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Conference Paper

Development of Antibacterial Gels Based on Sodium Alginate and Inclusion Complexes for Packaging Applications in Fruits and Vegetables

Desarrollo de geles antibacterianos a base de alginato de sodio y complejos de inclusión para aplicación en el envasado de frutas y verduras

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Abstract

Bacterial growth in fruits and vegetables causes a large percentage of the loss and waste of these foods worldwide. For this reason, the objective of this study was to develop antibacterial gels based on sodium alginate, β-cyclodextrin, and allyl isothiocyanate inclusion complexes (β-CD:AITC) for the potential packaging of fresh fruits and vegetables. The β-CD:AITC complexes were prepared by the co-precipitation method with a 1:1 molar ratio, and was further verified by FESEM microscopy. On the other hand, the antibacterial gels were prepared using the external gelation method, and the effect of the order of incorporation of the components, the conditions of the components, and the resting conditions of the dispersions on their visual appearance was evaluated. Furthermore, the antibacterial activity of the gels against *Escherichia coli* and *Listeria innocua* was evaluated using a headspace system. FESEM micrographs showed a crystalline block-like morphology in the inclusion complexes. The order of incorporation (1) glucone-delta-lactone (2) complexes (3) alginate allowed for obtaining more homogeneous gels with a smooth surface. The presence of glucono-delta-lactone, the concentration of 0.05 M $CaCl_2$, a cross-linking time of 20 hr, and the cooling of the dispersion allowed more uniform gels to be obtained. Finally, better antibacterial activity against *E. coli* was obtained with the gels loaded with 10% β-CD:AITC complexes. According to these results, the developed materials could be used as antimicrobial packaging materials for fresh fruits and vegetables.

Keywords: *β-cyclodextrin, allyl isothiocyanate, alginate, food packaging, antimicrobial.*

Resumen

El crecimiento bacteriano en frutas y verduras provoca un gran porcentaje de pérdida y desperdicio de estos alimentos a nivel mundial. Por esta razón, el objetivo de este estudio fue desarrollar geles antibacterianos a base de alginato de sodio y complejos de inclusión de β-ciclodextrina e isotiocianato de alilo (β-CD:AITC) para el potencial envasado de frutas y verduras frescas. Los complejos β-CD:AITC se prepararon mediante el método de co-precipitación con una relación molar 1:1, y su obtención se verificó mediante microscopía FESEM.

Por otro lado, los geles antibacterianos se prepararon mediante el método de gelificación externa, y se evaluó el efecto del orden de incorporación de los componentes, las condiciones de los componentes y de reposo de las dispersiones en su apariencia visual. Además, se evaluó la actividad antibacteriana de los geles frente a *Escherichia coli* y *Listeria innocua* mediante un sistema de espacio de cabeza. Las micrografías FESEM mostraron una morfología del tipo bloque cristalino en los complejos de inclusión. El orden de incorporación (1)Glucono-delta-lactona-(2)Complejos-(3)Alginato permitió obtener geles más homogéneos y con una superficie lisa. La presencia de la Glucono-delta-lactona, la concentración de 0,05 M de $CaCl_2$, un tiempo de entrecruzamiento de 20 horas y la refrigeración de la dispersión permitió obtener geles más uniformes. Finalmente, se obtuvo una mejor actividad antibacteriana frente a *E. coli* con los geles cargados con un 10% de complejos β-CD:AITC. De acuerdo con estos resultados, los materiales desarrollados podrían ser utilizados como materiales de envase antimicrobiano para frutas y verduras frescas.

Palabras Clave: *β-ciclodextrina, isotiocianato de alilo, alginato, envases de alimentos, antimicrobiano.*

1. Introduction

Fruits and vegetables are one of the most consumed food groups in the world, with a high capacity to perish. In 2016, the Food and Agriculture Organization of the United Nations (FAO) reported that approximately 715 million tons of fruits and vegetables produced for human consumption were lost or wasted in Latin America and the Caribbean (1). These values increased during 2020 and 2021 since the effect of the COVID-19 pandemic produced a change in eating patterns, causing greater consumption of these foods by consumers (2). Furthermore, the greatest loss of fruits and vegetables occurs at the household level, with values between 20 and 40%, and during transportation, with an average value of 30% (3).

