Área: Tecnologia de Alimentos

POTENTIAL USE OF STARCH FROM DIFFERENT SOURCES IN THE PREPARATION OF MUCO-ADHESIVE FILMS

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ABSTRACT – The characteristics of starch vary according to its origin. The knowledge of these peculiarities is of great importance for the industry to use it. Lately, the capability of starch to form biodegradable films has been discovered, which has aroused the interest of the food and pharmaceutical industry. This study aimed to develop mucoadhesive films using starches from different sources. The mucoadhesive films were made by casting methodology using corn, wheat, *Castanea sativa*, potato, and cassava starch; and analyzed the thickness, weight, surface pH, moisture, folding endurance, color, hydration index and erosion properties. The mucoadhesive films presented adequate thickness and homogeneous weight, showing uniformity in all formulations, except for cassava starch films. The pH variated when the films are elaborated with different types of starch, showing the lowest pH on corn starch films. The moisture of the films was around 20%, and it is a factor that will be improved in the future. Wheat and potato starch films had the highest percentage of erosion, while *Castanea sativa* and cassava starch showed the lowest. The hydration percentage varied according to the starch source, the higher hydration was found on cassava film after 240 min on pH 6.8, and the highest folding resistance was found on potato starch films. It was concluded that it is possible to develop mucoadhesive films from different sources of starch and that the choice of raw material must consider, as well as the drug that will be inserted in it due to the specific characteristics of each one.

Keywords: mucoadhesive film; starch; folding endurance; hydration index; percentage of erosion.

1 INTRODUCTION

Starch is a polysaccharide composed of glucose units that form the amylose and amylopectin fractions. It is easily found in nature and has a low cost to the market (KOCHKINA and LUKIN, 2020). The amylose/amylopectin ratio, the helical and lamellar conformation, crystalline structures, and the granular morphology are some of the variables that influence their physical, chemical, and technological applications (CHI et al., 2021). Another factor to be considered is the starch source. Main source as well alternatives sources as grains, tubers and seeds are rich in starch, but the composition of each one has its peculiarities (COLUSSI et al., 2020). Consequently, some starches show higher applications than others. Starch from different sources has a huge range of application beyond human consumption, as textile, chemical and package industry, and the main reason is due to its characteristics of biodegradability and biocompatibility (KOCHKINA and LUKIN, 2020). As starch from different sources has different characteristics, is possible to apply in different fields of the industry without any modification, making the process as "clean label", which has caught the attention of the population nowadays.

Due to its biocompatibility, starch has been investigated as source for production of mucoadhesive films (MIKSUSANTI et al., 2020; SOE et al., 2020). Mucoadhesive films e are targets for research by the pharmaceutical industry due to the advantages presented about absorption and ease of self-medication (ABRUZZO et al., 2017). The buccal mucosa is vastly vascularized and has an intense blood flow, in addition to being less thick than other mucous membranes of the digestive system and, due to the proximity of glands that produce and secrete saliva, they remain naturally humidified, an environment that facilitates the dissolution of hydrophilic compounds and thus the controlled release of compounds of interest (BISWAS and SAHOO, 2016).

The careful choice of the mucoadhesive polymer is fundamental for the success of the developed mucoadhesive system. The polymer and its degradation products must not be toxic or irritating to the mucous membrane and must adhere quickly to moist tissue. In addition, they must not decompose during the storage time of the pharmaceutical form and must allow easy incorporation of the drug; and should not cause any impediment to the release of the drug (SOUTO and LOPEZ, 2011). Thus, starch meets what is desired in a polymer intended to produce mucoadhesive, however, as far as we know, investigative studies have not yet been carried out to evaluate the best type of starch for making film mucoadhesive. In this away, this study aims to elaborate mucoadhesive film from corn, wheat, *Castanea sativa*, potato, and cassava starch and analyze the thickness, weight, surface pH, moisture, folding endurance, color, hydration index and erosion looking for future uses as medicine carrier.

