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Formulation of Alginate and Pectin-Based Beads Encapsulating Trichoderma for Sustainable and Efficient Agriculture †

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Abstract: The formulation of polysaccharides-based beads encapsulating Trichoderma spp. represents an eco-friendly strategy for promoting sustainable and efficient agriculture. Trichoderma, a beneficial fungus, is well-known for its ability to enhance plant growth, combat phytopathogens, and improve soil health. Encapsulating Trichoderma spores in a polysaccharide matrice provides a protective environment that ensures their viability and facilitates their controlled release into the soil. Alginate is a natural polymer found in various species of brown algae and certain bacteria. Pectin is an heteropolysaccharide present naturally in the cell walls of all higher plants. Due to their distinctive characteristics, alginate and pectin are regarded as promising carrier materials for the encapsulation of bioactive agents. In this work, alginate (Alg) beads, pectin (Pec) beads extracted from orange peel, and Alg/Pec composite beads in a 50/50 (*w*/*w*) ratio encapsulating Trichoderma S1 (1.83. 10^4 conidia/mL) and S2 (1.56.10^8 conidia/mL) were prepared using the ionic gelation method. The Moisture content of the prepared beads was evaluated. The size and shape of the beads were determined by analyzing images obtained by an optical microscope XE3910. The average size of the microcapsules (wet) varied from 1886 ± 6.557 µm to 1942 ± 28.688 µm. All samples were characterized by Fourier transform infrared spectroscopy (FTIR). The overall results demonstrated the successful encapsulation of Trichoderma spp. and highlighted the effects of the different formulations on the physicochemical properties of the beads.

Keywords: Alginate; pectin; Trichoderma; encapsulation; bioformulation

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1. Introduction

The development of sustainable agricultural practices, in line with the principles of the circular economy, is currently the focus of a number of initiatives aimed at reducing waste, enhancing resource efficiency, promoting the use of renewable materials and the integration of environmentally friendly technologies [1–3]. These efforts aimed to the protection of the environment, the human health and economic prosperity. In this context, the utilization of biodegradable materials, including alginate, a polysaccharide derived from brown algae, and pectin, a by-product of fruit processing, presents a interesting solution and a suitable option for enhancing agricultural productivity while reducing environmental impact [4,5]. Both alginate and pectin could be prepared by valorization of marine and vegetal biomass. Exploring these wastes as a potential resource for developing sustainable systems in agriculture is needed to address multiple future societal challenges.

Numerous studies reported the potential of alginate to form stable hydrogels [6,7]. When formulated as beads or capsules, they offer significant advantages in encapsulation techniques for the controlled release of bioactive agents, making them highly effective in

agricultural applications [4,8]. The combination of alginate beads with pectin results in the formation of composite beads that exhibit enhanced physicochemical properties, biodegradability and encapsulation efficiency [9,10]. Encapsulating beneficial microorganisms, such as Trichoderma species [11], within polymeric matrices offers a sustainable and eco-friendly strategy to promote crop health and productivity. In fact, It ensures a prolonged and controlled release of these bioagents, enabling continuous protection and growth stimulation for plants [11,12].

The principal goal of the present study was to design efficient encapsulation systems using alginate, extracted pectin and alginate-pectin composites to protect Trichoderma spores, for future agricultural applications. The beads were fully characterized using FT-IR, and the impact of the different formulations on the size and shape of the beads was determined.

2. Methods

2.1. Extraction of Pectin

The raw materials used for this end comprise fresh citrus peels of mandarin oranges, procured from a local market in the city of Sidi Bel Abbes (35°11′38′ N, 0°38′29′ W), Algeria. The extraction of pectin with citric acid was performed according to the experimental protocol described in our previous paper [13].

