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HEALTH SCIENCES

Production of HPMC-films for lactase administration

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Abstract: The present work aimed to develop and characterize HPMC-films (HFs) based on hydroxypropylmethylcellulose (HPMC) with the addition of lactase for later application in the reduction of lactose in whole milk. The HFs were produced by the casting technique, with the addition of 1, 3, 5 and 7 g of lactase per 100 g of filmogenic solution. All the formulations presented a high capacity of film formation, but the thickness and time of disintegration increased according to the added lactase concentration. Regardless of the formulation developed, HFs in whole milk, a reduction in the lactose content (~ 80%) occurred, classifying the product as zero lactose. Thus, the HPMC-films with the addition of lactase can be considered as a good alternative for solubilization in foods which have a high concentration of lactose.

Key words: hydroxypropylmethylcellulose, lactose, films, lactase, new product, solubilization.

INTRODUCTION

Lactose is a disaccharide (glucose and galactose) found in several dairy products, so for an adequate absorption of these products, the activity of the enzyme lactase is required. Lactase hydrolyzes the lactose, thus allowing the passage of carbohydrates through the intestine to reach the bloodstream (Domínguez-Jiménez & Fernández-Suárez 2017). Lactose intolerance is considered a disease that mainly affects children and has increased in recent years, significantly impacting those affected. This disease affects the intestinal mucosa, which is not capable of digesting lactose due to the deficiency of the enzyme lactase (Pereira Filho & Furlan 2004).

To minimize the effects caused by the absence or low efficiency of lactase, capsules and liquid medicines are available on the market (Pray 2000). However, some patients have difficulties in taking solid dosing drugs, especially in the case of pediatric and geriatric patients due to fear of suffocation, or suffering from dysphagia (Patel et al. 2010). Seager (1997) and Nagaraju et al. (2013) also reported difficulty in using solid dosage drugs for bedridden patients who are busy or traveling, especially those who do not have access to water.

As an alternative for drug administration, HPMC-films have been produced to dissolve and release the active component (Dixit & Puthli 2009). According to Saini et al. (2012), the production of films for oral disintegration can involve the use of polymers, plasticizers and active compounds, in addition to saliva stimulating agents, surfactant, sweetener and flavor. There is also the possibility of incorporating natural active compounds or pharmaceutical ingredients (Daud et al. 2011).

Takeuchi et al. (2017) reported that orally films are recommended because of their thinness and flexibility, making them suitable for all types of patients. The main use of oral films is as a carrier of pharmaceutical ingredients of synthetic origin, marketed as over-the-counter or prescription drugs (Reiner et al. 2010). Despite these characteristics, no reports were found in the literature of orally films used directly in food as a source of lactase.

Within this context, the present work aimed to develop films based on hydroxypropylmethylcellulose as a carrier of lactase. The developed HFs were characterized and their efficiency in reducing lactose (in whole milk) was verified.

MATERIALS AND METHODS

Material

Hydroxypropylmethylcellulose was purchased from Extratus Farma (Umuarama, PR) as the polymer, sorbitol (Vetec) as plasticizer and commercial lactase was donated by Biotech Brazil Ferments and Coagulants - LTDA. The reagents used were: sodium chloride (Quimex), potassium chloride (Nuclear), disodium phosphate (Synth), monopotassium phosphate (Synth), cupric sulfate (Synth) solutions, sodium and potassium tartrate (Anhydrol) and sodium (Nuclear), glacial acetic acid (Vetec) and methylene blue indicator (Nuclear). To verify the efficiency of HFs in reducing lactose, a commercial whole milk sample was selected as the model system.

Production of the HPMC-films

For the production of the films based on HPMC, the polymer was initially dispersed in distilled water (~60 °C) by magnetic stirring (Marconi, MA85) and after 30 minutes, the filmogenic solution was placed in a thermostatic bath (Marconi / MA159) at 90 °C for 10 min before the addition of the plasticizer under stirring. The concentration of the polymer (2 g per 100 g of filmogenic solution) and sorbitol (0.4 g per 100 g of filmogenic solution) was kept constant. Lactase was added under stirring at different concentrations (1, 3, 5 and 7 g per 100 g of filmogenic solution), after complete homogenization, the filmogenic solution was dispersed in plates and oven dried (Marconi, MA35) at 30 °C for 24 hours.