2 MATERIAL AND METHODS

2.1 Material

Corn, wheat, *Castanea sativa*, potato, and cassava starches were used. Corn, potato, and cassava starches were purchased in the city of Pelotas, RS, Brazil. *Castanea sativa* starch was extracted according to the methodology proposed by (CRUZ et al., 2013). Wheat starch was extracted according to the Knight and Olson (1984) methodology. All reagents used were analytical standard.

2.2 Methods

2.2.1 Development of mucoadhesive films

Mucoadhesive films were made using the casting method (COLUSSI, et al., 2017). 4 g of starch per 100 g of a film-forming solution was used. Glycerol was used as a plasticizer, being used at a concentration of 30% (starch base). The filmogenic solutions were heated to 90 °C and maintained for 30 minutes. Then, 24 g of each filmogenic solution was spread on circular acrylic plates and dried in an oven with air circulation at 40 ° C for 16 hours.

2.2.2 Thickness, weight, and surface pH

The mucoadhesive films were cut to 2 cm x 2 cm and weighed on a precision scale. The thickness was measured in three points of six different films of each formulation using a digital micrometer (INSIZE model). For pH determination, 0.2 g of film were soaked in water and homogenized. After 2h of soaking, the surface pH was measured using peagameter (Kasvi, k39-0014pa, Brazil).

2.2.3 Moisture

The moisture content was determined following the methodology of AACC (1995), where the films were weighed and then placed in an oven at 105ºC where they remained for 24 hours until their weight remained constant.

2.2.3 Folding endurance

The fold resistance of mucoadhesive films was determined manually by repeatedly folding the starch film in the same place until it broke. The number of folds required to break or crack the film was considered to be bending resistance. The experiments were in triplicate, and average values were reported (SAHNI et al., 2008).

2.2.4 Film color

The color of the mucoadhesive films was obtained through an average of 5 determinations, one in the center and the other in the perimeter, using a colorimeter (Minolta, CR 400, Osaka, Japan). The films were placed on a white plate defined as standard, and the CIE-Lab scale and daylight (D65) were used to measure the color of the starch films. The parameter ΔE was used to compare the total color change of mucoadhesive films. The ΔE color difference was determined by Equation 1.

$$\Delta E = [((\Delta L)2 + (\Delta a*)2 + (\Delta b*)2)0{,}5] \qquad (1)$$

Where:
ΔL = difference in lighter and darker (+ = lighter, - = darker).
Δa*= difference in red and green (+ = more red, - = more green).
Δb* = difference in yellow and blue (+ = more yellow, - = more blue).
ΔE = total color difference.

2.2.5 Hidratation index and erosion properties

The hydration index was conducted to study and compare the hydration characteristics of the mucoadhesive films made from different sources of starch. The films were weighed individually and placed separately in a Falcon tube containing 5 mL of pH 6.8 phosphate buffer. At regular intervals (60, 90, 120, 180, 240 min), the samples were removed from the tube, and the excess water was carefully removed using filter paper. The swollen films were weighed again. The swelling index for each system was calculated using Equation 2.

$$\text{Hidratation index} = \frac{\text{Wet film weight} - \text{Dry film weight}}{\text{Dry film weight}} \times 100 \qquad (2)$$

To quantify the percentage of erosion, after 90 min, the swollen films were removed, and the leached material was dried in an oven at 105 °C for 24 hours. The leachate material was then weighed. The percentage of erosion was calculated according to Equation 3.

$$\% \text{ Erosion} = \frac{\text{Dry film weight} - \text{Weight of leachate material}}{\text{Dry film weight}} \text{ x } 100 \quad (3)$$

2.2.6 Statistical Analysis

The analysis was performed in triplicate, and the results were submitted to analysis of variance (ANOVA) and comparison of averages by the Tukey test with a level of significance of 5%.

3 RESULTS AND DISCUS SION

Table 1 presents the values of thickness, weight, pH, and fold endurance of mucoadhesive films made with starch from different sources. Films made from corn, wheat, and chestnut starch did not show significant differences between them, for another hand the mucoadhesive film made from potato starch presented great thickness, and the film made with cassava starch was very hygroscopic with difficulties to handle, which is why its thickness has not determined. The thickness determination in mucoadhesive films is extremely important, since these mucoadhesive films will carry certain compounds for subsequent controlled release. Also, an ideal thickness is required to provide adequate adhesion. According to Nair et al. (2013), the "ideal" mucoadhesive film should exhibit a thickness value between 50 and 1000 μm. In this sense, the thickness of the films obtained is in agreement with what is recommended by the literature.

