Design of Pectin Microcapsules Protected with Shellac and Sodium Alginate with Sensibility of Degradation in Intestinal *p*H of Bovine

GUADALUPE TORIZ CERÓN[1], J. EFRÉN RAMÍREZ BRIBIESCA[1]*, LEONOR MIRANDA JIMENEZ[1], ROSY G. CRUZ MONTERROSA[2]*, HILDA A. ZAVALETA MANCERA[3], MARÍA M. CROSBY GALVÁN[1], JESÚS A. RAMOS-JUÁREZ[4], DAVID HERNÁNDEZ SÁNCHEZ[1]

[1]Posgrado En Recursos Genéticos Y Productividad-Ganadería, Campus Montecillo. Colegio De Postgraduados. Km. 36.5. Carretera México-Texcoco. 56230, Montecillo Edo. De México.

[2]Ciencia Y Tecnología De Los Alimentos. Universidad Autónoma Metropolitana. Unidad Lerma. Av. De Las Garzas 10, El Panteón, Lerma De Villada, Edo. De México. Cp 52005.

[3]Posgrado En Botánica. Colegio De Postgraduados. Carretera México-Texcoco, Km 36.5. Montecillo, Texcoco, Estado De México. Cp. 56230.

[4]Campus Tabasco Colegio De Postgraduados. Periférico S/N, 86500 Heroica Cárdenas, Tabasco. México.

Pectin is a polysaccharide present in food, its basic structure is an anhydro-D-galacturonic acid chain with α 1,4-bonds with methylated carboxyl groups (COOH) that allow the pectin to form a viscous gel and bind with the β-carotene in the intestine and decreases its absorption, consequently it could reduce the yellow color of the covering fat in the bovine carcass. The objective of the study was to design and evaluate pectin microcapsules at different pH. These were made with the extrusion technique using Sodium alginate (SA) and shellac (SH). The treatments were: T1: 8 g of pectin + 5.2 of SA + 2 g of SH (dissolved in 30 mL of ethyl alcohol) in 400 mL of deionized water + one SH layer by immersion. T2: Same ingredients of T1 + SH by aspersion. T3: 8 g of pectin + 5.2 of SA in 400 mL of deionized water + one SH layer by immersion. T4: Same ingredient as T3 + SH spray. T3 showed the highest release of D-galacturonic acid (70%) at pH 3 (P <0.05), without microscopic morphological differences between treatments. It is concluded that the elaboration of microcapsules with the described evaluation characteristics are stable and offers an alternative to capture β-carotene at the duodenum level.

Keywords: pectin, microcapsules, shellac, sodium alginate

The yellow pigmentation of the fat that covers the muscle of the cattle gives an appearance of unpleasant meat, limiting the commercialization in the national and international markets. This coloration is due to excessive ingestion of carotenoids present in tropical forages where bovines grow [1]. The consumed carotenoids reach the intestine, and along with the formation of chylomicrons are absorbed in the intestinal mucosa, passing into the bloodstream intact to be stored in the adipocytes [2]; β-carotene is one of the main chromogens, its excess is not all transformed into vitamin A [3]. A previous study, carried out by our research group, reports pectin as an antagonist of β-carotene absorption in the bovine intestine [4]. The disadvantage is that the pectin is easily degraded by the microorganisms of the rumen and cannot be administered with food supplements naturally.

The objective of this study was to encapsulate pectin, designing microcapsules with encapsulating materials that resist alkaline pH. Shellac and sodium alginate combinations were used to evaluate the degradation at acidic pH (simulating the intestine ~ 3) and at the pH tending to the alkalinity (simulating the physiological environment of rumen 6 to 7).

Experimental part

Material and methods

Assignment of treatments

The research was carried out at the Colegio de Postgraduados, Campus Montecillo, State of Mexico. The design of the pectin microcapsules was made with citrus Pectin (with 69% degree of esterification, Cargill®) and encapsulating agents such as sodium alginate (SA: C6H7O6Na, Sigma®) and shellac (SH: CEDROSA®). With the previous experience of several preliminary tests, the following treatments were proposed (each treatment had 11 replicates and 3 runs per treatment were made at different times):

Group 1. The SH was included in the preparation mixture and in the cover of the microcapsules: Treatment 1 (T1): 8 g of pectin + 5.2 of SA + 2 g of SH (dissolved in 30 mL C_2H_6O) were mixed in 400 mL of deionized water. Once the microcapsules were formed, they were covered with a layer of SH by immersion. Treatment 2 (T2): Microcapsules formed with the same ingredients of T1, were covered with SH by spray.

email: ramirezbribiesca@hotmail.com

Group 2. SH was not included in the preparation mixture, it was only used to cover the microcapsules: Treatment 3 (T3): 8 g pectin + 5.2 SA were mixed in 400 mL of deionized water. The microcapsules were formed and covered with a layer of SH by immersion. Treatment 4 (T4): Microcapsules formed with the same T3 ingredients, were covered with SH by spray.

