P-068	**Carboxymethylated flamboyant (*Delonix regia*) seed gum for microencapsulation of papain**	

Segura-Campos M., Pacheco-Aguirre J., Chel-Guerrero L. and Betancur-Ancona D. *#
Fac Ing Quím, Univ Autónoma de Yucatán. Mérida, Yucatán,México
*#bancona@uady.mx

INTRODUCTION AND OBJECTIVES

The use of modified polysaccharides with ionic charge to microencapsulate substances with biological activity (also known as nutraceuticals) has become increasingly popular in the nutraceutical food industry. Microencapsulation protects nutraceuticals, keeps them stable in storage at room temperature and ensures release of an appropriate dosage in a gastric or intestinal pH. In the food industry, the modified polysaccharides carboxymethylcellulose, carboxymethylated guar gum and carboxymethylated starch are the most frequently used to encapsulate nutraceutical substances. Their structural characteristic allow cross-linking action with metallic bivalent cations and capture of the active substance in its interior
Flamboyant (*Delonix regia*) trees are considered an ornamental species, and native gum from its seeds has not been used previously to microencapsulate nutraceutical substances in food and drugs. Nonetheless, flamboyant seed native gum (FNG) is potentially useful for microencapsulation because it contains galactomannan-type polysaccharides similar to those of guar gum (*Cyamopsis tetragonalobus*) and locust bean (*Prosopis chilensis*) gum. The few branched regions present in FNG consist of α-D-mannose (1→4) linkages and α-D-galactose (1→6) branches (mannose–galactose 2:1ratio). Its mannose and galactose proportions are similar to those of guar gum but differ in terms of the OH bond position in the main chain: flamboyant gum has α-D-mannose while guar gum has β-D-mannose.

The present study objective was to evaluate the capacity of carboxymethylated flamboyant (*Delonix regia*) seed gum, an underexploited tropical resource, to encapsulate papain as a model for producing controlled delivery of bioactive substances for potential food, nutraceutical and biotechnology applications.

MATERIALS AND METHODS

Flamboyant native gum (FNG) extraction was done following Azero et al. (2006), the endosperm flour was suspended in water (1:30 w/v, pH 7) and heated at 50 °C for 30 min, then was precipitated in 70% (w/v) ethanol, dried at 55 °C for 24 h and milled to an 80 mesh size.

FNG carboxymethylation was done following Bahamdan et al. (2005). FNG was swelled in 2-propanol. After, NaOH (40%) was added and the mixture left 30 min at room temperature to allow further swelling. Temperature was then raised to 70 °C for 1 h, and the reaction proceeds for 3 h. The carboxymethylated flamboyant gum (CFG) was recovered by filtration, washed with 99.9% methanol and dried.

Microcapsule preparation microcapsules were prepared based on Sankalia et al. (2004). A 2^3 factorial design with 4 central points was used. The tested factors and levels were gum concentrations of 1 and 3% (w/v); $FeCl_3$ concentrations of 0.05 and 0.15 M; and hardening times of 20 and 30 min. Response variables were amount of papain released and residual enzymatic activity.

Differential scanning calorimetry the amount of papain in the microcapsules and its interaction with the polysaccharide were monitored with differential scanning calorimetry (DSC) following Babu et al. (2002), 3 mg (db) sample were weighed out and water added until reaching a 20% (w/w) concentration. This mixture was crimped into a standard aluminum pan and heated from 30 to 200 °C at a 10 °C/min rate under constant nitrogen purging at 20 ml/min.

In vitro studies release capacity was evaluated according to the method of Takagi et al. (2003). Dry capsules were added to beakers containing HCl solution (pH 1.2) with 2% NaCl at 37 °C for 2 h, to simulate gastric pH (GpH). The microcapsules were recovered and then placed in beakers containing 0.25 M phosphate buffer (pH 6.8) at 37 °C for 3 h, to simulate intestinal pH (IpH). Residual enzymatic activity was quantified according to Anson (1938).

RESULTS AND DISCUSSION

The microcapsules produced in each treatment were grouped by morphology and size (Figure 1). No differences in particle size were observed between treatments, meaning the factors gum concentration, cationic solution and hardening time had no effect on this parameter.