The high deterioration of fresh fruits and vegetables is associated with poor or almost no packaging of these products. This fact causes these foods to suffer physical damage during transportation and, therefore, rapid decomposition during storage (4). On the other hand, the decomposition of fruits and vegetables is also because they are living organisms with high water activity, which generates optimal conditions for the growth of pathogenic microorganisms such as bacteria (5). In order to avoid these losses, the agri-food industry and academia have directed their objectives towards the development of technological strategies that prevent the growth of these microorganisms. One of these strategies has been the development of materials with antibacterial capacity that allow the development of functional packaging capable of protecting fresh fruits and vegetables and extending their useful lives. (6–8).

The development of antibacterial packaging materials has been achieved through the use of various active compounds and polymers (9). Among the most studied natural

compounds, the use of essential oils from rosemary, lavender, thyme, bergamot, clove, oregano, cinnamon, and mustard, among many others, stands out (10–12). Furthermore, it has been shown that the antibacterial capacity of these compounds correlates with their major constituents, such as terpenes, phenolic compounds, and isothiocyanates (13–15). For this reason, the most recent developments in antibacterial packaging materials have been mostly based on the use of allyl isothiocyanate (AITC), since this compound exhibits high antibacterial capacity at low concentrations.

AITC is a highly volatile organic sulfur compound with an acyclic aliphatic linear structure. This compound is found in the seeds, stems, leaves, and roots of plants such as mustard, wasabi, and horseradish (16–18). The high volatility of AITC has favored the gaseous antimicrobial treatment of different fruits and vegetables, such as lettuce, tomato, melon, spinach, cabbage, and strawberries, among others, before storage (13, 19, 20). However, the high volatility of this compound is also a disadvantage, since its rapid release from packaging materials prevents correct microbiological control during food storage (21). To solve this problem, the encapsulation of AITC through the formation of inclusion complexes with cyclodextrins has emerged as a novel strategy.

Cyclodextrins are nontoxic cyclic oligosaccharides that display a hollow, truncated conical shape formed by D-glucopyranose units linked by α-1,4 bonds (22). Due to the orientation of the primary and secondary hydroxyl groups (primary and secondary faces), the external wall of cyclodextrins is hydrophilic, which allows their solubility in water. On the other hand, glycosidic bonds oriented towards the internal cavity favor lower hydrophilicity in this area, favoring the hosting of hydrophobic compounds through the formation of inclusion complexes (23–25). One of the most studied cyclodextrins used in the formation of inclusion complexes is β-cyclodextrin (β-CD). This is due to its low cost, good availability, and the adequate size of its cavity, which allows it to host and protect hydrophobic compounds such as AITC(23, 26).

On the other hand, the use of polymers from petroleum for the development of plastic food packaging has caused a challenge to the industry since the large amount of this plastic waste has generated enormous pollution in the environment. For this reason, in recent years, academia has begun to develop packaging materials based on biodegradable and naturally abundant polymers, such as alginates (27).

Alginates constitute a group of natural polysaccharides that can be isolated from bacteria or extracted from some marine brown algae. These polysaccharides occur as binary copolymers made up of residues of β-D mannuronic acid (M) and α-L guluronic acid (G), linked by 1–4 glycosidic bonds and arranged in the form of blocks along the polymer chain (28, 29). Because alginates are “generally recognized as safe” (GRAS) by the Food and Drug Administration (FDA), they have been widely used in

the food industry as thickening agents, stabilizers, and emulsifiers (30). On the other hand, alginates can form very stable three-dimensional structures known as gels, which can carry active compounds inside. Alginate gels are obtained by introducing divalent cations such as Ca^{2+} ions into the polymer structure, which causes conformational changes such as the alignment of G blocks and the formation of the egg-crate pattern (31). This advantage that these polysaccharides present makes them potential polymers for the development of packaging materials such as active gels.

Based on this background, the objective of the present research was to develop antibacterial gels based on sodium alginate and inclusion complexes with allyl isothiocyanate as potential materials for the active packaging of fresh fruits and vegetables.

2. Materials and methods

2.1. Materials

2.1.1. Chemical reagents and microorganisms

Pharmaceutical grade β-cyclodextrin (Molecular weight = 1134.98 g mol^{-1}, 98.5% purity) was obtained from Cyclolab, Ltd. (Budapest, Hungary). The active compound allyl isothiocyanate (Molecular weight = 99.15 g mol^{-1}) with purity greater than 95% and calcium chloride dihydrate ($CaCl_2$) with purity ≥ 99% and ACS grade were obtained from Sigma Aldrich (Santiago, Chile). Sodium alginate from algae was obtained from Oregon Chem Group (Santiago, Chile). Glucono-delta-lactone (GDL) was obtained from Limited Transfer Food (Santiago, Chile).

