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Encapsulation of α-amylase in a starch matrix

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Abstract

In this study thermostable α-amylase was encapsulated in a pregelatinized potato starch to obtain a controlled degradation upon water uptake. Encapsulation was performed using kneading, a (thermo) mechanical process. The aim of the study was to evaluate the possibility to encapsulate a degrading enzyme for a system, which can be triggered to degrade upon water uptake instead of direct water contact. After the encapsulation process, the enzyme (α-amylase) activity in the samples was found to have 90 ± 5% of activity of the unprocessed enzyme. It was seen that there was initial degradation of the starch matrix during processing, proportional to the enzyme concentration. The encapsulated enzyme was inactive during storage under low humidity conditions (RH ≤ 60%) and could be reactivated upon water uptake by incubation of samples in humid atmosphere (90% RH). The reduction of the molecular weight, upon activation of the encapsulated enzyme was also proportional to the concentration of the encapsulated enzyme.

Keywords: Starch; Enzyme encapsulation; Triggered degradation

1. Introduction

One of the reasons to apply encapsulation technology is to obtain controlled release of active organic and inorganic substances for availability at desired site, time and at a specific rate (Pothakamury & Barbosa-Cánovas, 1995; Shahidi & Han, 1993). Among the frequently used matrix materials for encapsulation, starch presents some advantages, as it is cheap, available in large quantities, fully biodegradable, food grade and can be easily modified. Different methods and processes have been applied to prepare starch-containing formulations for a number of applications (Bergsma & Wierik, 1997; Carr, Wing, & Doane, 1991; Chen & Jane, 1995; Doane, 1993; Mauro, 1996; Trimnell & Shasha, 1988; Trimnell, Wing, Carr, & Doane, 1991; Qi & Xu, 1999).

In controlled release systems, degradation of the matrix material frequently occurs as a determining factor for the release of the encapsulant (Pothakamury & Barbosa-Cánovas, 1995). In such systems, unfortunately, the degradation is often dependent on uncontrolled external parameters, like e.g. microbial activity and environmental conditions (Arevalo-Niko et al., 1996; Dave, Rao, & Desai, 1997; Imam et al., 1998; Whitney, Swaffield, & Graffham, 1993). Recently a system was described in which an enzyme (α-amylase) was encapsulated to degrade the matrix surrounding it, when placed in a dissolution media. In this system, degradation started with swelling and dissolution of the device was associated with the enhancement of—burst release in most cases—the release (Dumolin, Cartilier, & Marteescu, 1999). However, this study does not provide sufficient information about the possibility of applying the described approach for systems where direct water content should be avoided. In order to obtain such a system, that the degradation occurs upon a trigger other than direct water content, the degrading enzyme is required to be encapsulated in an efficient way. It is also required that the enzyme maintains its activity while it will stay inactive during processing and storage. It is known that enzyme activity is dependant on factors like pH, temperature, substrate concentration and water activity (Mathewson, 1998). As temperature and pH can cause irreversible damage to the enzyme and substrate concentration is constant, keeping the water activity low can be used to control the enzyme activity during the course of processing and storage. When required, reactivation of the enzyme can be achieved by increasing the water activity upon water uptake. Thus, the objective of this study is to evaluate the possibility to encapsulate a degrading enzyme and to obtain degradation of the encapsulating matrix using water uptake as a trigger instead of direct water contact. As a processing method, a melt

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encapsulation method was chosen. As an encapsulation matrix, pregelatinized potato starch was selected. A thermostable enzyme was used in order to tolerate temperatures required for processing temperatures. Addition of extra water was avoided to keep the water activity as low as possible to minimize the matrix degradation during processing (Simpson, Ashie, & Smith, 1996).

