Case Study For Stabilization And Controlled Release Of Microorganisms Based On Fluidized Bed Technology

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INTRODUCTION AND OBJECTIVE

The food market has an increasing demand on functional food. Functional food contains substances, ingredients or additives which have a positive effect on human health. Such ingredients are for instance probiotics. Probiotics are living microorganisms and are beneficial for health. The positive effect of probiotic foodstuffs strongly depends on the probiotic culture itself and on the total number of living microorganisms in the final product. As a rough number at least 10^6 colonies forming units per gram or millilitre are required (Lourens-Hattingh et at. 2001).

To promote functional food their beneficial effect has to be proofed in a scientific study. Such studies are required by the Health-claims-regulation (Regulation 2006) by EFSA (European Food Safety Authority). The case study explained in this article focuses on the increase of survival rates of microorganisms and controlled release in human body.

The anatomy and physiology of the human gastro intestinal tract (GI-tract) is essential for the application of enteric coating. Because of that, the transition times and pH-values in GI-tract have to be taken into account in the product design phase.

MATERIALS AND METHODS

The delivery form Micropellet was used in the study. This concept of formulation allows optimization studies and ensures reproducible results. The spherical shape of the particle gives optimal properties for subsequent layering and functional coating. In Figure 1 the overall design of such a pellet is shown as well as the ingredients used in the case study.

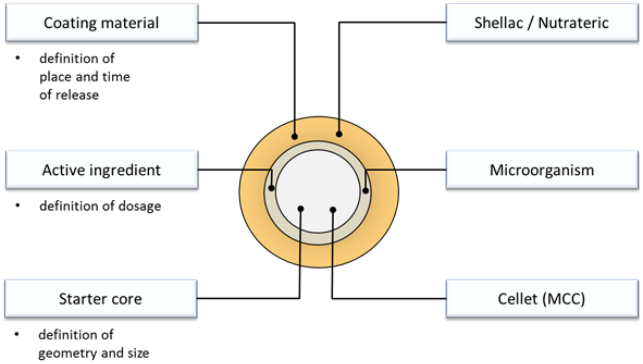

Figure 1: Delivery form - Micropellet

As starter cores so called Cellets 200 (supplier: Synthapharm/Germany) were used because of their very spherical shape. They are made from microcrystalline cellulose (MCC) and are inert, odourless and tasteless. The shape offers optimal conditions to be layered or coated with additional substances. As a typical microorganism the species Lactobacillus plantarum 299v was used. It is commercially available and was supplied as freeze dried material. For enteric coating a water based dispersion of Shellac (supplier: Harke Pharma/Germany) was used. Shellac dissolves at a pH-value higher than 7.2 and has excellent film forming properties. Additional, Nutrateric (supplier: Colorcon/UK) was used as a second option for enteric coating. Nutrateric consists of two components. The first Surelease, is a water-based dispersion of ethylcellulose and it is insoluble in the whole GI-tract. The second component, NS Enteric, is a pore forming agent and its release depends on the pH-value. As well as Shellac Nutrateric has food approval.

In the experimental study, various processing options were investigated to apply the different coating layers. For all processing steps, the modular fluidized bed apparatus ProCell Labsystem (supplier: Glatt Ingenieurtechnik GmbH/Germany) was used. The main advantage of that system is the unique flexibility and the wide range of processing conditions.

RESULTS AND DISCUSSION

At first the layer of microorganisms were applied on the starter cores by suspension layering using the ProCell 5 - processing insert. For that process option, the freeze dried microorganisms were transferred into a suspension which was comparable to the original fermentation step. In a possible industrial application the untreated fermentation liquid can be used.

The layer of microorganisms was characterized as very uniform and homogenously distributed on the surface of the starter core.

As an optimization study the survival rate of microorganisms during suspension layering was also investigated. Different formulations of the spray suspension were tested with and without protective additives. The use and influence of additives in fluidized bed processing was already discussed in literature (Stummer et.al. 2012). On basis of their results, skimmed milk powder was used in our case study as protective material.
A main target of the study was the immobilization of living microorganisms. Because of that, the survival

rate was investigated. The results of experiments are shown in Figure 2.