2.2. Preparation of Alginate (Alg), Pectin and Composite Beads Alg/Pec

The beads were prepared using the ionic gelation method. The alginate beads (Alg) were prepared by dissolving 1 g of sodium alginate (Mn: 195,000 and Mw: 350,000 g.mol^{-1}, polymolecular index (Ip): 1.8) in 100 mL of bi-distilled water and stirring for 48 h at ambient temperature. The alginate mixture was added dropwise to 250 mL of $CaCl_2$ (0.1M), with continious stirring. The resulting beads were left to stand in the solution overnight, then filtered, washed several times with bi-distilled water to remove excess $Cacl_2$ and dried at 30 °C for 24 to 48 h. The pectin beads were prepared following the same procedure, with the pH adjusted to 4 after the complete dissolution of pectin. The composite beads Alg/Pec were formulated as follows: a homogeneous polymer solution of 1% (*w*/*w*) alginate and pectin in water was prepared at an Alg/Pec ratio of 50/50 and kept under continuous magnetic stirring at room temperature for 24 h. The mixture was then introduced dropwise into a solution of $CaCl_2$ 0.1M with stirring maintained continuously. After 24h, the formed beads were filtered and dried at 30 °C.

2.3. Preparation of Trichoderma-Loaded Beads

The encapsulated fungus, Trichoderma gamsii, was supplied by the National Institute for Agronomic Research (INRA). It was selected following a screening of a collection of Trichoderma strains from various regions of Algeria for their inhibitory effects against multiple fungal pathogens [14]. Two spore suspensions of Trichoderma in sterile distilled water at concentrations of 1.83×10^4 conidia/mL (S1) and 1.56×10^8 conidia/mL (S2) were prepared for encapsulation into the beads. The Trichoderma-loaded beads were prepared as described above with minor modifications. A precise volume of the Trichoderma suspension was mixed with a 2% polysaccharide solution at a 1/4 (*v*/*v*) ratio under moderate stirring. The mixture was then dropped into a $CaCl_2$ 0.1 M and kept at room temperature for 30 min. The obtained beads were filtered, washed with sterile water and stored at 4 °C.

2.4. Beads Characterization

The size and shape of the beads were determined by analyzing images obtained by an optical microscope XE3910 equipped with a 3.2 Mpix digital camera and software for image capture and processing. The Moisture content (TH%) was determined gravimetrically, by calculating the weight difference before and after drying. The FTIR spectra were recorded on a Bruker ATR spectrometer in a wavelength range 500–4000 cm$^{-1.}$

3. Results and Discussion

3.1. Percentage Yield of Pectin Extraction and Degree of Methylesterification (DE)

The extraction yield obtained was 10.2%, this result is in agreement with values obtained by Awuchi et al. [15], the authors reported percentage yields of pectin extraction from orange ranging from 11.01 to 16.01. It should be noted that the present result is 2 times higher than that obtained in our previous paper [13], although the experimental conditions were identical. This could be due to the origin or the quality of the oranges. The DE of the extracted pectin ranged from 39.47 to 40.74%, indicating that it belongs to the category of low methoxyl pectin [15].

3.2. Image Analysis

Table 1 showed a smaler diameter of Alg/Tri S1 beads compared to Alg/Tri S2 one which can be explained by the lower concentration of the Trichoderma solution. Thus, The size increased as more Trichoderma spores were introduced. In addition, the encapsulated trichoderma wet beads showed high water content