The weight of the films did not vary significantly ($p < 0.05$), which means that the films dried homogeneously (Table 1). A variation in the weight of mucoadhesive films indicates the inefficiency of the method used, and it is likely that when the drug is added to the film, the content will not be uniform (NAIR et al., 2013).

The surface pH of mucoadhesive films showed variability, with the lower values observed in films made with corn starch. This result observed in mucoadhesive films made from corn starch could be explained by the extraction method used. As in this study the corn starch used was from a commercial source, we cannot explain the reason for this lower pH. The measurement of pH in mucoadhesive films is utmost importance since films with a very acid or basic pH affect the application area and can cause damage to the oral mucous membrane leading to patient discomfort because it can alter the action of it will carry. Likely, the chemical nature of the drug and the excipients to be carried will influence the pH of the prepared films (BOTTENBERG et al., 1991; MIKSUSANTI et al., 2020).

The flexibility of mouth adhesives is a relevant physical attribute necessary for easy application at the application site. The fold resistance varied significantly in the mucoadhesive films made with starches from different sources. The potato starch film showed the highest resistance and wheat starch the lesser resistance. According to Farajpour et al. (2020), starch films in general have high fragility as a deficiency, this deficiency has been confirmed by Sahni et al. (2008) for most of the films elaborated with tapioca tested their study. They verified that with the increase of zein nanoparticles, there are an increase on the resistance of the films. According to the results obtained in this study is possible to verify great variability on folding endurance in the different fonts of starch studied. Potato starch showed the highest values (≥300) following by *Castanea sativa* starch (110), corn starch (68) and wheat starch (30). Cassava starch films are not analyzed due its high stickiness. Suh et al. (2020) suggest that the amylose content influences the mechanical properties of starch films, making them more resistant when elaborated with starch with high amylose content. We emphasize that the data found by these authors are related to the production of films, aiming at application in packaging. Data regarding the application of these films as mucoadhesive are still scarce, therefore, in the future studies we intend to verify the importance of amylose in this parameter.

The moisture of the films produced did not vary according to the source ($p < 0.05$), however, all films produced have high moisture. Starch, regardless of its origin, has in its molecular structure several points where hydrogen bonding is facilitated, mainly in the free spaces generated by the conformation of amylopectin, where the water could interact. This can be advantageous for the formation of more resistant films, but also can favors high hygroscopicity and water trapping in the film (JHA et al., 2019).

Table 1. Thickness, weight, pH and fold resistance of mucoadhesive films made with starch from different sources.

Starch*	Thickness (mm)	Weight (g)	pH	Fold endurance	Moisture (%)
Corn	0.196±0.005 b	0.105±0.010 a	5.45±0.10 c	68.00±9.30 c	20.63±0.06 a
Wheat	0.189±0.011 b	0.092±0.009 a	6.58±0.06 a	30.00± 5.60 d	22.55±0.04 a
Castanea sativa	0.193±0.015 b	0.085±0.012 a	6.61±0.02 a	110.00±2.00 b	21.37±3.68 a
Potato	0.227±0.011 a	0.103±0.011 a	6.37±0.02 b	300.00±0.00 a	22.54±0.31 a
Cassava	ND	0.090±0.006 a	6.42±0.04 b	ND	20.35±0.32 a

*Different lowercase letters in the same column represent a significant difference between the means submitted to the Tukey test at 5% probability of error. ND: Not determined.

Table 2 presents the results of the colorimetric profile and percentage of erosion of mucoadhesive films made with starch from different sources. The films did not differ in brightness and color, however, in the other parameters of the profile, there was variation, this variation may be due to the matrix from which starch were extracted. This theory can explain the reason of corn starch film has a more intense yellowish color, possibly, due to the complexation of phenolic and carotenoid compounds (HU and XU, 2011). Veiga-Santos et al. (2005) found similar results where there was yellowing of the films. Unlike the preparation of films for application in packaging, where transparent films are sought, in the preparation of films to produce mucoadhesive, opacity can be an interesting factor since the opacity will mask and/or protect the drug ingredient that the film will carry.

