UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSTGRADO

BIOFORTIFICACIÓN DE TOMATE Y LECHUGA UTILIZANDO COMPLEJOS DE QUITOSÁN-YODO PARA REDUCIR SU VOLATILIZACIÓN Y AUMENTAR SU DISPONIBILIDAD

Tesis

Que presenta IRMA ESTHER DÁVILA RANGEL

como requisito parcial para obtener el Grado de

DOCTOR EN CIENCIAS EN AGRICULTURA PROTEGIDA

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INTRODUCCIÓN

El yodo es un elemento esencial para el ser humano, pues está involucrado en la síntesis de las hormonas tiroideas triyodotironina y tetrayodotironina. El déficit de yodo es uno de los más prevalentes en el mundo en comparación de otros nutrientes. La falta de yodo puede ocasionar distintos padecimientos de gran importancia tales como: bocio, cretinismo, hipotiroidismo, déficit mental, muerte al nacer. Mayormente conocidos como desórdenes por deficiencia de yodo (IDD por sus siglas en inglés). Desde el año de 1920 surgió la yodación universal de la sal de mesa en Suiza y partes de Italia (Fuge y Johnson, 2015). Aunque los esfuerzos por disminuir el número de personas expuestas a déficit de yodo han sido muchos, el 30 % de la población mundial aún no disponen de las cantidades adecuadas de yodo en su ingesta diaria (Ma y Skeaff, 2017).

A pesar de que el yodo no se considera esencial para las plantas, algunos estudios señalan que el metabolismo del yodo ha tenido efectos en la actividad antioxidante, biomasa y que su acumulación ocurre principalmente en partes comestibles (Medrano-Macías et al., 2016). Siendo este último punto un aspecto de gran importancia para el ser humano ya que constituye un factor que aumentaría el interés por aplicar yodo en los cultivos.

Otro factor importante por considerar es la volatilización del yodo. La mayor parte del yodo presente en suelos proviene de las formas químicas del yodo metilado originado por la volatilización del yodo presente en el océano, derivado principalmente de algas marinas. Una vez en la atmósfera, por medio de lluvia o deposición en seco entra al sistema suelo-planta (Shetaya *et al*., 2012). El yodo molecular es un elemento volátil, también lo son las especies I^- y IO_3^- presentes en el suelo, las cuales pueden volatilizarse como hidruros (Sheppard *et al*., 1994). El yodo puede fijarse o unirse al suelo a través de atracciones electrostáticas débiles, también se ha sugerido que su retención puede estar asociada físicamente con el suelo y la materia orgánica (Sheppard y Thibault, 1992). Considerando lo anterior se han propuesto diversas técnicas para mitigar la volatilización del yodo, entre ellas el uso de materia orgánica, materiales adsorbentes o la aplicación de biopolímeros como el quitosán (Medrano-Macías et al., 2016).

Objetivo general

Evaluar el efecto de la aplicación de los complejos de quitosán-yodo (Cs-I) en forma líquida en cultivos de lechuga y tomate.

Objetivos específicos

- Cuantificar la asimilación de yodo en lechuga y tomate en función de la especie química de yodo aplicada con/sin polímero de quitosán.
- Evaluar el efecto de los complejos de Cs-I y sales de yodo en el crecimiento de las plantas de lechuga y el efecto en los frutos y en la calidad de poscosecha en tomate.
- Evaluar el contenido de minerales al aplicar los complejos de Cs-I en comparación con sales de yodo en lechuga.
- Determinar el impacto de los complejos de Cs-I y las sales de yodo aplicados en las variables agronómicas y bioquímicas en tomate y lechuga.

Hipótesis

El complejo de Cs-I permite una liberación prolongada del yodo, disminuyendo la volatilización y aumentando su disponibilidad en cultivos de *Lactuca sativa* L. y *Solanum lycopersicum* L.

