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BRIEF REPORT

Weissella paramesenteroides encapsulation and its application in the use of fish waste

Carina Libonatti[a,*], **Daniela Agüeria**[a,b], **Cecilia García**[c], **Marina Basualdo**[d]

[a] *Departamento de Tecnología y Calidad de los Alimentos, Argentina*
[b] *Instituto Multidisciplinario sobre Ecosistemas y Desarrollo Sustentable, Argentina*
[c] *Departamento de Fisiopatología, Argentina*
[d] *Departamento de Producción Animal, Facultad de Ciencias Veterinarias, UNCPBA, Argentina*

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Abstract The goal of the present study was to evaluate the encapsulation of *Weissella paramesenteroides*, isolated from bee bread, as a technological tool for its use in biological fish silage. The pH decrease in fish silages using the bacteria encapsulated and in a non-encapsulated form was compared. *W. paramesenteroides* showed a good performance in the development of biological fish silage. The alginate encapsulation method showed an encapsulation efficacy of 85% and provides a reliable technological application.

PALABRAS CLAVE
Residuos;
Pescado;
Ensilado;
Encapsulación;
Weissella

Encapsulación de *Weissella paramesenteroides* y su aplicación en residuos de pescado

Resumen El objetivo del presente estudio fue evaluar la encapsulación de *Weissella paramesenteroides*, aislada a partir del pan de polen, como herramienta tecnológica para su uso en la elaboración de ensilado biológico de pescado. Se comparó el descenso de pH para los ensilados utilizando la bacteria encapsulada y no encapsulada. *W. paramesenteroides* mostró un buen desempeño en el desarrollo de ensilado biológico de pescado. El método de encapsulación con alginato mostró una eficacia del 85% y puede ser utilizado para su aplicación tecnológica.

* Corresponding author.
E-mail address: redlab@vet.unicen.edu.ar (C. Libonatti).

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Weissella genus belong to lactic acid bacteria group, *Firmicutes* phylum, class *Bacilli*, order *Lactobacillales* and family *Leuconostocaceae*. These bacteria are Gram-positive, catalase-negative, non-endospore forming cells with coccoid or rod-shaped morphology[3].

The genus *Weissella* belongs to a group of heterofermentative lactic acid bacteria (LAB) commonly associated with foods. *Weissella* occur in a great variety of habitats, including the skin, milk and feces of animals, saliva, breast milk, human feces, in plants and vegetables, as well as in a variety of fermented food such as European sourdoughs and Asian and African traditional fermented foods[10]. From a technological point of view, *Weissella* occur an important role in fermentation processes such as the production of silage, as well as in food fermentations based on vegetables or meat as substrate[4]. Fish silage waste is used in some countries and allows recovering nutrients contained in fish waste and their use provides a double benefit: reduces the contamination risk generated by untreated waste and reduces the costs of producing animal food[9]. *Weissella paramesenteroides* is one of the predominant LAB species in fresh vegetables as well as processed meat substrates, e.g. fermented sausages and cured meats[4]. *W. paramesenteroides* strains have been reported to produce bacteriocin Weisellin A Class II A with wide activity against some microorganism[15].

Encapsulation has been recognized as an effective way to enhance the LAB viability and has been used in the food industry[13]. There are a variety of encapsulation methods, however alginate is one of the most widely used encapsulating materials, which is a linear heteropolysaccharide composed of β-D-mannuronic and α-L-guluronic acids[11]. An encapsulated method for LAB isolated from fish intestine was investigated for potential use of silage preparation from *Cyprinus carpio* residues[14].

The aim of this study was to evaluate the encapsulation of *W. paramesenteroides* as a technological tool for its use in biological fish silage.

W. paramesenterorides used in this study was isolated from bee breads on the apiary located in the southeast of Province of Buenos Aires, Argentina (the isolated strain was previously identified obtaining a similarity grater than 99% with a strain *W. paramesenteorides* ATCC33313).

The capsules were prepared by using alginate as encapsulation material. The emulsification and gelation were performed according to Chang[6] method using a peristaltic pump. A solution of Na alginate (2.2%, w/w) and a solution of calcium chloride (0.1 M) were prepared and both solutions were sterilized for 15 min. The solution of sodium alginate with the bacterial suspension was mixed and was dropped into the solution of Cl_2Ca in agitation for 15 min obtaining the capsules[2,12].

