Influence of Different Encapsulating Materials on the Viability of *Pediococcus Pentosaceus* P107 Microencapsulated by Spray Drying

Fernanda Weber Bordini
UNICAMP: Universidade Estadual de Campinas
Michele Dutra Rosolen
UFPEL: Universidade Federal de Pelotas
Gabriela de Quadros da Luz
UFPEL: Universidade Federal de Pelotas
Patrícia Diaz de Oliveira
UFPEL: Universidade Federal de Pelotas
Fabrício Rochedo Conceição
UFPEL: Universidade Federal de Pelotas
Ângela Maria Fiorentini
UFPEL: Universidade Federal de Pelotas
Wladimir Padilha da Silva
UFPEL: Universidade Federal de Pelotas
Simone Pieniz (nutrisimone@yahoo.com.br)
Federal University of Pelotas: Universidade Federal de Pelotas https://orcid.org/0000-0002-7403-1577

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Abstract

This study evaluated the influence of whey isolated, and associated with pectin and xanthan gum as encapsulating agents for *Pediococcus pentosaceus* P107, by *spray drying*. In the storage test at temperatures of -20°C and 4°C the most viable microcapsule was WP (whey and pectin), but WX (whey and xanthan) presented better stability at 25°C, WX did not present itselft stable to ensure probiotic potential (< 6 Log CFU.mL^{-1}) for 110 days. In the passage through the simulated gastrointestinal tract, the WX microcapsule stood out in all tested conditions, presenting high cellular viability. For the thermal resistance test, WP was shown to be efficient in the protection of *P. pentosaceus* P107 cells. The three microcapsules produced were able to protect the cellular viability of the microorganisms, as well as the drying parameters were suitable for both microencapsulation.

Highlights

- Whey, pectin and xanthan were effective in protecting *P. pentosaceus* P107 from adverse conditions.
- All materials combined with spray drying technique offered high encapsulation efficiency encapsulation.
- Protection results show potential for industrial application

1. Introduction

In several fermented products and in the mammal's microbiota, *Pediococcus pentosaceus* - a bacterium belonging to the group of Lactic Acid Bacteria (BAL) - is detected [1]. It is used as a starter culture in fermentation, mainly of vegetable products and canned meats, increasing their conservation as well as having technological properties for the production of metabolites (pediocins) and for uses, such as a probiotic in food [1, 2].

Studies demonstrated that the probiotic potential of *Pediococcus spp.*, reaveling it´s ability to adhere to epithelial cells, and resistance to both gastric and intestinal passage [2, 3]. Nevertheless, this viability can be compromised during storage, food processing, or even by the passage through the gastrointestinal tract itself [4, 5]. For food products to be effective as probiotics, they depend on the number of viable microorganism cells in the product at the time of consumption, with at least 6–7 log of UFC.g^{-1} or mL^{-1} recommended [6].

In order to guarantee workability and offer protection to cells during storage, microencapsulation was sought, being a process that enables "packaging" of the object of protection, thus guarding it from undesirable adverse conditions [7, 8]. The *spray drying* technique is one of the most used on microencapsulation of microorganisms, due to its low cost and high efficiency [9].

For this process to take place, matrices containing different materials are used, for instance, polysaccharides (gums and maltodextrins), proteins (caseins, gelatin and albumins) and lipids (mono

and diglycerides) [10]. Among the materials, whey has been on the spotlight in recent years, due to its high nutritional capacity, containing proteins of high value (albumin) and low cost in parallel to other noble materials [11].

Another encapsulating material used is pectin, a homopolysaccharide, abundant in the cell wall of fruits. Used by the food industry as an additive, gelling and thickener instrument and on the pharmaceutical industry as coating for medicines. Such characteristics, along side with it´s insolubility in the digestive tract can assist in the protection of microorganisms [9, 12]. Beyond that, it´s fibrous property is appreciated from a dietetic point of view, contributing to consumer´s welfare [13].

Xanthan gum, on the other hand was also used as a material, extracellular heteropolysaccharide, produced by strains of *Xanthomonas*, and is considered a biopolymer for industrial use. It is a gum with high thickening power and viscosity can be relative, according to manipulation parameters, whereby temperature and pH [14]. Generally, it has high viscosity in low concentrations, which is why it is used in the food industry [15]. It´s pseudo-plastic capacity along side resistance to temperature and to some enzymes are positive factors to the microencapsulation (Argin et al.,2014).

The aim of the present study was to microencapsulate and characterize why microcapsules isolated or combined with pectin and xanthan by spray drying in the protection of *Pediococcus pentosaceus* P107 native from adverse conditions. For this the efficiency and yield of the drying process, viability of *P. pentosaceus* P107 under storage conditions 180 days at the temperature of -20, 4 e 25°C, as well as survival to the gastrointestinal tract, thermal resistance and morphology of the microcapsule.

2. Material And Methods

2.1 Microorganism and culture conditions

The *Pediococcus pentosaceus* P107 (*P. pentosaceus* P107) – was isolated of the baked sliced ham, identified and cataloged in previous studies, belonging to the Collection of probiotic bacterias and starter cultures of the Food Microbiology Laboratory - Department of Food Science and Technology (DCTA) / Federal University of Pelotas (UFPel) - For high-density cell culture, their multiplication was performed in two stages, as described by Rosolen et al., (2019), whey powder being used in both, with an average composition of 11% proteins, 1.5% fats, 6% mineral salts and 3% humidity, according to the manufacturer (Relat - Estação, RS, Brazil), being reconstituted in sterile distilled water at 6% (mv^{-1}) and after that, undergone heat treatment (65 ºC for 30 min).