REVISIÓN DE LITERATURA

El yodo en el ambiente

La volatilidad del yodo tiene un papel importante en su ciclo biogeoquímico. De los océanos ocurre transferencia a la atmósfera. La presencia del yodo en suelos es a través de la deposición de las lluvias y la nieve. El agua de mar, el principal reservorio de yodo contiene alrededor de 60 μg L^{-1} (Venturi, 2011). Diferentes estudios muestran que la presencia de I_2 en la atmósfera sobre los océanos puede tener relación con diversos factores; a) Las algas marinas bajo estrés oxidativo, pueden liberar I_2, b) el yodo es liberado de la superficie del océano por fotooxidación química, es decir cuando los iones yoduro son expuestos a la radiación solar, c) el O_3 atmosférico depositado en la superficie del mar reacciona con I^- de agua de mar para producir I_2 (Saiz-Lopez *et al*., 2012). En agua de mar el yodo se encuentra en forma de aniones como yoduro (I^-) y yodato (IO_3^-) con cantidades variables de complejos orgánicos (Wong, 1991).

A partir del metabolismo de las algas y microalgas se liberan a la atmósfera diversas formas de yodo tales como: yoduro de metilo o yodometano (CH_3I), yoduro de metileno o diyodometano (CH_2I_2), cloruro de yodometano (CH_2ICl) bromuro de yodometano (CH_2IBr), pero la mayor fuente de yodo atmosférico marino es el I_2 (Saiz y Glasow, 2012). Entre las especies de algas marinas, el género *Laminaria* metaboliza grandes cantidades de yodo; sin embargo, otros autores han reportado que especies como diatomeas son las mayores fuentes de yodo gaseoso (Manley y De La Cuesta, 1997). Los compuestos órgano-yodados una vez liberados en la atmósfera son fotodisociados produciendo compuestos como HI, INO_2, $IONO_2$, IO, OIO (Kaltsoyannis y Plane, 2008). La presencia del óxido de yodo en la atmósfera ha sido ligada a la formación de partículas ultra finas, importantes en la condensación de nubes (O'dowd *et al*., 2002). El yodo se encuentra en menor proporción en ríos, lagos y suelos. En el océano la presencia de yodo tiene un rango entre 45 y 60 μg L^{-1}, mientras que en la corteza terrestre su concentración es de aproximadamente 0.5 mg kg^{-1} y en la atmósfera la concentración varía entre 10 a 20 ng m^{-3} dependiendo de la cercanía al mar (Moreda-Piñeiro *et al*., 2011). El depósito en suelos es debido al desgaste de rocas, actividad volcánica, descomposición de la vegetación, lluvia, nieve y actividades antropogénicas. Los niveles en suelo representan un amplio

rango que va desde <0.1 a 150 mg kg^{-1} dependiendo del tipo de suelo y de la cercanía con el océano (Moreda-Piñeiro *et al*., 2011).

Impacto del yodo en la salud

El yodo es un elemento traza esencial para el ser humano, forma parte de las hormonas triyodotironina (T3) y tetrayodotironina (T4) producidas por la glándula tiroides. Dichas hormonas son esenciales para el metabolismo celular, crecimiento, desarrollo de estructuras del cuerpo, función y desarrollo neuronal (Zimmermann *et al*., 2008).

El requerimiento diario para el consumo de yodo, para niños o infantes de 0 a 59 meses es de 90 µg, para niños de 6 a 12 años es de 120 µg, en adultos y mayores de 12 años de 150 µg y para embarazadas y mujeres en lactancia es de 250 µg (WHO, 2007). Alrededor de 1.92 billones de personas en el mundo tienen un inadecuado consumo de yodo (Zimmermann y Andersson, 2012).

El yodo se absorbe en el ser humano a través de los pulmones y el tracto gastrointestinal (Black y Hounam, 1968; Morgan *et al*., 1968). El yodo en forma inorgánica es rápidamente absorbido cuando es inhalado en forma de vapor o en aerosol. El yodo absorbido o incorporado se ve reducido por alimentos que contienen goitrógenos como los tiocianatos, que se pueden encontrar en las especies brasicáceas (Ubom, 1991). El 90 % del yodo es eliminado por la orina y heces; mientras que, en la glándula tiroides el contenido total de yodo es de 10 mg (Hays, 2001). Sin embargo, también puede ser excretado en menor cantidad a través de la saliva, leche materna, sudor, lágrimas y al exhalar.

Uno de los principales problemas por el déficit de yodo es el bocio, el cual consiste en el agrandamiento de las glándula tiroides a consecuencia por los bajos niveles de T4 que a su vez provoca la síntesis de la hormona estimulante de la tiroides (TSH) y esto a su vez, la división de células foliculares (Gizak *et al*., 2017).