Group 3. Reference treatments: Treatment 5 (T5): Control. It included 8 g of pectin + 5.2 of SA without encapsulating in 400 mL of deionized water. Treatment 6 (T6). Blank: It included 5.2 SA without encapsulating in 400 mL of deionized water.

Design of the microcapsules

The microcapsules were prepared with the extrusion method, using a B-390 BUCHI encapsulator, at a frequency of 810 Hz, with 28.3 ° C and a pressure of 677 to 718 mbar. The microcapsules were captured in a $CaCl_2$ solution, drying at room temperature. 4 SH covered with spray (0.5 mL of SH by spray in 8 g of microcapsules) were applied after the microcapsules were formed constantly homogenizing the sample. While in the treatments with SH by immersion, and 8 g of microcapsules were introduced in 30 mL of the solution (C_2H_6O + SH) for 5 minutes.

Microscopic characterization of the microcapsules

The morphology of the microcapsules was evaluated with an ESEM FEI-QUANTA 200 (Fei- Termolab, USA) electronic microscope from the National Research Laboratory in Nanoscience and Nanotechnology (LINAN) operated in low vacuum mode and secondary electrons + backscattered electrons at 20 keV. The microcapsules were placed in the brass sample holder with a conductive double adhesive carbon tape and coated with gold/palladium in a metal ionizer (Ion Sputter JFC-1100).

Release of D-Galacturonic acid to different pH solutions

Initially the release of D-Galacturonic acid was evaluated at 15 and 60 minutes. However, the release response did not show significant differences in the first time, for this reason we decided to carry out the experimental development only with the last time. Buffers with pH 7 and 5 were prepared with McDougall saliva to bovine [5] + CH_3COOH. The solution at pH 3 (it was prepared with clarified duodenal bovine liquid (obtained from the slaughterhouse) + $C_8H_5KO_4$ + HCl). Subsequently, 0.5 g of the microcapsule samples were weighed, and 25 mL of each buffer was added and placed in the Daisy digester (ANKOM, Technology Fairport, NY-USA) at 39 °C (simulating the rumen) with circular agitation constant. Then, 2 aliquots of 250 µl (15 and 60 min) were taken and placed in an ice tray and a magnetic stir plate, making the acid digestion phase. Subsequently, 1 mL of H_2SO_4 was added to each aliquot and maintained in magnetic stirring twice for 10 min each time.

Two hundred µl of distilled water were added twice, shaking for 10 and 20 min, respectively. An aliquot of 0.1mL of the digested sample was added to each tube placed in an ice tray and 2 mL of the solution prepared with 0.5% of BNa_3O_3 and concentrated H_2SO_4 was added. The mixture was vortexed and placed on a tray with boiling water for 5 min and the tubes were quickly cooled (tray with ice); 40 µl of solution with 0.15% $C_{12}H_{10}O$ + 0.5% NaOH was added. Finally, all the samples were rested for 15 min at room temperature, the measurement reading was recorded with the absorbance of 520 nm using an ultraviolet light spectrophotometer (Cary UV-Vis 1-E). The calibration curve was prepared from 0 to 40 µ with D-galacturonic acid (Sigma®).

Analysis of information

The microscopic characterization of the microcapsules was described qualitatively. The release of acid D-galacturonic *in vitro* in the different pH solutions were performed with the repetitions for each treatment. The values obtained were adjusted with the reference blank treatment. The percentage of release of pectin (PRP) from each treatment was quantified with the total amount of D-galacturonic acid released, using the following formula:

$$\text{PRP} = \frac{amount\ of\ D-galacturonic\ released\ from\ microencapsulated\ pectin}{amount\ of\ D-galacturonic\ released\ from\ non-encapsulated\ pectin} \text{ x } 100$$

The PRP of treatments 1 to 4 were analyzed with a completely randomized design considering the pH factors (3, 5 and 7) between each of the treatments. The statistical model was the following:

Statistical model:

$$Y_{ij} = \mu + T_i + T_i\ (pH_j) + T_i \text{ x } pH_j + \varepsilon_{ij}$$

Where:
Y_{ij} = Response variable in PRP. μ = General average. T_i = Effect of the i-th treatment.

