Full Length Research Paper

Microencapsulation of *Aspergillus niger* phytases produced in triticale by solid fermentation and microencapsulates characterization

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Received 15 August, 2018; Accepted 18 April, 2019

This paper aimed to carry out the stabilization of phytase produced in solid-state fermentation by means of *Aspergillus niger* 7A-1 in triticale as substrate. The crude extract obtained in liquid state was stabilized with glycerol and maltose. These materials presented a synergic effect that improve phytase stability around 80% with ki of 0.031 and a shelf-time of 156 days (22.35 weeks) with glycerol 30% and maltose 20%. Besides, microencapsulation by spray drying was used as well to analyze the potential of whey and guar gum to obtain microencapsulates of phytase for monogastric animals. Statistical analysis allowed establishing the suitable temperature at 110°C with a flow of 10% (3.3 ml/min), guar gum concentration of 1% and yield process at 93%. Characterization allows us to observe that microencapsulates with whey and guar gum is a good alternative to be used in spray drying. SEM showed the formation of aggregates with pores. Thermogravimetric analysis demonstrated that thermal stability of guar gum is improved when it is mixed with whey. Infrared showed the signal of protein groups in samples with whey and guar gum. Stability assessment was performed with two different containers, Eppendorf and Ziploc bags, in which it was demonstrated that microencapsulation with whey and guar gum allow the retention of 85% for both containers. At 35°C, phytase activity decreases until 55%. In this study, color was evaluated as well in Ziploc bags with the color observing change during the 8 weeks; this change was not observed in Eppendorf container and it was possible to observe that color does not affect enzymatic stability. Finally, digestibility evaluation demonstrated that whey and guar gum provide protection in gastrointestinal conditions. After digestive system simulation, 90% of activity is retained which allows observing the potential of whey and guar gum to protect enzymes under gastrointestinal condition.

**Key words:** Phytases, phosphorus, phytate, guar gum, microencapsulation.

INTRODUCTION

After energy and protein, phosphorus is the third element more important in animal diets (Rodehutscord, 2013). It could be found in cereals as a compound known as phytate, which is formed of phytic acid with minerals or...
proteins. Phytate has a negative effect upon phosphorus' bioavailability. This leads to lower absorption of phosphorus and the needing of alternative phosphorous sources (Tang et al., 2018). Phytase (myo-inositol hexaphosphate phosphohydrolase) catalyzes the hydrolysis of phytate to inositol phosphates, myo-inositol, and inorganic phosphate. Phytase can be found in cereals but in low concentration, researches have been carried out to improve the expression of phytase genes and in this way promote the synthesis of this enzyme (Tan et al., 2017). Solid fermentation is the most used procedure to obtain this enzyme and then, the crude extract is recovered in aqueous solution. However, when proteins are in aqueous solution the hydration of the molecule and denaturalization of quaternary structure proteins is the main risk. One of the main challenges in livestock industry is to maintain the enzymatic activity and stability in aqueous solution. Stabilization with some additives as sugars, salts, amino acids, amines, polyols and buffers systems are as co-solvents which has been employed for enzyme stabilization and these additives have a positive effect upon the enzymes degradation.

High temperatures of ∼70–90°C are used in the feed-pelleting procedure; phytase is easily inactivated to 30% or less of its original activity. Microencapsulation is a process widely used due to its low cost and it is the most common method to dry liquid compositions. It provides protection by carriers and additives by means of spray drying and, in this way, avoiding enzyme inactivation. Nowadays, many different compounds have been dried by this method like some oils (Aghbashlo et al., 2012; Gangurde et al., 2015; Koç et al., 2015), lactobacillus (Eckert et al., 2017), DNA (Alexakis et al., 1995), food (Pérez-Alonso et al., 2009), volatile aromas (Brückner et al., 2007), enzymes (Anjani et al., 2007; Dutta and Bhattacharjee, 2017; Estevinho et al., 2014; Gupta et al., 2014; Jesus and Filho, 2014), among others. The greatest challenge in the spray drying of proteins is denaturalization due to heating. High temperatures are involved in this process; spray drying is based on generation of fine droplets by a nozzle or a rotary atomizer into a hot dry air stream normally at 180-220°C; and enzymes cannot support these temperatures. Nevertheless, there are carriers that can be used to protect enzymes. Spray drying technique consists of three steps: atomization, dehydration, and powder collection. The feed liquid, which include carriers, additives and compounds to encapsulate (like enzymes), is sprayed through an atomizing nozzle into a drying chamber (Ganju and Gogate, 2017; Hamin et al., 2017), in which a hot air flow dry water and finally the powder is recollected. There are few reports of phytase microencapsulation through spray drying with polysaccharides (Spier and Greiner, 2015).

Whey is considered as a waste for dairy industry, which is a by-product and it is produced in significant quantum due to the volume of required milk products (Ganju and Gogate, 2017). It is mainly composed of proteins, lactose, minerals and dry matter, and could be used in fermentations, beverages or additives in food in order to obtain a functional one (Teniza, 2008). Whey proteins concentrated and isolated have been recovered by the utilization of membranes, bio-catalysis or high performance tangential flow ultrafiltration. However, the residues of the separation of proteins remain considered as waste. For this reason, new alternatives for the utilization of whey are being searched for. When whey is dried, water can be promptly removed from the dry matter, minimizing heat damage and heating costs. Some components have been used as a carrier or additive to encapsulate different compounds. Guar meal is the main by-product of guar gum (GG) production with a high protein content (Ahmed and Abou-Elkhair, 2016). GG has been used as a carrier to encapsulate, and it has shown positive effects during drying, protecting the product even at high temperatures (Kuck and Noreña, 2016; Mehyar et al., 2014).

