Influence of hydrocolloid interactions on their encapsulation properties using spray-drying

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Introduction

A fundamental topic of probiotic food additives is to ensure the physiologic effectiveness of the product from the point of production, during storage and residence time in food, up to the time of consumption. Futhermore, it is necessary that the organisms survive the barriers of the upper gastro-intestinal tract (Goldin, 1998; Heller, 2001; Molin, 2001; Picot, 2003; Tuomola, 2001; Siuta-Cruce, 2001; Bezkorovainy, 2001). Because living cells are susceptible to degradative influences of the surroundings, they should be protected when used as a probiotic food additive to ensure their metabolic activity. Microencapsulation seems to be an appropriate technique to protect microorganisms from these harmful conditions (Park, 2000; Khalil, 1998; Lee, 2000; Shah, 2000).
The inclusion of living bacteria in gel capsules for biotechnological processes is examined by many researchers (Champagne, 1992; Champagne, 2000; Ertesvag, 1998; Fadnavis, 2003; Göksungur, 1999; Park, 2000; Serp, 2000; Szajani, 1996; Tal, 1999).Concerning the stabilisation of probiotic bacteria by means of gel capsules, there are fewer but promising publications (Khalil, 1998; Lee, 2000; Picot, 2003; Shah, 2000). Refering to the literature, spray-dried probiotic organisms coated with water-soluble hydrocolloids seem to be less protected compared to organisms encapsulated in gel capsules (Khalil, 1998; King, 1995; Lee, 2000; O'Riordan, 2001). This might be due to the fact that a water-soluble coating cannot fulfill all the needs of a protective shell material like building up a dense diffusion barrier (O'Riordan, 2001; Gardiner, 2000).

So, besides the choice of the encapsulation technique, a proper choice of capsule material can determine the functional properties of the microcapsule (Chen, 2005; McNamee, 2001; McMaster, 2005; Jackson, 1991; Pegg, 1999). Since single materials often cannot meet all demands, combinations of different capsule materials are chosen (Pegg, 1999).
Using mixtures of hydrocolloids, it should be taken into consideration that these substances might interact in an attractive or repulsive way, depending on their structure and net charge, leading to either a homogeneous or an inhomogeneous dispersion (Dickinson, 1995; Tolstoguzov, 2000a; Tolstoguzov, 2003; Tolstoguzov, 1993; Tolstoguzov, 2000b). Precipitates depending on attractive interactions are known as complex coacervates (Benichou, 2002; De Kruif, 2001; Tolstoguzov, 1998). Phase separations caused by repulsive interactions are characterised by a thermodynamic incompatibility leading to water-in-water-emulsions, when a critical concentration is reached (Capron, 2001; Gilsenan, 2003a; Gilsenan, 2003b; Tolstoguzov, 2000a). These themodynamically stable emulsions can be marked by the following features: low surface tension, low density and viscosity of the polymer-depleted interfacial layer between the phases, and high viscosities of the phases themselves. Because of these characteristics, the boundary layer can absorb, for example, hydrophobic particles or lipids (Tolstoguzov, 2000a; Tolstoguzov, 2002; Tolstoguzov, 1993; Tolstoguzov, 2003).
Due to their special properties, both kinds of interactions, attractive and repulsive, might be used to adjust the protective features of capsule material mixtures.

The aim of the present study was to determine the effect of production, storage, residence time in a model food system, and a simulated gastric juice on the viability of microencapsulated lactic acid bacteria encapsulated in different capsule materials, focussing on capsule material interaction.

Material and Methods

In this study *Lactobacillus reuteri* (DSM 20016) was used as the test organism. Cells from a 9 h culture were harvested by sedimentation, washed with 0,5 % maltose-solution, sedimented again, and resuspended in the capsule material dispersions.

The capsule materials investigated were pectin (Classic AF 707, Herbstreith & Fox) at a concentration of 3 % as a single material or mixed with 3 % of dextrin, modified (OSA-) starch (both from National starch & Chemical GmbH), gelatin (from pigskin, Merck) or soymilk powder (Vivasoy, JRS GmbH & Co), respectively.

The microorganism-capsule-material supensions were extruded into a 2 % Ca-lactate hardening bath. The resulting capsule dispersions were adjusted to a pH of 6.5 and spray-dried with an inlet air temperatue of 180°C and an outlet air temperature of 65°C (spray-dryer: Nubilosa LT-C). The powders were harvested every 5 minutes and stored in petri dishes.