At the end of the process, cell suspension was obtained at a concentration of approximately 14 Log CFU / mL^{-1} of P. *pentosaceus* P107, kept under refrigeration at 4 ºC.

2.2 Microencapsulation by spray drying

Whey used was isolated and associated with either pectin or xanthan gum, the encapsulating materials used in the production of the microcapsules. Aaerosil, the anti-caking agent (Pharma-chemistry; Malaga, Spain) was added to all prepared solutions at a concentration of 1.25%.

The prepared solutions were: (W): whey (6%) dissolved in sterile distilled water; (WX): whey (6%) and xanthan pruni synthesized by strain 101 of *Xanthomonas arboricola* pv pruni (1.25%) and produced at the Bioprocess Technology Laboratory (CDTec / UFPel), previously (24 h) dissolved in water sterilized at 100°C; (WP): whey (4%) and commercial pectin (Pharma-chemistry; Malaga, Spain) (2%) dissolved in sterile distilled water at 100°C. All solutions were subjected to heat treatment for 30 min at 65°C and kept under agitation in a orbital shaker.

After the cell suspension, P. *pentosaceus* P107 (14 Log CFU.mL^{-1}) was added to the respective encapsulating solutions and kept under agitation in a magnetic stirrer at 150 rpm at room temperature until the moment of application of the spray drying. All solutions were subjected to the drying process using *spray dryer* (LabMaq – MSDi 1.0, São Paulo, SP, Brasil). The parameters were 100 ºC outset, and 68 ºC ending, the feed flux, 0.25 L h^{-1} and 3.00 m^3 min^{-1} air flow. The final dry product was collected in sterile flasks and stored at different storage temperatures, -20°C, 4°C and 25°C.

2.3 Characterization of the microcapsules

The morphology of the microcapsules was determined in seven days of housing at -20 ºC. For this, samples were fixed with a double-sided carbon tape, spray coated with gold (Leica, model EM SCD 500, Wetzlar, Germany) and scanned by a electron microscopy (SEM) Jeol®, model JSM – 6610LV (Jeol®, Tokyo, Japan), in high vacuum mode to examine the morphology of the surface. The average particle size was determined with the aid of the Image J software, starting on 50 measurements.

Water activity (aw) was measured by a direct method, at 25°C, using the Labtouch-aW analyzer (Novasina, Neuheimstrasse, Switzerland).

The moisture present in the microcapsule was determined gravimetrically by oven drying, according to the guidelines of the Association of Official Analytical Chemists (AOAC, 2012), according to method No. 990.20.

Color analyses were determined using a colorimeter (Minolta Chroma Meter CR-300, Osaka, Japan), with a CIE 'L * a * b *' reading system, proposed by the Commission Internationale de l'Eclairage (CIE).

2.4 Microcapsule rupture test

To determine the disruption of the microcapsules, two solutions were used: Solution A: containing PBS buffer (100 mM, pH 7.4) and solution B: 0.5% saline solution (mv^{-1}) pH 2.5 containing 3 mg.mL^{-1} of pepsin in the times of 30, 60 and 120 min. For all microcapsules 0.01g of the powder was suspended again in 1 mL of both solutions (A and B), shaken in a vortex and maintained in an orbital shaker at 37 ºC, 150 rpm.

Viable cell count was determined by means of serial dilution in 0.15% peptone water (m v^{-1}) and rates were plated using the drop plate technique on MRS agar, incubated at 37 ºC for 48 h under anaerobic conditions and the concentration of viable cells was expressed in Log CFU.g^{-1}.

2.5 Encapsulation efficiency (EE) and process yield

To determine the encapsulation efficiency (EE), Eq. 1 was used described by Annan et al. [17] and presented as a percentage:

$$EE\ (\%) = \left(\frac{N}{N_0}\right) x\ 100 \quad (1)$$

Where N is the number of viable cells after the process and N0 is the number of viable cells before the process.

The yield was expressed as a percentage proposed by Gonsalvez et al. [18], calculated according to Eq. 2:

$$yield(\%) = \frac{final\ mass\ of\ the\ project}{theoretical\ initial\ mass}\ x\ 100 \quad (2)$$

2.6 Viability of *Pediococcus pentosaceus* P107 microencapsulated cells for storage at different temperatures

The viability of microcapsules containing P. *pentosaceus* P107 were carried out during 180 days of storage, in three different temperatures, -20°C ± 1°C (freezing), 4°C ± 1°C (refrigeration) and 25°C ± 1°C (ambient). To disrupt the WP and WX microcapsules, 0.01 g of powder was dispersed in 1 mL of 0.5% saline solution (mv^{-1}) pH 2.5, containing 3 mg.mL^{-1} of pepsin and kept in an orbital shaker at 150 rpm at 37 ° C for 30 min, according to Rosolen et al. (2019), with modifications. For microcapsule W, 0.01 g of the powder was suspended in 1 ml of PBS buffer (100 mM, pH 7.4) and kept in an orbital shaker at 150 rpm at 37°C for 30 min, according to Eckert et al. [11] with modifications. The viable cell count was determined according to item 2.4.