The poultry industry has become a specific area of producing enzymes for animals. The demand for complementary enzymes for animals diets have been increasing (Ahmed and Abou-Elkhair, 2016). Phytases are one of this example, which is estimated by Global Phytases Market Report (2017) with an annual growth rate of 2.78% from 2012 to 2013, implying 315 to 342 million dollars respectively (Global Phytases Market Report, 2017). There are predictions for 2021-2022 of 520 million dollars for phytases market. This is the reason why the huge intake of cereals and legumes in animal diets that are complemented with phytases allowing the development of a process to produce the enzyme, recover and purify this kind of enzymes with higher profitability. Phytases hydrolyze phytate producing inorganic phosphorous and myo-inositol (Neira et al., 2013). There are different products currently commercialized, most of which are presented in liquid, with transportation and availability in process of food pelleting as disadvantage (Menezes-Blackburn et al., 2015). Microencapsulation is the drying of liquid solutions by wrapping compounds with a thin polymeric coating to obtain micro particles powders with a normal size from 5 – 5000 μm. At present, microencapsulation has been widely used in textile, cosmetic, pharmaceutical and food industry. Depending on the method used, characteristics of microencapsulates will differ (Das et al., 2011). Chemical, physic and physico-chemical are methods of microencapsulation. Spray drying is a physico-chemical process suitable for use in industry due to its low cost commercial process. It is useful for compounds that are heat sensitive, such as proteins or peptides like enzymes; however, with the appropriate coating material, encapsulation could be accomplished (Nava et al., 2015). In addition, spray drying method is a rapid and reproducible method for microencapsulate and is easy to scale up. There are a lot of materials that can be used as
coating materials like gums, waxes, proteins, polysaccharides, carbohydrates, etc. (Eckert et al., 2017; Lupo et al., 2012; Meyyar et al., 2014). Nevertheless, aspects such as properties materials, feed rate, viscosity, glassy transition temperature, inlet and outlet temperature, vacuum, equipment conditions, uniformity as well as the compound to be encapsulated, must be considered in drying process. These aspects will have an influence not only on the objective desired, but also in the physical characteristics. Techniques of characterization which are great tools to elucidate physical characteristics, thermogravimetric analysis, X-Rays, infrared, scanning electronic microscopy, among others has been used. In this way, it is possible to determine physical structures of microcapsules. All products tend to deteriorate during storage, but in different rates (Sousa-Gallagher et al., 2016). For this, storage assessment is important, as it is possible to determine the time in which products will preserve their qualities that make them a stable product. Products can suffer different changes during storage like enzymatic browning, non-enzymatic browning, oxidation-reduction reaction, microbial damage, change in color, etc. Another important element to take into consideration is the choice of packing material as well, so that the integrity of the product could be preserved for a long duration. This is the reason why a suitable assessment of storage should be carried out. Furthermore, digestibility evaluation will provide relevant information about the bioavailability of microencapsulates in gastrointestinal conditions. Digestibility is defined as the percentage of foodstuffs available to be absorbed by the organism. Poultry have a gastrointestinal condition like those of humans. α-amylase, pepsin and pancreatic enzymes (α-amylase, lipase and proteases) are enzymes presented in gastrointestinal tract. Nowadays, there are different models for simulation of gastrointestinal condition, and they could be applied according to the purpose of interest.

The aim of this work was the stabilization of phytase liquid extract using maltose and glycerol to improve its shelf-time. Besides, evaluation of whey and guar gum intended to be used as materials for phytase encapsulation in spray drying was carried out as well, to determine physical characteristics of phytase microencapsulates obtained under the conditions selected and to evaluate the digestibility of the microencapsulates in an in vitro system simulating gastrointestinal conditions.

**METHODOLOGY**

**Microorganism**

The microorganism used to produce phytase was a fungus of the genus *Aspergillus*, the *niger* species, provided by Nanobioscience Group of the Autonomous University of Coahuila, Mexico with code 7A-1. *A. niger* 7A-1 strain was selected by Neira et al., (2013).

**Phytase production**

Phytase was produced in solid state fermentation (SSF) following the methodology used by Costa et al., (2010) and modified by Neira et al., (2013). The strain was grown in potato dextrose agar (PDA) at 37°C for 5 days. Spores were recovered with 0.1% Tween 80 and stirred for 15 min. The liquid was filtrated, and spores concentration quantified. The inoculum was prepared with dextrose (20 g/L) and yeast extract (4 g/L), spores were added into solution in concentration of 1×10^6 spores per millilitre and stirred at 180 rpm for 36 h. The triticale substrate used was selected by Neira et al., (2013). Triticale was used for 159 days in a proportion of 3:1 spike-stem and was submerged in sodium hypochlorite (5 mL/L) for 5 min. Substrate was rinsed with distilled water and ground in a grain mill electric Thomas Willey with mesh size of 0.5 mm. Triticale was used as substrate and it was provided by the Antonio Narro Autonomous Agrarian University (UAAAN), Mexico. 5 g of substrate were placed in Petri dish and sterilized at 121°C for 30 min. After cooling, 3 ml inoculum and 2.5 ml (NH₄)_2SO₄ were added to each Petri dish and incubated at 37°C for 5 days. In order to analyze the growing, a kinetic was performed for seven days and every day one Petri dish was sacrificed, with sugars, proteins and phytase activity evaluated. Crude extract was obtained by adding 5 ml H₂O per gram of dry substrate and stirred at 200 rpm for 30 min. The liquid was centrifugated at 10,000 rpm for 10 min. The supernatant was recollected, filtrated to eliminate spore and stored at 4°C.

**Phytase activity**

Phytase activity was measured spectrophotometrically according to the technique used by Harland and Harland (1980). The reaction mixture consisted of addition of 1 ml of 100 mM MgSO₄ in 200 mM acetate buffer (pH 5.15); 2.4 ml of 6.82 mM phytic acid prepared in 200 mM acetate buffer (pH 5.15) and 0.6 ml of crude extract containing phytase. The reaction mixture was stirred and incubated for 60 min at 55°C. After this time, reaction was stopped with the addition of 0.5 ml of trichloroacetic acid 10%. 1 ml of distilled water was added to adjust volume, and 2.4 ml of freshly prepared reagent of Tausky-Schorr (10 mL of 10 N H₂SO₄, 1 g of (NH₄)₂MoO₄·2H₂O and 5 g of FeSO₄·7H₂O diluted to the mark in 100-ml flask with distilled water) to generate a blue chromophore were added. The contents were mixed and incubated for 30 min and the inorganic phosphate released was measured at 660 nm. One unit of phytase activity (U) was expressed as the amount of enzyme releasing 1 μmol of phosphorus per minute under standard assay conditions. Calibration curve was performed by triplicate in a range of 0.04 μmol/ml of inorganic phosphorus with KH₂PO₄. 28 mg of KH₂PO₄ was used, dissolved in acetates buffer (0.2 M pH 5.15) and diluted to 100 ml.

