Examination of capsule material – active substance interactions during spray-drying encapsulation of enzymes

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Introduction

The encapsulation of food ingredients by means of spray drying is an available and cheap and therefore commonly used technique (Ré, 1998; Reineccius, 2001). The suitability of the resulting products is very much influenced by the choice of the encapsulating material. Relevant publications point out that the encapsulating material might have the biggest influence on the resulting stability of the active substances (Vega, 2006; Reineccius, 2001). Most of the investigations concerning the influence of capsule materials on the stability of the spray dried products deal with parameters known to have a strong impact on the active substance like dry mass and viscosity of the feed emulsion, emulsion droplet size and DE-values of the materials (Drusch, 2006; Bangs, 1990; Madene, 2006; Ré, 1998). However, direct interactions between active substance and capsule material are often not taken into consideration, neglecting possible strong forces that might protect the active substance .

There are a lot of substances that might interact with suitable capsule materials like any kind of protein. Most of the proteins tend to react with other substances with a net charge because of their high counts of functional groups and their pH-dependent net charge. The interactions of proteins with polysaccharides are well known and used in the formulation and texturising of foods, like dairy products and ice cream. In the field of microencapsulation the technique of complex coacervation uses electrostatic interactions of oppositely charged polymers for the inclusion of the active substance (Benichou, 2002; de Kruif, 2001; Doublier, 2000; Tolstoguzov, 2003; Turgeon, 2003).
The utilisation of interactions between core and shell material might influence the protective effect and the release properties of spray dried formulations. To verify the effect of interactions between core and shell suitable model substances have to be chosen. Enzymes are proteins which can easily be detected and evaluated considering their activity. As an example the reaction velocity of the specific enzyme-catalysed reaction can be taken as a measure for the strength of interaction of the enzyme with the capsule material and the protective effect of these interactions during drying and storage.

This study presents a systematic investigation of the influence of the interplay between capsule material and active substance on the stability of the spray dried active substance during production of the powders, using an α-amylase as a model protein.

Material and Methods

In this study a α-amylase of the organism *Aspergillus oryzae* (generously given by SternEnzym GmbH&CoKG) was used as a model protein.

Soluble starch (Zulkowsky starch, Merck) was used as substrate. Substrate degradation velocity was measured as absorbance of the starch-jodine complex during a reaction time of 10 minutes at 578 nm with a spectrophotometer.
The concentration of the starch solution was 0,2 g/l. 4,9 ml of the starch solution was coloured with 100 µl of a saturated jodine solution, respectively. The concentration of enzyme used for the experiments was 0,5 g/l for the examination of the standards as well as for the determination of the activity of the spray dried powders. 100 µl of this enzyme solution was given to 5 ml of starch-jodine reaction mixture and homogenized. Measurements were done 30, 60, 180, 300 and 600 seconds after addition of the enzyme.

The capsule materials investigated were alginate (Sigma-Aldrich), gelatin from pigskin (Fluka), gum arabic (Sigma-Aldrich), dextrin and modified (OSA-) starch (both from National starch & Chemical GmbH), either as single material or as material combination. The material mixtures consisted of 1 % alginate and 4 % gelatine, gum arabic or starch. The enzyme-capsule material ratio was kept constant for all samples at 1:40, respectively.

One part of the enzyme-capsule-material suspensions were directly dried; the other parts were extruded into a 2 % Ca-lactate hardening bath. The resulting capsule dispersions were adjusted to a pH of 3 and spray-dried with an inlet air temperatue of 180°C and an outlet air temperature of 90°C (spray-dryer: Nubilosa LT-C). The powders were harvested every 5 minutes and stored in glass pots.

Process stability was determined by measuring the starch degradation velocity of the amylase after redispersing the spray dried powders in a pH 3,5 and 6,5 solution.

The starch degradation velocity was calculated from the slope of the double reciprocal plot of the starch concentration against the reaction time.