The microorganisms evaluated were E. coli ATCC 25922 and L. innocua ATCC 33090, provided by the Laboratory of Biotechnology and Applied Microbiology at the University of Santiago de Chile (Santiago, Chile).

2.2. Methodology

2.2.1. Preparation of inclusion complexes

The synthesis of β-CD inclusion complexes with AITC was performed by the co-precipitation method. The molar ratio 1:1 (β-CD: AITC) was considered according to the results obtained in a previous study (32). Five grams of pure β-CD were placed in an amber flask containing 50 mL of a 1:2 (v/v) water: ethanol solvent mixture and stirred at 50 °C for 2.5 h. Then, AITC was added to the flask, and the flask was sealed hermetically to prevent volatilization of the compound. The mixture was stirred for

30 minutes at 50 °C, and then it was cooled to room temperature and refrigerated overnight. Subsequently, the mixture was subjected to a filtration process to recover the solid phase, and it was washed with a 10% (v/v) ethanol solution in order to remove the surface AITC. The solid phase was dried in a Cool Safe 55-4 Pro freeze dryer (Lillerod, Denmark) and sieved using a Tyler #325 sieve to obtain the fraction under 45 µm. The inclusion complexes were stored in an airtight glass bottle inside a desiccator at 0% relative humidity until use.

2.2.2. Characterization of inclusion complexes

Obtaining the inclusion complexes was verified by field emission scanning electron microscopy (FESEM). The complexes were placed on an adhesive carbon sheet and coated with a carbon layer. The surface morphology of the complexes was observed using a FESEM Zeiss Merlin VP compact microscope (Jena, Germany) at a magnification of 150×.

2.2.3. Preparation of antibacterial gels

The antibacterial gels were obtained using the external gelation method, following the procedure described by Li et al. (2016) with some modifications (33). First, two grams of sodium alginate were dispersed in 100 mL of water, and the inclusion complexes were incorporated. This process was carried out while maintaining constant stirring at 30 °C for one hour. Then, the dispersions were allowed to sit overnight in order to eliminate bubbles. Finally, the dispersions were immersed in a $CaCl_2$ solution for 15 minutes to obtain the antibacterial gels. However, the successful production of sodium alginate gels depends on several factors. These are the order of addition of components, the concentration of the $CaCl_2$ solution (34, 35), the presence of a hydrolyzing agent such as GDL capable of controlling the release of the cross-linking cation ($Ca^{2+)}$ (36), the cross-linking time, the rest conditions of the dispersions, and the presence of other particles. For this reason, these effects were studied during the preparation of antibacterial gels, with the aim of obtaining materials with a homogeneous structure.

2.2.4. Effect of order on addition of components

This study was carried out with the purpose of establishing the order of addition of the different components (inclusion complexes, GDL, and sodium alginate) in the dispersion. For this, the conditions described in Table I were tested.

Tabla 1

Effect of the addition of components in the preparation of antibacterial gels.

Dispersion	Component incorporation order
CGA	Inclusion complexes GDL Sodium alginate
GAC	GLD Sodium alginate Inclusion complexes
GCA	GDL Inclusion complexes Sodium alginate

1.1.1.1. Effect of components

This study consisted of evaluating the effects of the presence of the hydrolyzing agent GDL, the concentration of the $CaCl_2$ cross-linking solution, the cross-linking time, and the presence of the inclusion complexes. The conditions tested are detailed in Table II.

Tabla 2

Effect of components in the preparation of antibacterial gels.

Factors	Specifications
Presence of GDL	With GDL Without GDL
Cross-linking solution concentration	0,01 M 0,05 M 0,10 M
Cross-linking time	4 hours 20 hours
Presence of inclusion complex	With inclusion complexes Without inclusion complexes

1.1.1.2. Effect of rest conditions

This study was based on the evaluation of rest time and the presence of inclusion complexes in dispersion, whose conditions are detailed in Table III.

Tabla 3

Effect of rest conditions in the preparation of antibacterial gels.