**Stabilization of phytase**

Glycerol (Jalmek, Mexico) and maltose (Sigma-Alrich, Japan) were employed to study the stability of the crude extract containing phytase. The study was performed under two conditions for glycerol (10 and 30%) and maltose (0 and 20%) in a statistic design of 2^2. Twenty-seven samples were prepared and stored at 4°C for eight weeks. Each week, three samples were sacrificed to determine phytase activity according to Harland and Harland method described previously. To predict the stability of the enzymes, the inactivation constants were determined using the Arrhenius equation. Shelf-time was calculated with equation number 1.

\[
t_{1/2} = \frac{\ln(2)}{k}
\]
Shelf-time equation for first order reactions (Brown et al., 2004).

Phytases microencapsulation

Characterization of whey

Whey was acquired from the company “Quesos de Saltillo”, Coahuila, México. Whey were analyzed for physicochemical parameters according to Association of Analytical Communities (AOAC, 1990). Total solids were evaluated using Thermodanalysis OHAUS MB23 at 105°C (method No. 990.20), ash using a muffle furnace Felisa FE-340 at 550°C (method No. 968.08), fat by using the Soxhlet method (method No. 922.06), protein by using the Kjeldahl method (method No. 991.20) and carbohydrates by using the difference method (method No. 986.25). pH was evaluated using a HANNA HI2550 potentiometer whereas samples were by triplicate.

Liquid feed, dryer equipment and operating conditions

Guar gum has been shown to be a good carrier for protecting different compounds in spray drying. According to literature, more than 1% of guar gum increases the viscosity of the liquid and makes it more difficult to dry samples. In addition, there are some reports where it is mentioned that more than 7.5 - 10% of guar meal in animal diets can cause severe growth depression and sticky feces (AOAC, 1990; Jellouli et al., 2011). Even though guar meal is a by-product of guar gum production, guar gum can present a few effect when more than 5% of it is used (Rao et al., 2016). Therefore, 0.1 and 1% were GG concentration in the liquid feed which contains 80% of whey/GG and 20% of extract crude containing phytase.

Phytase microencapsulation process

Spray drying was carried out in a Mini Spray Dryer B-290 (Büchi Labortechnik AG, Flawil, Switzerland). The liquid was fed into the atomizer under two-flow condition: 10 and 30%, respectively. To determine the suitable flow for this specific liquid, every sample was 100 ml and analyzed in triplicate. 100-ml liquid was dried, time was measured with a chronometer and flow rate was calculated. Nozzle specification was 0.7 mm. Previous experiments were conducted at 150°C according to the results obtained, and the inlet temperatures evaluated were 110 and 130°C. Central points were 0.55% for guar gum concentration, 120°C for temperature and 20% for flow. There are some important factors involved in drying, which can lead to a stress state for protein, dehydration stress, temperature and moisture. This study was performed by a factorial design with one central point.

Microencapsulates characterization

Enzymatic activity

100 mg of the powder were dissolved in 10 ml of saline solution to liberate enzyme and stirred. Phytase activity was evaluated by Harland and Harland (1980) method previously described.

Yield of encapsulation

Yield was calculated using total solids amount as reference and determined in the same way described before. The yield was calculated with the Equation 2.

\[
Yield = \frac{\text{Powder recovered (grams)}}{\text{Total solids (grams)}} \times 100
\]

This is recovery yield equation.

Product moisture content and water activity

Moisture content of the samples was calculated from weight loss after heating the sample at 60°C for 24 h. Water activity (Aw) was measured by direct reading in electronic meter (Aqualab 3TE-Decagon, Pullman). Both parameters were evaluated according to AOAC (1990). The analyses were performed in triplicate.

Color assessment of microencapsulates

Color of the microencapsulated powders was measured using a NH310 Portable Colorimeter. CIETLAB system define L*: a*, b*; where L* indicates light-ness (0 = black and 100 = white), a* and b* are coordinates for green (-a*)/red (+a*), and blue (-b*)/yellow (+b*) (Kuck and Noreña, 2016).

Statistical analysis

The data produced were analyzed for mean, standard deviation and standard error using Microsoft Office Excel (Tukey test) and Stat Graphics (ANOVA). All the results are expressed as mean of three replicates, unless otherwise stated.

Storage availability assessment

Temperature and container were the parameters evaluated to determine the storage-availability. Nowadays phytase is commercialized in bottles and bags; for this reason, microcapsules were placed in Ziploc bags and Eppendorf tubes. Samples were stored at 4°C, which represents refrigeration temperature (25°C), as well as room temperature (35°C), according to Tonon et al. (2010); this temperature is recommended for storage assessment. Every,
week one sample was taken until the 8th week. Phytase activity as well as color was evaluated. To predict the stability of the enzymes the inactivation constants were determined using Arrhenius equation and shelf life was also calculated.

**Digestibility and bioavailability assessment**

To study the effect of the simulated digestive tract conditions upon the stability of the microencapsulates, the conditions described by Madureira et al. (2011) were followed. Samples were prepared by dissolving microencapsulates with food and without food; as control, microencapsulates without enzyme was dissolved in a saline solution. Mouth digestion simulation was carried out adjusting pH in all tested solutions to values between 5.6 and 6.9, using HCl 1 M. Artificial saliva was simulated by using amylase (Novozymes, Denmark) at 100 U/mL and added to solutions digestion. Incubation was made within 1 min at 37°C and 200 rpm. pH was adjusted to 2.0 using HCl 1 M to simulate stomach digestion. Gastric juice was simulated by dissolving pepsin (Sigma-Aldrich Chemistry, USA) (25 mg/mL). Incubation lasted 60 min (that is, short digestion) at 37°C and 130 rpm. Simulation of gut conditions was performed as follows: intestinal juice was simulated by dissolving 2 g/L of pancreatic enzymes (Sigma-Aldrich Chemistry, USA); and pH was adjusted to 6.0 using NaHCO₃ 1 M. All assays were performed in duplicate. Samples were submitted to an extraction process to evaluate phosphorus release during the GIT, were thereafter stored at 4°C and subsequently analyzed.