T_i (pH_j) = Effect of the j-th pH within i-th treatment. T_i x pH_j= Interaction i-th treatment with j-th pH. ε = Random error

The comparison of the statistical means was performed with the Tukey-test using the statistical package IBM-SPSS ver. 21 [6].

Results and discussions

Release of pectin as a function of pH

There was statistical interaction between pH and treatments (P <0.001). The pH 3, regardless of the type of treatment, improved the PRP by 50% (*p*H = 3: 32.14 vs. *p*H = 5: 16.43 and *p*H= 7: 10.86%). Table 1 shows the PRP in each treatment associated with *p*H. Only T3 at pH 3 had the highest PRP value (P <0.001), indicated as acceptable.

Table 1
PERCENTAGES OF PECTIN RELEASE AS D-GALACTURONIC ACID IN FOUR MICROCAPSULE PREPARATIONS

Treatments	pH	Release	SD	Significance	*P* =
1	3	9.85	0.06	3 vs. 5	0.001
	5	14.47	0.70	3 vs. 7	0.846
	7	9.39	1.89	5 vs. 7	0.000
2	3	20.85	0.04	3 vs. 5	0.044
	5	16.42	3.76	3 vs. 7	0.024
	7	15.81	0.30	5 vs. 7	0.919
3	3	76.30	6.52	3 vs. 5	0.000
	5	25.88	3.06	3 vs. 7	0.000
	7	10.93	1.91	5 vs. 7	0.108
4	3	21.57	0.06	3 vs. 5	0.000
	5	8.96	0.12	3 vs. 7	0.000
	7	7.30	0.20	5 vs. 7	0.000

SD =Standard deviation.

The release of ac. D-galacturonic was higher at pH 3 in T2, T3 and T4 (P <0.05), contrary to T1 presented the highest release of ac. D-galacturonic with *p*H 5 (P <0.001). In general, the results indicate that the maximum release of pectin from the microcapsules was at *p*H 3, indicating that the agglutinates and the encapsulation process have a marked influence on the release of pectin. The SA is a good protector in the preparation of microcapsules [7] and SH is soluble in alkaline solutions, resisting water. This is a moldable compound under conditions of pressure, temperature and dissolution in volatile organic solvents, for this reason it is used in the preparation of coatings with hardness and durability [8,9]. *Lactobacillus reuteri* [10] and *Lactobacillus paracasei* [11] were encapsulated using powdered whey and SA, both combined with SH; the results of survival of the probiotic with conditions of pH 2 improved the survival in 77%. The results relate to the resinous characteristic of SH, indicating that microcapsules with SA and SH can be used as an encapsulation system to release a lipophilic compound at the intestinal level.

Sustained-release theophylline microcapsules coated with SH have also been developed [9] which were tested at pH 1.2, finding that none of the formulations showed release of more than 4% of the dose in 120 min confirming gastric resistance. Microcapsules have been prepared to be administered to bovines, these were designed with spray drying, using porous starch as core material and a triple coated Eudragit E100 and SH. These were incubated with cultures of ruminal microorganisms, and the release efficiency of the microcapsules was 85% in 30 minutes at pH 3 (similar to the abomasum fluid) [12.13]. In our study, the extrusion procedure is simpler than those published and the ideal one was T3, where only SH with cover by immersion was included. As is known, SH contains polymers that cause an increase in porosity, there being an association between size and porosity (simulating the structure of blackberries) [14]. Therefore, the morphology of the microcapsules becomes porous when SH was included in the matrix; which is why the release of D-galacturonic acid decreased. While the coating of the microcapsules with SH provided better protection at alkaline pH and was properly released at acidic *p*H [15].