2. Materials and methods

2.1. Materials

Pregelatinized potato starch with 10% w/w water content (amorphous, Flocgel LVW) was supplied by AVEBE, The Netherlands. Glycerol was purchased from Chemproha Chemical Distributors BV, The Netherlands and soy lecithin (Topcitin 50) was purchased from Lucas Meyer, The Netherlands. Applied thermostable α-amylase preparation (Termamyl 120L) was purchased from Novo Nordisk A/S, Denmark. This enzyme is produced by a genetically modified strain of *Bacillus licheniformis* with an enzyme activity of 120 KNU/g (1 KNU = amount of enzyme which breaks down 5.26 g. starch, at 37 °C and pH 5.6 per hour, to dextrins and oligosaccharides). All other reagents used in the experiments were of analytical grade.

2.2. Premix preparation

In the composition of the premix, pregelatinized starch was used as a matrix material. Addition of extra water was avoided and glycerol was used as a plasticizer which also worked as a stabilizer for the enzyme (Copeland, 1996), to minimize degradation of the matrix.

To obtain homogeneous premix of the matrix formulation, pregelatinized starch, glycerol (20% w/w, on dry starch basis) and lecithin (3% w/w, on dry starch basis) were randomly mixed in a beaker with blade type agitator for 5 min at room temperature before being fed into the kneading device.

2.3. Encapsulation process

Processing was performed using a kneading device (a lab scale torque rheometer, Haake-Rheocord 90. Haake Inc., USA, with a Haake Rheomix 600 mixing device). The screw speed was kept constant at 70 rpm and total feed of premix was 50 g. The barrel temperature was 80 °C. Operation time was 13–15 min. A homogeneous melt was obtained after kneading for 10 min and the enzyme was introduced into the kneader. The applied enzyme concentrations varied between 0 and 8% w/w on the dry base of starch. Before the liquid enzyme preparation was introduced into the kneader, 70 ppm $CaCl_2$ (on the dry base of starch) was added. After the enzyme was added kneading was continued for 3–5 min to obtain a homogeneous mixture with the melt. Mixing of the melt and the liquid enzyme was screened via the torque values. The mixing was assumed to be complete with the recovery of the torque value, which decreased after the addition of the enzyme. After the process was completed, samples were removed from the kneader and cooled down under ambient conditions for an hour prior to grinding.

2.3.1. Sample preparation for analysis

Prior to further analysis, the samples were cryogenically ground in liquid nitrogen using Polymix A 10 (Switzerland) grinder. Ground samples were sieved using a sieve having a mesh size of 212 μm. Powder fraction, which goes through this sieve was stored in closed containers at 4 °C before further analysis to be used.

2.4. Analytical methods

2.4.1. Determination of alpha amylase activity after encapsulation process

Enzyme activity of the samples was determined based on a comparison of processed enzyme extracted from the samples and unprocessed enzyme based on an iodine staining (amyloclastic) method. Ground samples with encapsulated alpha amylase (1 g), were added to 9 ml of 0.1 M Sodium acetate buffer (pH 5.6) and were continuously stirred using a magnetic stirrer at 4 °C and complete extraction was achieved in 16 h. Then the suspension was centrifuged at 14,000 rpm, for 3 min by using Eppendorf centrifuge 5415 and the supernatant was used for the determination of the enzymatic activity. The appropriately diluted supernatant solution (0.5 ml) was added to 5.0 ml of substrate solution (20 mg of soluble starch per ml in sodium acetate buffer, pH 5.60) and kept at 25 °C for 10 min. To stop the enzymatic reactions, 0.5 ml of the digest was added to 5.0 ml of 0.1 M HCl. After mixing, 0.5 ml of this mixture was added to 5.0 ml of the iodine solution (0.5 mg of iodine and 0.05 g. KI in 100 ml demi-water). The intensity of the blue color was measured in a spectrophotometer (660 nm). The activity of alpha amylase was given as arbitrary units defined in terms of a specified decrease in intensity of iodine strain (Bajpai & Bajpai, 1989.