**RESULTS AND DISCUSSION**

**Fermentation process**

Figure 1 shows a fluctuating graphic for sugars. The higher sugar amount was observed in the first day, then, as long as time is going on, the amount of sugar presents a fluctuating behavior. In day three and four, sugar amount increase and from day four, sugar amount decreases. This behavior could be because the microorganism produces sugars or hydrolyzes them from the substrate used. The level decreased in the first day, in association with microbial growth wherein microorganism consumes sugar presented from medium; however, in the following days it was fluctuating. This may be related to the exponential phase in which the microorganism synthetizes a great quantity of metabolites such as sugars. Besides, protein production is increased in the first day. Neira et al., (2013) observed the same behavior and asserted that in the third day there could be a process of adaptation, which can promote the production of enzymes. In addition, Neira et al., (2013) commented that this sugar production come from the hydrolysis of vegetal material, with each other, sugars from cell wall and other structures. There are reports that phytases are involved in dephytinization process in cereals, in which inorganic phosphorus, proteins and reducing sugars are being released (Turki et al., 2011). Therefore, this fluctuating behavior could be related to sugar produced by the metabolism of microorganism and the releasing of sugars from the substrate.

Figure 2 shows the results related with the fermentation process. It was possible to observe the protein production in the first day which was increasing until the maximum production in the fifth day. In this stage, the microorganism is found in the exponential phase, where different kinds of protein are produced, like enzymes, to get used to the medium. According to Costa et al., (2010), the microorganism uses the proteins produced as a nutrient, which could be used as nitrogen source. This value is similar to the phytase activity (Figure 2), which is related to the content of protein. It was possible to
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Figure 2. Phytase activity and protein plot in relation with time for eight days.

Figure 3. Phytase stability for 8 weeks. G30/M20: Glycerol 30% (v/v)/Maltose 20% (w/v), G10/M20: Glycerol 10% (v/v)/Maltose 20% (w/v), G30/M0: Glycerol 30% (v/v)/Maltose 0% (w/v), G10/M0: Glycerol 10% (v/v)/Maltose 0% (w/v).

corroborate what Neira et al., (2013) found and selected as the maximum production on the fifth day.

Phytase stabilization

Stabilization was carried out with glycerol (G) and maltose (M) as Figure 3 shows the behavior during the 8 weeks. It was possible to observe that for G30/M20 the activity was lost gradually until 62% from his initial activity. G10/M20 retained an activity of 78% after 8 weeks. Both samples contain maltose 20% (w/v) which can be related to this behavior. It has been reported that some sugars allow the stability of proteins by the creation of bonds among the enzyme and glycerol (Crowe, 2002; Singh et al., 2015). Though there is a difference when maltose is not in the sample.

In week eight, G30/M0 lost a little bit more than 90% from his initial activity, and G10/M0 lost 25%. This could be due to lack of maltose. The great difference among
Figure 4. Linearization chart using Arrhenius equation. G30/M20: Glycerol 30% (v/v)/Maltose 20% (w/v), G10/M20: Glycerol 10% (v/v)/Maltose 20% (w/v).

Table 1. $k_i$ and half-time ($t_{1/2}$) for the stability treatments that present a first order kinetic.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$k_i$</th>
<th>$t_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>G30/M20</td>
<td>0.031</td>
<td>22.35</td>
</tr>
<tr>
<td>G10/M20</td>
<td>0.036</td>
<td>19.46</td>
</tr>
</tbody>
</table>

...this compared to samples is bigger and this allow determination of the influence of maltose and its interaction with glycerol upon the stability of phytase.

The samples with maltose and glycerol were selected according to regression value where both samples showed straight lines with high regression coefficient values ($R^2 > 0.95$) (Figure 4). This demonstrates that thermal inactivation kinetics for both preparations follow a first-order kinetic behavior in which concentration decrease proportional to time. $k_i$ and half time ($t_{1/2}$) were calculated and shown in Table 1. The values for $k_i$ are similar among them and it was possible to deduce that maltose together with glycerol could be the elements that provides this stability to phytase.

Rodríguez-Fernández et al. (2013) described that the inactivation of the phytase could be owing to irreversible chemical degradation process that is related to the amount of water in the samples. When the enzyme is in liquid or solution, proteins can get hydrated through the formation of hydrogen bounds (Costa et al., 2002). Neira et al. (2018) also reported similar $k_i$; they used glycerol, maltose and phenyl methyl sulphonyl fluoride (PSMF), with the last one acting as proteases inhibitor. The present study shows values similar to Neira et al., (2018) without the addition of PSMF. They attribute it to peptides folding and stabilization of third and quaternary structure, due to the formation of stronger bounds among enzyme, glycerol and maltose, while PSMF inhibits the effect of proteases in the medium (Beg and Gupta, 2003).

Half-life results indicate that both samples present small differences, which can be due to the presence of maltose. Mensink et al. (2017) proposed two ways of how sugar protects proteins in solution. Vitrification and water replacement are the two ways, but the complete mechanism has not yet been understood. It is possible to determine that sugar could create a rigid matrix around the protein which avoids the movement of enzyme and preserves the structure. It has been shown that smaller and molecular oligosaccharides are better able to stabilize due to its molecular flexibility. Sugars produce stronger interactions and a tighter packing reducing free volume (Kamerzell et al., 2011).

One possible reason of this may be related to the fact that smaller sugars fill smaller cavities in the protein structures (Mensink et al., 2017). Besides, sugar can also interact with aromatic protein groups presented in enzymes. However, although there are two possible explanations on sugar stabilization of proteins, Grasmeijer et al. (2013) proposes that these two explanations are related with glass transition temperature (Tg); vitrification mechanism is presented if the temperature used is below Tg. Above Tg, protein mobility is reduced and water...
Phytase microencapsulation

Whey characterization

In order to determine the whey elemental composition and evaluate the properties that make it affordable for microencapsulation, physicochemical analysis was carried out. Table 2 allows observing variations with previous reports. These variations could be related with the origin of whey. There are reports which indicate that variations in whey compositions are as a result of animal feed, animal growth stage, milk composition and kind of cheese produced (Poveda, 2013).

For this study, lactose, protein, pH and ashes (mainly NaCl and CaCl) (Tsakali et al., 2010) were the elements more important in whey composition. This is because whey is characterized by high protein content, which is beneficial to be used for different purposes (Ganju and Gogate, 2017). Both isolated and concentrated whey protein have been used as binders (Aghbashlo et al., 2012; Koç et al., 2015; Mehyar et al., 2014). Lactose has been used as a stabilizer and has shown a great effect over the encapsulated material (Gonçalves et al., 2013). Whey contains minerals as NaCl and CaCl, which have been used as stabilizer as well (Pescuma et al., 2015). According to Neira et al., (2018), phytase from A. niger has an optimum pH of 4.5-5.5. Whey pH is suitable for maintaining the phytase bioavailability. This study shows the potential of the use of whole whey.