Yodo en alimentos

El yodo puede estar presente de forma natural en plantas en concentraciones menores que las dosis recomendadas en la ingesta diaria recomendada. Adicional a esto algunos vegetales contienen inhibidores de su absorción tales como los tiocianatos y sus

precursores como los glucosinolatos que pueden reaccionar con el yodo y reducir su biodisponibilidad (Moreda-Piñeiro et al., 2011).

En países industrializados la fuente más importante de yodo son los alimentos fortificados, tales como: leche de vaca de (27 a 47 μg kg^{-1}), huevos (93 μg kg^{-1}), granos y cereales (47 μg kg^{-1}), en menor proporción en pescado de agua dulce (30 μg kg^{-1}), carne y aves (50 μg kg^{-1}) y frutas (18 μg kg^{-1}) (WHO, 1996; Longman, 2003).

El uso del yodo/yodóforos como desinfectantes especialmente en la industria lechera, han contribuido a que ésta sea una fuente importante de este elemento (Pearce et al., 2004).

Aplicaciones y beneficios del uso de polímeros de quitosán

El quitosán (Cs), es un biopolímero de poli(N-acetil-D-glucosamina), es comercialmente preparado por la desacetilación de la quitina, la cual es obtenida del exoesqueleto de crustáceos marinos (Hong & Meyers, 1995; Ortega-Ortíz et al., 2003). El Cs tiene distintas propiedades, muchas de ellas con aplicación en el área agronómica, tales como:

Propiedades antimicrobianas. Esto depende del tipo de quitosán (nativo o modificado), su grado de polimerización, el cultivo, la composición química, la nutrición de las plantas, así como la temperatura y humedad del ambiente (Pospieszny *et al*., 1991; Rabea *et al*., 2003). Inhibe el crecimiento de un amplio número de bacterias (Muzzarelli *et al*., 1990).

Efecto antiviral. Inhibe la propagación de virus y viroides en plantas (Rabea *et al*., 2003). El nivel de supresión es de acuerdo con el peso molecular. Algunos ejemplos virus X de la papa, virus del mosaico de tabaco y de pepino.

Efecto insecticida. Varios derivados de Cs (p. ej. N-alquil- y N-bencil quitosanos) tienen actividad insecticida a dosis desde 0.32 hasta 5 g kg^{-1} en una dieta artificial en insectos (Rabea *et al*., 2005).

Agente de revestimiento de semillas. Se ha estudiado que el Cs aplicado como revestimiento de semillas, mejora el vigor de plántulas de maíz (Shao *et al*., 2005).

Quelación de nutrientes. El Cs puede ser un agente quelante esto quiere decir que puede secuestrar nutrientes y minerales como Fe y Cu entre otros, como consecuencia estos nutrientes no están disponibles para los patógenos (El Hadrami *et al*., 2010).

El efecto quelante del Cs, permite que los metales o elementos que sean volátiles se estabilicen formando complejos, permitiendo que estén en forma disponible para las

plantas por un mayor tiempo. De esta manera, a lo largo del ciclo del cultivo la planta pueda tener disponible los elementos tomándolos cuando así lo requiera, esto significa que no estarían interactuando con otras especies químicas que se encuentren en la solución del suelo.

Biofortificación de cultivos aplicando complejos Cs-I y sales de yodo

La biofortificación es una práctica agronómica que se usa para añadir a los cultivos, elementos traza esenciales para el ser humano. White y Broadley (2005) la definen como el proceso de incrementar la concentración de elementos esenciales en la parte comestible de los productos cosechados mediante la intervención agronómica. Varios estudios toman como modelo a especies hortícolas como la lechuga, repollo, tomate, cebolla, pepino, y zanahoria para la biofortificación (Medrano-Macías et al., 2016).

