Introduction

The benefits of consuming probiotics and farmamiotics 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 probiotic and farmamiotic industry has, in this stage of development, is the decrease of viability of the ingested bacterial products when passing through the intestinal tract.

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 top of genetic modifications 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 dryer 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 atomized, leading to 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 CaCl₂ (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 was made using a semi industrial Niro dryer having a centrifugal nozzle and spray drying. The maximum dimension of this microcapsules was 9.6 μm, but none of the measured particles exceeded 20μm.

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).

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.

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

For this simulation were used solutions simulating the gastric juice (HCl pH 1.5, 1 % NaCl), the pancreatic juice (270mg/ml pancreatin) and a bile solution (20 mg/ml dry bovic 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 again (8000rpm/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-NNL medium.

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 microcapsulation procedure reduces the number of free measurable bacteria (countable on MRS-NNLP medium) from 10⁷ to 10⁸. 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 at all type of products, until the treatment with pancreatic juice. In this case the recorded viability decreases 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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