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PHYSICOCHEMICAL CHARACTERIZATION OF FILMS FORMULATED FROM ACHATINA FULICA MUCUS FED WITH MEDICINAL PLANTS

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ABSTRACT

The cutaneous mucus released by tropical mollusks of the *Achatina fulica* species, in front of a plasticizer, forms a network that provides stability to the mucus thus forming a biopolymer film and its derivatives have recognized fungicidal, bactericidal and healing properties. To evaluate this product with therapeutic potential, the characterization of mucus was performed with protein determination, pH measurement, chemical composition, molecular analysis by polyacrylamide gel electrophoresis, colorimetry assays, scanning electron microscopy, degree of swelling and water vapor transmission. The results show the percentage of protein found to be 78.76% in the mucus of the snail. The pH range between 4.0 and 7.0 is considered ideal for medical formulations. The determination of proteins reveals similar bands of proteins and the chemical constituents present in the mucus. Scanning electron microscopy demonstrates the high quality of biopolymer networks, ideal for the formulation of topical pharmaceutical forms. The degree of numbness reflects the expected since, the mucus proteins against pH tending to neutrality, does not present alteration of their structures. The water vapor transmission reveals that the porosity of a film with a scar healing purpose is fundamental that besides allowing the incorporation of drugs. the analysis of mucus characterization and the respective *Achatina fulica* film have demonstrated that being able to be used with beneficial effects in the repair of tissue lesions, they have great viability of use in the pharmaceutical and food industry.

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INTRODUCTION

Film production is a way to obtain new polymeric materials without the need to invest in the development of new monomers or new polymerization processes (Spadaro *et al.*, 1996). Several biomaterials are being used in the tissue repair process, which can be processed in innumerable forms, such as leaves, sponges, dispersions, gels, films, injectable solutions, each type being suitable according to its properties and characteristics (Aoyagi *et al.*, 2016). The use of films composed of natural biopolymers has been very widespread because they have the capacity to interact with the cells of the damaged tissue and not only cover the wound like most bands, sending them signals that control their functional activities allowing the tissue regeneration . In this way, this technology tends to imitate the cellular environment providing a better recovery (Kaos, 2003). Bioactive films that are absorbed and degraded have the ability to support the growth of new blood vessels and allow the nutrition of the cells that fill the affected tissue and the access of the defense cells to the site, that is, they allow adhesion, Migration and cell proliferation (Girardi, 2005). Although proteins of plant origin are more extensively studied (Aydt, 1991; Gennadios, 1993; Gernpad, 1995; Gnanasambandam, 1997; Brandenburg, 2003), probably due to their abundance and low cost in developed countries, Animals demonstrate excellent bioactive film-forming ability (Noles *et*

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al., 2010). The mucoglycoproic substance released by the tropical mollusc of the *Achatina fulica* species has a high protein content, which in front of a plasticizer forms a web giving stability to the mucus forming in this way a film. Currently, with the field of biotechnology, the use of malacofauna in the production of films has been widely diffused with the aim of increasing the possibility of therapeutic arsenal with products of natural origin (Sírio *et al*., 2008). The potential of cicatrization found in the mucus (Lorenzi and Martins, 2008) and the ability to incorporate the properties of plants with therapeutic actions through feeding, enables the production of new pharmaceutical forms among them the film, which according to Diniz (2006) Presents an excellent cicatrizant activity from this material mucoglicoproteico enriched with chemical compounds acquired from the herbs. The objective of this study was to characterize physically-chemically processed film with *Achatina fulica* mucus fed with medicinal herbs.

MATERIAL AND METHODS

Biological material

The *A. fulica* snails were collected in the urban area of Sergipe/Brazil, according to the method described by Fischer and Colley (2005), after approval by the Ethics Committee (Protocol 101107). Initially, the faeces and mucus of the snails were submitted to parasitological evaluation (Hoffman *et al*., 1934) to guarantee the absence of parasitic infections. The animals were divided into three groups: LSG - Control, thirty animals fed a diet with *Lactuca sativa* (lettuce), SOG - thirty animals fed a diet with *Symphytum officinale* L (confrei) and PGG - thirty animals fed a diet with *Punica granatum* (pomegranate). The animals were fed *ad libitum* and kept in the laboratory under normal conditions of temperature and humidity.