The percentage of erosion in the polymer says a lot about how much mass it will lose when, after contacting the simulated salivary solution, extrapolate its swelling limit (SOE et al., 2020). The mucoadhesive films made from starch from different sources differed in the percentage of erosion because each starch has a maximum amount of water absorption (LI et al., 2020). The percentage of erosion can predict how much the film will erode and will deliver the drug. Depending on the greater or lesser speed of drug delivery, erosion may be desired. Thus, we can suggest for a faster delivery the films made with wheat starch, and for a slower delivery the films made with corn, *Castanea sativa* and cassava starch.

Table 2. Color parameters, L, a *, b *, ΔE and % erosion of mucoadhesive films made with starches from different sources.

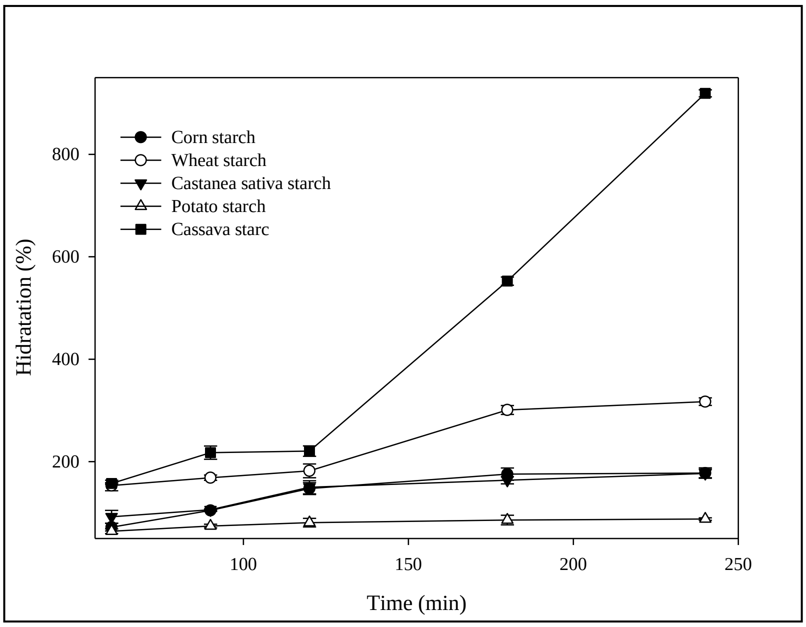
Starch	L	a*	b*	ΔE	% Erosion
Corn	93.16 ±0.28 a	-1.32±0.02 a	5.94±0.13 a	93.36±0.28 a	26.08±4.40 bc
Wheat	93.53±0.19 a	-0.79±0.01 b	4.44±0.10 c	93.64±0.18 a	43.83±5.75 a
Castanea sativa	92.75±0.18 a	-0.56±0.03 e	5.92±0.11 a	92.94±0.18 a	16.68±0.72 c
Potato	93.84±0.20 a	-0.74±0.02 c	4.43±0.04 c	93.94±0.20 a	35.40±0.01 ab
Cassava	92.99±1.37 a	-0.61±0.02 d	5.06±0.32 b	93.13±1.36 a	18.76±0.87 c

*Different lowercase letters in the same column represent a significant difference between the means submitted to the Tukey test at 5% probability of error.

Swelling properties is one of the most critical factors affecting the bioadhesive properties of the polymer and very important to predict the drug release mechanism. As the films absorb water, swelling begins, bonding begins, and adhesion occurs. Initially, the bond formed will be weak, but it increases with hydration. However, it finally reaches a point where excessive hydration leads to the unraveling and distortion of polymer molecules at the interface and decreases adhesion (PEH and WONG, 1999). The swelling index of mucoadhesive films made with starch from different sources are presented in Figure 1.

