Probiotics viability improvement – encapsulation by ultrasonic nozzle atomization

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Introduction

The benefits of consuming probiotics and farmabiotics containing live lactic bacteria have been considered well known, from the start of our studies. Functional aspects of this food supplements like improving of colon health state, prevention of colon cancer or decreasing of the cholesterol level in blood are considered modern themes of discussion and of research in the last 30 years.

One of the main problems the the probioc and farmabiotic industry has, in this stage of development, is the decrease of viability of the ingested bacterial products when passing through the intestinal tractus.

During the last years methods of improving the viability of the probiotic bacteria have been taken into consideration, starting from simple microbiological methods and reaching the tops of genetics, for developing genetically modified microorganisms. We consider genetics as being the future as long as we would be able to completely understand and control the mechanisms implied.

A simpler and safer method was considered to be the protection of lactic bacteria using the microencapsulation technology. This study focused on the methods of encapsulating Bifidobacterium Bb-12, especially on the method based on using a spray drier with ultrasonic atomization nozzle and on comparing this method with more traditional methods of encapsulating like extrusion, gelification and spray drying with centrifugal and stationary dual-fluid nozzles.

The advantages of using the ultrasonic nozzles come from the way in which the liquid drops are obtained with this technology, resulting smaller and more uniform drops comparing to the traditional spray drying process.

Materials and methods

Extrusion.

A suspension of lactic bacteria in a sodium alginate solution have been used. The strengthening solution used was $CaCl_2$ (Fluka 21108) 0,05mMol/l. The tests were made with a 10 ml syringe pump with a needle 0,8 x 40 mm. The strengthening time was 30 minutes.

Emulsioning/gelification.

A solution of 2% natrium alginate is prepared. In 20 ml of this solution a suspension of Bb-12 in ¼ strength Ringer solution is added. Over this mixture were added 5 ml calcium ions suspension (500mM Ca^{2+}), vegetal oil and SPAN80. Everything mixes at 250rpm for 15 min. A W/O emulsion was obtained. After the forming of the emulsion, 20 ml of vegetal oil with 0,5 mol acetic acid wass added, for releasing of the calcium ions, necessary for the ionotropic gelification of the alginate solution. The phase inversion was made with 150 $CaCl_2$ 0,05mMol/l. After the phase separation the gel beads were washed with a TWEEN 1% solution.

Spray drying.

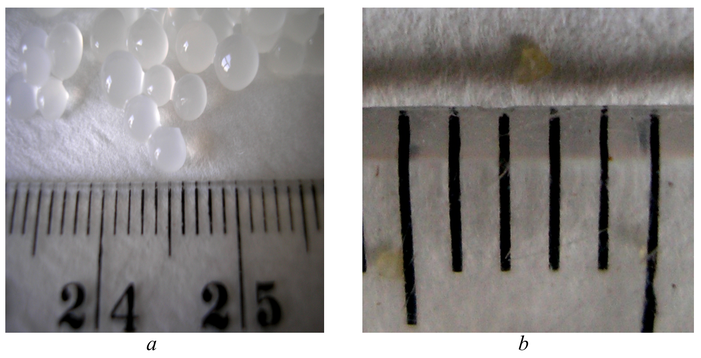
Three distinct equipments were used, being interested especially in the way of atomization (nozzle type). First spray drying tests were made with SonoDry 750 a laboratory scale spray dryer with ultrasonic nozzle. For comparison were used a semi industrial Niro dryer having a centrifugal nozzle and Büchi B290 with a two fluid stationary nozzle. For this tests it was used a suspension of Bifidobacterium Bb-12 in a sodium alginate–maltodextrin solution. The maltodextrins were used both for increasing the dry content and as a prebiotic.

The methods of analysis used were chemical (dry matter, water activity, viscosity), microscopical (optical and SEM), as well as a simulation of intestinal tractus.

For this simulation were used solutions simulating the gastric juice (HCl pH 1,5, 1% NaCl), the pancreatic juice (26mg/ml pancreatin) and a bile solution (20 mg/ml dry bovine bile). 0,1 g from the analyzed product were put into 1,5ml Epi container and 0,9 ml of skimmed sterilized milk were added. After 30 minutes at 37°C anaerobiosis, the samples were taken out and centrifuged (8000rpm/ 10 min). After taking away the supernatant, 0,9 ml simulated gastric juice were added. The samples were maintained at 37°C anaerobiosis, 120 minutes, centrifuged at 8000 rpm for 10 min and the supernatant was taken away. Samples were retained for analysis. In the same way the bile solution was tested. For the simulated pancreatic juice the time was reduced to 60 minutes. In all the stages counting the number of free bifidobacteria was made using MRS-NNLP medium.

Results

The attempts of encapsulating using extrusion and emulsioning/gelification had unsatisfying results. The maximum dimension of 30µm was exceeded up to 100 times. The extruded wet particles had medium diameter of 2,3 mm, and the particles obtained by emulsioning/gelification a diameters in a range from 0,01 mm up to 2 mm (medium diameter for such a disperse value range was considered ineloquent).



a *b*

Fig. 1. Capsules resulted by extrusion (a-wet, b-dried)

In case of spray drying using the semi industrial Niro atomizer the medium diameter of obtained capsules was 36.2 µm, but with very large dimensions' distribution.