Characterization of mucus

Protein determination

Protein determination of the three samples (LSG, SOG and PGG) was performed by the Lowry method using albumin as standard. The experiment was performed under any light conditions. The absorbance was recorded at 750 nm using a spectrophotometer (SIGMA k415, RJ, Brazil DUR 708).

Determination of pH

To determine pH of the mucus in pHmetro (VOLTEX 5000, model W3B, SP, Brazil), the mucus diluted in distilled water (1:1) was used. The experiment was performed in triplicate with 20 mL for each reading.

Chemical composition

The samples were submitted to the analysis of ash, moisture and lipids. The moisture content was determined by drying the samples at 105 ± 5 ° C, followed by recommendations described by Nagakura (1972). Total lipids (LT) used the cold extraction method of Bligh and Dyer (1959). Ash percentage of the samples was determined according to the method of Nagakura (1972), by incineration in muffle at a temperature of 550°C.

Polyacrylamide gel electrophoresis

The molecular weight of the proteins was analyzed by a 12.5% and 4% dodecyl sulfate polyacrylamide gel (SDS-PAGE) as described by (Laemmli, 1970). The molecular weights of the protein markers in the method are: bovine albumin: 66 kDa; Ovalbumin: 45 kDa; Dehydrogenase glyceraldehyde-3-phosphate: 36 kDa; Carbonic anhydrase: 29 kDa; Trypsinogen: 24; Trypsin inhibitor: 20.1; A-lactalbumin:14.2. Proteins were visualized by staining with the silver satin procedure.

Development of biological films

The snails had massaged pelvic gland, whose objective was to obtain the mucoglicoprotein secretion released by the specimen. The films were prepared by casting method with 20 ml of mucus and 20% of plasticizer (polyethylene glycol-PEG 400 LOT ISOFAR, 021423). This dispersion was melted on an acrylic plate and allowed to dry at room temperature to obtain the films.

Biopolymer Films

Biopolimer Films Characterization

This analysis was performed through subjective evaluation, assigning scores classified as poor (+), good (++) and excellent (+++). Only the subjective analysis of an evaluator was performed. The formulations were subjected to macroscopic analysis using preliminary criteria to evaluate the biopolymer film: continuity (no breaks and fractures after drying), homogeneity (absence of insoluble particles or visible to the naked eye, or areas of opacity or different colors), Management capacity (ability to be handled without risks of rupture) and the ability to form a continuous film (Monterrey and Sobral, 1999).

Colorimetry

The color of the films was determined with a Minolta colorimeter (CR 400; Minolta, Japan). The values of L *, a *, b * of each film were evaluated by means of measurements of reflectance. For the calibration of the instrument, a sheet of A4 paper type CHAMEX was used as background for the color readings of the films (Garcia and Sobral, 2005). The readings were obtained in two-minute intervals, collecting data in the digital colorimeter. The analyzes were carried out at the Laboratory of Food Engineering of vegetal origin, at the Federal University of Sergipe. Data were collected in triplicate.

Scanning electron microscopy

The Scanning Electron Microscopy (SEM) was performed using a model apparatus (lXA - JEOL JSM-6360-LV) coupled to a scanning electron microscope, University of Campinas-UNICAMP. The films were mounted in aluminum tubes, coated with a thin layer of gold and visualized, with an acceleration voltage of 20 kV, realized at the University of Campinas - UNICAMP / SP.