2.7 Survival of microencapsulated cells exposed to *in vitro* simulated gastrointestinal conditions

Prior to seven days of storage at -20°C, microcapsules were subjected to 60 min of exposure to gastric fluids and 240 min to simulated intestinal fluids. The test was performed according to the method proposed by Rosolen et al. [15]. For this, 0.1 g of the microcapsules were exposed to 1 mL of simulated gastric juice, containing 3.0 mg.mL^{-1} of pepsin and 0.5% saline solution (m.v^{-1}), at pH 2.0, 2.5 and 3.0. For the simulated intestinal juice, the solution was prepared with 1.0 mg mL^{-1} of pancreatin and 0.5% saline (m.v^{-1}), at pH 8.0, with or without 0.5% bovine bile. Both were sterilized by filtration through 0.22

µm membranes (Sartorius Stedim Biotech, GmbH, Goettingen, Germany). Rates were collected and the viable cell count was performed according to item 2.4.

2.8 Thermal resistance

The thermal resistance was performed according to Ilha et al. [19] with modifications. Using 1 g of microcapsules in 9 mL of 0.1% peptone water (mv^{-1}) in test tubes and subjected to conditions of 65 ºC for 30 min and 72 ºC for 15 sec in a thermostatic bath (QUIMIS, Brazil), followed by an immersion ice bath for 10 min. After that, rates were collected and counts were performed, as described in item 2.4.

2.9 Statistical analysis

The data was submitted to variance analysis (ANOVA) using the Graph pad Prism 7 program followed by the Tukey's test to compare the averages at a significance level of 95% ($p < 0.05$). The analyses were performed in triple, and the results of the microencapsulation were calculated with the averages of three independent experiments.

3. Results And Discussion

3.1 Characterization of microcapsules of *Pediococcus pentosaceus* P107

As for the moisture content for probiotic cultures must be constant and below 5%, in order to obtain stability in long-term storage [19, 20]. In the present study, the microcapsules showed humidity values of 0.86% (± 0.01), 1.93% (± 0.02), and 3.40% (± 0.01) for W, WX and WP respectively, being considered adequate according to the ideal value mentioned above.The different values can be attributed to differences in the wall materials of each microcapsule and to the total content of encapsulating material, since it facilitates the formation of more compact walls, which may restrict the diffusion of water vapor from the interior of the microcapsule to the surface during spray drying [21].

In literature, a value ratio above the recommended 5% or 5 g $100g^{-1}$ was observed. Fávaro-Trindade & Grosso [22] found moisture content between 3.2 and 5.3 g $100g^{-1}$ in the powders obtained from the microencapsulation of *Lactobaillus acidophylus* La-05 and *Bifidobacterium lactis* Bb-2 spray dried. Likewise, Rajam & Anandharamakrishnan [21], observed values that ranged from 5.52 to 9.47%, in microcapsules containing fructooligosaccharides or whey proteins [21]. Determining points such as equipment parameters and choice of wall material must be considered [23, 24].

The water activity values of the microcapsules obtained in this study, 0.233 ± 0.01, 0.244 ± 0.01 and 0.265 ± 0.02, respectively, are within the normal range (less than 0.6) for atomized product and the minimum required to maintain cell viability [25]. The literature reported that the water activity values of less than 0.3 increases the stability of the dried product, due to the lower amount of free water available for biochemical reactions, ie, longer life [11, 25, 26].

As for the color attribute, all microcapsules showed high values for L *, 95.65, 94.24 and 96.12 respectively for W, WX and WP, indicating light colors. Negative values for a *, -1.67, -0.56 and − 0.24 respectively for W, WX and WP, indicating shades of green and finally b * positive values, 5.68, 7.19 and 4.82 also respectively for W, WX and WP, indicating trend in yellow. These parameters can be assigned to the predominant use of whey for the three solutions produced microcapsules, since it has a light yellow color, explaining the tendency of microcapsules to yellow [11, 27]. The final color may impact the food matrix to be applied, white pattern microcapsules have convenient features for applications in different formulations [28].

Figure 1 shows Scanning Electron Microscopy images (SEM) of the microcapsules W, containing whey (ab), WX, containing xanthan and whey (bc), WP, containing whey and pectin (a). The microcapsules containing whey (W), and whey with xanthan gum (WX) are symmetrical and rounded with average sizes of 12.32 µm ± 0.80, 9.87 ± 0.76, respectively. In contrast, microcapsules with whey and pectin (WP) had an average size of 6.99 ± 0.67 µm and some deformations on it´s surface, which may be due to the interaction of the material with the high pressure exerted by spraying and to shrinkage during the process and cooling [29].

Considering the size of the microcapsules obtained, they are adequate (up to 80 µm) to be inserted in foods because small particles ensure a homogeneous and high quality product without affecting the sensory properties [9]. Rosolen et al. [15] observed midst values of 12.73 µm for L. *lactis* R7 microcapsules as whey and inulin. Otherwise, Nunes et al. [4] obtained microcapsules *Lactobacillus acidophilus* with inulin, trehalose, and Hi-maize by spraying, having sizes between 6.68 µm and 7.30 µm

3.1 Microcapsule rupture test, viability, encapsulation efficiency (EE) and process yield

Table 1 shows the plug effect of 100 mM PBS (pH 7.4) and the saline solution (0.5% pH. 2.5 with 3 pepsin $mg.mL^{-1}$), referred to from now on as PBS buffer and gastric solution, respectively, about the disruption of the microcapsules studied.

Table 1
Release of bacterial cells from microcapsules when immersed into the PBS buffer or simulated gastric fluids with various exposure times at 37 ° C.