2.4.2. Water content and water uptake of the samples

Starch samples containing different concentrations of encapsulated enzyme and a reference sample without any enzyme encapsulated (referred to as blank sample) were at the different relative humidities (30, 60, 90% RH) at 20 °C and changes in weight were recorded as a function of time. The water content of the humidified samples after equilibrium (no further water gain as determined by weight control) was reached, with and without encapsulated enzyme were determined by difference in weight before and after freeze-drying.

2.4.3. Molecular weight determination of the samples

Average molecular weights and molecular weight

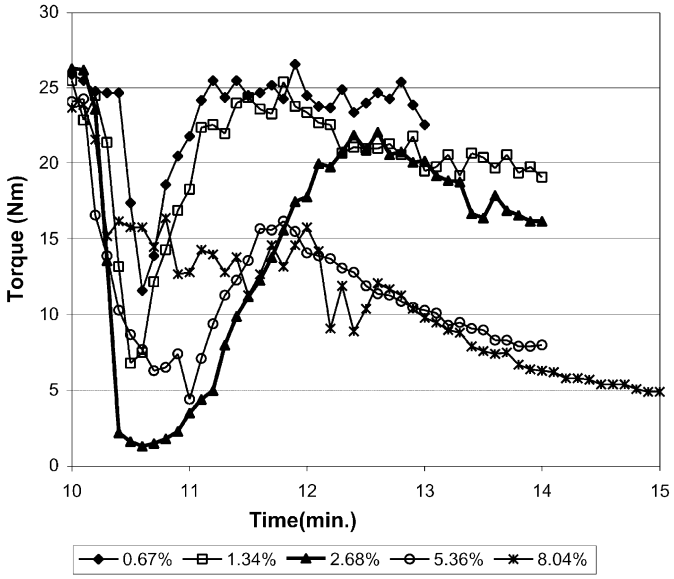

Fig. 1. Torque values after the addition of α-amylase as a function of the enzyme concentration (w/w%, on dry starch basis).

distribution of the starch matrix were determined with a high performance size-exclusion chromatography unit (HPSEC-MALLS-RI) with a multi-angle laser-light-scattering detector and differential refractometer. 25 mg of ground sample and (enzyme concentration in the sample × 0.50 mg), were dissolved in 0.25 M NaOH solution, and diluted into phosphate buffer (pH 8.0). ethlenediaminetetraacetic acid (EDTA) was added to inhibit enzymatic activity and to stop further degradation of the matrix. Final concentration of the dilution was 5.0 mg ml^{-1}. Diluted samples were filtered through a membrane filter (Millipore-0.45 μm) and injected into a sample loop (200 μl). Together with the eluant (phosphate buffer at pH 8.0), the sample was pumped into a guard column (TSK PWH-PE perd-guard, 7.5 × 7.5 mm, Beckman) followed by six main columns (Spherogel-TSK 1000PW, 2000PW, 3000PW, 5000PW, 5000PWHR and 6000PW, 30 × 7.5 mm). Analysis of the eluant was performed by a Dawn-F multi-angle laser photometer (Wyatt technology), equipped with an Argon-Ion-Laser operating at 488 nm and with detectors in different angular ranges between 15 and 151° by a RI detector (Waters 410 differential refractometer). The measurements were performed at a constant temperature of 50 °C. A d*n*/d*c* value of 0.145 ml g^{-1} was applied to calculate the molecular weights (Veelaert, 1996).

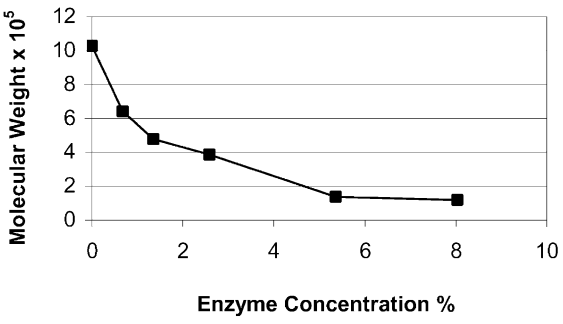

Fig. 2. Effect on enzyme concentrations (w/w% on dry starch basis) on the molecular weight of starch matrix at the end of encapsulation process.