Degree of swelling

The film-to-film ratio was determined using samples of films of size 2x3 cm in triplicate. Samples were weighed before and

after swelling in containers containing 100 mL of 0.1 N HCl and distilled water. In pre-determined periods the mass of the films was obtained in an analytical balance (SHIMADZU AY220, São Paulo, Brazil), with a precision of 0.0001. Before each weighing the excess solution was removed with absorbent paper and the manipulation of the films in this analysis was performed with the aid of a forceps. The calculation of the degree of hydration was performed using the following equation 1: (% I = 100 x Ma/Ms (1) where% I is the percentage of swelling; Ma is the mass of water absorbed; Ms is the mass of the dry film degree of swelling.

Water vapor transmission

The permeability of the films was determined by the loss of water vapor in a vessel by the gravimetric method film sealed in known RH given by solutions saturated in contact with the non dissolved salt (ZnSO4 90% RH) placed in a desiccator containing silica. Each experiment was performed with eight replicates, for 48 h. The permeability was calculated by: (WVT ¼ w = A - h) where WVT is the water vapor transmission in mg mm3, w is lost mass in mg, A is the film area in mm^2, and his film thickness in Mm. (P ¼ WVT = t) where P is the permeability in mg mm 3 H and T is the time in hours.

RESULTS AND DISCUSSION

Characterization of mucus

Protein determination

The results of the protein dosage on the *Achatina fulica* mucus samples revealed that the feed did not influence the protein concentration. A difference was observed between the mucus composition of the Aracaju-SE species of 90%, as shown in Table 1.

Table 1. Concentration of mucus protein of A. fulica fed with different medicinal plants

Groups	Protein Concentration(mg/mL)	Protein Concentration (%)
LSG	0.380±0.078	90.82±2.01
SOG	0.370±0.072	90.60±1.97
PGG	0.374±0.068	90.76±1.78

Compared to the results of samples obtained by Sirio (2008) from Pirassununga-SP, where the percentage of protein found Was 78.76% in the mucus of the snail. This difference in results reproduces the differentiation of the species themselves, and may be directly related to the environmental factors where the specimens were collected (Kubota, 1985). The other components found in the samples were presented in Table 2.

Table 2. Chemical composition of the mucus produced by A. fulica snail fed with medicinal plants

Groups	Humidity (%)	Ashes (%)	Lipids (%)
LSG	99.03	0.42	0.11
SOG	98.82	0.78	0.05
PGG	98.72	0.51	0.19

As mucus samples revealed the high moisture content. The moisture content present not mucus facilitates the process of forming the polymer network when incorporated into the plasticizer.

This level of information is linked to the number of multicellular exocrine glands that secrete their products through departments on the surface of the body or in the shells of the snail. In addition, mucin, a glycoprotein present in the mucus, complexes water to form a viscous liquid, the mucus, which protects and lubricates the surfaces of the specimen's structure, serving as a barrier against microorganisms and dehydration (Mitra *et al*., 1987). For the pharmaceutical industry the development of pharmaceutical forms with controlled release has been the object of high investments (Das and Das, 2003). Among the wide variety of systems, transdermal systems, which act as modulating agents for drug release, are included (Lordi, 2001).

Determination of pH of *Achatina fulica* mucus

The pH values of the samples were within the neutral range (Table 3).

Table 3. Determination of pH of A. fulica mucus

Groups	pH
LSG	7.92
SOG	7.12
PGG	6.87

The neutral mucus pH of *A. fulica*, even for groups fed with medicinal plants, suggests that the chemical constituents present in the herbs were not able to alter the hydrogen ionic potential of the mucus. The pH range between 4.0 and 7.0 is considered ideal for medical formulations since the chemical stability of the sample directly reflects on the compatibility of the tissue pH and can resect the skin making it more susceptible to external aggressions. The functional pH of the skin is an important regulator of lactic acid production, which gives the skin surface a protection called "cutaneous acid mantle" (Leonardi *et al*. 2002).