The hydration of the mucoadhesive films varied significantly depending on the source used. The highest values are obtained from cassava films, which, as already reported, presented high stickiness and hygroscopicity which justifies the high hydration; however, it is noteworthy that the greatest hydration occurred after 120 min, until this period the cassava starch films had the same behavior as the other films. In contrast, the films elaborated with potato starch showed the lowest hydration after 240 min of the analysis. This low hydration could result in a very low release of the drug, during the application of the mucoadhesive. Miksusanti et al., (2020) elaborated chitosan–tapioca starch composite as polymer in the formulation of gingival mucoadhesive patch film and verified that with the increase of starch in the formulation there is an increase on hydration. The results obtained in their study are similar to those found for potato starch in this study, it means that the cassava, wheat, *Castanea sativa* and corn starch shows a high hydration index for application on muchoadhesive film elaboration. According to Mortazav and Smart, (1993), hydration of the polymer is essential for the relaxation and interpenetration of polymer chains, but excess hydration generally leads to decreased mucoadhesion or retention due to the formation of slippery mucilage, in this away an alternative is to modify the starches for reduce their hygroscopicity.

Figure 1. Swelling index of mucoadhesive films made with starch from different sources.



4 CONCLUSIONS

It is possible to make mucoadhesive films with starch from different sources. All elaborated starch films presented desirable characteristics for mucoadhesive application, however, the film produced from corn starch has a combination of high flexibility, intermediated indexes of hydration and a low percentage of erosion showing the best characteristics for mucoadhesive films applicability.

Based on this results, future studies will be carried out to evaluate the use of native and modified corn starches in the production of mucoadhesive films, as well as to evaluate the transport and controlled release performance.

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6 REFERENCES

ABRUZZO, A.; NICOLETTA, F. P.; DALENA, F.; CERCHIARA, T.; LUPPI, B.; BIGUCCI, F. Bilayered buccal films as child-appropriate dosage form for systemic administration of propranolol. **International Journal of Pharmaceutics**, v. 531, n. 1, p. 257-265, 2017.

BISWAS, N.; SAHOO, R. K. Tapioca starch blended alginate mucoadhesive-floating beads for intragastric delivery of Metoprolol Tartrate. **International Journal of Biological Macromolecules**, v. 83, p. 61-70, 2016.

BOTTENBERG, P.; CLEYMAET, R.; MUYNCK, C.; REMON, J.P.; COOMANS, D.; MICHOTTE, Y.; SLOP, D. Development and testing of bioadhesive, fluoride-containing slow-release tablets for oral use, **Journal of Pharmacy and Pharmacology.** v. 43, n.1, p. 457–464, 1991.

CHI, C.; LI, X.; ZHANG, Y.; CHEN, L.; LI, L.; MIAO, S. Progress in tailoring starch intrinsic structures to improve its nutritional value. **Food Hydrocolloids**, v. 113, 106447, 2021.

COLUSSI, R.; KRINGEL, D.; KAUR, L.; ZAVAREZE, E. R.; DIAS, A. R. G.; SINGH, J. Dual modification of potato starch: Effects of heat-moisture and high pressure treatments on starch structure and functionalities. **Food Chemistry**, v. 318, 126475, 2020.

COLUSSI, R.; PINTO, V. Z.; HALLAL, S. L. M.; BIDUSKI, B.; PRIETTO, L. CASTILHOS, D. D.; ZAVAREZE, E. R.; DIAS, A. R. G. Acetylated rice starches films with different levels of amylose: Mechanical, water vapor barrier, thermal, and biodegradability properties. **Food Chemistry**, v.221, n.1, p.1614-1620.

CRUZ, B. R.; ABRAÃO, A. S.; LEMOS, A. M.; NUNES, F. M. Chemical composition and functional properties of native chestnut starch (*Castanea sativa Mill*). **Carbohydrate Polymers**, v. 94, n. 1, p. 594-602, 2013.

FARAJPOUR, R.; DJOMEH, Z. E.; MOEINI, S.; TAVAKOLIPOUR, H.; SAFAYAN, S. Structural and physico-mechanical properties of potato starch-olive oil edible films reinforced with zein nanoparticles. **International Journal of Biological Macromolecules**, v. 149, p. 941-950, 2020.