Factors	Specifications
Dispersion rest condition	No rest Rest for 4 hours at room temperature. Rest for 4 hours in the refrigerator
Presence of inclusion complex	With inclusion complexes Without inclusion complexes

2.2.5. Characterization of antibacterial gels

Visual evaluation

The effect of the order of addition of components, component conditions, and rest conditions on obtaining antibacterial gels was evaluated visually. In this sense, photographs were taken of the samples, and the stability of the dispersions after stirring and the visual appearance of the structures formed were analyzed.

2.2.6. Determination of mass loss and gelation percentage.

The mass loss of gels and the percentage of gelation were determined by equations (1) and (2), respectively.

$$Mass\ loss = (m_i - m_f)\ x\ 100 (1)$$

Where:

m_i = dispersion mass before rest [g]

m_f = dispersion mass at the end of rest [g]

$$Gelled\ dispersion = (m_T - m_g)\ x\ 100 (2)$$

Where:

m_T = dispersion mass prior to gelation [g]

m_g = gel mass [g]

2.2.7. Antibacterial activity of gels

To evaluate the antibacterial activity of the gels, the preparation conditions that resulted in the best morphological appearance were chosen. Gels were prepared with 5 and 10% (relative to the final material) of inclusion complexes and were microbiologically evaluated against *E. coli* and *L. innocua* using an in vitro headspace system. To do this, cell cultures of each bacterium were prepared in tryptic soy broth until they reached their exponential phase. With a cell concentration of ≈107 colony-forming units per milliliter (CFU/mL), dilutions of each bacterium were prepared at ≈106 CFU/mL. Subsequently, 200 μL of this bacterial solution was seeded in petri dishes with trypticase soy agar (TSA), and one gram of active gel was placed on the lid of the plate. The plates were sealed with Parafilm® and incubated inverted at 37°C. In addition, TSA plates without active gel and with non-active gel were used as controls. The results of the antibacterial activity were determined visually. Establishing “full growth” means the presence of a bacterial lawn on the surface of the agar; “partial growth” means the presence of spaces

between the bacterial lawn present on the surface of the agar. Also, “no growth” means the non-presence of the bacterial lawn on the surface of the agar.

2.2.8. Statistical analysis

Statistical analysis of mass loss and percentage of gelation of gels was performed using analysis of variance (one-way ANOVA) to detect differences between samples with a 95% confidence level. Subsequently, a comparison of samples was carried out through the Tukey test, using the InfoStat statistical program student version (Córdoba, Argentina).

3. Results and discussion

3.1. Obtaining inclusion complexes

Obtaining the β-CD: AITC inclusion complexes was verified by FESEM microscopy. Figure **1** shows that the complexes presented a compact structure similar to crystalline blocks with defined edges and a certain thickness. Furthermore, these structures exhibited high uniformity in their structure and regularity in their shapes. Previous studies have obtained similar results; for example, Yao et al. showed that inclusion complexes of β-CD and cardanol appeared as a block-stacked structure (37). Likewise, inclusion complexes of β-CD and hydroxypropyl-β-cyclodextrin with chlorothalonil exhibited a prismatic crystal morphology with defined edges (38). Similarly, inclusion complexes of β-CD and geraniol have also shown a crystalline block morphology with great uniformity and regularity (39).

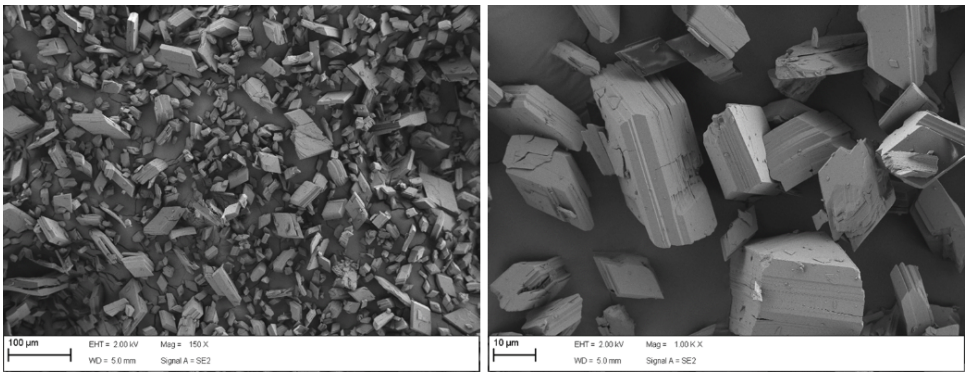


Figura 1

FESEM micrograph of β-CD:AITC inclusion complexes.