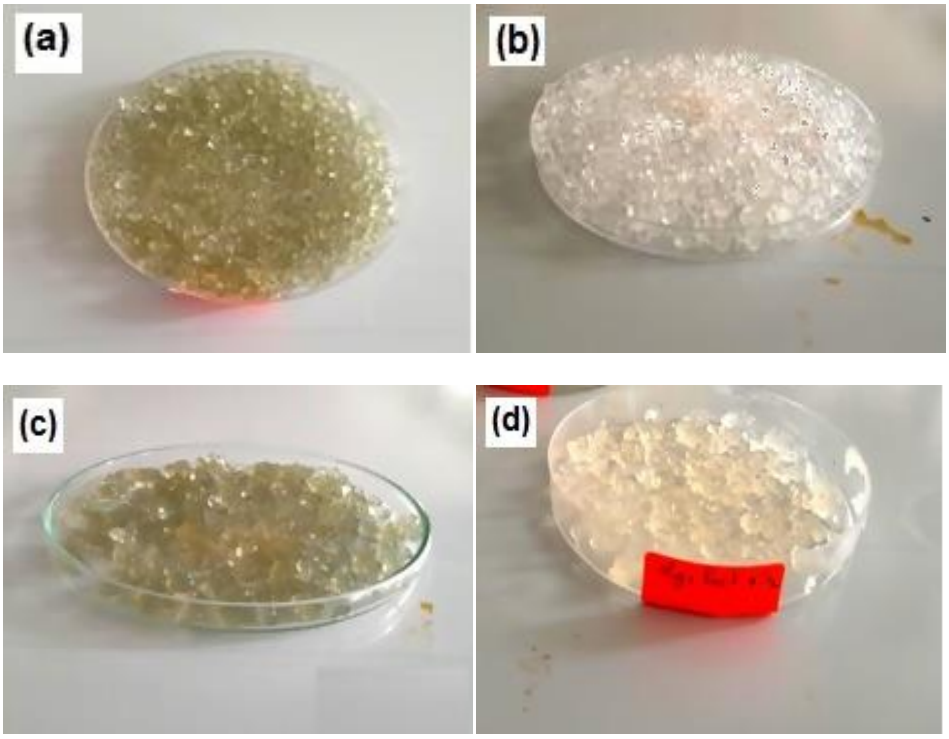

Figure 1. Wet samples of the formulated beads (**a**) Alg/Tri S1, (**b**) Alg/Tri S2, (**c**) Alg/Pec/Tri S1, (**d**) Alg/Pec/Tri S2.

Table 1. Diameter (μm) of the formulated beads and their water content.

Bead	D1	D2	D3	D ± Sd	TH(%)
Alg/Tri S1	1879	1892	1887	1886 ± 6.557	98.71
Alg/Tri S2	1975	1923	1928	1942 ± 28.688	98.67

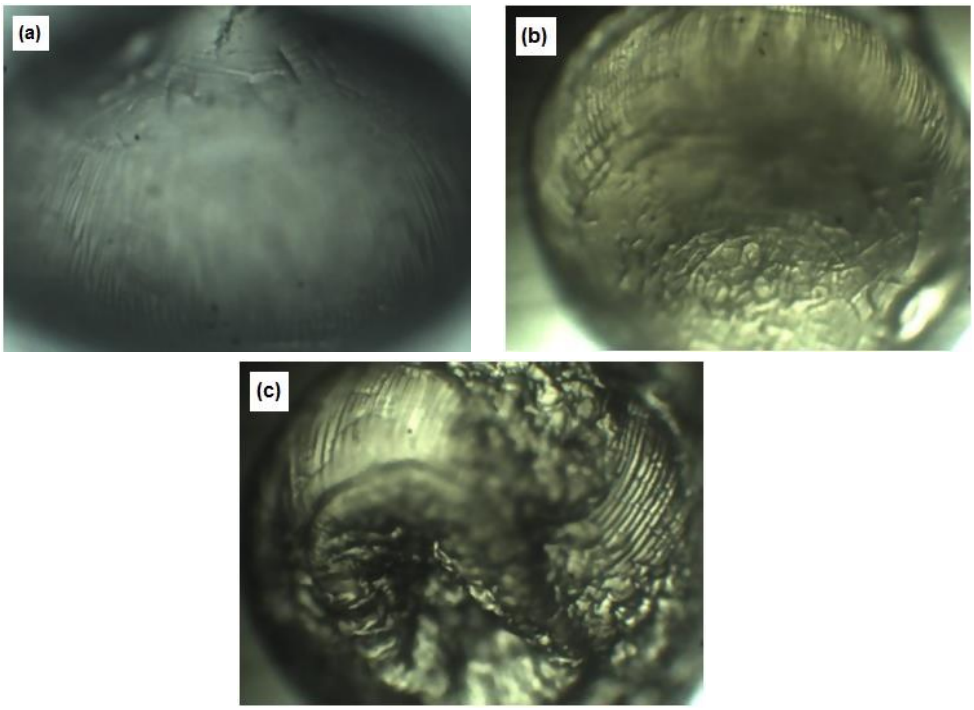

Figure 2. Microscopic Images of (**a**) Alg, (**b**) Alg/Tri S1, (**c**) Alg/Tri S2 beads.