Polyacrylamide gel electrophoresis

SDS-PAGE results (Figure. 1)

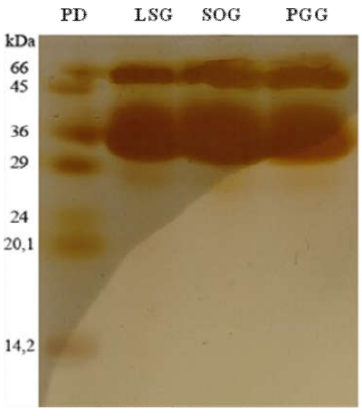

Figure 1. Electrophoresis gel - A. fulica mucus polyacrylamide with differentiated feeding

showed *A. fulica* mucus protein bands with molecular weight equivalent to standards (bovine albumin: 66 kDa, ovalbumin: 45 kDa, glyceraldehyde-3-phosphate dehydrogenase: 36 kDa, carbonic anhydrase: 29 kDa; trypsinogen). The LSG, SOG and PGG groups showed similar bands of proteins, showing that the chemical constituents present in the feed offered to them were not able to alter the structural conformation of the protein.

Biopolymer Films

Biopolimer Films Characterization

Macroscopic evaluation of biopolymer films

The formulation described in the methodology allowed the formation of the films, which are exposed in Figure 2.

Lactuca sativa (LSG)

Symphytum officinale L. (SOG)

Punica granatum (PGG)

Figure 2. Biopololymer films produced from *A. fulica* mucus fed with different medicinal plants

The films were evaluated as homogeneous, continuous, easy to handle continuous film formation, as shown in Table 4. Films based on *Achatina fulica* mucus fed with *Lactuca sativa* (LSG) showed clear and non-pigmented staining, while the films of the fed groups *Symphytum officinale* L. (GSO) and *Punica granatum* (PGG) showed yellowish. The macroscopic analysis of the films allows the evaluation of the quality of the developed films, since the appearance of the films can interfere in the acceptability of the product (Matta Jr, 2009). Colorimetry .

The transparency of a film is a desirable property since the consumer wants to see clearly the aspect of the product that the film will cover (Cuq *et al*., 1996; Rhim *et al*. 2006). All films showed luminosity and transparency tending to yellow, with characteristics of transparency. This transparency (low opacity) is important in situations where the film is applied in the form of dressings, since it allows the treated area to be visualized in its histological alterations, besides allowing greater commercial acceptance due to the combination of good visual quality and high level (Matta Jr *et al*., 2011). The results of the color measurements are presented in Tabela V. The films were transparent, with a coloration ranging from slightly yellowish to yellowish. With respect to the luminosity (L*) of *Achatina fulica* films fed with medicinal plants, it is possible to observe that these values are within the luminosity pattern for films produced from natural products, when compared to films obtained by Rhim *et al*. (2006), for which the range of values found was between 66.8 and 85.8. On the other hand, the analysis for the value of (L*) found for the PGG group ($p = <0,001$) indicates a decrease in brightness and an increase in film opacity. The parameters of (a*) and (b*) are parameters that describe the color of films starting from the white taken as parameter for calibration to the natural color of the film, being this a color coordinate that influences the total color difference Park *et al*. (2002). The values obtained in the (a*) and (b*) reading for the SLG, SOG groups show statistically different results as compared to the PGG group ($p = <0,001$), reaffirms the intensification of the yellow color of the *Achatina fulica* film fed with *Punica granatum* in relation to the other feeds. The difference observed in the coloration of the films suggests that feeding the mollusks interferes with the staining of both the mucus and the respective film.

Biopolimer Films Characterization

The micrographs of surface (a) and fracture (b) images of the films shown in Figure 3. exhibit structures that help in understanding the behavior of films against water vapor permeability.