**Liquid feed, dryer equipment and operating conditions**

Aimed at observing the condition in which whey with guar gum should be dried, a preliminary test was carried out and results obtained (Table 3). Whey without guar gum was well dried with a considerable yield, but when guar gum was added and dried, it was not possible to obtain microencapsulates. Glass transition temperature (Tg) could be defined as the temperature at which a material turns from a hard material to a glassy material and it must be considered when microencapsulation is used. When drying temperature is higher than Tg, a sticky solution will appear in spray cylinder. Nevertheless, it is possible to avoid this phenomenon, as mixing materials with high Tg could increase the protection and prevent the sticky solution formation. Also, since it was not possible to observe that in this material GG has a Tg of 120°C, (Mudgil et al., 2012), it was then necessary to decrease inlet temperature.

Taking these results into consideration, this experiment was performed by factorial design with one central point. Statistical analysis allows determining the better conditions to dry samples. From numbers 1 – 8, values

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**Table 2.** Elemental composition for whey compared with literature.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result (%)</th>
<th>Range (%)</th>
<th>Reported (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>L = 60.66 ± 0.2</td>
<td>-</td>
<td>55-70</td>
<td>Hernández-Rojas and Vélez-Ruiz (2014)</td>
</tr>
<tr>
<td>Fat</td>
<td>6.7 ± 0.02</td>
<td>6-8</td>
<td>-</td>
<td>Teniza (2008),</td>
</tr>
<tr>
<td>Total solids</td>
<td>8.6 ± 0.01</td>
<td>4-5</td>
<td>-</td>
<td>Poveda (2013)</td>
</tr>
<tr>
<td>Protein</td>
<td>1.73 ± 0.25</td>
<td>0.7 - 1.2</td>
<td>0.1 - 10</td>
<td>De Jesus and Maciel Filho (2014)</td>
</tr>
<tr>
<td>Ashes</td>
<td>0.63 ± 0.01</td>
<td>0.6 - 0.8</td>
<td>-</td>
<td>Gupta et al. (2014)</td>
</tr>
<tr>
<td>pH</td>
<td>4.9 ± 0</td>
<td>&lt;5.0</td>
<td>4.5 - 5.5</td>
<td>Neira (2017)</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.89 ± 0.005</td>
<td>4 - 5</td>
<td>5</td>
<td>Gupta et al. (2014)</td>
</tr>
</tbody>
</table>

**Table 3.** Preliminary test in which it was evaluated whey and guar gum at 150°C to observe the response at this temperature.

<table>
<thead>
<tr>
<th>Treatment (%)</th>
<th>Temperature/pump</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey/GG 0.0</td>
<td>150°C/30%</td>
<td>85% ±0.91</td>
</tr>
<tr>
<td>Whey/GG 0.25</td>
<td>150°C/20%</td>
<td>NY</td>
</tr>
<tr>
<td>Whey/GG 0.50</td>
<td>150°C/20%</td>
<td>NY</td>
</tr>
<tr>
<td>Whey/GG 1.0</td>
<td>150°C/20%</td>
<td>NY</td>
</tr>
</tbody>
</table>

NY: No yield.
from odd numbers treatments (ONT) are samples dried at 130°C, even numbers treatments (ENT) are samples dried at 110°C and central point treatments (CPT) is number nine (120°C). Results are shown in Figure 5.

**Humidity and water activity**

Humidity results are shown in Figure 5a. Microencapsulates presents low humidity no more than 3.5% and is related to the drying, which is one of the advantages of spray drying process. This value is important because higher humidity values could promote the growing of microorganism and deteriorate encapsulate (De Castro-Cislaghi et al., 2012). In the Figure 5b, it can be observed that the result of water activity (Aw) is an important data as it allows predicting possible changes in the product as Maillard reaction. Some authors described that lower values to 0.6 could promote a long-time storage availability (Beg and Gupta, 2003; Poveda, 2013). In this study, the values of all samples were below the reference. Outcomes obtained can be a favorable factor for preserving phytase due to growth of microorganisms when water activity exceeds 0.8, wherein fungus and bacteria tend to grow.

**Yield**

In the case of yield, treatment numbers 4, 7, 8 and 9 had a yield above 60% (Figure 5c). A yield above 60% represents an efficient drying. This could be due to the flow applied. Microencapsulation by means of spray drying of enzymes comes with great challenges due to some materials that must be dried at high temperatures to obtain higher yields. However, proteins like enzymes, cannot resist high temperature; hence it is necessary that low temperatures be used, and is the reason why higher yields are difficult to obtain. Some examples of yield are presented in Table 4.

There is a report on phytase microencapsulation in which the maximum yield obtained was 68%, different materials were tested and yield obtained was from 35 - 68%, with maltodextrin the most unsuitable material due to low yield at low temperatures (Spier and Greiner, 2015).
Table 4. Spray drying process of enzymes.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Carrier or binder</th>
<th>Temperature inlet/outlet (°C)</th>
<th>Yield (%)</th>
<th>Enzymatic activity (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylanase</td>
<td>Magnesium sulfate and malt extract</td>
<td>140/65.2</td>
<td>62.14</td>
<td>99.4</td>
<td>Gupta et al. (2014)</td>
</tr>
<tr>
<td>α-Amylase</td>
<td>None</td>
<td>145/90</td>
<td>Data not mentioned</td>
<td>83</td>
<td>De Jesus and Maciel Filho et al. (2014)</td>
</tr>
<tr>
<td>Lipase</td>
<td>Skim milk powder, Maltodextrin, Arabic gum</td>
<td>160/85</td>
<td>100</td>
<td>64</td>
<td>Souza et al. (2014)</td>
</tr>
<tr>
<td>β-galactosidase</td>
<td>Arabic gum</td>
<td>115/58</td>
<td>56</td>
<td>20%</td>
<td>Estevinho et al. (2014)</td>
</tr>
<tr>
<td>Phytase</td>
<td>Starch</td>
<td>100/68</td>
<td>63.1</td>
<td>41.2</td>
<td>Spier and Greiner (2015)</td>
</tr>
</tbody>
</table>