Las respuestas de la biofortificación con yodo dependerán de múltiples factores tales como: especie química de yodo, concentraciones aplicadas, cultivo y factor ambiental. Diversos estudios señalan las ventajas de biofortificar con sales de yodo, aunque a la fecha no se encontraron estudios sobre biofortificación con complejos de Cs-I; éstos sólo han sido evaluados como aplicaciones de películas comestibles. Se ha visto que con aplicaciones foliares de KIO_3 se aumenta la acumulación de yodo con respecto a la fertilización al suelo (Smoleń *et al*., 2011). En película comestible de Cs-I aplicado en frutos de tomate, el complejo extendió la vida de anaquel (Limchoowong *et al*., 2016). En *Capsicum* la película de Cs-I, no tuvo efectos en las actividades enzimáticas, no modificó la frescura y mantuvo una buena vida de anaquel (Limchoowong *et al*., 2018). En lechuga biofortificada con IO_3^- se incrementó la actividad de las enzimas superóxido dismutasa (SOD), ascorbato peroxidasa (APX), catalasa (CAT) y la concentración de ácido ascórbico, los cuales tienen la propiedad de aumentar el sistema de defensa contra patógenos o radicales libre (Blasco et al., 2011). En otro estudio, se indicó que el yodo tiene una alta absorción y eficiencia cuando se combina con ácidos húmicos y fúlvicos los cuales funcionan como agentes acomplejantes, sin mostrar efectos negativos en el rendimiento de espinaca (Smoleń *et al*., 2016). Al biofortificar con I^- aumentó el contenido de fenoles, flavonoides, antocianinas y ascorbato en contraste con IO_3^- en lechuga (Blasco et al., 2008). Al biofortificar con IO_3^- se promovió el crecimiento más que

con I^-; es recomendado como un compuesto benéfico contra el estrés por cadmio, ya que incrementó los sólidos solubles, fructosa, glucosa, ácidos ascórbico y fenoles, sin afectar o provocar daños en frutos de tomate (Kiferle *et al*., 2013; Gupta *et al*., 2015;).

Transporte de yodo

El yodo puede ser absorbido a través de las células de la epidermis de la raíz, en sus formas biodisponibles como I^- o IO_3^- que pueden estar presentes en solución del suelo o ser añadidas en solución. Sin embargo, para poder ser transportados a los diversos órganos de las plantas, el IO_3^- tiene que ser previamente reducido a I^-, esto se logra a través de la enzima yodato reductasa (Kato *et al*., 2013) o nitrato reductasa (Medrano-Macías *et al*., 2016), esto significa que el IO_3^- funciona como un sustrato para estas enzimas y por lo tanto lo vuelve menos tóxico que el I^-. En el caso del I^-, no se requiere ningún proceso previo, de tal forma que si en el suelo o solución nutritiva hay grandes cantidades de yodo en esta forma iónica pueden acumularse en la planta causando diversos efectos fitotóxicos. Una vez que el yodo es absorbido, puede estar presente como yodo orgánico o I^- (Humphrey *et al*., 2019), éste es transportado vía xilema a tallos, hojas, frutos y semillas. Además, se sabe que el yodo inorgánico y compuestos organoyodados (CH_3I y CH_2I_2 entre otros) se transportan por vías de simplasto y apoplasto. En un estudio en espinaca, el 90% de yodo se encontró en el apoplasto como yodo orgánico y el remanente como I^- (Humphrey *et al*., 2019). Los autores de ese estudio sugieren que existe una rápida conversión de yodo inorgánico a yodo orgánico y que ésta toma lugar en las raíces. Otro mecanismo de absorción de yodo por raíces ha sido estudiado en arroz, señalando que el yodo puede ser absorbido en forma de yodo molecular (I_2) oxidado, gracias al poder oxidante de las raíces, esto ocurre cuando perciben un exceso del I^-, usando este mecanismo para no verse afectadas, puesto que su ambiente es reductor debido a los suelos inundados (Yamada *et al*., 2005).

En la actualidad, han sido poco estudiado los mecanismos implicados en el transporte de yodo. A la fecha, se sabe que, en las células de la raíz, el yodo puede fluir a través de las membranas por canales del ion cloruro (Cl^-), transportadores de H^+/haluro o por medio de los transportadores de aniones (White & Broadley, 2001; Landini et al., 2012; Medrano-

Macías et al., 2016) e inclusive el transporte de yodo de la raíz a brotes puede estar regulado por los mismos (Kato *et al*., 2013).