Process stability was determined by examining the colony forming units (CFU) of *Lactobacillus reuteri* before (feed) and after the spray-drying process (powder).
Tests regarding the storage stability were conducted by placing the petri dishes in the refrigerator (5°C) and taking samples in an irregular mode for 120 days.
Apple juice (100 % juice, Jacobi Scherbening GmbH & Co KG) was chosen as a model food system. The juice containing the different encapsulated bacteria and the stock culture, respectively, were stored at 5°C. Samples were taken every 4 days.
The resistance to gastric juices was scrutinised by dissolving 1 % of the stock culture and the powder in a hydrochloric acid solution with a pH of 1.5 and a temperature of 37°C to achieve a simulation of the gastric milieu, and keeping the organisms under these conditions for 3 hours. CFU were enumerated before and after this procedure.

The colony forming units were counted after incubating MRS-agar plates inoculated with the samples, which were dissolved with the help of a 3 % tri-K-citrate-solution, in order to destroy the Ca-pectinate gel complexes, at 37°C for 48 hours.
All results were calculated on the basis of the solid content (SC)

$$\mathrm{CFU/g\ SC} = \mathrm{CFU/mL} \cdot \frac{1}{\mathrm{g\ SC/mL}}$$

to make the obtained data comparable.

Results and Discussion

Due to the different kinds of interactions between capsule materials and because of the resulting phase separation behaviour above definite concentrations, various mixtures of capsule materials might show different protective effects. This study was conceived to investigate the influence of capsule material interactions on the protection of sensitive core materials. Because microorganisms show a high susceptibility to every step of processing, *Lactobacillus reuteri* was chosen as a model core substance. The capsule materials and concentrations are selected to ensure that diffent kinds of interactions occur (Tolstoguzov, 2002). The extrusion process is applied to secure that the structures, developed because of the specific interactions, are fixed by gelling one part of the capsule material mixture (Gilsenan, 2003b; Picout, 2000b; Picout, 2000a). Furthermore this process leads to a limited solubility of the capsules in aqueous solutions like apple juice or hydrochloric acid, ensuring that the stability of the microorganisms can be attributed to the diffusion properties of the resulting structures.

The following table (table 1) lists the results of the CFU determination of the samples pectin (Pec), pektin with dextrin (Pec + Dex), pectin with starch (Pec + Sta), pectin with gelatin (Pec + Gel), and pectin with soymilkpowder (Pec + Soy) in dependence on the step of examination, respectively.
The mortality rates were calculated by adopting a linear regression to the linear range of the obtained death curve. The rates are expressed as loss of reproductive cells (CFU) per day. The mortality during the spray-drying process and the acid treatment are expressed as CFU after the process in relation to CFU at the beginning.

	CFU feed per g SC	**Mortality during spray-drying / %**	**Motality rate during storage CFU / d**	**Mortality rate in applejuice CFU / d**	**Mortality during acid treatment / %**
stock culture	n.d.	n.d.	n.d.	-0.128	100.00
Pec	$4{,}3 \cdot 10^{10}$	99.19	-0.026	-0.264	90.43
Pec + Dex	$1{,}6 \cdot 10^{10}$	99.49	-0.023	-0.291	98.11
Pec + Sta	$1{,}8 \cdot 10^{10}$	99.22	-0.008	-0.127	99.27
Pec + Gel	$2{,}2 \cdot 10^{10}$	99.23	-0.016	-0.123	88,05
Pec + Soy	$1{,}7 \cdot 10^{10}$	99.12	-0.009	-0.090	83.81

Table 1: Influence of processing steps and digestion model on the CFU of the investigated samples; n.d.: not determined

Comparing the results it can be seen, that the spray-dying process has the most degradative influence on the cells. The production of the powders leads to a decrease of viable miroorganisms of circa 99 % whereupon no difference concerning the protective effect of the variousmaterial mixtures can be observed. Gardiner and O'Riordan desrcibe losses of one decimal power during spray-drying of bacteria (Gardiner, 2000; O'Riordan, 2001) which is a much lower loss compared to the results of this study. Due to the big differences in heat sensitivity of different microorganisms comparisons witrh other studies are scarcely concise. One possible explanation for the high mortality during spray-drying in this study might be the drying properties of the gel structures. The gel structure of the Ca-pectinate matrices could lead to an obstructed transport of the water vapor to the capsule surface during the drying process (Ré, 1998). As a result, the particles might need longer drying times, causing a higher heat stress to the microorganisms.