3.3. FTIR Results

The FTIR spectra of the formulated beads are presented in Figure 3. As it can be seen, a substantial absorption band within the range of 3000–3400 cm^{-1} was discerned for all samples, that can be ascribed to the stretching vibration of hydroxyl groups –OH [7]. The bands observed at 1100–1018 cm^{-1} are attributed to COC-stretching vibrations and confirmed the saccharide structure [6]. The peaks at 1725 –1605 correspond to the vibrations of the carbonyl group C=0. The characteristic peaks at 1725, 1640 and 1441 cm^{-1} for pectin are related to the C=O stretching vibration of -COOMe, the asymmetric C=O stretching vibration of -COOH, and the symmetric C=O stretching vibration of $-COO^{-}$, respectively [16].

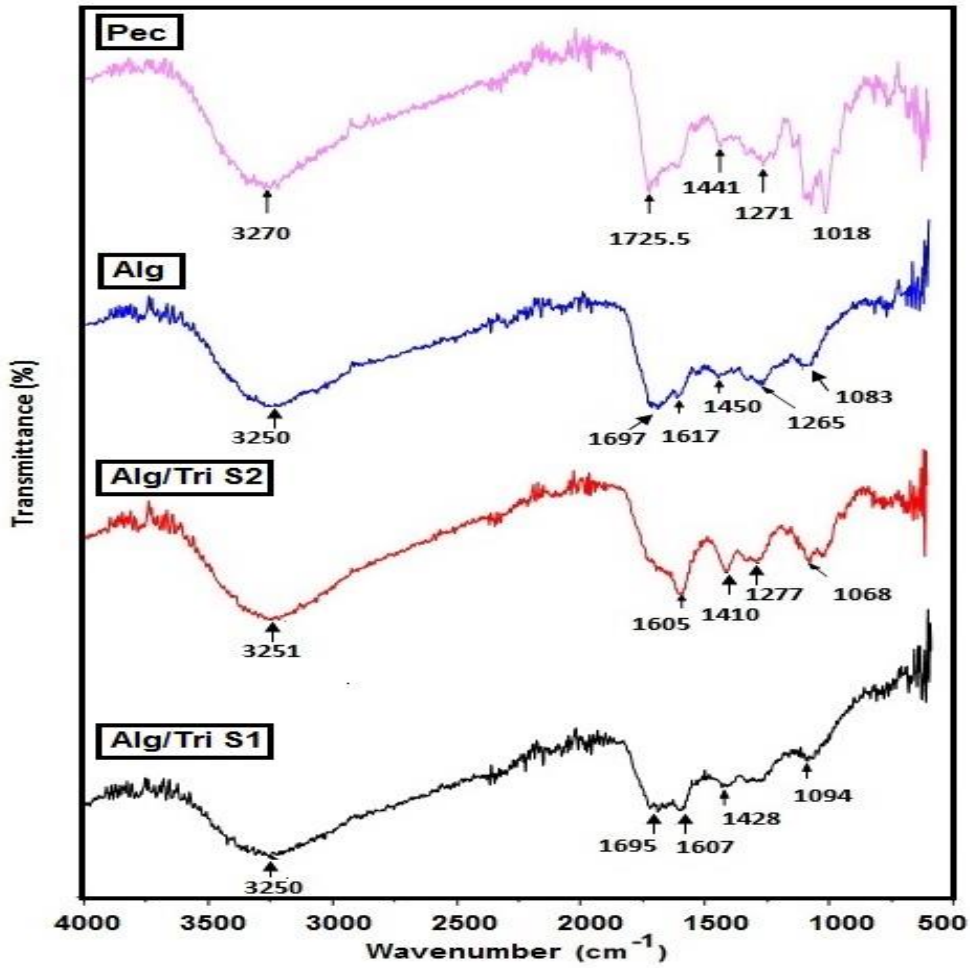

Figure 3. FTIR spectra of alginate and pectin beads and Trichoderma-loaded alginate beads.

4. Conclusions

Beads composed of alginate, pectin extracted from orange peel, and alginate-pectin composites were successfully prepared using the ionic gelation method. These beads were effectively utilized for the encapsulation of *Trichoderma gamsii*, for the first time. The preliminary findings of this study are promising, demonstrating the potential of these materials for efficient encapsulation. Further research will be conducted to explore and optimize the encapsulation process and to evaluate the long-term stability and performance of the beads in agriculture

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