Time (min)	PBS buffer 100 mM pH 7.4	Gastric Solution (saline solution 0.5% pH 2.5 and pepsin 3 mg.mL^{-1})
	Log CFU g^{-1}	
	W WX WP W WX WP	
30	12.85 ± 0.34^{a,A} 7.09 ± 0.13^{c,B} 0.00 ± 0.00^{a,B}	8.92 ± 0.07^{a,B} 12.99 ± 0.20^{a,A} 13.63 ± 0.15^{a,A}
60	12.69 ± 0.10^{a,A} 8.15 ± 0.15^{b,B} 0.00 ± 0.00^{a,B}	8.76 ± 0.26^{a,B} 11.58 ± 0.51^{b,A} 9.15 ± 0.15^{b,A}
120	11.87 ± 0.67^{a,A} 9.15 ± 0.15^{a,B} 0.00 ± 0.00^{a,B}	8.44 ± 0.39^{a,B} 11.15 ± 0.15^{b,A} 9.00 ± 0.10^{b,A}
W: microcapsule whey;		
WX: microcapsule whey and xanthan;		
WP: microcapsule whey and pectin;		
Results represent the mean (standard deviations), n = 3.		
$^{a-c}$ Different superscript lowercase letters in the same column represent statistical difference ($p < 0.05$).		
Means with different superscript capital letters represent a defined difference ($p < 0.05$) between the solutions tested for the same microcapsule.		

The PBS plug at a concentration of 100 mM has been described in the literature as a microcapsule tear solution [4, 11, 26, 30]. Conforming these results, the whey (W) microcapsule of the present study, when exposed to the PBS plug over 30 min time showed a viable cell concentration of 12.85 Log CFU g^{-1}. In contrast, the same plug promoted partial disruption of microcapsules in WX and was not able to rupture the microcapsules WP and cause total exposure of the microorganism.

When the gastric solution was analyzed, higher cell concentrations were obtained at 30 min for WX (12.99 Log CFU.g^{-1}) and WP (13.63 Log CFU.g^{-1}), reducing significantly ($p < 0.05$) the cell count depending on exposure time. It is noteworthy that both pectin as xanthan thickeners have properties that are strongly influenced by acidic pH and by the temperature of 37 ° C, compromising the structure of the microcapsule [16, 31].

Understanding the tearing process of microcapsules and delivery of its content is complex, as it involves several factors. There are few reports in the literature which explore the tearing properties of encapsulated

bacteria, being some of the mechanisms described diffusion, erosion and fragmentation. In addition, factors such as changes in pH, activity of proteolytic enzymes, osmotic stress and time staying in a tearing solution, contribute to the process [32].

Whey proteins when bound to pectin polysaccharides form complexes through non-covalent interactions, as electrostatic forces in both solution and interfaces, hydrogen bonding, and hydrophobic interactions under various conditions. Xanthan, when in acid solution (pH 3.0) is negatively charged while whey proteins are positive, when this repulsive electrostatic force cancels out, the particles coated with both materials quickly separate, causing ruptures of the microcapsule and exposing the microorganism [33].

The initial viable cell count for W capsules, WX and WP was 13.50 Log CFU.g^{-1}, 13.67 Log CFU.g^{-1} and 13.76 Log CFU.g^{-1}, respectively. After the drying process the microcapsules W and WX reduced 1.05 Log CFU.g^{-1} and 1.28 Log CFU.g^{-1}, respectively, showing no significant difference ($p > 0.05$). The WP microcapsules obtained the lowest cell reduction (0.13 Log CFU.g^{-1}) showing the highest encapsulation efficiency of 99.05% in relation to the others, as shown in Table 2.

Table 2
Cell viability, encapsulation efficiency and yield of the process of *Pediococcus pentosaceus* P107 microencapsulated using different materials before and after spray drying.

	Number of viable cells (Log CFU.g^{-1})		**Reduction (Log CFU.g^{-1})**	**Encapsulation Efficiency (EE%)**	**Yield (%)**
	Before *spray drying*	After *spray drying*			
W	13.50 ± 0.20	12.45 ± 0.71	1.05 ± 0.21^{a}	92.22 ± 0.50^{b}	17.37 ± 1.05^{a}
WX	13.67 ± 0.50	12.39 ± 0.80	1.28 ± 0.30^{a}	90.63 ± 0.25^{c}	9.58 ± 0.98^{b}
WP	13.76 ± 0.22	13.63 ± 0.50	0.13 ± 0.28^{b}	99.05 ± 0.20^{a}	15.23 ± 0.67^{a}
W: microcapsule whey;					
WX: microcapsule whey and xanthan;					
WP: microcapsule whey and pectin;					
Results represent the mean (standard deviations), n = 3.					
$^{a-c}$Means with different superscript lowercase letters in the same column represent statistical difference ($p < 0.05$).					

The combination of whey and pectin culminated in the best encapsulation efficiency, which can be explained in the fact that this polysaccharide a nano-porous polymer, (2–50 nm) allowing only water and smaller particles to diffuse into the produced microcapsules. *P. pentosaceus* P107 has an average size ranging from 2–8 μm, making it comparatively larger than the pores of the complex used, allowing the retention of a large number of bacteria inside, when compared to other encapsulating materials [34].

Whey proteins have the ability to interact with glycoproteins present on the bacterial surface, making them a biocompatible material due to the adhesive potential in the protection of the microorganism. When mixed solutions of whey proteins and xanthan polysaccharides are heated there is competition between the gelling and separation processes of phases. Once gelling occurs the basic structure of the gel is established and therefore the separation of phases is delayed, which contributes to the formation of a continuous and stable microcapsule to the drying process [35, 36].

