International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277-209X (Online)
An Online International Journal Available at http://www.cibtech.org/jfav.htm
2012 Vol. 2 (3) May-August pp.161-166/Kumar et al.
Research Article

CHARACTERISATION OF BLENDED SODIUM ALGINATE MICROCAPSULES FOR CONTROLLED RELEASE OF ANIMAL FEED SUPPLEMENTS IN THE GIT

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ABSTRACT
The controlled release of bioactive substances to their site of action in the Gastro Intestinal Tract (GIT) is essential for effective functioning of these bioactive compounds such as probiotics, feed enzymes etc. The major obstacles to retain the activity for the bioactive compounds is to overcome stomach acidity and bile salts. An *in vitro* study has been conducted to study the protective effect of sodium alginate and its combinations with other compounds such as xanthan gum, carboxy methyl cellulose (CMC) and carboxy methyl starch (CMS) as microencapsulating agents to protect bioactive compounds such as feed enzymes namely amylase and xylanase against the different extreme conditions in GIT of animals. The optical sensitive feed enzyme amylase of known optical density was microencapsulated with 1 % (w/v) and 2 % (w/v) sodium alginate, sodium alginate + xanthan gum (1: 0.50) and sodium alginate + carboxy methyl cellulose (1: 0.75). Xylanase enzyme was encapsulated with sodium alginate + carboxy methyl starch (1: 0.75). These capsules were incubated in simulated gastric fluid (SGF) of pH 2 and simulated intestinal fluid (SIF) of pH 7.5. The release of the amylase and xylanase at different time intervals from various capsules was measured. In alginate CMC capsules, enzyme release was rapid in SGF, with a release of 56 percent of the enzyme in the first forty five minutes of incubation period when compared with other microcapsules. Alginate xanthan capsules showed good tolerance to SGF, only 12.8 % of amylase was released after 35 minutes of incubation and 24 % of amylase was released after 90 minutes of incubation which was very low when compared with other capsules with a release level of 61 % from alginate CMC capsules, 46.8 % of xylanase from alginate CMS capsules and 43.6 % from 2 % alginate capsules and 52.4 % from 1% alginate capsules. Whereas in SIF, alginate CMS capsules showed good tolerance when compared with other capsules, with only 56 % of xylanase was released after 90 minutes of incubation when compared to 61 % percent of amylase was released from alginate CMC capsules and alginate xanthan capsules, 100 % after 45 minutes of incubation from 1 % alginate capsules and 100 % after 90 minutes of incubation from 2 % alginate micro capsules.

Key Words: *Encapsulation, amylase and GIT*

INTRODUCTION
Currently, enzymes are being used as supplements in both pig and poultry feed. Enzymes are catalytic proteins with their active sites housed inside a 3D structure, making them sensitive to some physico-chemical conditions (Pérez-Portabella and Roura, 2001) such as pH, hydrothermal conditioning, frictional forces and to the heavy metals that are added to certain animal feeds (Steen, 2001). Enzyme supplementation improves performance and nutrient digestibility of broilers fed diets containing high levels of grains rich in Non Starch Polysaccharides (NSP). Fibrolytic enzymes, proteases, lipases and phytases play an important role in efficient utilization of fibre and other nutrients in diet. Hong *et al*. (2002) found that the use of an enzyme cocktail that has xylanase, amylase and protease activities improved the digestibility of corn soya based diets. Improvement in weight gain, efficiency of feed utilization and reduced sticky droppings in broilers by supplementing the diet with enzymes has been documented (Raghavan, 1990). The reason is not totally clear but has been related to a decrease in

intestinal viscosity, which may improve nutrient digestibility and increase feed intake (La´zaro *et al.*, 2003).

However, during the passage through the gastro intestinal tract, feed enzymes are required to tolerate the low pH of the stomach, and the activity of bile salts. Similarly native enzymes may lose their three dimensional structure which affects their activity. It is important to find methods for retaining the activity of bioactive feed enzyme supplements in the digestive tract. Micro encapsulation is packaging active materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions according to (Shahidi and Han 1993). Several studies have shown that microencapsulation with alginate at different concentrations or other gels protects them against acid stress, allowing the bioactive supplements to be active in the stomach and to be delivered in the intestine (Crittenden et al. 2006). Moreover encapsulation with alginate will not affect the structural integrity in the encapsulated condition or during the relase of enzyme. The pH of the stomach has been reported to be 1 to 3 during fasting and changes upon the bolous entry (Ekmekcioglu, 2002). Sufficient published work is not available regarding the effect of encapsulation of enzymes and subsequent release after ingesting in the GIT. Thus an experiment was conducted with the following objectives to study the effect of encapsulation of feed enzymes. In the present study micro encapsulation of amylase and xylanase using alginate and with combination of other compounds has been attempted to overcome the effect of acidity and adverse conditions of gastro intestinal tract on bioactive feed enzymes and an assessment was made to understand the controlled release of bioactive feed enzymes (amylase and xylanase) in simulated gastric fluid (SIF) and simulated intestinal fluids (SIF).