In order to determine the encapsulation efficacy of *Weissella*, one gram of the capsules was added to 9 ml of phosphate buffer 0.2 M. The suspension was homogenized for 30 min in a rotary shaker to release the content in order to determine the percentage of entrapment[7]. Once the capsules were dissolved, dilutions were prepared in sterile physiological solution in order to quantify the bacterial viability, using MRS agar in microaerophilic conditions at 30 °C for 48 h[12].

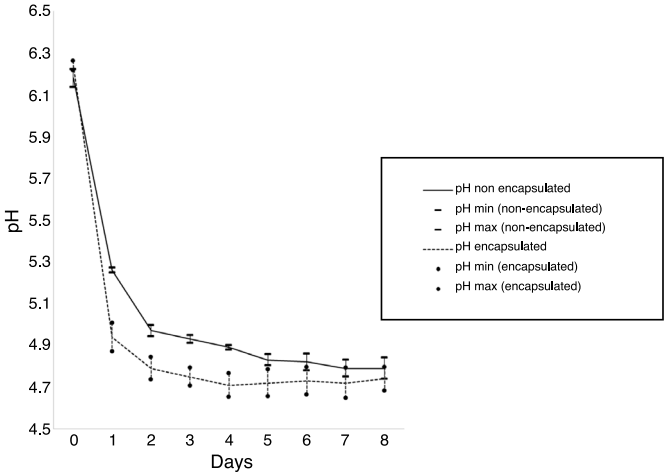

Figure 1 Values of pH of *Cyprinus carpio* silage using *Weisella paramensenteroides* non-encapsulated and encapsulated form during incubation period.

The encapsulation efficacy of capsules was calculated as:

$$E = \frac{N}{N_0} \times 100$$

where N is the number of viable cells encapsulated and N_0 is the number of viable cells used for encapsulation[5].

Capsules morphology was investigated using optical microscope (Olympus BX 40 F4) and the size (diameter) was measured with Pinnacle Systems Software Image Pro.

Biological fish silage was produced from common carp (*C. carpio*). Sugar as substrate (20%, w/w), sorbic acid (0.25%, w/w) and the bacterial non encapsulated inoculum (1%, v/w) was added (treatment 1), or its equivalent encapsulated bacterial based on encapsulation efficacy (treatment 2). Biological fish silage was prepared in triplicate with the encapsulated and non-encapsulated strain, and it was stored at 30 °C during 8 days. pH was determined by means of a pHmeter (TESTO) daily.

Bacterial counts in fish silage, including aerobic mesophilic, total coliforms, molds, yeasts and *Salmonella* spp. were performed at the end of the trial[1,12].

Statistical analysis was performed using procedures from SAS V9.3 (SAS, Institute Inc., Cary, NC, USA).

The results showed that the *W. paremesenteroides* could be encapsulated with the alginate method achieving an encapsulation efficacy of 85% representing 10^7 CFU/ml.

Capsules with regular homogeneous spherical shape were obtained. The mean diameter was 2.09 ± 0.05 mm.

When the pH of the silages produced using *W. paremesenteroides* for both treatments were compared, no significant differences were found ($p = 0.2042$). However, it was detected interaction between the treatments and days ($p < 0.0001$).

The initial values of pH obtained for ensilage fish (day 0) were 6.24 ± 0.023 and 6.18 ± 0.044 for non-encapsulated and encapsulated bacterial strain, respectively (Fig. 1). The pH values for both treatments significantly decrease ($p < 0.05$) at 48 h of incubation (Fig. 1).

In both cases the pH decreased and inhibited the development of pathogen and spoilage microorganisms, responsible of liquefaction in fish silage[8]. The pH is the most important parameter to be controlled during the silage elaboration and storage, because it evidences the development of the process, the quality of the silage and shows any change that could affect the product[16]. However the pH decrease was similar for both treatments, the encapsulation method keep bacterial viability throughout the time and it is an advantage for technological application.

Cai et al.[5] using aliginate-$CaCO_3$ for *Lactobacillus acidophilus* CGMCC1.2686 encapsulation found a 37.9% of efficacy. Our results show a good encapsulation efficacy (85%) indicating that it is an effective method to enhance the *W. paremesenteroides* viability and could be a reliable alternative to ferment fish residues.

In addition, the fact that there was no growth of spoilage and pathogen microorganism during silage storage indicates that it could be effective method to protect the fish silage.

It is the first report of the use of *W. paramesenteroides* isolated from bee bread and its encapsulation for fish silage production.

Conflict of interest

The authors declare that they have no conflicts of interest.

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