De acuerdo a varios estudios, el I^- es la especie química más fácilmente absorbida por las plantas. Sin embargo varios autores coinciden que puede llegar a ser fitotóxico e inclusive a concentraciones similares al IO_3^- (Mackowiak *et al*., 2005; Weng *et al*., 2008). Se ha visto que la toxicidad del I^- es consecuencia de la oxidación intracelular o pérdida de electrones convirtiéndose en yodo molecular, que puede unirse a componentes celulares incluyendo la clorofila (Mynett y Wain, 1973). La tasa de absorción del yoduro es diferente en cultivos sin suelo y con suelo. En general parece que el I^- es rápidamente disponible para las plantas en cultivos sin suelo, mientras que bajo condiciones de campo, está sujeto a más pérdidas que el IO_3^- *(*Lawson *et al*., 2015).

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Artículo

COMPARISON OF IODIDE, IODATE, AND IODINE-CHITOSAN COMPLEXES FOR THE BIOFORTIFICATION OF LETTUCE

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Comparison of Iodide, Iodate, and Iodine-Chitosan Complexes for the Biofortification of Lettuce

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Featured Application: Use of Cs-I complexes as material to biofortify soilless crops with iodine. under conditions of protected agriculture.

Abstract: Iodine is an essential trace nutrient for humans; its deficit can affect motor and cognitive development. Biofortifying crops with iodine is a way of promoting the adequate intake of this element. The uses of chitosan-iodine complexes for crop biofortification have not been previously studied. The present work evaluated the effects of KIO_3 and KI salts, chitosan-KIO_3 complex (Cs-KIO_3), and chitosan-KI complex (Cs-KI) application on lettuce, with a chitosan-only treatment as a control and water as the absolute control. Each treatment involved the application of 0, 5, and 25 mg I kg^{-1} soil applied before transplanting or 25 mg I kg^{-1} soil applied as split doses of 12.5 mg kg^{-1}, once immediately before transplanting and the second application 15 days later. Single application of Cs-KIO_3 at 5 and 25 mg I kg^{-1} increased lettuce biomass while the split-dose application (SDA) of Cs-KI (25 mg I kg^{-1}) led to a decrease in biomass. Maximum accumulation of iodine in lettuce was observed after the application of KIO_3 (25 mg I kg^{-1}) in two parts. This study shows that the use of chitosan complexes, especially Cs-KIO_3, may be a viable alternative for crop biofortification with iodine without affecting crop yields.

Keywords: trace element; biopolymer; iodine; complex; biofortification

1. Introduction

Iodine is an essential trace element for humans. Iodine deficit can cause iodine deficiency disorders (IDD), which, according to some estimates, affect around two billion people [1]. Recommended daily iodine intake levels are 90 µg for preschool children (0 to 59 months), 120 µg for children 6 to 12 years old, 150 µg for adults over 12 years old, and 200 µg for pregnant and nursing women, according to the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), and the

International Council for Control of Iodine Deficiency Disorders (ICCIDD) [2]. Some experts believe that doses of 1–2 mg per day are probably safe for most people, although not all [3,4]. A worldwide initiative in 1920 led to the universal iodization of table salt, which led to a significant increase in iodine intake. Unfortunately, inorganic iodine salts have a disadvantage in that up to 20% can be lost through volatilization, on top of the percentages that are lost depending on the type of cooking used [5–7]. The WHO recommends that salt consumption not surpass more than 5 g per day, equivalent to >2 g of sodium per day, as high salt intake combined with insufficient potassium intake (<3.5 g per day) can provoke high blood pressure. Because of that, the WHO aims to reduce salt intake by 30% in the worlds' population by 2025 [8]. Thus, alternative options for adequate iodine intake while reducing salt consumption are necessary.

The iodine supplementation of foods or the biofortification of plant-based foods would be one alternative for maintaining iodine intake while reducing table salt consumption. The advantage of biofortification is that the iodine is found in organic forms that have greater bioavailability and stability against volatilization than the inorganic salt forms. Another advantage of biofortification is that it can be applied to frequently consumed or traditional crops in certain regions [9].

Iodine is not considered an essential nutrient for terrestrial plants; however, it plays an important part of algae metabolism [9]. The chemical forms of iodine most commonly used for crop biofortification are potassium iodide and potassium iodate. In lettuce, biofortification with different doses of iodide (I^-) and iodate (IO_3^-) cause changes in biomass, enzymatic activity, and antioxidant activity, suggesting they might cause toxicity in plants [10–14]. The type of iodine application, either sprayed on the foliage or applied directly to the growth substrate or soil, can affect the final concentrations of iodine in the edible parts of the plants. Some studies have found foliar iodine applications to be more effective than soil applications [11,15]. However, foliar applications are reportedly less effective for biofortifying fruits or seeds, supposedly due to lower iodine mobility through phloem when applied via foliar spray [9,16,17]. When iodine is applied to soil or substrate, its bioavailability depends on the volatilization rate and its interaction with other soil components like metal oxides or hydroxides. For example, $Fe(OH)_3$, $Al(OH)_3$, and MnO_2, can play an important role in the determination of iodine behavior in soil, both through inorganic iodine adsorption and iodine oxidation [18–20].