MATERIALS AND METHODS

Experimental design

The protective nature of the alginate capsules was determined by assessing the retention time of the enzyme feed supplements in the capsule at different ionic strengths.

Procurement of enzymes

The feed enzymes amylase and xylanase was procured from Kaypeeyes Biotech Private Limited, Mysore.

Determination of absorption maxima (λ_{max}) of the bioactive feed supplements

Generally, the pure protein enzymes have the absorption maxima at 280 nm but these commercial enzymes while processing may have added with traces of other compounds. So, the absorption maximum (λmax) of the enzymes was determined using UV-Visible Spectrophotometer.

Encapsulation of Enzymes (Extrusion Technique)

Enzymes were added into a hydrocolloid solution (alginate) and then the cell suspension was extruded through a nozzle with pressure of 1.5 bars around it to form fine droplets, which free-fall into a hardening solution ($CaCl_2$) or setting bath. The concentrations of alginate used for encapsulating amylase was 1% and 2% to form a gel with 0.5M $CaCl_2$ (Krasaekoopt *et al.*, 2003) and its combinations alginate-xanthan in the ration 1:0.5 and alginate CMC in the ratio 1: 0.75. Xylanase was encapsulated with alginate-CMS in the ratio 1: 0.75

Micro capsule size measurement

The wet capsule's size distribution was measured using a laser diffraction instrument, Particle Size Analyzer (Horiba Instruments, Japan). The dried capsules size was measured with scanning electron microscope at Ruska labs, Rajendranagar, Hyderabad.

Buffers of different ionic strength

Simulated gastric juice was used with a pH of 2 and simulated intestinal juice was used with a pH of 7.5. Buffers of pH 2 and pH 7.5 were used to simulate gastric juice and intestinal juices.

In vitro assay of release of bioactive compounds from alginate micro capsules

An experiment was designed mimicking the temperature, peristaltic moments using orbital shaker instrument and pH variations in the GIT of mono gastric was mimicked using simulated gastric fluid

(SGF) and simulated intestinal fluid (SIF) to determine the release rate of enzymes from the enzyme micro capsules of 1% and 2% alginate and their combinations. The enzyme capsules were placed in these buffer solutions and kept in orbital shaker for incubation. Temperature was set to 39^0C and the oscillations were set to 120 rpm to mimic the peristaltic movements of GIT. The absorbance of the buffer solution was read at timely intervals using an UV visible spectrophotometer against the blank of buffer and capsules without enzyme. The absorbance is considered as directly proportional to the amount of enzyme released (Pomsaksriamomsak and Sunghthongjeen, 2007).

RESULTS & DISCUSSION

Encapsulated enzymes particle size

The wet 2% alginate enzymes micro capsules were of the size 200 micrometers and after drying these encapsulated enzyme capsules were of the size 100 micrometers which was measured using scanning electron microscope. The dried sodium alginate + xanthan gum (1: 0.50) were of the size 50 micrometers, sodium alginate + carboxy methyl cellulose (1: 0.75) were of the size 20 micrometers diameter and sodium alginate + carboxy methyl starch (1: 0.75) were of the size 50 micrometers (fig 1-4).