HU, Q-P; XU, J-G. Profiles of Carotenoids, Anthocyanins, Phenolics, and Antioxidant Activity of Selected Color Waxy Corn Grains during Maturation. **Journal of Agricultural and Food Chemistry**, v. 59, n. 5, p. 2026-2033, 2011.

JHA, P.; DHARMALINGAM, K.; NISHIZU, K.; KATSUNO, N.; ANANDALAKSHMI, R. Effect of Amylose–Amylopectin Ratios on Physical, Mechanical, and Thermal Properties of Starch-Based Bionanocomposite Films Incorporated with CMC and Nanoclay. **Starch**, v. 72, n. 1-2, 1900121, 2020.

KNIGHT, J. W., OLSON, R.M. Wheat starch: Production, modification, and uses. In Whistler, RL, BeMiller, JN & Paschall EF (Eds.), **Starch: Chemistry and technology** (2nd ed., pp. 491–505). Orlando: Academic Press. 1984.

KOCHKINA, N. E.; LUKIN, N. D. Structure and properties of biodegradable maize starch/chitosan composite films as affected by PVA additions. **International Journal of Biological Macromolecules**, v. 157, p. 377-384, 2020.

LI, C.; DHITAL, S.; GILBERT, R. G.; GIDLEY, M. J. High-amylose wheat starch: Structural basis for water absorption and pasting properties. **Carbohydrate Polymers**, v. 245, 116557, 2020.

LI, H.; QI, Y.; ZHAO,Y.; CHI, J.; CHENG, Z. Starch and its derivatives for paper coatings: A review. **Progress in Organic Coatings**, v. 135, p. 213-227, 2019.

MIKSUSANTI; FITHRI, A. N.; HERLINA; WIJAYA, D. P.; TAHER, T. Optimization of chitosan–tapioca starch composite as polymer in the formulation of gingival mucoadhesive patch film for delivery of gambier (*Uncaria gambir* Roxb) leaf extract. **International Journal of Biological Macromolecules**, v. 144, p. 289-295, 2020.

MORTAZAVI, S. A.; SMART, J. An investigation into the role of water movement and mucus gel dehydration in mucoadhesion. **Journal of Controlled Release**, v. 25, n. 3, p. 197-203, 1993.

NAIR, A. B.; KUMRIA, R.; HARSHA, S.; ATTIMARAD, S.; AL-DHUBIAB, B. E.; ALHAIDER, I. A. In vitro techniques to evaluate buccal films. **Journal of Controlled Release**, v. 166, n.1, p. 10–21, 2013.

PEH, K. K.; WONG, C. F. Polymeric films as vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties. **Journal of Pharmacy and Pharmaceutical Sciences**, v. 2, p. 53-61, 1999.
SAHNI, J.; RAJ, S.; AHMAD, F. J.; KHAR, R. K. Design and In Vitro Characterization of Buccoadhesive Drug Delivery System of Insulin. **Indian Journal of Pharmaceutical Sciences**, v. 70, n. 1, p. 61-65, 2008.
SOE, M. T.; CHITROPAS, P.; PONGJANYAKUL, T.; LIMPONGSA, E.; JAIPAKDEE, N. Thai glutinous rice starch modified by ball milling and its application as a mucoadhesive polymer. **Carbohydrate Polymer**, v. 232, 115812, 2020.
SOUTO, E.; LOPES, C. **Novas formas farmacêuticas para administração de fármacos**. Edições Universidade Fernando Pessoa, p. 105- 138, 2011.
SUH, J. H.; OCK, S.Y.; PARK, G. D.; LEE, M. H.; PARK, H. J. Effect of moisture content on the heat-sealing property of starch films from different botanical sources. **Polymer Testing**, v. 89, 106612, 2020.
VEIGA-SANTOS, P.; SUZIKI, C. K.; CEREDA, M. P.; SCAMPARINI, A. R. P. Microstructure and color of starch–gum films: Effect of gum deacetylation and additives. Part 2. **Food Hydrocolloids**, v. 19, n. 6, p. 1064-1073, 2005.