Chitosan (Cs) is a biodegradable, biological polymer that functions as a metal and trace metal complexing agent. Chitosan also possesses a wide variety of properties: it is a plant elicitor as it induces phytoalexin production; it protects plants from pathogen diseases as it inhibits microbial growth; it activates MAP-kinases and provokes chromatin alterations; and it participates in the synthesis of alkaloids and plant growth regulators. It is also biodegradable, biocompatible, induces several plant defense genes, and induces proteinase inhibitors. Responses to its application will depend on the concentration, plant species, and growth phase [21–24].

The electrostatic interactions between the amino group (positively charged; $–NH_2$) and iodate (negatively charged; $–IO_3$) are quite pronounced [7,25]. An increase in the degree of deacetylation of chitin leads to a greater complexing capacity, as that ability is related to the content of NH_2 [26]. To date, the exact mechanism by which the rhizosphere operates with the chitosan-iodine complexes is unknown. Iodide or iodate ions can be absorbed by the roots because they are bound to Cs only by electrostatic interactions that are weak [27]. It is possible that exchange reactions occur between the natural chelates of the roots (organic acids) and the chitosan-iodine complexes, this is due to the high molecular weight of the Cs that may not be absorbed. A study in sunflower with iron chelated by humic acids of low and high molecular weight refers to this [28]. On the other hand, it has been shown that Cs and Cs nanoparticles can be in the cell walls of the subsidiary cells of the lower epidermis [29]. However, no reports were found that indicated the similar effect on roots.

The present study was undertaken with the hypothesis that it would be possible to increase the absorption of iodine applied to substrate if it was applied as an iodine-chitosan complex. The objective was to determine if potassium iodide or iodate complexed with chitosan led to more effective lettuce biofortification than either salt in their uncomplexed forms.

2. Materials and Methods

2.1. Preparation of Cs-KI, Cs-KIO_3 Complexes and Iodine Salts

The Cs-KI and Cs-KIO_3 complexes were prepared in the Center for Applied Chemistry Research (CIQA). The chitosan used had a viscometric molecular weight of 200,000 g/mol and a 98% degree of deacetylation. First, a 1% Cs solution in 1% acetic acid (AcOH) was prepared by adding the Cs little by little over 3 h, with stirring at 300 rpm until completely dissolved, at a temperature of 60–65 °C. The resulting solution was filtered and adjusted to 1 L, for later use as the Cs controls.

For the iodine complexes, solutions of 0.1 mol dm^{-3} potassium iodine (KI) and 0.1 mol dm^{-3} potassium iodate (KIO_3) were dissolved in 1% Cs solution and adjusted to obtain complexes with Cs:I molar ratios of 5. Thus, complexes with 1.06 mg I per milliliter of complex solution were obtained.

For iodine salt-only treatments, solutions of 0.025 mol dm^{-3} KI and 0.025 mol dm^{-3} KIO_3 were prepared in 1 L deionized water. Each solution contained 3.17 mg I per milliliter.

2.2. Vegetable Material and Applied Treatments

The experimental work was performed in August 2017, in a greenhouse belonging to the Department of Horticulture, Agrarian Autonomous University Antonio Narro, in Saltillo, Mexico. Lettuce (*Lactuca sativa*), cv "Great Lakes," seeds were sown in 200-cell polystyrene trays filled with peat moss/perlite (1:1 *v/v*) mix. When the seedlings reached a size of approximately 15 cm, they were transplanted to 4 L, black polyethylene pots filled with peat moss and perlite mix (1:1 *v/v*). Before applying any treatments, a field capacity test was performed to evaluate the weight of the substrate. On average, a weight of 3 kg for substrate moistened to field capacity was obtained for each pot. The iodine concentrations used were 0, 5, and 25 mg kg^{-1} wet substrate, applied once, as well as 25 mg I kg^{-1} wet substrate, applied as two doses of 12.5 mg kg^{-1}: before and 15 days after transplanting (d.a.t).