Table 5. Color assessment from factorial design treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>c*</th>
<th>h*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>71.95 ± 0.44</td>
<td>5.61 ± 0.12</td>
<td>18.16 ± 0.29</td>
<td>19.00 ± 0.30</td>
<td>72.83 ± 0.92</td>
</tr>
<tr>
<td>2</td>
<td>60.06 ± 0.68</td>
<td>7.57 ± 0.13</td>
<td>22.15 ± 1.03</td>
<td>23.40 ± 1.10</td>
<td>71.12 ± 0.34</td>
</tr>
<tr>
<td>3</td>
<td>71.64 ± 0.90</td>
<td>5.64 ± 0.60</td>
<td>17.97 ± 1.08</td>
<td>18.84 ± 1.49</td>
<td>72.58 ± 1.75</td>
</tr>
<tr>
<td>4</td>
<td>54.61 ± 0.67</td>
<td>8.47 ± 1.37</td>
<td>23.60 ± 0.81</td>
<td>25.08 ± 1.5</td>
<td>70.27 ± 1.55</td>
</tr>
<tr>
<td>5</td>
<td>74.27 ± 1.17</td>
<td>5.11 ± 1.29</td>
<td>16.66 ± 0.95</td>
<td>17.43 ± 1.75</td>
<td>72.97 ± 1.15</td>
</tr>
<tr>
<td>6</td>
<td>60.24 ± 0.91</td>
<td>7.21 ± 1.39</td>
<td>21.94 ± 0.65</td>
<td>23.10 ± 1.42</td>
<td>71.83 ± 1.68</td>
</tr>
<tr>
<td>7</td>
<td>74.52 ± 1.27</td>
<td>4.65 ± 1.25</td>
<td>15.15 ± 1.46</td>
<td>15.85 ± 0.85</td>
<td>72.91 ± 0.94</td>
</tr>
<tr>
<td>8</td>
<td>50.84 ± 1.12</td>
<td>8.00 ± 0.32</td>
<td>21.9 ± 1.54</td>
<td>23.32 ± 1.64</td>
<td>69.92 ± 1.30</td>
</tr>
<tr>
<td>9</td>
<td>72.65 ± 1.69</td>
<td>5.47 ± 0.57</td>
<td>16.28 ± 1.22</td>
<td>17.23 ± 0.69</td>
<td>71.54 ± 0.59</td>
</tr>
</tbody>
</table>

L* indicates light-ness (0 = black and 100 = white), a* and b* are coordinates for green (-a*)/red (+a*), and blue (-b*)/yellow (+b*). Hue angle (H* = tan⁻¹ b*/a*).

In this study, the Treatment 7 shows higher yield. Protein was measured, and results are shown in Figure 5d. High protein content was observed, which is normal considering that the majority of whey composition is protein. Besides, the protein was related with whey. The crude extract used is not purified; also, the presence of other enzymes could be the reason why this protein content is considerable. A. niger produces phytases, lipases, cellulases, xylanases, β-xylosidase, tannase, among others, however, the process used promote the production mostly of phytase, without the exclusion of other enzymes.

**Color assessment**

The color was also evaluated using the color values result shown in Table 5. L* value is scaled from 0-100, in which 0 is black color and 100 is white color. It was possible to obtain results from 50-70. Compared with what was observed in elemental composition, this value is similar to the one after spray drying. a* values tend from green (-) to red (+) while b* values tend from blue (-) to yellow (+), with results in a yellow range, according to (De Castro-Cislaghi et al. 2012), this color presented in the samples may be indicative of Mallard reaction due to the presence of lactose. Due to the purpose of this work which was to produce a highly drying process with high enzymatic activity, phytase activity was taken into consideration during statistical analysis (Figure 6).

Pareto chart shows that all factors and their interactions influenced phytase activity (Figure 7). Statistical analysis indicates the most suitable conditions for temperature as well as flow in low values, whereas for guar gum concentration, the higher values are better.

During drying, proteins are submitted to high shear forces while atomization is being carried out along with high-temperature air flow. These conditions result in an irreversible thermal denaturation. In this way, it was possible to set the better conditions, in which temperature is 110°C, 10% flow and guar gum 1%.

**Microencapsulates characterization**

**Morphology**

Figure 8 shows the microencapsulates micrographs, in which it is possible to observe the formation of aggregates and irregular shapes that are characteristic of...
Figure 6. Phytase activity remnant for microencapsulates of experimental design.

Figure 7. Pareto chart for phytase activity drying condition.

spray drying process. In Figure 8a and 8b, microencapsulates present soft surfaces and with the presence of pores; whereas in Figure 8c and 8d, the surface is smooth, but aggregates are in more compact form than microencapsulates at 130°C. This kind of shapes are typical of spray drying process (Favaro-Trindade et al., 2010). Rodriguez et al. (2005) commented that temperature and material are the reasons why these shapes are presented at low temperatures and carbohydrates, and agglomerates are promoted to be formed (Rodríguez-Huezo et al., 2007). This differences in surfaces, between microencapsulates dried at 110 and 130°C could be due to condition process or that the material make the formation of a tight matrix difficult owing to quick evaporation of water (Favaro-Trindade et al., 2010). However, Cunha et al., (2005) observed that GG tend to form microencapsulates with pores, which is because there is a cross-linking formed between hydrogen bonds and enzyme, which could protect the enzyme from being encapsulated (Cunha et al., 2005).

Currently, the reason why formation of aggregates is not well known may be that the phenomenon is influenced by different factors as earlier mentioned. The knowledge of this process could be useful for physicochemical explanation. However, Benavent et al. (2018) worked on a study in which he observed the formation of aggregates around some capsules. They
commented that the formation of aggregates is due to the interaction among materials used (Benavent-Gil et al., 2018; Cunha et al., 2005). These results could be compared with the explanation by Costa et al., (2002) who worked with starch and gelatin and observed the formation of aggregates as well. They attributed this to the presence of gelatin protein, which leads to this observation and pointed out that when starch without gelatin is dried, microencapsulates are more scattered but, when gelatin protein is added, the formation of aggregates are induced (Beirão-Da-Costa et al., 2011). This was mentioned previously by Zhao and Whistler (1994), who demonstrated that these aggregates are produced when suspensions are submitted to spray-drying if bonding agents are presented like polysaccharides or proteins at any temperature.