Considering the yield of the process is influenced by drying parameters, added to environmental and physical-chemical properties of the encapsulating material, the yield obtained in the present study for W was 17.37%, 9.58% for WX and 15.23% for WP, with W and WP being significantly larger. Much of the work brings the yield factor as process yield, ie, the efficiency of the same. Being comparative studies only a few, Arslan-Tontul & Erbas [37] found that yields ranged from 39.33 g to 54.65 g to 100g when microencapsulating *Saccharomyces cerevisiae* var: *Boulardii* with different wall materials by spraying.

3.2 Feasibility of storage of microencapsulated cells of P. pentosaceus P107 at different temperature

In this study, the microcapsules were evaluated by observing their ability to maintain the viability of P. *pentosaceus* P107 during storage at different temperatures (-20, 4 and 25°C) (Table 3).

Table 3
Viability of *Pediococcus pentosaceus* P107 microencapsulated for 180 days.

***Temperature**									
Time	-20°C			4°C			25°C		
(days)	W	WX	WP	W	WX	WP	W	WX	WP
0	12.45 ± 0.71^{a}	12.39 ± 1.00^{a}	13.63 ± 0.50^{a}	12.45 ± 0.71^{a}	12.39 ± 1.00^{a}	13.63 ± 0.50^{a}	12.45 ± 0.71^{a}	12.39 ± 1.00^{a}	13.63 ± 0.50^{a}
7	10.95 ± 0.04^{b}	10.40 ± 0.80^{b}	13.54 ± 0.06^{a}	10.00 ± 0.00^{b}	9.95 ± 0.42^{b}	13.54 ± 0.06^{a}	11.25 ± 0.24^{b}	8.16 ± 0.15^{c}	13.39 ± 0.08^{a}
14	9.15 ± 0.15^{c}	10.58 ± 0.51^{b}	13.00 ± 0.00^{a}	8.80 ± 0.06^{b}	9.58 ± 0.51^{b}	13.51 ± 0.20^{a}	7.90 ± 0.05^{b}	7.16 ± 0.15^{c}	13.25 ± 0.24^{a}
21	8.52 ± 0.60^{c}	10.00 ± 0.00^{b}	12.69 ± 0.35^{a}	8.58 ± 0.51^{c}	9.99 ± 0.61^{b}	12.00 ± 0.00^{a}	7.62 ± 0.15^{b}	VC	13.15 ± 0.15^{a}
30	8.00 ± 0.00^{c}	9.88 ± 0.51^{b}	12.58 ± 0.51^{a}	8.58 ± 0.55^{b}	9.89 ± 0.78^{b}	12.00 ± 0.03^{a}	7.60 ± 0.00^{b}	VC	13.00 ± 0.12^{a}
45	7.15 ± 0.15^{b}	10.00 ± 0.01^{a}	8.88 ± 0.78^{a}	7.47 ± 0.02^{c}	9.00 ± 0.22^{b}	11.59 ± 0.11^{a}	7.56 ± 0.24^{b}	VC	10.69 ± 0.35^{a}
60	7.77 ± 0.01^{b}	9.58 ± 0.51^{a}	8.15 ± 0.15^{b}	7.54 ± 0.06^{b}	8.72 ± 0.12^{a}	7.69 ± 0.35^{b}	7.54 ± 0.06^{a}	VC	6.44 ± 0.39^{b}
75	7.44 ± 0.39^{a}	8.12 ± 0.11^{b}	7.95 ± 0.04^{a,b}	7.44 ± 0.39^{a}	8.00 ± 0.00^{a}	7.88 ± 0.78^{a}	7.47 ± 0.00^{a}	VC	VC
110	7.84 ± 0.01^{a}	7.85 ± 0.15^{a}	7.77 ± 0.68^{a}	7.59 ± 0.27^{a}	7.58 ± 0.51^{a}	7.85 ± 0.15^{a}	7.47 ± 0.02^{a}	VC	VC
140	7.84 ± 0.06^{a}	7.54 ± 0.06^{b}	7.74 ± 0.17^{a,b}	7.46 ± 0.15^{a}	7.52 ± 0.04^{a}	7.52 ± 0.04^{a}	7.18 ± 0.14^{a}		VC
180	7.58 ± 0.21^{a}	7.27 ± 0.02^{a}	7.59 ± 0.11^{a}	7.15 ± 0.15^{a}	7.27 ± 0.02^{a}	7.15 ± 0.15^{a}	6.13 ± 0.39^{a}		

*Storage at freezing: -20°C; refrigeration: 4°C, and ambient temperatures: 25°C.

$^{a-c}$ Means ± standard deviation with different superscript capital letters indicate significant difference ($p < 0.05$) between microcapsules in the same period and storage temperature.

VC = Viable cells < 6 log CFU.g^{-1};

W: microcapsule with only whey;

WX: microcapsule with whey and xanthan;

WP: microcapsule with whey and pectin;

The Cell viability presented was > 6 log CFU.g^{-1} in W microcapsules, at the end of 180 days for the three storage temperatures. Similar results were shown by the WX microcapsules (7.27, 7.27 log CFU.g^{-1}) and WP (7.59, 7.15 log CFU.g^{-1}) but at temperatures of -20 ° C and 4 ° C, respectively. Likewise, Liao et al. [38] evaluated the feasibility to storage at temperatures of -20°C, 4°C, 25°C and 37°C *Lactobacillus casei* K1, microencapsulated by spray drying. The authors reported a high viability when stored at -20°C over the other temperatures. According to De Castro- Cislaghi et al. [30] the stability of the microencapsulated probiotic is increased in low temperatures, such as refrigeration and freezing temperature.