Characterization of the HPMC-films

Visual aspect and thickness

The visual evaluation of the HFs was performed according to Garcia et al. (2017) in relation to homogeneity, (characterized as the absence of insoluble particles), capacity of formation (absence of discontinuity zones after the drying step) and handling (ease of removal of the oral film from the carrier). The thickness of the films was determined, at ten random points, using a digital micrometer (Western).

Disintegration time

To determine the disintegration time, the HFs were fixed in a slide frame and placed in Petri dishes, then the buffer solution (200 µL) was deposited on the surface of the film with the aid of a pipette. The buffer solution was prepared according to Föger et al. (2008) using 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCL), 1.536 g of disodium phosphate (Na_2HPO_4) and 0.2 g of monopotassium phosphate (KH_2PO_4) per liter of distilled water. The time required for the drop to dissolve the film and form a hole was determined as the disintegration time (Garsuch & Breitkreutz 2010).

Surface pH

The surface pH was determined using a digital pH meter (Gehaka, PG2000). HFs were placed in a container containing 1 mL of phosphate buffer solution (pH 6.8). The pH of the solution was recorded after 2 minutes (Manhar & Suresh 2013) of the HFs in contact with the solution.

Evaluation of the addition of HFs to lactose reduction in whole milk

The lactose was measured according to Brazil's Ministry of Agriculture, Livestock and Food Supply guidelines (MAPA 2013) by the Lane-Eynon method. Whole milk (15 mL) was used as a positive control and lactose-free yogurt (15 mL) as a negative control. The HFs with lactase were solubilized in whole milk (15 mL) on a magnetic stirrer (Marconi, MA85). For the positive control, negative control and the whole milk with the solubilized HFs, 20 mL of distilled water (60 °C) was added separately, then the solution was precipitated with glacial acetic acid (90%) and kept in a water bath (Kacil, BM- 02) at 60-65 °C for 5 minutes. The obtained solution was cooled, filtered (Unifil) and transferred to a burette. The Fehling A and Fehling B solutions were transferred into the Redutec apparatus (Marconi, MA-087) and 100 mL of distilled water was added. After the solution boiled, 1 mL of methylene blue solution (1%) was added for titration. The spent volume of solution () was recorded. The lactose reducing glycerides (%) was calculated according to Eq. 1.

$$\text{Lactose reducing glycerides (\%)} = \frac{VS_i . VS_f . C_F}{V_S . V_F} \qquad (1)$$

Where: VS_i - volume of the inverted sucrose solution used in the measurement of solution A; VS_f - final volume of the sample solution; C_F- conversion factor; V_S - volume of sample used; V_F - volume of filtrate spent in titration.

Statistical analysis

The analyzes were performed in triplicate and the results expressed as mean ± standard deviation. The comparison of means was performed by analysis of variance (ANOVA), followed by the Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

Visual aspect

Table I shows the visual characteristics of the HFs. The films produced based on HPMC without lactase and with addition of lactase (1, 3, 5 and 7 g per 100 g of filmogenic solution), were homogeneous, with the absence of bubbles and ease of plaque removal, indicating that the increase of the lactase concentration did not influence the film-forming capacity. Garsuch & Breitkreutz (2010), Dinge & Nagarsenker (2008) and Sabar (2013) also reported that HPMC is efficient for film production with high film-forming capacity.

The thickness of the films varied from 0.08 to 0.14 mm, with an increase in the thickness of the HFs with increasing lactase incorporation due to the higher concentration of solids available per plate. Takeuchi et al. (2013) reported that in the production of films with the addition of pharmaceutical ingredients, such as acetaminophen and microcrystalline celulose, the increased concentration of these compounds also led to an increase in the thickness of the HFs.

The characteristics observed in Table I corroborate with that observed visually in Figure 1.