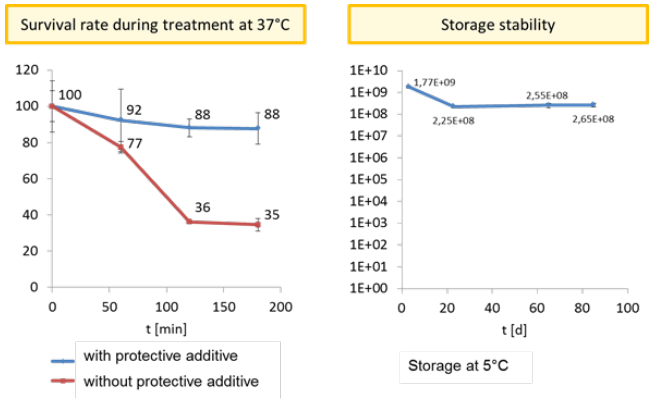

Figure 2: Results of survival rate analysis

At first, the samples produced by suspension layering of microorganisms on starter cores were treated at 37°C in a batch fluidized bed without spraying. It was the aim of the trial to determine the influence of processing time and of the protective additive (skimmed milk powder) on the survival rate. Additionally the long-time storage stability of the samples at 5°C was investigated. The quality of the samples was defined by total viable cell count. The left diagram in Figure 2 indicates the positive influence of skimmed milk powder as stabilizer in the formulation. With protective additive the decrease of the survival rate was much lower than without. Significant differences have also been observed in the most interesting processing time of about 40 to 60 minutes. The stability of the samples at 5°C storage was very good as there was no further decrease of the total number of colony forming units (CFU) after about 25 days.

To investigate the coating quality and the release a simulation of the human GI-tract was carried out using a bioreactor system BIOSTAT B-DCU II (supplier: Sartorius/Germany). The stomach was simulated as a one-hour treatment of the samples at a pH-value of 1.2 in the reactor. After that period the pH-value was changed to 7.5 or 6.8 respectively and the samples stayed in the system for additional three hours. During the simulation, samples have been taken and the viable cell count was determined.
The both types of enteric coating substances Shellac and Nutrateric were applied on the outer layer of microorganisms using Wurster-coating. The Shellac coated pellet structure is spherical, uniform and no cracks are visible. The samples produced using Nutrateric had an increased amount of agglomerates due to the much higher stickiness of the coating liquid. That is why the spray rate had to be significantly reduced and more processing time was needed. Due to that situation all final GI-tract simulations were carried out only with the Shellac samples. In GI-tract simulation the uncoated samples were used as a reference. As expected, the amount of living microorganisms dropped down to zero extremely fast in gastric fluid. That implies that a use of those probiotics in dry product forms is absolutely not useful without a final functional coating.
As typical examples in Figure 3 the results of samples characterized by 25% and 40% Shellac coating level are shown. In the first 60 minutes of residence in artificial gastric fluid, no living cells were detected in both cases. That was expected as the organisms were protected by enteric coating. After changing pH to 7.5 or 6.8 respectively the enteric coating films start to release. After about 2 hours of releasing time, stable and constant values were achieved.

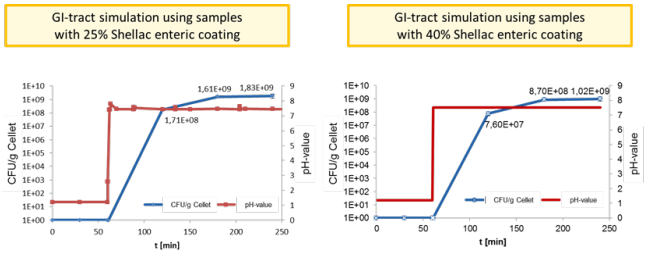

Figure 3: Results of GI-tract simulation using Shellac enteric coating

CONCLUSIONS

It could be shown, that the immobilization of microorganisms using fluidized bed technology is possible with a sufficient survival. The use of protective additives like skimmed milk powder significantly improves the yield and the survival rate of microorganisms. Moreover probiotics can be reproducible protected against the influence of gastric fluids by enteric coating using Wurster-coating. The release of the microorganisms depending on pH-value was proofed by in-vitro simulation. The quality of enteric coating achieved using 25% Shellac was optimal compared to 40% Shellac and to all test using Nutrateric.

REFERENCES

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