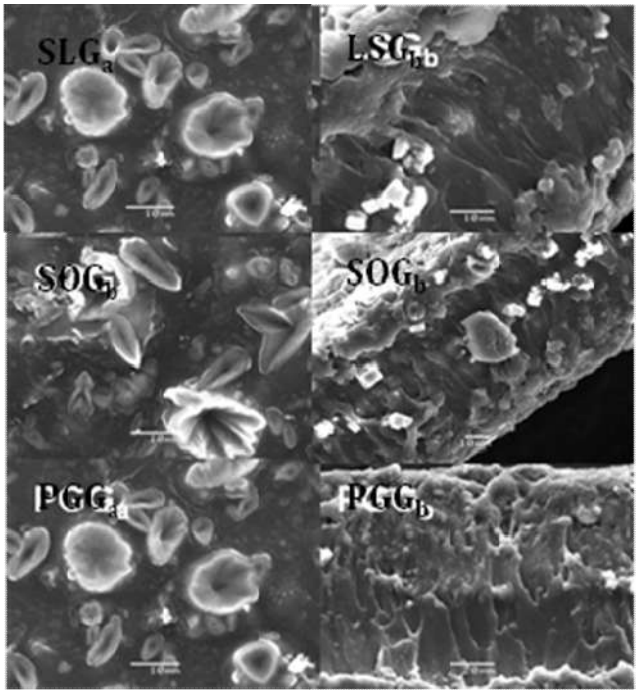

Figure 3. Micrograph of the films of A. fulica

The photomicrographs of the surface (a) of the films seen under the microscope, presents as a disordered arrangement of fragments. In all groups, the photomicrographs revealed an extensive and amorphous mass, with the presence of reliefs or depressions similar to flowers. These reliefs may arise from the interference of circulating air in the formation of clumps not totally plastified in the medium of the mucus. These "floral" lumps more on the surface are observed in simple relief or with furrows around. The latter may have been formed as a result of drying. The shape of the lump found on the surface of this film differs from that reported in the literature, ie, rounded, oval and irregular forms (Hedley *et al*., 2002, Themeier *et al*., 2005). The cross-sectional aspect of the films was similar for all groups (fracture (b)), presenting different phases per hour more or less homogeneous. This phase division is probably due to the rate of deposition or drying of the components of the film-forming solution (Hariharam *et al*., 1994). It was also observed the presence of juices forming channels, which certainly provided the permeability to water vapor and oxygen (Colombo *et al*., 2000).

Degree of swelling

Some of the parameters investigated in film production include the water penetration rate in the system (Wan *et al*., 1991), the rate of hydration and the extent of swelling of the polymer (Wan *et al*., 1995). As the water-polymer interactions increase, the interaction forces between the polymer chains decrease. The water entering the system fills the spaces between the polymer chains and diffuses into the denser regions of the polymer, "forcing" the remaining polymer chains to separate. Consequently, the chains gain rotational freedom and begin to occupy more space, remaining in a new solvated state (Colombo *et al*., 2000). In a macroscopic observation of the swelling process progressing from the center to the matrix, the rate of water penetration in the matrix system conditions the mode of drug release, since the swelling behavior of the hydrophilic matrices, resulting from a chain relaxation process (Bettini *et al*., 1994, 1997, 1998; Colombo *et al*., 1995, 1996). The LSG, SOG and PGG films submerged in medium containing 0.1 N HCl and distilled water presented different behavior Figure 4.

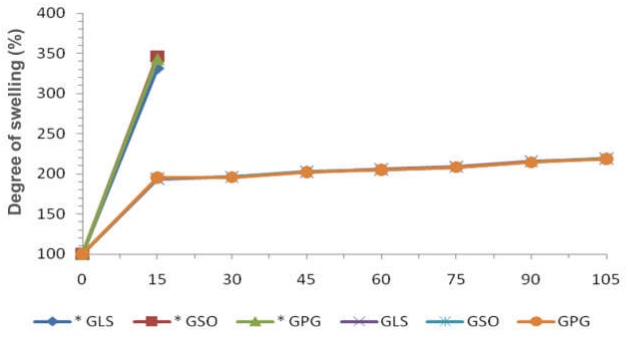

Figure 4. Swelling index of the A. fulica flies fed with Lactuca sativa (LSG) Symphytum officinale L. (SOG) and Punica granatum (PGG)