3. Results and discussion

3.1. Encapsulation process

A homogeneous melt of the starch matrix was obtained by kneading for 10 min. Torque value was 26 Nm and melt temperature increased from 80 to 93 °C during this kneading process. Addition of the enzyme preparation, which was a liquid formulation, into the kneader, resulted in a decrease in the torque values. The mixing process of the liquid enzyme preparation and the starch melt was followed via the torque values and was assumed to be complete when the torque values increased up to a constant level (Fig. 1).

It was possible to obtain samples having enzyme concentrations up to 10.7% (w/w) on the dry base of starch. When the enzyme concentration was greater than 10.7% (w/w), a homogeneous mix could not be obtained, probably due to excessive degradation of the starch matrix (data not shown).

The required time for mixing α-amylase with the starch melt in the kneader and the recovered torque values were affected by the concentration of α-amylase added, as given in Fig. 1. When the concentration of the enzyme was increased, more time was required for a complete mixing and the recovered torque values at the end of the process decreased indicating a lower viscosity of the melt. Although the addition of extra water was avoided, low viscosity of the

Table 1
Water content (w/w% based on total weight) of the samples after 14 days of storage at various humidities at 20 °C

Enzyme concentration (w/w%, on dry starch basis)	$t = 0$ days	$t = 14$ days		
		30% RH	60% RH	90% RH
Blank	9.3	6.3	10.9	23.6
0.67	9.1	6.1	11.0	25.1
1.34	9.0	6.0	11.5	26.4
2.68	11.0	5.9	11.3	26.8
5.36	10.0	5.8	11.7	26.6
8.04	10.0	5.7	11.9	26.8

melt indicated degradation of starch during processing. Indeed the molecular weight of the starch matrix decreased in course of processing and this decrease was proportional to the enzyme concentration (Fig. 2).

The remaining enzyme activity in the samples after the encapsulation process was determined and found to be 90 ± 5% of its original activity. After 2.5 months of storage (30 and 60% RH, at 20 °C), the remaining enzyme activity was found to be 80 ± 5% of the activity of unprocessed enzyme for all samples.

3.2. Water content and water uptake of the samples

The samples were in equilibrium (water content of samples was constant during storage) after 48–72 h of incubation under different conditions. When the samples were incubated in 30% RH, all samples dehydrated loosing between 1.5 and 5% (w/w) of their weight in water (Table 1). For the samples, which were stored at 60% RH, 20 °C, the water uptake was minor, between 1.5 and 2.5% (w/w). However, the water uptake was up to 25–26% (w/w) when the samples stored at 90% RH, at 20 °C (Fig. 3).

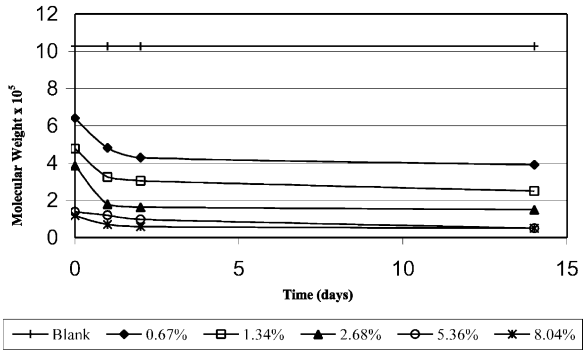

Fig. 4. Effect of the enzyme concentration (w/w%, on dry starch basis) on molecular weight change of the starch matrix during storage at 90% RH and at 20 °C.