During storage at 25 ° C, there was loss of cell viability from the 21st day for WX and 75th days for WP, showing that the temperature has negative effects on the wall of the microcapsule and thus, on the micro-organism protection. The whey used alone plays an important role as the encapsulating agent to maintain the viability of *P. pentosaceus* P107 during storage. Moreover, as many LAB are indigenous in milk, the whey is considered a suitable matrix, making the microcapsules an environment with chemical-physical and biological characteristics adequate to maintain these microorganisms [39].

Oliveira et al., [24] found higher viability losses at 37°C when compared to 7°C for microencapsulated *Bifidobacterium lactis* by coacervation with casein and pectin, followed by atomization. Just like Sagardia-Vega et al. [40] that encapsulated *Lactobacillus fermentum* UCO-979C with alginate and xanthan, added or not of oil and found high viability at 4°C when compared to 25°C. The adaptation to the environment, such as heat stress and moisture content are important conditions for probiotics to survive at high temperatures [41]. A greater reduction in the number of encapsulated microorganisms is observed in the stored at 25°C, because at this temperature the metabolic activity is higher and nutrients are consumed quickly. Similarly other studies have shown that microencapsulation using thermoprotective agents increases the survival of microorganisms during storage because mechanical, oxidative and osmotic stress is minimized [42].

3.3 Survival of microencapsulated *Pediococcus pentosaceus* P107 passage the gastrointestinal tract simulated

One of the main barriers for oral administration of probiotic bacteria is the low pH of the stomach and associated high concentration of hydrochloric acid, the encapsulation technique being a useful tool to increase the protection of such micro-organisms when exposed to these conditions.

In the present study, significant protection by the WX microcapsules, demonstrated by cell viability higher than 10 Log CFU.g^{-1} of P. *pentosaceus* P107, 120 min after the passage in the simulated gastric tract at different pH (2.0, 2.5) (Fig. 2a, 2b) and greater than 9 Log CFU.g^{-1} at pH 3.0 (Fig. 2c). It can be explained that the very low pH of electrostatic bonds between xanthan and whey begin to disappear once amine groups deionize, while carboxyl groups retain a negative charge. The network formed by the encapsulant

material is capable of expanding and absorbing water to buffer the acid compounds present in the gastric fluid, as they penetrate the microcapsules [43].

In contrast, both W and WP microcapsules, after the same period (120 days) had significantly lower values (< 9 Log CFU.g^{-1}) demonstrating that the enzyme action time and acid concentration are crucial for the integrity of the microcapsule and protection of the micro-organism. It has been reported that the dense network of hydrogel formed by combining whey to xanthan or pectin reduces the diffusion rate from the microcapsule, thereby reducing the exposure of the microorganism to the acidic medium which it is exposed [39, 44].

The presence or absence of bile salts in the simulated intestinal juice did not affect the viability of P. *pentosaceus* P107 (Fig. 2d). In the absence of bile salts, the microcapsules showed no significant difference. However, the presence of bile salts promoted microcapsule rupture and exposure of the microorganism, showing a reduction of 4.16, 3.41, 5.94 Log CFU.g^{-1} to W, WX and WP, respectively.

Chen et al. [43] demonstrated that xanthan microcapsules linked with chitosan, when exposed to 1% bile salts for 120 min presented a reduction in 2.06 Log CFU.g^{-1}. On the other hand, Rosolen et al. [15] found a high cell viability in cells of *Lactococcus lactis* subsp. *lactis* R7 microencapsulated, after 240 min exposure to simulated intestinal tract both in the presence (reduction of 2.3 Log CFU.g^{-1}) and in the absence of bile salts (2.88 Log CFU.g^{-1}).

However, results may be affected by the encapsulating material used or by the metabolic interaction of the microorganism or because the natural resistance of these bacteria to different pH and digestive enzymes [45].

3.4 Evaluation of heat resistance

The survival of P. *pentosaceus* P107 microencapsulated exposed at temperatures of 65 ° C for 30 min and 72 ° C for 15s, is shown in Table 4.

Table 4
Viability and percentage of survival of *Pediococcus pentosaceus* P107 microencapsulated and subjected to heat treatment at different temperatures

Microcapsule	**Survival rate (%)**	
	65°C by 30 min	72°C by 15 s
Whey (W)	84.37 ± 1.12^{c}	90.75 ± 0.25^{b}
Whey and Xanthan (WX)	87.91 ± 0.18^{b}	99.18 ± 0.18^{a}
Whey and Pectin (WP)	92.46 ± 0.78^{a}	99.31 ± 0.69^{a}
W: whey microcapsule;		
WX: whey and xanthan microcapsule;		
WP: m whey and pectin icrocapsule;		
Results represent the mean (standard deviations), n = 3.		
$^{a-c}$ Means with different superscript lowercase letters in the same column represent statistical difference ($p < 0.05$).		

The results showed that the microencapsulated cell with whey (W) was significantly more sensitive to heat treatment ($p < 0.05$), but still obtained counts that demonstrate probiotic potential (> 6 Log $CFU.g^{-1}$) in both of the tests. For heat treatment of 65°C for 30 min the best results were presented by WP microcapsules with a survival rate of 92.46%, followed by WX with 87.91% survival. It can be observed that there was no change in the survival of *P. pentosaceus* P107 (WP and WX) when subjected to 72°C for 15s.