Morphology of the microcapsules

The micrographs of the pectin microcapsules, in the different treatments showed irregular shape with rough and porous texture on the surface (Figure 1). The morphology was similar to that found by other authors [16,17], who

microencapsulated *Lactobacillus acidophilus* with SA and SH as polymer, using the extrusion and co-extrusion technique. The shape, surface and sizes found in all the treatments studied were similar, with irregularly shaped microcapsules and rough surfaces, with sizes from 600 to 1000 μm [16]. The morphology is attributed mainly to the technique [18] and the conditions of the encapsulation process [19], generally the microcapsules dried by bed-fluidized have spherical shape and rigid structure, while the dried microcapsules by lyophilization they tend to have a quasi- spherical shape [17].

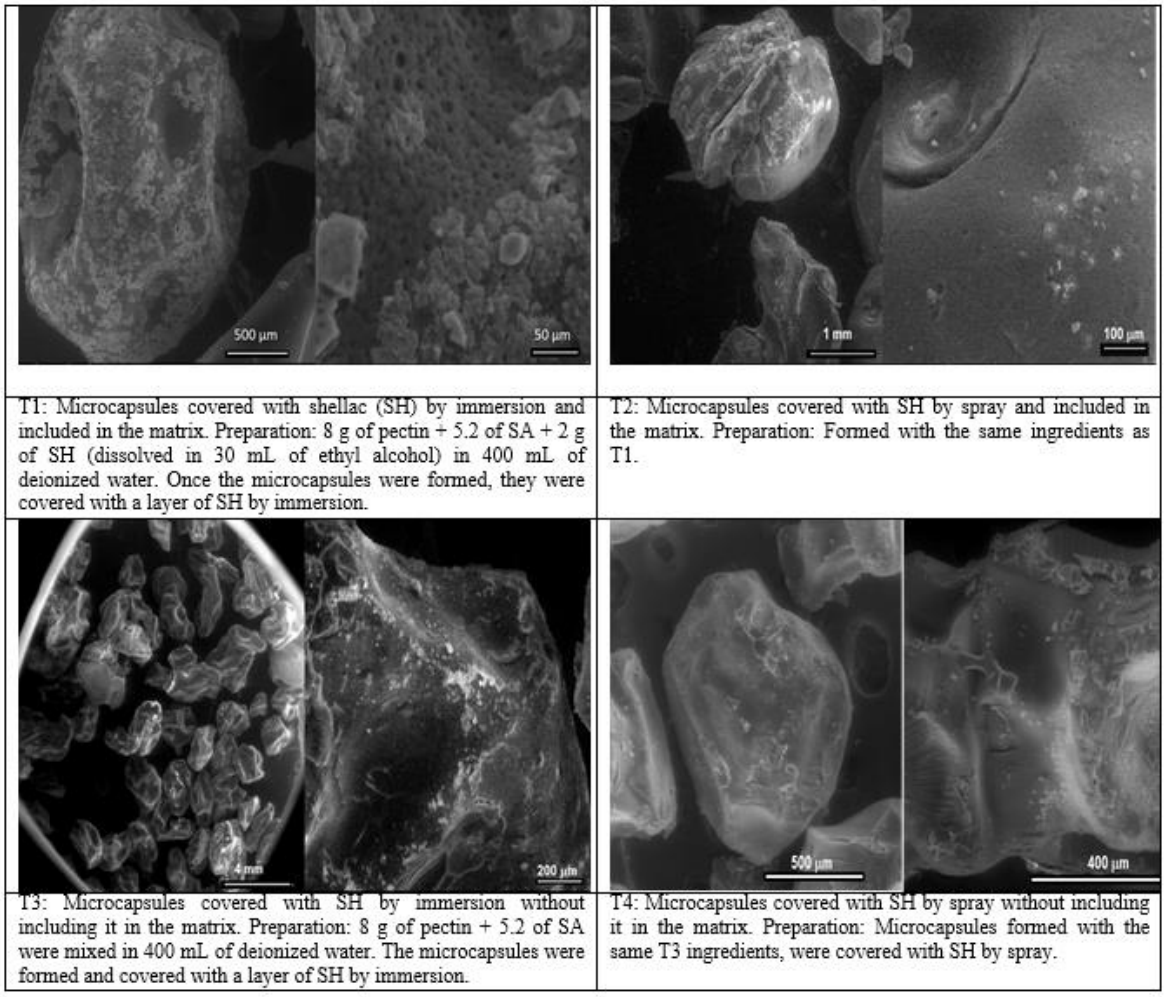

Fig. 1.