Figure 1: SEM photograph of 2 percent alginate microcapsule

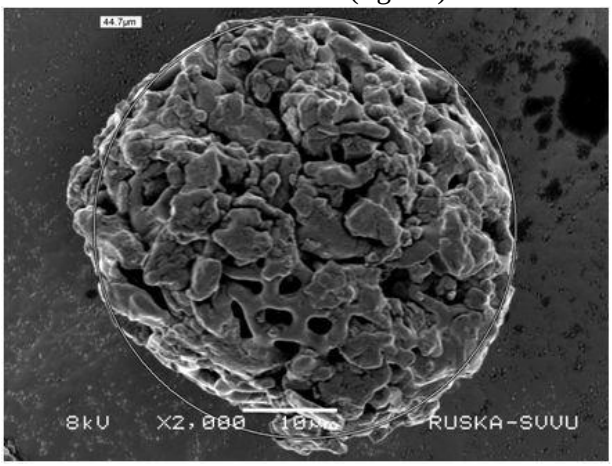

Figure 2: SEM of photograph of alginate-xanthan microcapsule

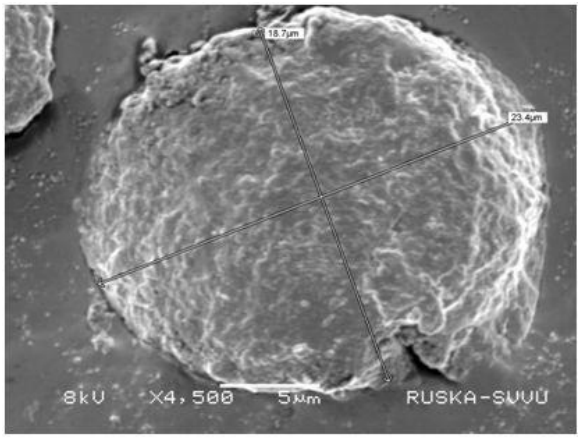

Figure 3: SEM photograph of alginate-CMC microcapsule

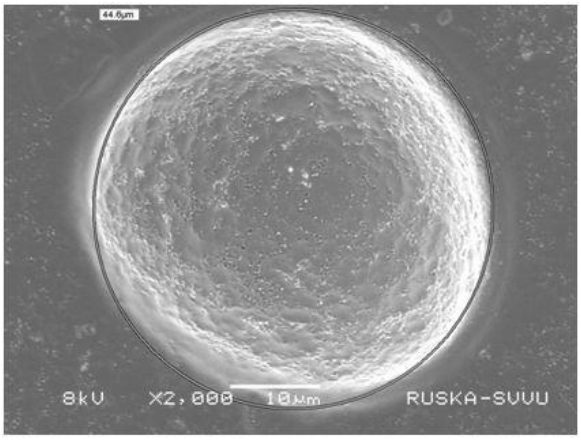

Figure 4: SEM photograph of alginate-CMC microcapsule

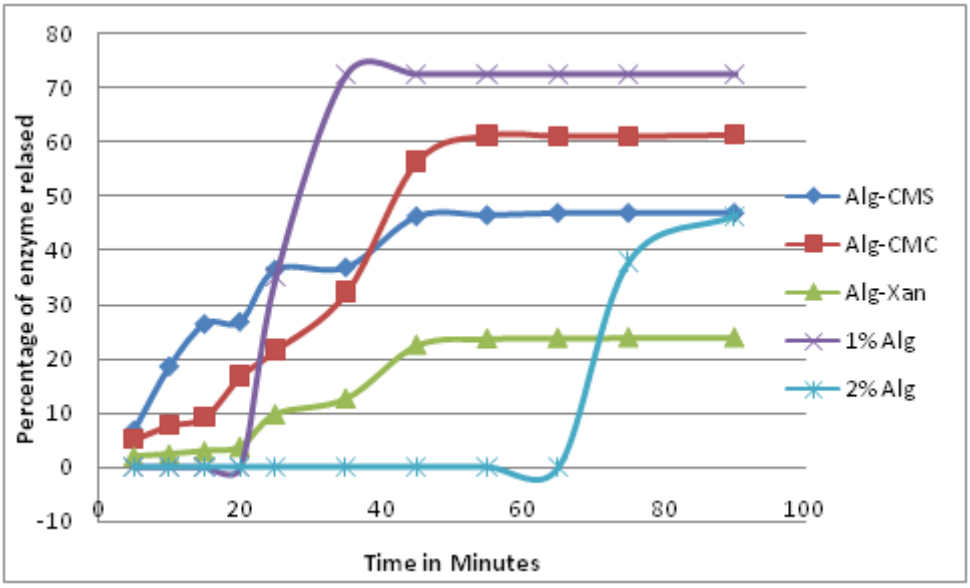

Figure 5: Enzyme release pattern in the Simulated Gastric Fluid (SGF)