3.2. Obtaining antibacterial gels

Obtaining sodium alginate gels with a homogeneous morphology depends on various factors. These are the order of addition of components, concentration of cross-linking solution, cross-linking time, rest time of dispersions, and the presence of other particles and agents. Figure **2** shows the results after adding the components that are part of the alginate dispersion in a different order.

As shown in Figure **2**a, the CGA dispersion exhibited a separation of the oil phase, corresponding to the active compound AITC. This result demonstrated that the incorporation of β-CD: AITC complexes in water at the beginning of the process caused them to solubilize, therefore favoring the start of the release of the active compound into the medium. Furthermore, the subsequent addition of GDL to the medium produced its hydrolysis, thus promoting a decrease in the pH of the dispersion and further favoring the release of AITC from the inclusion complexes (40).

On the other hand, Figure **2**b shows that the GAC dispersion maintained an agglomeration of the inclusion complexes. In this case, the amount of alginate used generated a highly viscous dispersion with a high degree of difficulty in stirring. Therefore, the subsequent incorporation of the β-CD: AITC complexes into the medium hindered their distribution in the dispersion, giving rise to the surface agglomerations detected in the photograph. Unlike the preparation of these two dispersions, Figure **2**c shows that the GCA dispersion was completely homogeneous, without the presence of agglomerations of the inclusion complexes or separation of the phases. Furthermore, compared to the GAC dispersion (Figure **2**b), this dispersion exhibited a white coloration. This fact demonstrated that AITC was still complexed within β-cyclodextrin.

Based on these results, the GCA dispersion (1. GDL → 2. Complexes → 3. Sodium alginate) was selected to continue with the evaluation of the other factors that influence the preparation of homogeneous gels.

Figura 2

Scatter photographs: a) CGA (Complexes + GDL + Alginate), b) GAC (GDL + Alginate + Complexes), and c) GCA (GDL + Complexes + Alginate).

Figure **2** shows the results of the evaluation of the effects of the presence of GDL, $CaCl_2$ concentration, cross-linking time, and inclusion complexes.

The use of a 0.01 M $CaCl_2$ solution did not favor the gelation of the alginate dispersion, regardless of the cross-linking time and the presence of GDL and inclusion complexes. This result was associated with the ability of calcium ions to cross-link alginate due to their low availability in the medium (41). On the contrary, a higher concentration of $CaCl_2$ (0.05 and 0.10 M) favored the formation of gels in the presence or absence of GDL and inclusion complexes. Despite this, Figure **2** shows that the 0.10 M concentration of CaCl2 produced a contraction in the formed gel structure. Giving rise to structures with a spherical shape (without GDL) or swollen in the center (with GDL), regardless of the presence of complexes and cross-linking time. Therefore, it was evident that the hydrolyzing agent positively influenced the macrostructure of the formed gels, since dispersion contraction towards the center of the container mold was avoided during cross-linking, giving rise to more homogeneous gels in thickness. The positive effect of GDL on the formation of uniform gels is attributed to its ability to reduce the release rate of calcium ions into the alginate dispersion, favoring a more controlled gelation (42). On the other hand, alginate dispersions in the presence of GDL and cross-linked with 0.05 M $CaCl_2$ solution generated flatter gels, regardless of the presence or not of the inclusion complexes (Figure **2**).

The cross-linking time of the dispersion also influenced the morphology of the gels. A cross-linking time of four hours produced a wrinkled or transparent surface in the center of the gel, which corresponded to a fraction of the polymer dispersion not yet gelled. On the contrary, increasing the cross-linking time to 20 hours promoted complete gelation of the dispersion, therefore obtaining flat structures with a smooth surface.

According to these results, it was decided to incorporate GDL during the preparation of the alginate gels and use a 0.05 M concentration of the cross-linking solution and a cross-linking time of 20 hours.

Figure 2. Appearance of ALG gels formed under different conditions. Table IV shows the results of the evaluation of the conditions of the dispersion rest time prior to cross-linking. The non-resting effect of the dispersion promoted the formation of gels with an irregular surface due to the instability of the dispersion due to stirring. On the contrary, the application of resting the gels allowed us to obtain structures with a uniform surface. On the other hand, the application of a cooling temperature during the rest of the dispersion favored the formation of flat gels with a homogeneous surface. This result could probably be associated with a slowdown during crossing-over. Furthermore, this effect is consistent with a significantly lower gelation percentage value compared to the values obtained for dispersion without rest or with rest at room temperature (Table IV).