Results and Discussion

The main goal of a well conceived spray-drying-process is the maximum activity of the core substance. To reach this it might be suitable to investigate the influence of shell-core interactions to optimize the protection of the shell.
Enzymes are proteins that show a characteristic activity which can work as a measure for their condition. They can therefore be employed as model substances for interacting core substances.
This study was developed to investigate the influence of capsule material – active substance interactions during the spray drying encapsulating of water soluble substances. The capsule materials were chosen to ensure different strengths of interactions between core and shell material (Tolstoguzov, 2002). The extrusion process was applied to gell one part of the capsule material in order to develop structures that might influence the processing and the release of the enzyme.

The following table (table 1) depicts the results of the determination of the starch degradation velocity calculated from the slope of the double reciprocal plot of the starch concentration against the reaction time for the redispered spray-dried samples. The samples are: dextrin as a neutral carrier without interaction with the enzyme; alginate (Alg) as a negatively charged encapsulant; Alginate mixed with starch (Alg+Starch), gum arabic (Alg+GA) and gelatine (Alg+Gel) respectively to alter the strength of interaction of positively charged enzyme and negatively charged alginate. The samples containing alginate were extruded (extr.) to change possible interactions between core and shell material by creating a Ca-alginate gel. Preexperiments were carried out to determine the isoelectic point of the enzyme at pH 5,2 by conductometric titration. Therefore

investigations on enzyme activity were carried out at two different pH-values, 3,5 and 6,5 to alter the net charge of the enzyme and thereby the interaction between enzyme and capsule material.

Comparing the results in table 1 it can be seen, that the degradation during spray-drying depends on the choice of capsule material and structure of the feed. There is an obvious difference between the spray-dired and the extruded and spray-dried samples which can be attributed to the gel structure of the extruded samples.

	Degradation velocity at	
Sample	**pH 3,5 (%·s^{-1})**	**pH 6,5 (%·s^{-1})**
Dextrin	3,900	4,050
Alginate	0,630	2,205
Alg+Starch	1,337	4,077
Alg+GA	1,001	4,058
Alg+Gel	0,444	11,351
Alginate extr.	0,133	0,965
Alg+Starch extr.	0,187	0,718
Alg+GA extr.	0,386	1,029
Alg+Gel extr.	2,721	3,063

Table 1: Starch degradation velocity of the samples after spray-drying in media of pH 3,5 and 6,5.

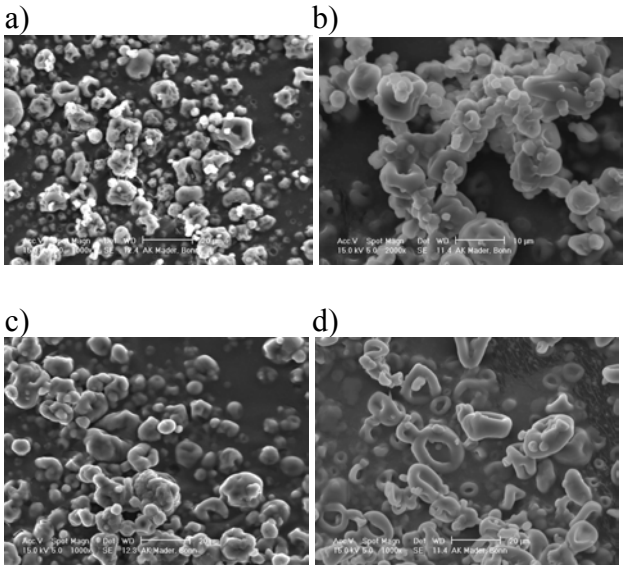

Figure 1: SEM-pictures of the samples a) Alg+ GA; b) Alg+Gel; c) Alg+GA extr.; d) Alg+Gel extr.

Because of their three-dimensional structures, which capture the water, gels obstruct the transport of the water vapour to the capsule surface during the drying process (Ré, 1998). This results in longer drying times causing a greater heat stress to the core. Only the samples consisting of alginate and gelatine diverge from these observations. Under the conditions used for the sample preparation (i.e. pH 3,5) before drying, coacervate-like structures between enzyme and alginate and gelatine and alginate may develop, thereby including the enzyme in a protective matrix. If this mixture is extruded into a Ca-lactate hardening bath, the coacervate like structures are substituted by an interpenetrating network made by Ca-alginate as the first and gelatine as a second network (Tolstoguzov, 2000). These structures show a high protection during drying of the enzyme allthough the gel structure should here lead to a stronger heat stress, too.
The SEM-pictures in figure 1b and 1d show a lot of imperfections for the capsules made from the material mixture alginate and gelatine, pointing out, that this material mixture is not optimal for the spray-drying process even if the starch degradation velocity of the enzyme is comparatively high. An optimization of the formula, for example by adding fillers with good drying properties like dextrins, could lead to a very stable product.