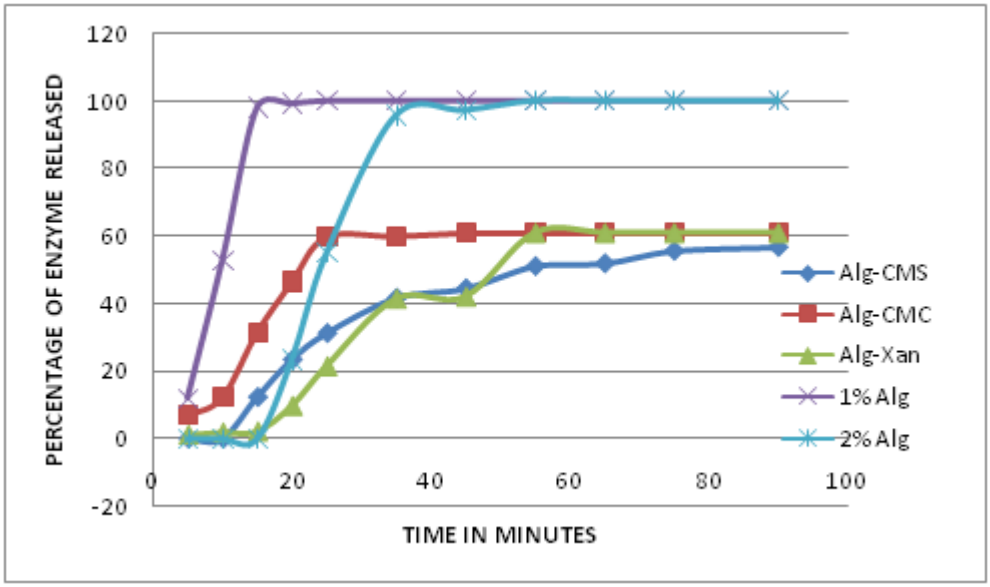

Figure 6: Enzyme release pattern in the Simulated Gastric Fluid (SGF)

Absorption maxima (λ_{max}) of bioactive compounds (enzymes)

The absorption maximum of the amylase enzyme was found to be 273 nm and xylanase was found to be 278 nm which are slightly different from the absorbance of the standard proteins of 280 nm. This may be due to the fact that, while manufacturing and harvesting of the microbial enzymes some traces of other compounds such as culture broth or some nutrients etc., may also be retained with the pure enzyme.

In vitro release of bio active compounds in SGF

Two percent alginate micro capsules showed good resistance towards acidic pH 2 only and 46.36 percent of amylase was released when compared with 1 % alginate micro capsules which showed 72 percent of amylase release (Fig 5). At this pH there may be opening of the capsules (Ellenton 1998) allowing the amylase to get dissolved in the buffer. About 52% of the amylase released during first 45 minutes of incubation. Similar results were obtained by Houria *et al.,* 2012; in which they have used 1% alginate to encapsulate probiotic and incubated in SGF. Zoe and Maria (2006) also concluded that alginate capsules prepared from 1% (w/v) alginate were fragile and difficult to handle and released significant amount of bioactive compounds into the culture medium. Capsules of alginate xanthan gum combination showed better resistance (24 percent amylase release) than alginate CMC (46 percent amylase release) and alginate CMS capsules (64 percent xylanase release). Similar conclusions were made by Soma and Lo (2009) in which they reported the protective effect of encapsulation with xanthan and chitosan in SGF and concluded that xanthan chitosan are effective in releasing of cells especially for targeted delivery in intestines. Mankan *et al.,* (2011) reported only 20% of release from the microcapsules of alginate with xanthan gum (1:1). Moreover xanthan showed high resistance towards acidic conditions especially gastric juice and taurocholic acid (Ding and Shah 2009). Similar conclusions were made by Lee and Heo (2000) in which Bifidobacterium *longum* encapsulated in calcium alginate containing 2.0, 3.0, and 4.0% (w/v) sodium alginate tolerated significant incubation time in a simulated gastric juice (pH 1.5) better than free cells.

In vitro release of bio active compounds in SIF

The proportion of amylase released in SIF after 90 minutes is 61.6 % and 61 % for alginate xanthan, alginate CMC and 56.6 % of xylanase was released from alginate CMS capsules (Fig 6). Two percent alginate micro capsules showed good resistance than the 1% alginate micro capsules. The leakage from the alginate capsules may also be attributed to porous nature of the alginate capsules (Gouin 2004) which may be of around 7 nm (Klein et al 1983). So the capsules become fragile and the contents in the capsules start dispersing into the medium. As alginate concentration in the capsules is around 60 percent only, the enzyme present in the alginate capsules might have been dispersed. In another experiment conducted by Reddy and Reddy (2010), 51.4 percent drug release was observed from alginate CMC capsules after 90 minutes of incubation.

When compared with the basic conditions the acidic conditions are more deteriorative for the capsules. This may be due to the fact that depletion of calcium ions from alginate capsules in acidic conditions during the first 30 minutes of incubation forms alginic acid which is soluble (Sabitha et al, 2012) and also may be due to the fact that the ions used for stabilizing alginate beads i.e calcium which is highly susceptible to the mono valent ions (Smidsrod and Skjak-Braek, 1990) and chelating agents (Ellenton 1998).

ACKNOWLEDGEMENTS

The above research work was supported by the Department of Biotechnology, Govt of India (BT/PR-14851/AAQ/01/457/2010 Dt.22/3/2011).

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