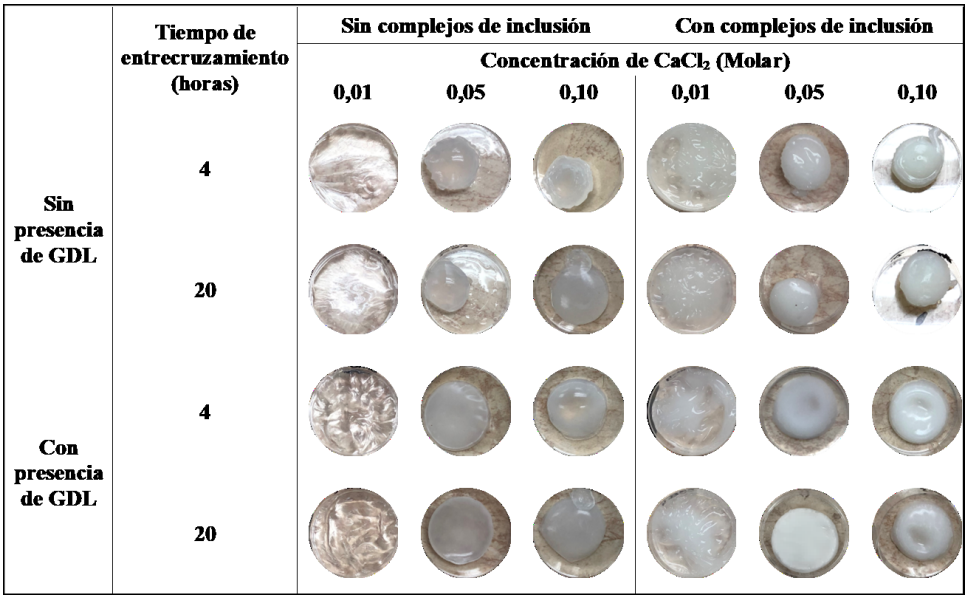


Figura 3

Appearance of ALG gels formed under different conditions.

Additionally, the use of low temperatures during the rest of the dispersion promoted minimal or no mass loss. This fact is notable since this condition would prevent the possible evaporation of the AITC compound from the gels.

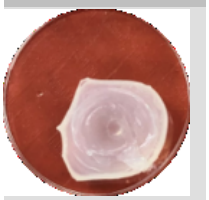
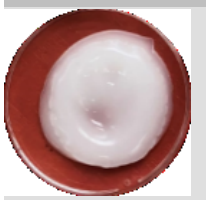
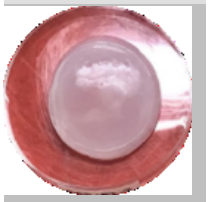
3.3. Antibacterial activity of gels

Figure **4** shows the results of the antibacterial activity of gels against a Gram(-) E. coli bacteria and a Gram(+) L. *innocua*. The control samples without active gel and with non-active gel (0% inclusion complexes) exhibited the presence of a bacterial lawn on the surface of the agar, evidencing the total growth of the microorganisms. On the other hand, the incorporation of *β*-CD:AITC inclusion complexes in gels promoted the antibacterial activity of the materials. As shown in Figure **4**, antibacterial gels loaded with 5% complexes produced partial growth in both bacteria, as gaps were observed in the microbial lawn. However, increasing the concentration to 10% produced different results. In this case, the E. coli bacteria was highly susceptible to the antibacterial activity of AITC since there was no microbial growth on the agar. On the other hand, for the bacteria *L. innocua*, this concentration of inclusion complexes promoted partial growth of the microorganism.

The different antibacterial activity against these bacteria was associated with their different cell envelope structures. Gram(-) bacteria consist of a complex cell wall composed of a triple-layered envelope, a thin peptidoglycan layer, and an outer

Tabla 4

Appearance of alginate gels formed with dispersions rested at different conditions.