In the study of Ilha et al. [19] the *Lactobacillus paracasei* FNU cells microencapsulated with skim milk and whey by spray drying were subjected to thermal testing at 65°C for 30 min and showed a percentage of survival of approximately 70%. Etchepare et al. [46] evaluated the viability of *L. acidophillus* microencapsulated in multiple layers by ion gelling using calcium alginate and whey. When the particle containing alginate is submitted to the thermal resistance test (72°C for 15 s) 77% survival was observed. When the particle receives a layer of whey the survival increases to 82%, demonstrating the effect of the coatings used in the protection of the microorganism. However the values are lower than those obtained in the present study

Few studies evaluate the protection the microcapsule can provide the microorganism when exposed to lethal temperatures. The high temperature and short time (72°C for 15 sec) is preferred for products containing encapsulated probiotics because the microcapsule is capable of protecting from the generated damage, such as protein denaturation and destruction of nucleic acids, which would lead to cellular apoptosis [47]. The viscoelastic properties of polysaccharides associated with the colloidal

protein system improve electrostatic interactions between wall materials determining the level of thermotolerance of the same [46, 48].

The use of heat due to the microencapsulation process or heat treatment during food processing provides in matrices such as whey the release of sulfur amino acids, reducing redox potential, thus aiding probiotic survival [49]. There is a long way to go in research that relates wall materials to the thermal resistance of microencapsulated probiotics.

4. Conclusions

The present study demonstrated that whey and the association with pectin and xanthan as encapsulating materials were able to protect *P. pentosaceus* P107 during microencapsulation by spray drying. It should be noted that the food matrix in which the microcapsule will be applied will define the choice of the encapsulating material.

In this context, during storage of -20, 4 and 25 ° C the microcapsule maintained the viability of the cells for 180 days, while WP and WX remained stable at temperatures of -20 and 4 ° C during the same period. The WX microcapsule was the best in protecting cell viability when submitted to the simulated gastrointestinal tract. WP, on the other hand, showed the greatest survival when subjected to heat treatment.

The combination of proteins and polymers in the composition of probiotic microcapsules promotes an improvement in survival and maintenance of cell viability. It can be inferred that the microcapsules produced presented specific morphology, simulated gastrointestinal resistance and thermal, in addition to low solubility in water, which demonstrates great potential for application in food.

Declarations

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Author contributions: FB, WS, and SP conceived and designed this research. AF, PO, FC contributed new reagents or analytical tools. FB, GL and MR performed experiments. All authors analyzed data. FB and MD wrote the manuscript. All authors read and approved the final version of the manuscript.

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Figures

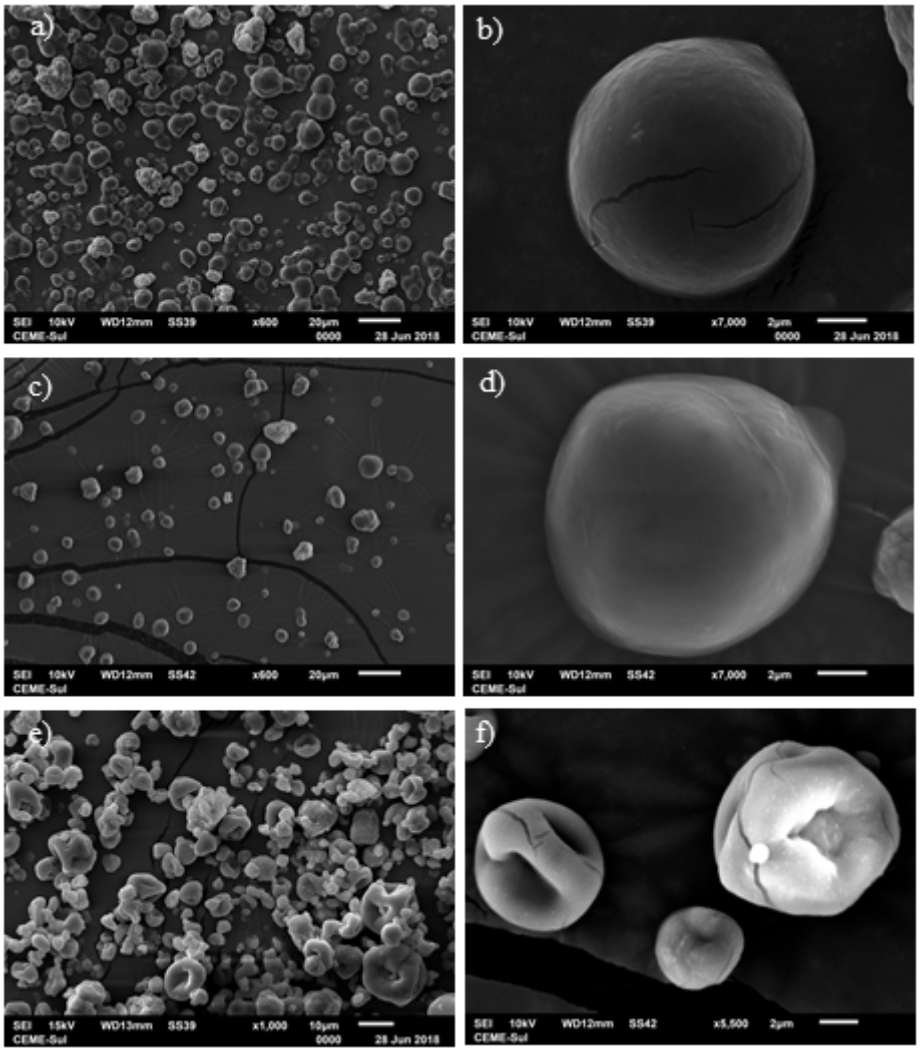

Figure 1

SEM images of microcapsules of Pediococcus pentosaceus P107 obtained by spray drying whey W (a) 600 x and (b) 7000 x; whey and xanthan WX (c) 600 x and (d) 7000x; and whey and pectin WP (e) 1000 x and (f) 5000 x