Table I. Visual aspect and thickness of HPMC-films without and with different concentrations of lactase.

Formulation	Lactase (g)	Homogeneity	Capacity of formation	Handling	Thickness (mm)
F1	-	+	+	+	0.08±0.01 [a]
F2	1	+	+	+	0.09±0.01 [a]
F3	3	+	+	+	0.10±0.02 [a]
F4	5	+	+	+	0.12±0.02 [ab]
F5	7	+	+	+	0.14±0.02 [b]

*** Means with the same lowercase letter in the same column did not differ significantly among themselves at the 5% level of significance by the Tukey test.**

Disintegration time

According to Figure 2, the HPMC-films with the highest lactase concentration, showed the longest disintegration time, which can be justified by the increase in thickness after the addition of different lactase concentrations (Table I), with the longest observed disintegration (~ 70 seconds) for the F5 formulation which had the greatest thickness (0.14 mm). Possibly, the addition of lactase resulted in an increase in the hydrophobic characteristics of the HPMC films, consequently, the disintegration time was longer, since the technique used simulates the saliva pH (6.7), however, as observed in thickness determination, the disintegration time did not differ significantly from F4. According to Preis et al. (2012) the disintegration behavior varies according to the thickness of the films, in which the films with greater thickness have a longer disintegration time.

Tedesco et al. (2016) reported that the orally films of HPMC showed a disintegration time of 34.43 seconds (0.050 mm), indicating greater affinity with water when compared to films of other macromolecules like gelatin. Liew et al. (2012) reported a mean thickness of 0.358 mm at a time of 43.83 seconds for HPMC-films. Moreover, Jyoti et al. (2011) suggested that fast-release films that have a disintegration time of less than 60 seconds, regardless of the formulation.

Surface pH

In addition, the HFs with lactase showed a surface pH of 6.76 (F1), 6.70 (F2), 6.87 (F3), 6.84 (F4) and 6.88 (F5), not differing significantly from each other, regardless of the lactase concentration. All formulations presented a pH close to neutrality (6.8), indicating a pH close to that of the mouth, which would not cause discomfort if it comes in contact with the oral mucosa.

Lactase has maximum activity at pH 6.5 (Rossetto et al. 2012), close to that of the HFs. Factors such as temperature and pH directly influence milk stability (Wang et al. 2017), with the pH of milk varying from 6.6 to 6.8 (Brasil 2017), which is close to the pH of the films and does not cause changes in the pH taste of food. As the data regarding visual evaluation, disintegration time and surface pH was similar for the F4 and F5 formulations, the formulation with the highest lactase concentration was selected to verify the efficiency for lactose reduction.

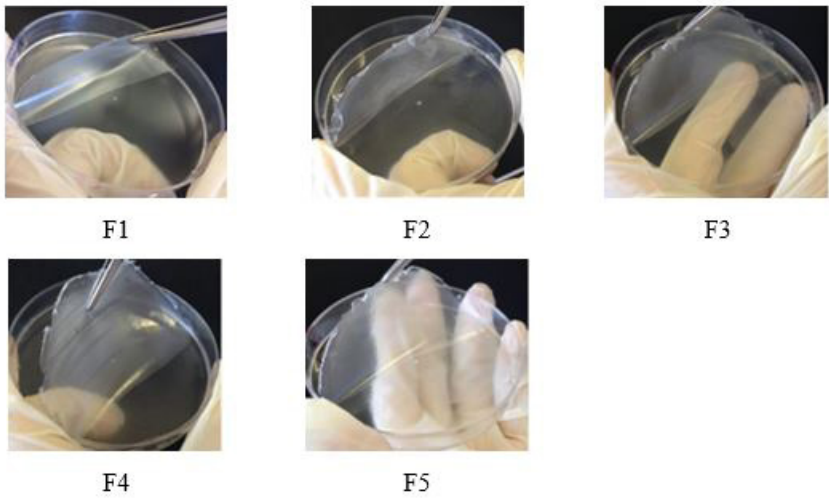

Figure 1. HPMC-films produced without lactase and with different concentrations of lactase per 100 g of filmogenic solution: (F1) 0 g; (F2) 1 g; (F3) 3 g; (F4) 5 g and (F5) 7 g.