Dispersion Rest	Without inclusion complexes			With inclusion complexes		
	Appearance	Gelled dispersion (%)	Mass loss (g)	Appearance	Gelled dispersion (%)	Mass loss (g)
No rest		55,1 ± 0,6^{a}	-		53,8 ± 2,6^{ab}	-
4 hours at room temperature		61,3 ± 4,3^{a}	4,1 ± 0,1^{a}		56,8 ± 1,8^{a}	4,4 ± 0,1^{a}
4 hours in refrigeration		49,6 ± 2,3^{b}	0,1 ± 0,1^{b}		48,6 ± 3,6^{ab}	0,1 ± 0,0^{b}

Means with a common letter within a column indicate statistical similarity ($p > 0.05$) according to an analysis of variance and the Tukey test.

membrane composed of proteins, lipids, and polysaccharides. In contrast, the cell envelope of Gram(+) bacteria contains a thick layer of peptidoglycan and a plasma membrane (43, 44). nst Gram (-) bacteria. Lu et. al. (2106) observed that a concentration of 1000 ppm AITC reduced 3.7 log bacteria of E. coli and 1 log bacteria of *Staphylococcus aureus* (45). Likewise, Gram (-) bacteria such as *Salmonella Montevideo* and E. coli O157:H7 were more susceptible to the antibacterial attack of AITC compared to *Listeria monocytogenes* (46).

On the other hand, the antibacterial activity of this active compound can be expressed through different mechanisms of action. In this sense, the following AITC mechanisms have mainly been reported: damage to the cell membrane of microorganisms, favoring greater permeability and leakage of cellular metabolites, and inhibition of cellular proteins (46).

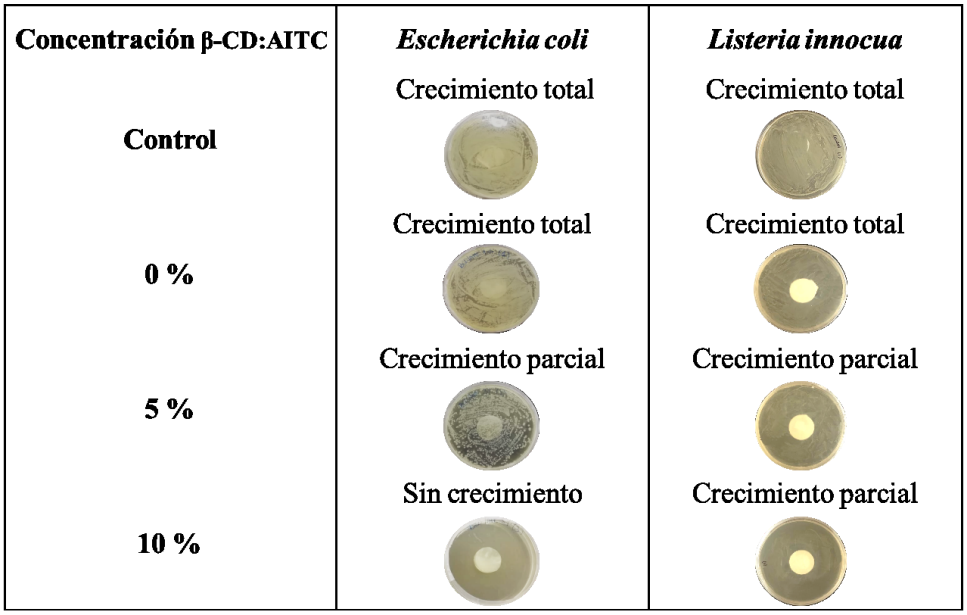


Figura 4

Antibacterial activity of the gels.

4. Conclusion

Inclusion complexes of β-cyclodextrin and allyl isothiocyanate with a 1:1 molar ratio was successfully obtained. The β-CD: AITC inclusion complexes exhibited a compact structure with a crystalline block-type morphology. On the other hand, more suitable conditions were identified for obtaining antibacterial alginate gels with a homogeneous structure. Such as the order of addition of the components and the rest temperature of the dispersion, the incorporation of an acidulant, the concentration of the cross-linking solution, and the cross-linking time. It should be noted that a packaging material with a homogeneous structure facilitates its applicability and, in addition, generates acceptability to the consumer. Regarding antibacterial activity, it was evidenced that gels loaded with 5% and 10% inclusion complexes inhibit the growth of pathogenic microorganisms that commonly cause the deterioration of fresh fruits and vegetables. This is the reason why the materials developed in this study could potentially be applied in a packaging system.

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Conflict of Interests

The authors declare not to have any interest conflicts.

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