3.3. Reduction of the molecular weight during storage period

It was observed that the molecular weight of the samples did not change when stored at 30% RH or at 60% RH humidity (data not shown). On the other hand, when the samples were stored at 90% RH, 20 °C, the molecular weight of the starch matrix decreased in time (Fig. 4). The molecular weight of the starch matrix with 0.67% (w/w) of enzyme load on dry base of starch, the molecular weight decreased towards 53% of its original value, during the storage period at 90% RH, at 20 °C of 24 h. After 14 days of enzymatic hydrolysis under these conditions, the molecular weight of the starch matrix had decreased towards 62% of its original value (Fig. 4). At lower enzyme loads of 0.014–0.14% (on dry starch basis, w/w) applied, molecular weight of the starch matrices decreased from 5 to 28% of the original value, respectively, during storage period of 14 days at 90% RH, 20 °C (data not shown). It can be seen that the decrease of the molecular weight of the samples correlates with the amount of enzyme, which is encapsulated (Fig. 4). However, the enzyme concentrations above 2.68% do not result in a significant decrease in the

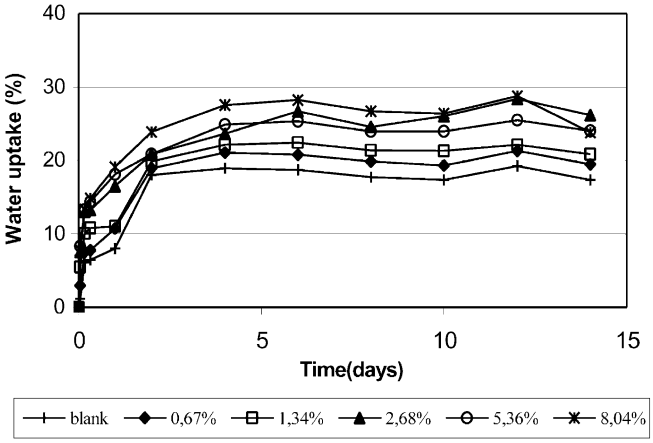

Fig. 3. Effect of the enzyme concentration (w/w%, on dry starch basis) on water uptake of the samples when stored at 90% RH. (experimental error ≤ 3%).

molecular weight of the starch matrix during the course of this storage (90% RH at 20 °C). This was explained as a consequence of excessive degradation of the starch matrix already during processing at these higher enzyme concentrations.

As can be seen from the results and observations, it is required that the samples are stored in an environment with a sufficient humidity, so that upon water uptake, the encapsulated enzyme can be reactivated. Apparently storage at 30–60% RH is not enough to meet this requirement, since the molecular weight of starch remained unchanged indicating that the hydrolysis did not occur. However, when the samples were stored at 90% RH, the molecular weight of starch did decrease due to the sufficient water uptake followed by the reactivation of the enzyme. These results and observations support the hypothesis that the activity of the enzyme can be controlled via the relative humidity and the reactivation of the enzyme can be achieved upon sufficient water uptake when the samples are stored in a sufficiently humid environment.

It was expected that the degradation rate would be constant, as no loss of activity of the enzyme was detected. The degradation rate decreased in the first 48 h of storage for all samples and the molecular weight of the starch matrices did not show a significant decrease after this period (Fig. 4). This observation is not yet fully understood, but is thought to be an effect of changing water activity due to production of lower molecular weight compounds which partially bounded the water in the system.

4. Conclusions

The results show that the encapsulation of a degrading enzyme is successfully performed, using thermoplastic processing methods with no significant loss of activity.

The results support the hypothesis that it is possible to obtain a system, which can be triggered to degrade, when the water activity is increased upon water uptake, which results in reactivation of the encapsulated enzyme. It is concluded that the water activity can indeed be used as a tool to inhibit the enzyme activity during storage under low humidity conditions. However, the results indicated that during processing avoiding the addition of water for keeping the water activity as low as possible, is not enough. It is also necessary to choose the suitable enzyme concentration to avoid excess degradation of the matrix.

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