The films tested in 0.1N HCl solution had a higher swelling index (347% in relation to the initial weight), when compared to the films evaluated in distilled water, which presented (220% in relation to the initial weight), forming a plateau , and only after 105 minutes, presented characteristics suggestive of dissolution of the film. The LSG, SOG and PGG films were dissolved in medium containing 0.1 N HCl after 15 minutes. This behavior is justified by the acidity of the medium to which the film was subjected. In front of the acid medium, the structure of the proteins present in the mucus, undergoes destructuring, exposing its bridges of bonds that exert affinity for the molecule of the water, resulting in an immediate swelling. In environment with neutrality (distilled water) pH, the films of the respective groups remained intact throughout the experimental time (105 minutes). This result, reflects the expected since, the mucus proteins against pH tending to neutrality, does not present alteration of their structures.

Water vapor transmission

The permeability of *A. fulica* mucus films showed permeability between 7.0 and 9.2 (Table 4).

Table 4. Macroscopic evaluation of biopolymer films

Formulations	Homogeneity	Continuity	Handling	Formation
LSG	+	+	++	+++
SOG	++	++	++	+++
PGG	++	++	++	+++

poor (+) - good (++) - excellent (+++)

Table 5. Colorimetric reading of A. fulica films with different feeds

Groups	L*	a*	b*
LSG	73.2±0.2	6.06±0.2	16.8±0.1
SOG	73.13±0.3	5.63±0.1	17.4±1.1
PGG	66.36±0.7	8.7±0.2	31.2±0.9

There was no difference between LSG, SOG and PGG films. The permeability observed in the control groups corresponds to that expected. Among the groups of films, the PGG was more permeable than the SOG and LSG films.

Table 6. Determination of the Water vapor transmission (WVTP) of A. fulica films

Groups	WVTP (g.mm/m^2.h.Kpa)
LSG	7.93±0.95
SOG	7.04±0.50
PGG	9.21±0.61
CTR NEG	65.55±19.56
CTR POS	0.35±0.04

This small difference is justified by the presence of porosity found in this film, which can be observed in the micrograph image of the film. The porosity of a film with a scar healing purpose is fundamental that besides allowing the incorporation of drugs into the filmogenic mixture, there is an air exchange between the isolated medium and the environment.

Conclusion

The results suggest that the physico-chemical properties of the *Achatina fulica* films fed with medicinal plants show similarity in the results for the SLG and SOG groups whose feeding were *Lactuca sativa* and *Symphytum oficinale* respectively. The results found for the PGG group fed with *Punica granatum* showed different results from the previously mentioned groups. This study suggests that the latter group was influenced by food, since the presence of chemical constituents of the pomegranate may have caused some alteration in the structure of the polymers present in the mucus.

In addition, the analysis of mucus characterization and the respective *Achatina fulica* film have demonstrated that besides being able to be used with beneficial effects in the repair of tissue lesions, they have great viability of use in the pharmaceutical and food industry. However, more accurate assays need to be performed to determine the applicability of *Achatina fulica* mucus products on a production scale. Finally, the research and application of the mollusk and its referred derivatives allows a better understanding of the interaction of the human being with the environment, besides allowing the elaboration of adequate strategies for the conservation of the natural resources.

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REFERENCES

Aoagi, S., Onishi, H., Machida, Y. 2007. Novel chitosan wound dressing loaded with minocycline for the treatment of severe burn wounds. *International Journal of Pharmaceutics*, v. 330, n. 1, p. 138-145. AYDT, T. P.,

Bettini, R., Colombo, P. and Peppas, N.A. 1997. Drug concentration profiles during release from hydrophilic matrices. International symposium on cont rolledrel ease of bioactivematerials. 24., Stockholm, Swe d e n. Proceedings. Deerfield: Controlled Release Society, 1997. v. 24, p. 635-636.