Conclusions

The encapsulation of pectin by the extrusion method was feasible. The process of covering them with SH by immersion was feasible to release the pectin (quantified by D-galacturonic acid) in more than 70% at pH 3. The novelty of this *In vitro study* is the viability of the microcapsules in pH 5.6 to 6.7 (ruminal pH). The disintegration of microcapsules in a pH like the duodenum (~ 3) may release the intact pectin and agglutinate with the *β-carotenes* of the food ingested by the cattle, limiting the intestinal absorption. Consequently, the problem of yellow fat in meat may decrease. Now our research group is interested in continuing with an experiment with live cattle.

Acknowledgements: The authors thank CONACyT for the master scholarship of the first author and likewise we appreciate the facilities granted by the Laboratorio Nacional de Investigaciones en Nanociencias y Nanotecnología (LINAN) associated with the División de Materiales Avanzados (DMAv) del Instituto Potosino de Investigación Científica y Tecnológica (IPICYT) for the observations made in the ESE FEI-QUANTA-200 Microscope.

References

1.REYNOSO, C.R., MORA, O., NIEVES, V., SHIMADA, A., GONZÁLEZ DE MEJÍA, A., Anim. Feed Sci. Technol., **113**, 2004, p. 183
2.BRENES, A. Nutr. Anim. Trop, **8**, 2014, p. 21
3.BARRÓN, S., MORA, O., CASTAÑO, V., SHIMADA, A., Téc. Pec. Méx., **44**, 2006, p. 231
4.CRUZ- MONTERROSA, R. G., RAMÍREZ, J.E., GUERRERO, M.I., ZINN, R.A., Anim. Feed Sci. Technol., **207**, 2015, p. 274-77.
5.MCDOUGALL, E. I., Biochem. J., **43**, 1948, p. 99
6.IBM-SPSS. IBM-CORPORATION, SPSS Statistics 21. IBM Corporation, Armonk, NY: IBM, 2012

7.OLIVAREZ-ROMERO, R., FAUSTINO-VEGA, A., MIRANDA-CALDERÓN, J.E., GONZÁLEZ-VÁZQUEZ, R., AZAOLA-ESPINOSA, A., Rev. Mex. Ing. Quím., **17**, 2018, p. 641
8.DEL PIANO, M., CARMAGNOLA, S., BALLARÈ, M., SARTORI, M., ORSELLO, M., BALZARINI, M., PAGLIARULO, R., TALI, A. ANDERLONI, A., STROZZI, G.P., MOGNA, L., SFORZA, F., CAPURSO, L., Gut Microb., **2**, 2011, p. 120
9.FARAG, Y., LEOPOLD, C.S., Eur. J. Pharm. Sci. **42**, 2011, p. 400
10.SCHELL, D., BEERMANN, C., Food Res. Int., **62**, 2014, p. 308
11.SILVA, M.P., TULINI, F.L., MARTINS, E., PENNING, M., FÁVARO, C.S., TRINDADE, C.M., PONCELET, D., Food Res. Int., **89**, 2016, p. 582
12.TETSURO, Y., TAKAHASHI, H., MATSUMOTO, K. J., -Fac. Agric. Kyushu Univ., **44**, 2000, p. 359
13.TETSURO, Y, MASAHIKO, S., TETSUO, F., MATSUMOTO, K. J., Agric. Food Chem., **47**, 1999, p. 554
14.RHYS J., FRANK, B., Renew. Sust. Energy Rev. Elsevier., **48**, 2015, p. 79
15.STUMMER, S. SALAR-BEHZADI, S., UNGER., F., Food Res. Int., **43**, 2010, p. 1312
16.ROSTAMI, Z., TABARSA, M., YOU, S., REZAEI, M., Proc. Biochem., **58**, 2017, p. 289
17.SILVA, M.P., TULINI, F.L., RIBAS, M.M., PENNING, M., FÁVARO- TRINDADE, C.M., PONCELET, D., LWT- Food Sci. Technol., **89**, 2018, p. 392
18.FUENTES-ORTEGA, T., MARTÍNEZ-VARGAS, S.L., CORTÉS-CAMARGO, S., GUADARRAMA-LEZAMA, A.Y., GALLARDO-RIVERA, R. BAEZA-JIMÉNEZ, PÉREZ-ALONSO, C., Rev. Mex. Ing. Quím., **16**, 2017, p. 477
19. SMRDEL, P., Scientia Pharm., **76**, 2008, p. 77

Manuscript received: 14.12.2019