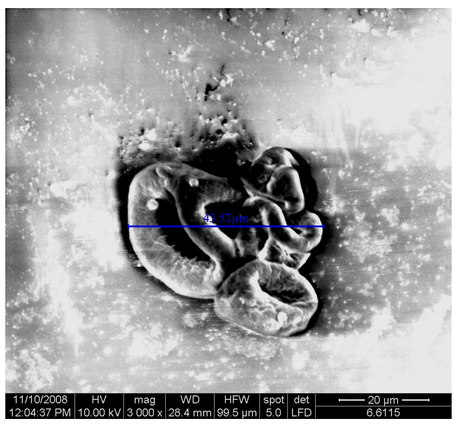
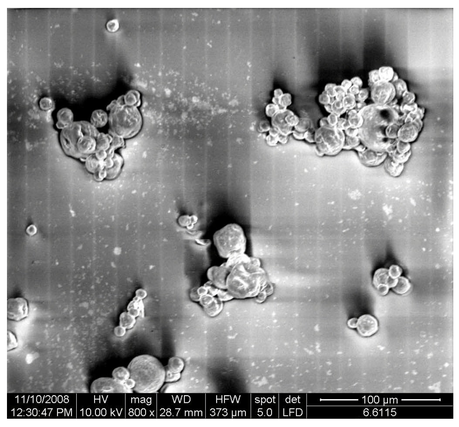


Fig. 2 Microcapsules obtained using Niro semi industrial atomizer

Relatively big particles can also be considered the one obtained with Buchi B290. In this case the medium diameter was 27.8 µm.

Fig.3. Microcapsules obtained using Buchi B290



Best results were achieved with SonoDry750, using an ultrasonic nozzle with the frequency of 120kHz. The medium diameter of this microcapsules was 9.6 µm, but none of the measured particles exceeded 20µm.

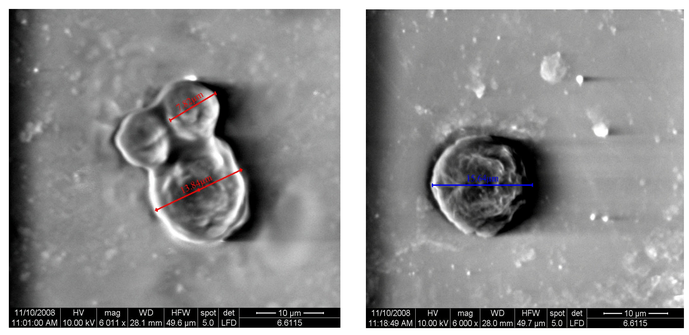


Fig.4. Microcapsules obtained using SonoDry750

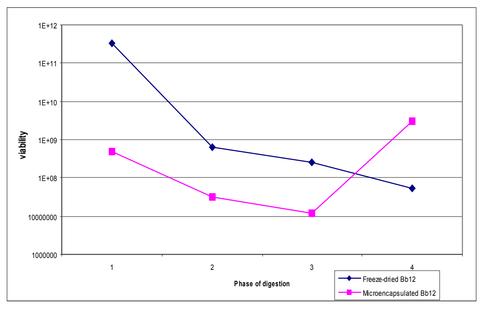


Fig. 5. Viability of freeze-dried and microencapsulated Bb12 in simulated gastrointestinal conditions (1-after skimmed milk, 2-after simulated gastric juice, 3-after bile solution, 4-after simulated pancreatic juice)

Comparing the number of viable probiotic bacteria counted in the microencapsulated product before and after passing the product through the simulation of gastric and intestinal juices with the number of viable probiotic bacteria from a freeze dried product who passed similar test, we can conclude the following:

- The microencapsulation procedure reduces the number of free measurable bacteria (countable on MRS-NNLP medium) from 10^{11} to 10^{8}. This could be considered as normal, due to the fact that on MRS-NNLP can be counted the free bacteria, but not the encapsulated one.
- During the simulation tests, a relative similar decease of the number of countable probiotic bacteria can be observed, for both type of products, until the treatment with pancreatic juice. In this case the recorded viability deceases because of the action of the gastric juice and of the bile solution over the free bacterial cells.
- The simulated pancreatic juice realizes the release of the bacteria from the microcapsules. In the case of microencapsulated Bb-12 a increase of the number of viable free bacteria was recorded. In the other case, the freeze dried bacteria continues a loss of viable cells

Conclusion

The best microencapsulation method, among the five tested, was the spray drying using the atomization with ultrasonic nozzles in SonoDry750.

The number of Bb-12 CFUs counted in case of the microencapsulated product, after the simulation of gastrointestinal conditions is 100 times bigger comparing with the results obtained for the freeze dried product. Of course it is noticeable a loss of viability also at the microencapsulated product, as the initial number of viable bacteria per gram was the same, the freeze dried product being used both in direct test and for obtaining the microcapsules.

Future studies will be done to ensure a lower loss of viability during the microencapsulation process, and a better percentage of encapsulated bacteria

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