their subsequent application in functional foods. While spray drying is an economical process for the large-scale preparation of these cultures, and is commonly used for the preparation of food ingredients, it suffers from the disadvantage of causing bacterial cell injury and death, which has been attributed primarily to the effects of heat and dehydration leading to destruction of the properties and performance characteristics of probiotic cultures (Ross et all. 2005). One approach used by a number of workers to improve probiotic performance in food systems is the addition of protectants to the media prior to drying.

In order to produce high quality and high viability probiotic powders, one must establish conditions suitable for the product. The probiotic bacteria need to be dried at low temperature and within a short time, therefore a vacuum environment and narrow sized droplet distribution is required. The encapsulation technology presented in this paper is based on ultrasonic vacuum spray drying process. Using this technique the heating is gentle and the vacuum in the drier space reduces significantly the temperature of the product as well as the particles residence time (Sadykhov et all. 1997).

Material and method

Lactobacillus paracasei were dissolved in maltodextrin solution prior to spray drying. The patented Dryer includes an ultrasonic atomizer, which can operate in a vacuum environment, and a vacuum chamber with adjustable heating zones. The atomized spray was directed into a vacuum chamber whose internal temperature control was set according to the specific task required. The drying was performed through two stages. At the first stage the homogeneous drops fall free in the vacuum chamber within 4-5 seconds and lose 90-95% of the free water, and the drops temperature does not exceed 20-30 ^{0}C. The remaining free water and any parts of coupling water evaporate during 20-60 min., at the second drying stage in a fluidized-bed. After this stage the product was removed from the collector without stopping the process.

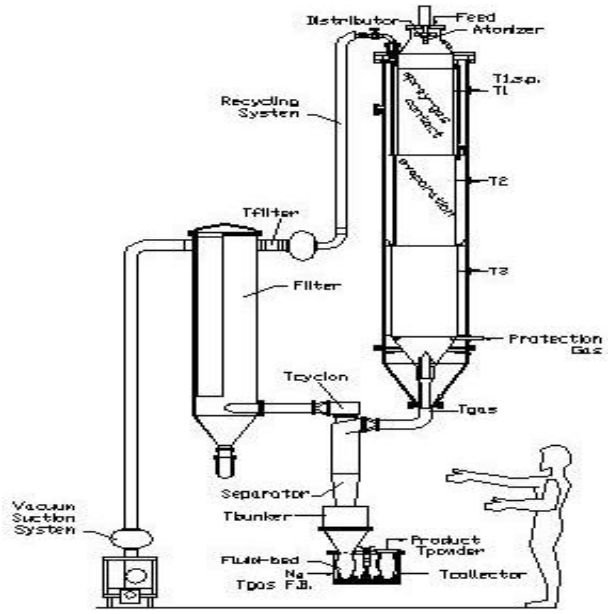

Figure 1 – Scheme of the Ultrasonic Vacuum Spray Dryer.
Dryer comprises three main technical components: (1) Liquid handling and Spraying system, (2) Vacuum drying chamber - contains 3 heat controlled zones (T1-T2) and a special vacuum system, (3) Powder collection site.

Determination of probiotic viability in spray-dried powders. The viability of the probiotic *Lactobacillus paracasei* in the maltodextrin solution before spray drying and in the resulting powders was measured by spread plating on MRS agar (Difco) plates. Encapsulated cell samples in triplicate (100-300 mg) were dissolved in 5 ml saline (0.85% NaCl), than serially diluted 10^{-1} to 10^{-4}, in saline, and 0.1 ml of the samples from the appropriate dilutions were spread plated onto MRS agar. Viable cells count was determined after 48 hours incubation under anaerobic conditions at 37^0C. The percent survival at each of the outlet temperatures tested was calculated as follows: Viability = $(N / N_0 \times 100)$, where N_0 is the number of bacteria per gram of dry matter before drying and N is the number of bacteria per gram of dry matter in the powder.

Results and Discussion

The dried *Lactobacillus paracasei* powder had features like high flow ability, mono-dispersive, fast and easy solubility, good handling properties and high porosity surface. The dried powder had particle size ranging from 10 to 50 microns, depend on solids concentration in sprayed solution, which is suitable for further coating using the fluidized bed technology.