A comparison of the mortality rates during storage shows that the protective prperties of the capsule materials might be different. Since the processing conditions and the solid content of the capsules are the same for all material mixtures, these effects can be ascribed to capsule material interactions. Refering to the literature, the kinds of interactions and the resulting phase-separation behaviour before the spray drying process should be:

- pectin with dextrin: homogeneous system based on weak repulsive interactions beneath the phase-separation threshold.
- pectin with modified starch: phase separated system based upon repulsive interactions near to or above the phase-separation threshold.
- pectin with gelatine: phase-separated system because of attractive interactions leading to coacervate-like structures.

- pectin with soymilk powder: homogeneous very complex system specified by repulsive interactions near to the phase-separation threshold of the main components pectin and soy-protein (Tolstoguzov, 2002).

The homogeneously structured capsules made from pectin and pectin with dextrin show similar mortality rates leading to the conclusion that the cells are randomly distributed within the capsules and for that reason poorly protected, especially in the peripheral regions of the spheres.
The systems with strong intermolecular interactions show clearly higher protective properties. The mixture of pectin with gelatin is a coacervate-like mixed gel, which might have included the bacteria before the extrusion process and was then structurally fixed and maybe reconstructed during extrusion which might lead to an interpenetrating gel-network (Tolstoguzov, 2000a). The resulting protective effect is clearly higher than that gained by the single material gel capsule.
Because of the properties of the interfacial layers in phase-separated repulsive systems, it can be assumed that microorganisms tend to adsorb preferentially to these layers (Gehrke, 1998; Tolstoguzov, 2002; Tolstoguzov, 2003; Tolstoguzov, 2004). Because addition of salts intensifies the thermodynamic incompatibility, the extrusion process might lead to a phase-separation in the system of pectin with soymilk powder (Tolstoguzov, 2002). Due to the comparably low mortality rate in the capsules with pectin and starch and pectin with soymilk powder during storage at 5 °C, it can be assumed that in these systems water-in water-emulsions were formed and that the bacteria were adsorbed to the interfacial layers. The Ca-lactate hardening bath and the spray-drying probably fixed these structures and generated protective areas away from the peripheral regions of the spheres in which the bacteria might be enclosed.

Apple juice and gastric juices are both marked by more or less aggressive acids which might attack the bacteria. A protective shell should therefore limit the diffusion of agressive acids into the interior regions of the capsule and thereby prolongate the the time until the microorganisms get exposed to the acids. Furthermore, the acidic surroundings can change the type of interaction from repulsive to attractive, for example, when the pH-value of the solution drops below the isoelectric point of a protein,which is probably the case in the material mixture with soymilk powder (Tolstoguzov, 2002). Looking at the results it can be seen, that the capsule material mixture pectin with soymilk powder showed the highest protective effect in acidic surroundings like apple juice and hydrochloric acid. It might be assumed that the reason for this phenomenon is the attractive interaction between pectin and soy protein at a pH below its isoelectric point, because the other attractive system – pectin with gelatin – likewise proved to be outstandingly protective. The attractive interaction might here lead to embedded microorganisms which are strongly protected by the materials building up coacervate-like structures (Benichou, 2002). The interfacial layers of the repulsive systems (pectin with starch) have less protective properties with respect to acids because once the acids have crossed through the outer layers they can probably diffuse within these layers, leading to a fast distributuion of the degradative substance (Abdulmola, 2000).

Conclusions

The current study presents a brief survey on the influence of capsule material interactions on the protection of sensitive core materials in different processing steps. It is outlined that every section of the production might have a degradative effect, which can influence the product quality. Additionally, it can be concluded that the medium the capsules are dispersed in might change the kind of material interactions from repulsiv to attractive or vice versa thereby leading to increased or decreased stability of the core material. Further research should be conducted on the cumulation of degradative effects in every production section until the product reaches the consumer to guarantee the physiological or technological value of sensitive core materials.

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