Bettini, R., Colombo, P., Massimo, G., Santi, P., Catellani, P.L., Peppas, N.A. 1994. Moving fronts and drugrelea sefrom hydrophi licmatrices . International symposium on controlled release of bioactive materials. 21., Nice, France, 1994. Proceedings. Deerfield: *Controlled Release Society*, v. 21, p. 19-20.

Bettini, R., Peppas, N.A. and Colombo, P. 1998. Polymer relaxation in swellable matrices contributes to drugrelease". *International Symposium on Cont Rol Led Rel Eas E of B Ioac T Ive Materials.* 25., Las Vegas, Nevada, USA, 1998. Proceedings. Deerfield: *Controlled Release Society,* v. 25, p. 36-37.

Brandenburg, A. H., Weller, C. L. and Testin, R. F. 1993. Edible films and coatings from soy protein. *Journal of Food Science, v. 58, n. 5, p. 1086-1089.*

Colombo, P., Bettini, R. and Santi, P. 2000. Swellable matrices for controlled drug delivery: gel-layer behaviour, mechanisms and optimal performance. PSTT, New York, v. 3, n. 6, p. 198-204.

Colombo, P., Bettini, R., Massimo, G., Catellani, P.L., Santi, P., PEPPAS, N.A. 1995 Drug diffusion front movement is important in drug release control from swellable matrix tablets. *J. Pharm. Sci, New York*, v. 84, n. 8, p. 991-997.

Colombo, P., Bettini, R., Santi, P., De Ascentis, A. and Peppas, N.A. 1996. Analysis of the swelling and release mechanisms from drug delivery systems with emphasis on drug solubility and water transport. *J. Control. Release, Amsterdam*, v. 39, n. 2-3, p. 231-237.

Cuq, B., Gontard, N., Cuq, J. L.,Guilber, S. 1996. Functional properties of myofibrillar protein based biopackaging as affected by film thickness. *Journal of Food Science*, v 61 n 3 p 580–584.

Das, N. G. and Das, S.K. 2003. Controlled-release of oral dosage forms. Formulation, Fill and Finish – a Technology Primer– a supplement to *Pharm. Tec*, v. 27, p. 10-16.

Diniz, G. 2006. Quase uma nova pele. *Rev. Ciência Hoje, Paraná*. jan, p. 36- 39.

Garcı´a, F. T., Sobral, P. J. A. 2005. Effect of the thermal treatment of the filmogenic solution on the mechanical properties, color and opacity of films based on muscle proteins of two varieties of Tilapia. LWT - *Food Science and Technology*, 38, 289–296.

Gennadios, A., Brandenburg, A. H., Weller, C. L., Testin, R. F. 1993. Effect of pH on properties of wheat gluten and soy protein isolate films. *Journal of Agricultural and Food Chemistry*, v. 41, n. 11, p. 1835-1839.

Gennadios, A., Mchugh, T. H., Weller, C. L., Krochta, J. M. 1995. Edible coatings and films based on proteins Journal of Agricultural and Food Chemistry, v. 47, n. 16, p. 15-19.

Ghorpade, V. M., Gennadios, A., Hanna, M. A., Weller, C. L. 1995. *Soy protein isolate/poly (ethylene oxide) films. Cereal Chemistry*, v. 72, n. 6, p. 559-563.

Girardi, R. C. G. 2005. Comportamento de matrizes de colágeno utilizadas no tratamento de feridas planas induzidas em pele de rato. Dissertação (Mestrado em Farmácia) Universidade de São Carlos, São Paulo 196pp.

Gnanasambandam, R., Hettiarachchy, N. S., Coleman, M. 1997. Mechanical and barrier properties of rice bran films. *Journal of Food Science*, v. 6, n. 3, p. 392-398.

Hariharam, D., Peppas, N.A., Bettini, R.;Colombo, P.(1994) Mathematical analysis of drug delivery from swellable systems with parcial physical restrictions or impermeable oatings. *Int. J. Pharm*., *Amsterdam*, v.112 n. 1 p. 47-54.