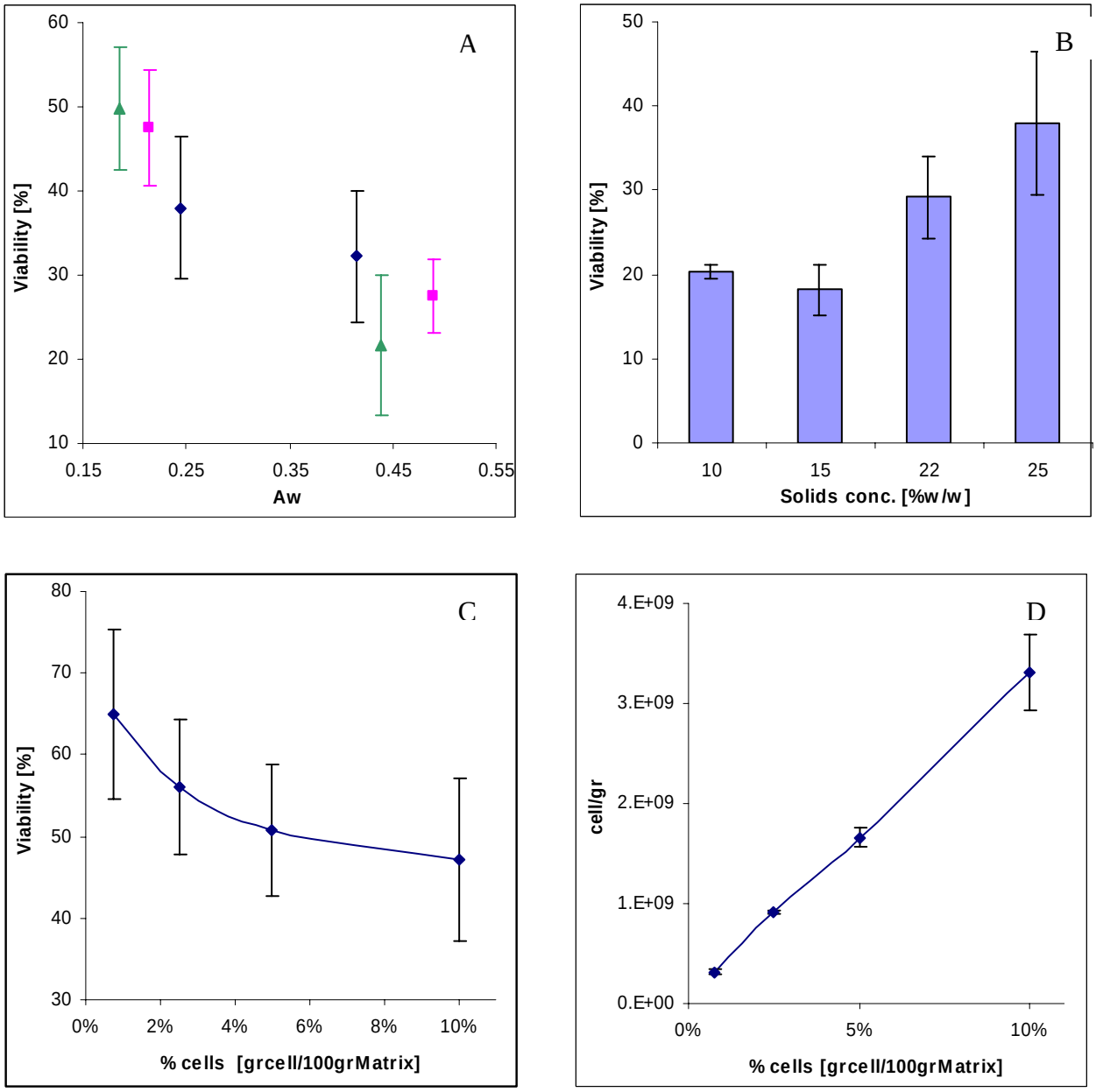

Figure 2 – *Lactobacillus paracasei* viability after ultrasonic vacuum spray drying process in correlation to: (A) water activity, (B) solids concentration in sprayed solution, (C) and (D) CFU concentration in the sprayed solution.

A number of factors influence the survival rate of *Lactobacillus paracasei* during ultrasonic vacuum spray drying process (Fig 2): final water activity in the dried product, solids and CFU concentration in the sprayed solution. Final water activity affects the viability of dried bacteria (Fig 2, A). Three different solutions were dried under the same conditions, except the drying time at the second drying stage, in the fluidized-bed. It is evident that higher water activity reduces the viability of

dried Lactobacilli. At low water activity (<0.25) the viability is higher than in high water activity (>0.35). We have also examined the influence of solids concentration in the feed solution (Fig 2, B), and found that in concentrated solutions the survival rate was higher than in low concentrations. This result may be explained by a shorter drying period required for removing lower water amounts, and thus reducing the time required for the drop to become a glassy state particle. In the next step we checked the influence of Lactobacilli concentration on their survival rate. There was a decrease of viability, from ~65 to ~47 percent, with the increase in cell concentration (Fig 2, D). Despite the decreased viability we were able to encapsulate more than 3×10^9 CFU/gr with over 50% survival.

Conclusions

The probiotic encapsulation by novel ultrasonic vacuum spray drying process provides us with much higher survival rates than conventional thermal spray drying process (Ross et all. 2005). Parameters like final water activity in the dried product, solids and CFU concentration in the sprayed solution have significant influence on the survival rate. Our latest experiments with improved formulas have shown up to 70% viability of the probiotic bacteria after encapsulation by ultrasonic vacuum spray dryer.

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