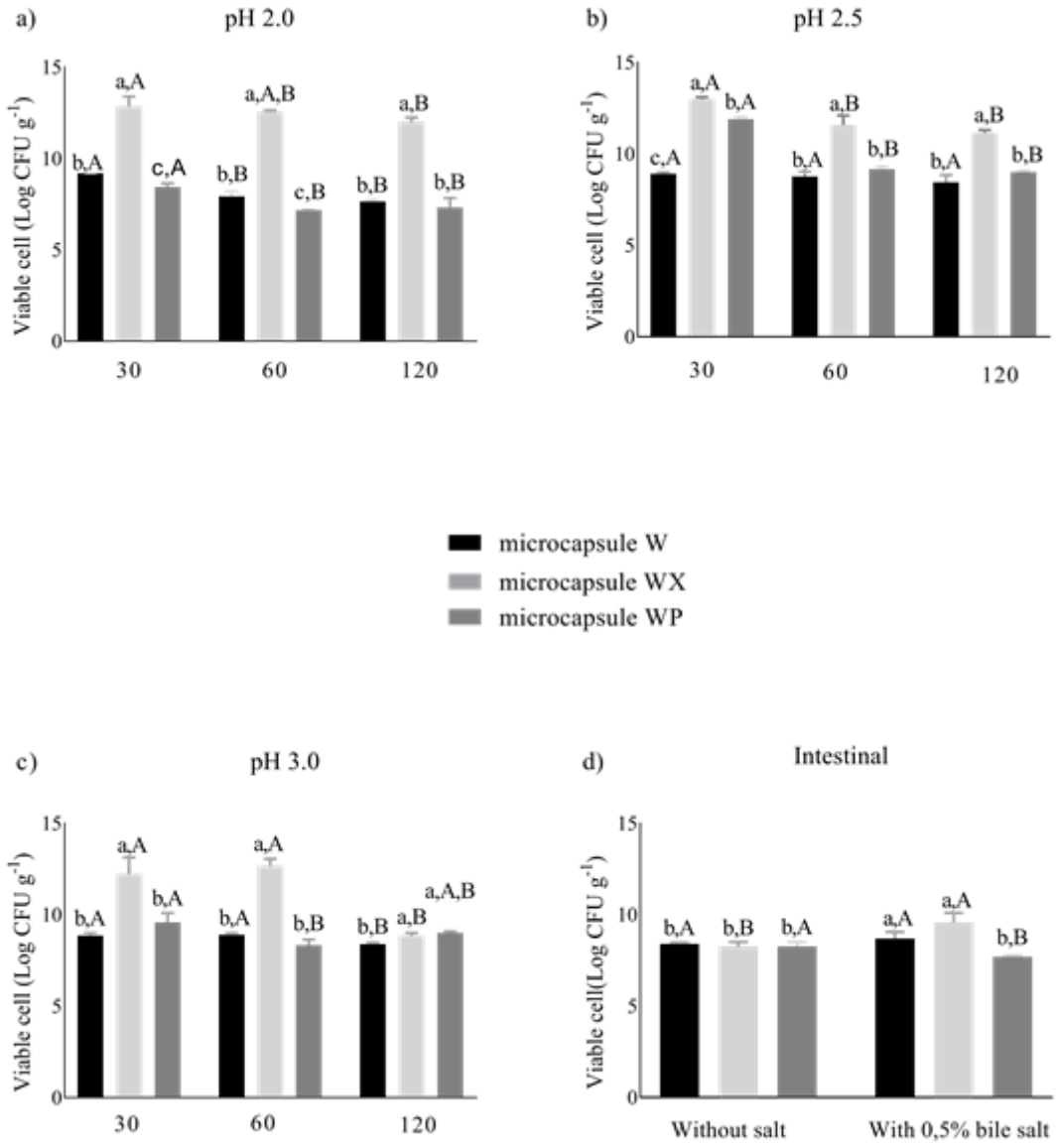

Figure 2

Pediococcus pentosaceus P107 microencapsulated viability up to 7 days storage at -20 °C during exposure to simulated gastrointestinal tract (A) gastric fluid pH 2.0, (b) gastric fluid pH 2.5 (c) Gastric fluid pH 3.0, both at times of 30, 60 and 120 minutes. (D) intestinal fluid pH 8.0 with 0.5% with bile salts and without, both by 240 min a-c Means ± standard deviation with different superscript letters indicate lower significant difference between the microcapsules for the same time and pH ($p < 0.05$). A-C Means ± standard deviation with different superscript capital letters denote significant differences between the same microcapsule in the different analysis times for the same pH ($p < 0.05$). W: cheese whey microcapsule; WX: cheese whey with xanthan microcapsule; WP: cheese whey with pectin microcapsule.

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