Comparing the results in dependence on the pH it can clearly be observed that specific interactions between enzyme and alginate occur. Alginate can bind the enzyme electrostatically, but being a highly viscous substance, it has poor drying properties. Adding a component with good drying properties, like gum arabic or modified starch, to the alginate enzyme complex, the loss in enzyme activity during drying can be reduced to an extent comparable to an inert substance (dextrin). By

means of such formulas a controlled pH-dependant release can be achieved while avoiding the disadvantages of poorly drying gels.
Regarding the SEM pictures (figure 1a and 1c) it can be seen that the alginate/gum arabic samples show very rough surfaces with lots of indentations. This formula needs to be optimised, too, by varying the ratio of alginate to gum arabic in such a way, that there is enough alginate to bind the enzyme electrostatically, without increasing the viscosity to an extend that the drying is obstructed.

Conclusions

The current study presents a short insight into the possibilities that the usage of capsule material-active substance interactions might offer. It is outlined that the degradative effect of spray drying might be decreased by a prudent choice of capsule material. Additionally, the choice of capsule material can – even using the spray drying technique – be done in respect of the desired release of the active substance. According to the basic requirements of the process, the main material should have good drying characteristics, so that a low viscosity at high solids can be achieved. Hence, stable products with on-off-release properties can be produced by means of spray drying.

References

1. Bangs, W. E. et al. (1990) *Characterization of Selected Materials for Lemon Oil Encapsulation by Spray Drying*. Journal of Food Science, 55, 1356-1358.
2. Benichou, A. et al. (2002) *Protein-polysaccharide interactions for stabilization of food emulsions*. Journal of Dispersion Science and Technology, 23, 93-123.
3. de Kruif, C. G. et al. (2001) *Polysaccharide protein interactions*. Food Hydrocolloids, 15, 555-563.
4. Doublier, J. L. et al. (2000) *Protein-polysaccharide interactions*. Current Opinion in Colloid & Interface Science, 5, 202-214.
5. Drusch, S. et al. (2006) *Microencapsulation properties of two different types of n-octenylsuccinate-derivatised starch*. European Food Research and Technology, 222, 155-164.
6. Madene, A. et al. (2006) *Flavour encapsulation and controlled release - a review*. International Journal of Food Science and Technology, 41, 1-21.
7. Ré, M. I. (1998) *Microencapsulation by Spray Drying*. Drying Technology, 16, 1195-1236.
8. Reineccius, G. A. (2001) *The spray drying of food ingredients*. In: *Microencapsulation of Food Ingredients* (ed. by P. Vilstrup), pp. 151-185. Leatherhead Publishing, Leatherhead, Surrey.
9. Tolstoguzov, V. (2002) *Thermodynamic aspects of biopolymer functionality in biological systems, foods, and beverages*. Critical Reviews in Biotechnology, 22, 89-174.
10. Tolstoguzov, V. (2000) *Foods as dispersed systems; Thermodynamic aspects of composition-property relationships in formulated food*. Journal of Thermal Analysis and Calorimetry, 61, 397-409.
11. Tolstoguzov, V. (2003) *Some thermodynamic considerations in food formulation*. Food Hydrocolloids, 17, 1-23.
12. Turgeon, S. L. et al. (2003) *Protein-polysaccharide interactions: phase-ordering kinetics, thermodynamic and structural aspects*. Current Opinion in Colloid & Interface Science, 8, 401-414.
13. Vega, C. et al. (2006) *Invited review: Spray-dried dairy and dairy-like - emulsions compositional considerations*. Journal of Dairy Science, 89, 383-401.