Hedley, C. L., Bogracheva, Y. T.Wang, T. L. 2002. Correlation of characteristics of gel extrusion module (GEM) tablet formulation rate. *J. Control, Release, Amsterd.*, v 5 n 4 p.235-245

Kaos and John, 2003. Bandage could repair damaged tissues. *Rev. Dermage Tissue*. v. 10, p. 10-15.

Kubota, Y. *et al*., 1985. Purificação e caracterização do fator antibacteriano do muco de escargots. Comp. *Biochem. Physiol.*, v. 82, n 2, p. 345-348.

Lammli, UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature v 4 n2 p 680-685.

Lisboa: Ed. Fundação Calouste Gulbenkia. cap. II, p.737-781.

Lordi, N.G. 2001. Formas farmacêuticas de liberação prolongada In: Lachman, L., Lieberman, H. A., KANIE, J. L. (Eds.). Teoria e prática na indústria farmacêutica.

Lorenzi, A. T., Martins, M. F. 2008. Análise colorimétrica e espectroscópica do muco de caracóis terrestres Achatina sp alimentados com ração diferenciada. Revista Brasileira de Zootecnia, São Paulo, v. 37, n. 3, p.572-579.

Matta Junior, M. D. 2009. Caracterização de biofilmes obtidos a partir de amido de ervilha (pisum sativum) associado à goma xantana e glicerol., Dissertação de Mestrado, Universidade de São Paulo, Brasil 102pp.

Matta, JR.D.I. Sarmentol, S.B. S., Claire I. G. L. and Silvio, S. 2011. Propriedades de barreira e solubilidade de filmes de amido de ervilha associado com goma xantana e glicerol. *Polímeros*, v. 21 n 1, p. 67-72.

Mitra, D. and Sarkar, M. 1987. Further characterization of the cold agglutinin from the snail *Achatina fulica Biochem.* v24 n2, p 331-338

Noles, S.E. and Tulour, V. 2010. Evaluation of traditional animal protein in treatment of thermal burn wounds in

fuzzy rats. *Journal of Burn Care and Rehabilitation*, v. 5, n. 1, p. 519-522.

Park, S. Y., Marsh, K. S. and Rhim, J. W. 2002. Characteristics of different molecular weight chitosan films affected by the type of organic solvents. *Journal of Food Science*, 67 n 1p 194–197.

Rhim, J., Hong, S., Park, H., Perry, K.W. 2006. Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. *Journal of Agricultural and Food Chemistry*, v 5 n 4 p 5814–5822.

Sírio, J. O. 2008. Verificação da potencialização do efeito cicatrizante do muco de caracóis do gênero *Achatina* promovida por dieta à base de Confrei (*Symphytum officinale L.*). Dissertação, Mestrado. Programa de Pós-Graduação em Nutrição e Produção Animal. Faculdade de Medicina Veterinária. Universidade de São Paulo (USP). Pirassununga, 87pp.

Spadaro, G., Acierno, D., Dispenza; Caderaro, E. and Valenza, A. 1996. Physical and Structural Characterization of Blends Made Polyimide 6 and gamma Irradiated Polyethylene, *Radiat. Phys, Chem*, v.48 p-23-32.

Themeier, H., Hollmann, J., Neese, U. and Lindahauer, M. G. 2005. *Carbohydrats films Polym*, v6 n1 p.72-79.

Wan, L.S.C., Heng, P.W.S. and Wong, L.F. 1991. The effect of hydroxypropyl methylcellulose on water penetration into a matrix system. *Int. J. Pharm., Amsterdam*, v. 73, n. 2, p. 111-116.

Wan, L.S.C., HENG, P.W.S., Wong, L.F. 1995. Matrix swelling:a simple model describing extent of swelling of HPMC matrices. *Int. J. Pharm., Amsterdam*, v. 116, n. 2, p. 159-168.

Weller, C. L. and Testin, R. F. 1991. Mechanical and barrier properties of edible corn and wheat protein films. *Transactions of the ASAE*, v. 34, n. 1, p. 207-211.

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