**Determination of weight loss by thermogravimetric analysis**

Thermal analysis demonstrated that for whey and whey with guar gum samples, the thermal behavior was similar; however, for the enzyme, extract and guar gum was completely different. Both samples suffered the first weight loss around 120-200°C, mostly water; whereas the second weight loss from 200 to 450°C was slow and could be related to thermal decomposition with the formation of volatile reaction products. The next mass loss takes place between 450 to 550°C, with a residue of 15%. Guar gum thermogram showed the first loss around 75 to 150°C, probably due to the amount of humidity. The second loss for guar gum is very fast, beginning from 290°C and ending at 320°C, and was attributed to
polymer decomposition. Gliko-Kabir (1999) reported values for this mass loss in a range of 280 to 310°C (with some differences of 10 to 30°C), in which lower temperature is required to cause 65% of weight loss (Gliko-Kabir et al., 1999). The last mass loss is observed from 330 to 500°C with a low residual of 5% which is due to guar gum composition that is mainly of organic compounds that are quickly lost and burns almost completely. For enzyme extract crude, it was possible to observe three mass losses: the first one related with water from 70 to 200°C; the second was less than the other samples and was from 200 to 500°C; and the third was from 500 to 600°C with a remnant of 20% which could be related to minerals and inorganic carbon presented in sample.

According to outcomes, it was possible to observe that the interaction of whey with guar gum improve the stability of guar gum without changing thermal behavior of whey. However, enzyme extract behavior is not improved or maintained, which may be due to the presence of minerals and residues of fermentation process, it is worth highlighting that enzyme extract was obtained by lyophilization after the recovery in solid state fermentation.

The thermal stability is important because in some cases during the process in which microencapsulates are applied, high temperatures are involved (Cunha et al., 2005). For this reason, the knowledge of thermal behavior is an important characteristic for materials used in biological process.

**X-Ray diffraction**

Diffractogram for this samples indicate that samples analyzed have amorphous structure (Figure 10). Guar gum diffractogram show a signal but this is not enough to affirm that guar gum has a crystalline structure. It is worth highlighting that when whey and enzymatic extract was added, the signal observed in guar gum decreased in microencapsulates; however there are not differences among samples with whey and whey/GG. Costa et al., (2011) found that when starch and material considered as semi-crystalline was examined with XRD it was possible to observe clear peaks; but when gelatin was added, the shape and intensity of some peaks undergo small changes for gelatin (Beirão-Da-Costa et al., 2011). Gelatin is mainly composed of fibrous protein by means of denaturation of collagen (Jellouli et al., 2011).

Protein content in gelatin could be different among materials, approximately from 8.8 - 12.8% (Silva et al., 2014); this protein is responsible for this decrease in shapes and intensity. Whey is formed by proteins, mainly β-lactoglobulin and α-lactalbumin (Ganju and Gogate,
García et al. (2017), and is the reason why the shape and intensity decreased. The interaction of whey protein and guar gum is reflected in the diffractogram. It was possible to observe the effect of protein over the semi-crystallinity structure of guar gum.

**Infrared**

It is possible to observe in IR spectrums (Figure 11) an absorption band at around 3450 to 3250 cm\(^{-1}\) for the O-H stretching vibration. At 2950 to 2940 cm\(^{-1}\), a signal was observed that corresponded to C-H stretching vibration. Then, guar gum structure (Figure 12) present a plane bending, which can be observed in 1423.55 cm\(^{-1}\), with the stretching vibration of alcoholic group of guar gum at 1249 cm\(^{-1}\) (Gliko-Kabir et al., 1999; Zhao and Whistler, 1994). These peaks are found in GG and whey/GG, through which it is possible to confirm the presence of GG in microencapsulates.

Signal for proteins are from 1800 to 800 cm\(^{-1}\). Ven et al. (2002) characterized casein and whey and found that...
both samples present same signal around 1700 to 1485 cm\(^{-1}\), values that may be related with proteins (casein and whey proteins) (Van der Ven et al., 2002). Forato et al., (1998) investigated the IR of proteins, in which they found that the secondary structure is preserved by means of the observation of amides (A, I, II and III) (Forato et al., 1998). Chanphai (2017) observed the amide I around 1660–1650 cm\(^{-1}\) and the amide II at 1550–1530 cm\(^{-1}\) (Chanphai and Tajmir-Riahi, 2017). These signals are presented in IR spectra, specifically in enzyme extract, which could be due to enzymes in the extract. There are a few reports of whey protein, due to there being a great variety of proteins, and every protein is characterized by a specific structure.

Storage availability

In Figure 13, the graphics for storage assessment are presented. Continuous lines belong to Eppendorf containers, while discontinued lines belong to Ziploc bags. It is possible to observe that for both containers a similar behavior was observed at each temperature. For 4°C (blue lines), microencapsulates retain 80% of remnant activity after 8 weeks, while for 25°C temperature (orange lines), only around 75% is retained for both
systems. Some authors have reported phytases shelf life of 14 to 20 days at 4°C (Mudgil et al., 2012; Sweeti and Sawarkar, 2015; Van der Ven et al., 2002). Spier et al., (2015) preserves phytase testing of different materials. Microencapsulates with Na-alginate retained 52% at room temperature for 60 days. Outcomes of the present investigation were better as the use of whey and guar gum is a promising alternative as protective material for phytase. It was expected that for 35°C, the loss of enzymatic activity was less than the other temperatures; however, it was possible to observe 55% of remnant activity.

Color assessment during storage analysis

In Figure 14, the color analysis in which continuous lines belong to Eppendorf containers, whereas discontinued lines belong to Ziploc bags is shown. Microencapsulates in Eppendorf container does not present a color variety, as for three temperatures evaluated, the color is kept as in the beginning. Different results are presented in Ziploc bags for 4°C as there is no difference with Eppendorf container; but for 25 and 35°C, color tend to change slowly from white to light yellow (Figure 15). The change in color is attributed mainly to Maillard reactions. At low temperatures, Maillard reactions are not promoted, while at other temperature (like 25°C room temperature and 35°C), Maillard reaction appear. This is owing to the lactose present in whey (Van Boekel, 1998). It is noted that whey was used completely without separating its components. Some of the major consequences of this reaction includes agglomeration of proteins causing inactivation and browning process (Zin El-Din and Aoki, 1993). The material properties, like permeability may be the reason why the color changes. Results shown in this work demonstrated that although color is different between both containers, phytase activity is not affected by this phenomenon. For this reason, there is no difference in which containers phytase is stored. However, there are other factors that could affect the stability of phytase microencapsulates as permeability. Ziploc bags are made of low density polyethylene (LDPE), a material that is very common in packing, but does not prevent permeability against humidity which could affect stability and appearance of microencapsulates. Furthermore, Eppendorf containers are made of polypropylene (PP), which is a material more high than LDPE and PP is impermeable to humidity (Huanchi, 2013).