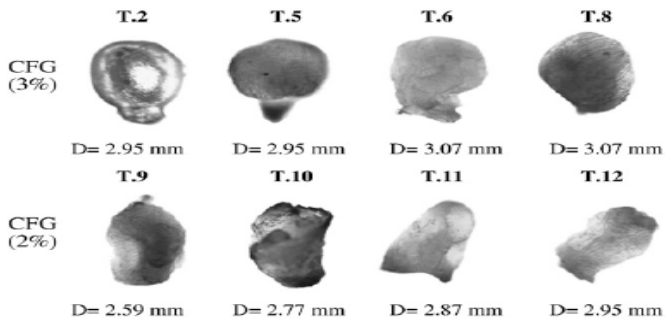

Figure 1: Morphology of carboxymethylated flamboyant gum (CFG) microcapsules at 2 and 3% gum concentrations. D: Diameter, T: Treatments.

Microcapsules containing papain exhibited melting temperatures different from those without papain (Table 1). In treatments 1, 3, 4 and 7, microcapsules did not form and therefore a zero melting temperature value was used in the statistical analysis. Thermal transition for microcapsules with and without papain was lower than for papain alone, which had two different thermal stability domains.

Table 1: Thermal transitions of carboxymethylated flamboyant gum microcapsules containing papain, blank microcapsules and papain alone.

Treatments	Factors[a]			Thermal transition (°C)
	A (%)	B (M)	C (min)	
1	1.0	0.05	20	0
2	3.0	0.05	20	75.92
3	1.0	0.15	20	0
4	1.0	0.05	30	0
5	3.0	0.15	20	80.15
6	3.0	0.05	30	81.52
7	1.0	0.15	30	0
8	3.0	0.15	30	81.37
9-12 (Central points)	2.0	0.10	25	78.14
Blank capsules	2.0	0.10	25	68.96
Papain				95.76 and 103.58

[a]A: Gum Concentration, B: $FeCl_3$ Concentration, C: Hardening Time

A higher gum concentration provided greater protection to the papain and produced consequent lower release levels under GpH and IpH conditions (Table 2) with the lowest levels in treatments 5 (GpH) and 2 (IpH). Residual enzymatic activity was affected by gum concentration and produced consequent lower release of papain under both conditions, with the highest levels of enzymatic activity in treatment 6 under GpH and IpH conditions.

Table 2: Papain release (%) and residual enzymatic activity (%) in CFG microcapsules and carboxymethylated guar gum (CGG) microcapsules under GpH) and IpH conditions.

Treatments	Papain Release (%)		Residual enzymatic activity (%)	
	GpH	IpH	GpH	IpH
2	59.72	27.57	3.20	26.80
5	19.54	28.83	1.16	33.61
6	22.07	37.79	3.46	37.69
8	35.85	32.89	1.13	21.42
Central points	36.72	29.80	2.41	25.71
CGG	18.48	18.34	4.77	11.49

CONCLUSIONS

Papain was microencapsulated with CFG at concentrations of 1 and 3%. Optimum papain release conditions in a simulated intestinal system were attained using CFG concentration as the main factor, indicating higher gum concentration leads to increased papain release, regardless of cationic solution concentration and hardening time. Carboxymethylated flamboyant gum is effective in encapsulating papain, suggesting it could be an effective delivery system in anti-inflammatory or preventative treatment of gastric or duodenal ulcers. This non-conventional gum source provides an effective microencapsulation system which could be used with similar nutraceutical or therapeutic agents.

REFERENCES

- Anson, M.L. (1938) *The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin*. Journal of General Physiology 22 79-89.
- Azero, E.G. et al. (2006) *Characterization of Prosopis juliflora seed gum and the effect of its addition to κ-carrageenan systems*. Journal of the Brazilian Chemical Society 17 844-850.
- Babu, G.V. et al. (2002). *In vivo* evaluation of modified gum karaya as a carrier for improving the oral bioavailability of a poorly water soluble drug, Nimodipine. AAPS Pharmaceutical Science Technology 3 1-9.
- Bahamdan, A. et al. (2005) *Poly(mxyalkylene) grafts/guar gum with applications in hydraulic fracturing fluids*. Polymers for Advanced Technologies 17 679-681.
- Sankalia, M.G. et al. (2004) *Evaluation and simultaneous optimization of papain entrapped in crosslinked alginate beads using 3^3 factorial design and desirability function*. Ars Pharmaceutica 45 253-279.
- Takagi, K. et al. (2003) *Comparative study of in vitro digestibility of food proteins and effect of preheating on the digestion.* Pharmaceutical Society of Japan. Biological Pharmaceutics Bulletin 26 969-973.