INTENSIFICATION OF PROBIOTIC MICROORGANISMS VIABILITY BY MICROENCAPSULATION USING ULTRASONIC ATOMIZER

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Uscarea prin pulverizare este o metodă frecvent folosită la încapsularea microorganismelor probiotice. În prezentul studiu a fost utilizat duză ultrasonică de 120 kHz pentru a obţine granule foarte fine, care pot fi folosite în produse lactate probiotice. Celule de bacterii lactice au fost încapsulate în alginat şi maltodextrină prin atomizare cu ultrasunete. Încapsularea a fost realizată pentru a întări viabilitatea celulelor bacteriene în condiţiile aparatului digestiv: în suc gastric şi intestinal.

The process of spray drying is a commonly used method to encapsulate probiotic microorganisms. In this study was used 120 kHz ultrasonic nozzle to obtain very fine drops, which can be used in probiotic dairy products. Lactic acid bacterial cells were encapsulated in alginate and maltodextrin by ultrasonic atomization. Encapsulation was executed to enhance viability of bacterial cells in digestive conditions: in gastric and intestinal juices.

Keywords: probiotics, ultrasonic, spray drying, microcapsules, viability

1. Introduction

Probiotics are live microorganisms (bacteria or yeast) which when ingested or applied locally, confer demonstrated health benefits to the host. The standard for probiotic foods follows the FAO/WHO recommendation: any food sold with health claims with addition of probiotics must contain at least 10^6 to 10^7 CFU viable bacteria per gram [1, 2].

Species of lactobacilli survive poorly under simulated gastrointestinal conditions [3, 4]. The physical protection of probiotics by microencapsulation is a

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new approach to improve the probiotic survival [5]. Microencapsulation by spray drying is considered the most popular, cost effective and good long-term preservation method for lactic acid and probiotic bacteria [6]. This process is readily available, easy to operate and energy efficient [7, 8].

Cîrîc [9] encapsulated probiotic bacteria by spray drying in sodium alginate and maltodextrin with high survival rate during the process.

Alginate is the most commonly used polymer for immobilizing viable cells and it has been used in various food applications. Maltodextrin functions as an osmotically inactive bulking compound, which causes spacing of the bacterial cells and strengthening of the glassy matrix [7].

2. Experimental

2.1. Microorganism and its maintenance

A pre-culture of strain *Lactobacillus brevis* isolated from traditional manufactured cheese in 250 mL of MRS broth (this medium is named by its inventors: de Man, Rogosa and Sharpe, developed in 1960, it was designed to favour the growth of *Lactobacilli* for lab study) was incubated at 37°C for 48 h, the cultured broth was centrifuged at 5000 rpm (d=12 cm) for 20 min. The resulting pellet was resuspended in 500 mL MRS broth and cultivated for 48 h, at 37°C. Before spray-drying cultured broth was centrifuged at 5000 rpm (d=12 cm) for 20 min and pellet was resuspended in 10 mL of 8 g/L NaCl solution.

2.2. Microencapsulation of the probiotic microorganisms

2.2.1. Preparation of the suspension

Bacterial suspension was mixed with sterilized Ringer solution, this suspension was agitated with magnetic stirrer. Separatelly 4.8 g sodium alginate was mixed with 0.8 g maltodextrin, this mixture was omogenized well, contrary in case of making suspension with water, will form clusters of alginate, hard to dissolve subsequently. The obtained mixture is dissolved in 30 mL of Ringer solution, to this suspension is added the bacterial suspension under strong stirring. The obtained suspension is cooled to 4°C for 1-24 h to remove bubbles [10].

2.2.2. Encapsulation by spray drying

The spray-drying process of the probiotic bacteria was undertaken in a laboratory-scale spray dryer (SonoDry 750, Sonotek, USA). The feed solution was ultrasonical atomized (120 kHz ultrasonic nozzle, 1.7 W) in a drying chamber at constant flow rate: 2.5 mL/min, air temperature: 130°C, 80 m^3/h. The dried powder was collected in a cyclone separator.

2.3. Size distribution analysis

Particle size distribution was measured by Mastersizer 2000 (Malvern) laser diffraction particle analyser in liquid phase (HCl solution in water) at pH 0.5 to maintain microcapsules in original form (to prevent depolymerization).

2.4. Evaluation of microcapsules

Microencapsulated beads (0.1 g) was mixed in Falcon tubes (12 mL) containing 5 mL of depolymerization solution (28 mL of 0.2 M solution NaH_2PO_4 and 72 mL of 0.2 M Na_2HPO_4 adjusted to 200 mL volume with distilled water, pH 7.1±0.1, sterilized). The mixture was vortexed at high speed for breaking the polymer formed and releasing completely the encapsulated culture into the buffer. The released cells were enumerated using MRS agar media, incubated for 48 h at 37°C [3].

3. Results and discussions

3.1. Size distribution and microscopic view of the obtained microcapsules

Microcapsules obtained from alginate-maltodextrin and bacterial cells suspension in Ringer solution by spray drying were observed with optical microscope (Fig. 1.).

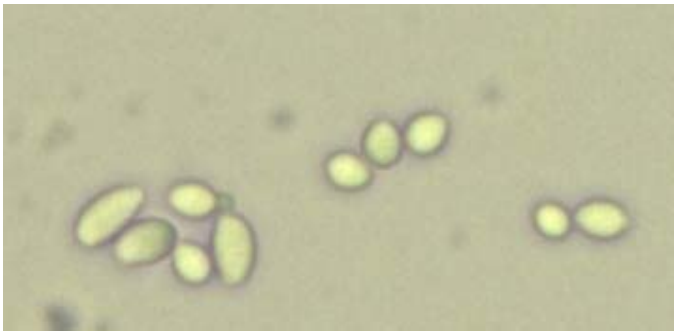

Fig. 1. Microscopical view of microcapsules

Particle size distribution of microcapsules was measured by laser diffraction particle analyser and the average size of the obtained microcapsules was determined. The average volume weighted mean is 95.167 μm, surface weighted mean is 33.585 μm and d (0.5) = 47.543 μm in case of microcapsules obtained by spray drying by ultrasonic nozzle atomization (Table 1).