Linearization of stability data and determination of $k_i$ and $t_{1/2}$

When data is linearized, it is possible to observe reaction of first order, in which concentration is decreasing with time. Once this data is obtained, $k_i$ is calculated, showing low values and, when shelf time was calculated ($t_{1/2}$), the number of weeks in which stability will be preserved was determined. These outcomes are presented in Table 6. $k_i$ values are a little higher than those obtained by Spier et al., (2015) in which $k_i$ of 0.0052 day$^{-1}$ was calculated with a half-life of 132.10 days (18.9 weeks). Although values are in days, it is possible to observe that results presented in this work with whey and guar gum could be used on industrial process as carrier for spray drying.

In Table 7, the currently commercialized phytases is presented. It is possible to observe that more superior products with higher shelf-time can be obtained based on
this study. Nevertheless, there are other enzymes with similar values and at the same temperature, which make it competitive against those currently sold.

Digestibility and bioavailability assessment

Figure 16 shows the results for digestibility evaluation. Digestibility is the amount of food that is available to be absorbed; this factor is very important due to gastrointestinal condition that could affect the food or product, preventing the bioavailability of the compound.

In mouth phase, for both samples (microencapsulates and microencapsulates with food), a low decrease in phytase activity of around 97% was observed; nevertheless, this activity is retained in stomach phase at low pH. At the end of stomach phase, phytase activity decreased to 95%. After this phase, a gradual decrease until end of intestinal phase could be observed. Around 90% of enzymatic activity is preserved after digestion process. It is worth highlighting that digestive simulation was for monogastric animals like poultry. It is well known that phytases in presence of proteases will be inactivated (Neira et al., 2018); for this reason, evaluation of phytases

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Table 6. Values for $R^2$, $k$, and $t_{1/2}$ for storage stability assessment.

<table>
<thead>
<tr>
<th>$T \degree C$</th>
<th>Eppendorf</th>
<th>B. Ziploc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>$k_{1/2}$</td>
</tr>
<tr>
<td>4</td>
<td>0.9342</td>
<td>0.0258</td>
</tr>
<tr>
<td>25</td>
<td>0.9690</td>
<td>0.0389</td>
</tr>
<tr>
<td>35</td>
<td>0.9714</td>
<td>0.0643</td>
</tr>
</tbody>
</table>

Table 7. Commercialized phytases in powder presentation.

<table>
<thead>
<tr>
<th>Study</th>
<th>Time (weeks)</th>
<th>Temperature</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probioway$^a$</td>
<td>52</td>
<td>Room temperature</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Yonkong$^b$</td>
<td>26</td>
<td>4$\degree$ C</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Habio$^c$</td>
<td>26</td>
<td>4$\degree$ C in absence of light and humidity</td>
<td>Light yellow</td>
</tr>
<tr>
<td>This work</td>
<td>27</td>
<td>4$\degree$ C</td>
<td>Light yellow</td>
</tr>
</tbody>
</table>

---

in gastro-intestinal system was not considered. Menezes et al. (2015) evaluated seven commercial phytases in an in vitro simulation of poultry digestive tract. Six phytases evaluated were in liquid presentation, with only one of them in powder presentation. This last one, after the assessment, presented just 47% of its initial activity. They attributed these results to the susceptibility of phytases to proteolytic cleavage by pepsin (Menezes-Blackburn et al., 2015). Currently, GG has been used to delay the releasing of drugs in pharmacy industry with good results (Patel et al., 2014). It has been demonstrated that GG can be used to carry drugs until colon releases the drug with higher availability (Spier et al., 2015). Li et al. (2011) observed that whey and guar gum at different pH retain the structure due to the uneasily dissociated shells formed by hydrogen bonding and Van der Waals force. Pepsin can accelerate the releasing of the compound encapsulated by means of enzymolysis. They observed that the addition of polysaccharides which are absorbed on the surface of microencapsulates forms a barrier that blocks the penetration of pepsin preventing the dissociation of microcapsules (Li et al., 2011).

It has been found that whey protein with gums can generate many lipid–water bonds in other systems (Li et al., 2011) allowing the releasing of compounds microencapsulated in intestinal phase.

**Conclusion**

It was possible to set conditions to dry proteins like phytases with great enzymatic activity after drying. Besides, materials used provide protection in gastro-intestinal conditions. As seen from results, it is possible to use phytase solution with some sugars and polyols, making it a stable product. Outcomes demonstrated that glycerol and maltose is a great and promising cryopreservation method, besides being a reducer of aqueous activity in molecular level and in this way, prevent protein denaturalization. This study demonstrated the use of whey as a material for microencapsulation because of its composition. Currently, whey protein isolated (WPI) has been used as carrier in spray drying; however, the purification process to obtain WPI causes waste which continue to serve as an environmental pollutant. This work proposes the use of whey without purification. Results demonstrated that it is possible to combine whey with other materials as guar gum. Microencapsulation with guar gum and whey is an alternative for improving stabilization of enzymes due to the ability to protect enzyme and obtain higher yields.

Overall, it was possible to observe interactions between whey and guar gum. Functional groups of guar gum and proteins was seen in IR, which affirms the possibility of using guar gum as carrier and retain its identity together with whey protein. Characterization indicated that the microencapsulation of phytases with guar gum and whey is possible and produces higher results than other authors. Besides, storage availability assessment demonstrates the potential to obtain a product that can compete with those currently commercialized; however, the evaluation of materials or even minerals which help to improve stability is necessary. It was possible to affirm that color, promoted by Maillard reaction, do not affect

![Figure 16. Remnant activities for microencapsulates and microencapsulates with food in a gastrointestinal system in vitro.](image-url)
enzymatic activity. Digestive evaluation in vitro showed that whey with guar gum can preserve phytases against condition of gastro-intestinal system. These results have important contributions for the microencapsulation process of enzymes, which continues to present serious challenges to the enzymes. The effect of whey and guar gum was examined and the bioavailability of phytases is improved in order to promote the hydrolysis of phytate on intestinal phase, allowing the absorption of native phosphorus in cereals. These contributions may be reflected in environmental aspects, due to phytate causingeutrophication; nevertheless, the study of how microencapsulates are being released in vivo is necessary for future studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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