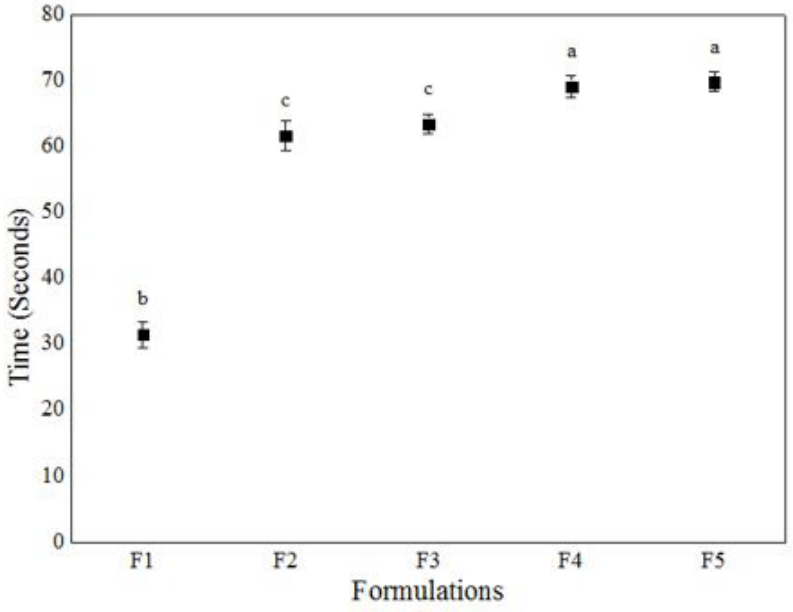

Figure 2. Effect of lactose concentration of HPMC-films. Being, (F1) HPMC; (F2) HPMC + 1 g of lactase; (F3) HPMC + 3 g of lactase; (F4) HPMC + 5 g of lactase; (F5) HPMC + 7 g of lactase. Means with the same lowercase letter did not differ significantly among themselves at the 5% level of significance by the Tukey test.

Table II. Determination of lactose content in whole milk.

Formulation	Film mass (g)[1]	Lactose content (%)
Positive control[2]	-	3.174±0.027
Negative control[3]	-	0.062±0.001
Whole milk	0.022	0.840±0.004
	0.026	0.605±0.001

[1]F5 (HPMC-films with addition of 7 grams of lactase); [2]Whole milk; [3]Zero lactose yoghurt.

Evaluation of the addition of HFs to lactose reduction in whole milk

Table II presents the results obtained from lactose content in whole milk after solubilization of the HFs (F5). The solubilization of HPMC-films in milk reduced the lactose content from 0.84 to 0.60%. According to RDC 135/2017, foods with a lactose content equal to or less than 100 milligrams per 100 grams or milliliters are defined as zero lactose and foods that contain an amount of lactose greater than 100 milligrams and equal to or less than 1 gram per 100 grams or milliliters are defined as low lactose. Thus, milk after the solubilization of the HFs with 7 g of lactase can be considered zero lactose and be consumed by people with a lactose intolerance. According to Vrese et al. (2015), the degree of hydrolysis of lactose depends on the amount of lactase administered. As shown in Table II, the mass of milk-solubilized HFs is proportional, since the increase in film mass is associated with a reduction in lactose content. Lactose is present in a high concentration in mammalian milk, about 3 to 7% (Troise et al. 2016), but was reduced by the solubilization of HFs with lactase produced in this study.

CONCLUSION

HPMC-films showed high film-forming capacity, easy manipulation and pH close to neutrality independent of the concentration of added lactase. After the rapid solubilization of the orally film containing 7 g of lactase in milk, an approximately 80% reduction in lactose content was observed, classifying the product as zero lactose. Therefore, films produced with hydroxypropylmethylcellulose with the addition of lactase are a viable alternative for people with lactose intolerance for administration of liquid foods.

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