Table 1

Measured data of microcapsules size distribution.

Nr.	Volume weighted mean [µm]	Uniformity	Specific surface area [m^{-2}/g]	Surface weighted mean [µm]	d (0.1) [µm]	d (0.5) [µm]	d (0.9) [µm]
1	110.635	1.59	0.171	35.186	16.955	51.434	292.436
2	88.235	1.27	0.181	33.134	16.303	47.701	194.371
3	86.632	1.29	0.185	32.437	16.060	46.494	169.327

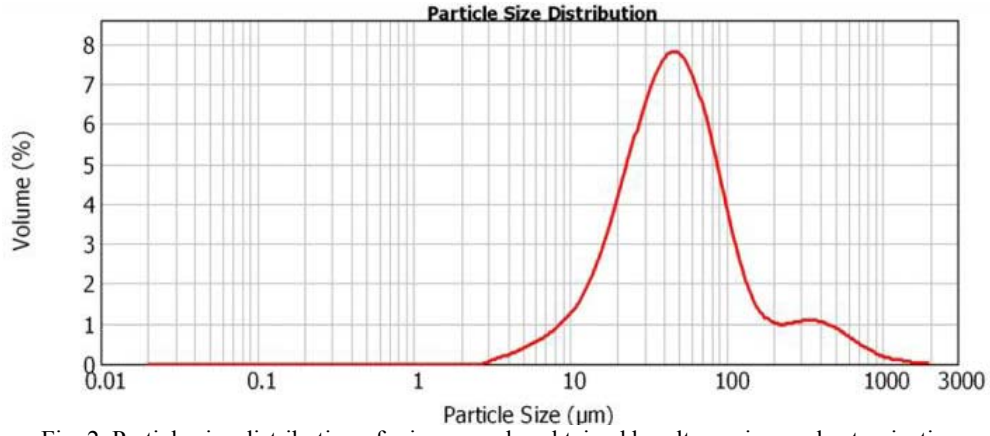

Fig. 2. Particle size distribution of microcapsules obtained by ultrasonic nozzle atomization

3.2. Survival during spray drying

Survival rate during spray drying at 130°C was calculated comparing log CFU (colony forming unit)/g dryed product initial with log CFU/g dryed product final (Fig. 3.). Initial CFU/g product was determined from alginate-maltodextrin water suspension before drying, final CFU/g product was calculated after evaluation of microcapsules in phosphate buffer. Survival rate is good: cell loss/g product is 1.25 log unit (average of tree measures).

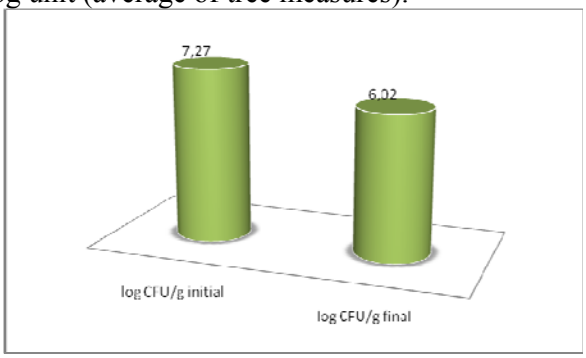

Fig. 3. Comparation of initial and final cell number at spray drying process

3.3. Comparation of microencapsulated and non-encapsulated cells viability in gastrointestinal conditions *in vitro*

Viability of encapsulated and non-encapsulated cells were compared after exposure to digestive juices. Obtained results demonstrate the protective effect of encapsulation in alginate: cell loss, in case of encapsulated cells is lower (1.64 log unit) compared to non-encapsulated bacterial cells, where cell loss after exposure to artificial digestive juices is very high. The results expressed are shown in Fig. 4.

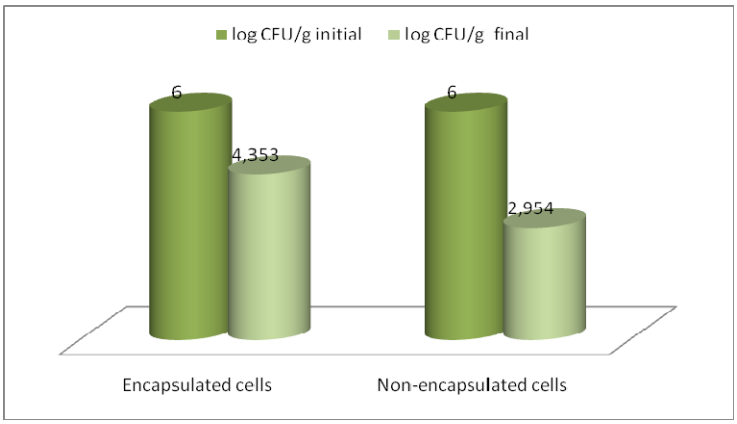

Fig. 4. Viability of encapsulated and non-encapsulated bacterial cells in digestive conditions.

6. Conclusions

This study demonstrates the possibility of producing encapsulated lactic acid bacterial cells using ultrasonic nozzle atomization at 130°C with high survival rate. *Lactobacillus brevis* isolated from traditional manufactured cheese microencapsulated in sodium alginate and maltodextrin shows higher viability in digestive conditions compared to non-encapsulated bacterial cells. Protection of bacterial cells by microencapsulation to maintain their viability was the main objective of our study.

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