2.2.1. Treatments Applied before Transplant

For the treatments applied prior to transplanting, the total volume of substrate per pot was divided into three parts, while the iodine salt, Cs-KI, and Cs-KIO_3 treatments were split into two parts. The first part of each treatment was applied over the first third of substrate in the pot. Another third of substrate was added on top and the remaining half of the treatment was applied over the top. Afterwards, the remaining third of substrate was added to fill the pot. The lettuce seedlings were transplanted to those pots. We assume this procedure allows the plant roots to have I^- and IO_3^- as salts or Cs complexes available as they grow.

Absolute controls (AC) only had water applied during the pot-filling process. Cs controls (CsC) had a total of 70.3 mL 1% Cs solution applied, to reach 0.2 g Cs per kg of substrate. Each treatment received the same final volume of Cs.

For the 5 mg I kg^{-1} substrate iodine salt treatments, a total of 4.7 mL of 0.025 mol dm^{-3} KIO_3 and 0.025 mol dm^{-3} KI, respectively, were added per pot. The 25 mg I treatments required the addition of 23.6 mL of each respective solution per pot.

A total of 14.07 mL of 0.1 mol dm^{-3} Cs-KI and 0.1 mol dm^{-3} Cs-KIO_3 solutions were added to the respective pots for the 5 mg I kg^{-1} treatments. To this, 56.2 mL 1% Cs solution per pot were also added in order to have the same Cs volume as in the controls. For the 25 mg I treatments, 70.34 mL of 0.1 mol dm^{-3} Cs-KI or 0.1 mol dm^{-3} Cs-KIO_3 was added per pot.

2.2.2. Treatments Applied before and after Transplant

For each split dose application-SDA (12.5 and 12.5 mg kg^{-1}) treatment, the same pot-filling procedure described above was used. All treatments received concentrations of 25 mg I kg^{-1} substrate. The treatment volumes were divided in two, with each half applied while filling the pots by thirds. The last half of the treatment was applied after adding the second third of substrate, then the pot

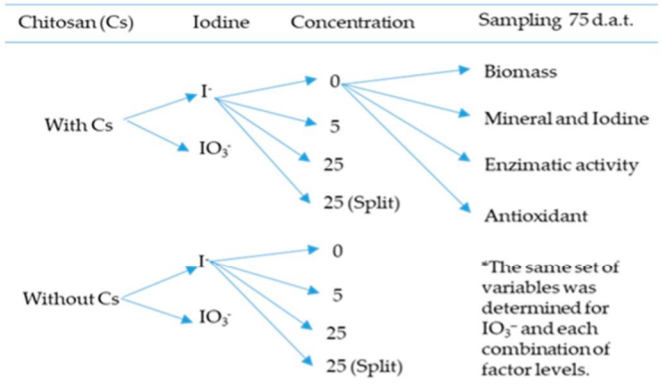
was filled by adding the last third of substrate. Afterwards, transplanting of the lettuce seedlings was performed as normal. The second application of the different treatments was applied by drench directly to the substrate at 15 d.a.t.

The experimental factors were arranged into a 2 × 2 × 4 factorial array (iodine species × Cs polymer × concentration) and a totally randomized experimental design was utilized. The treatments thus evaluated were: (1) Control-Cs (CsC); (2) absolute control (AC); (3) KIO_3 5 mg I kg^{-1} with Cs; (4) KIO_3 25 mg I kg^{-1} with Cs; (5) KIO_3 25 mg I kg^{-1} with Cs, split-dose application; (6) KIO_3 5 mg I kg^{-1} without Cs; (7) KIO_3 25 mg I kg^{-1} without Cs; (8) KIO_3 25 mg I kg^{-1} without Cs, split-dose application; (9) KI 5 mg I kg^{-1} with Cs; (10) KI 25 mg I kg^{-1} with Cs; (11) KI 25 mg I kg^{-1} with Cs, split-dose application; (12) KI 5 mg I kg^{-1} without Cs; (13) KI 25 mg I kg^{-1} without Cs; and, (14) KI 25 mg I kg^{-1} without Cs, split-dose application. Two Cs controls and two absolute controls were also included, for 16 total treatments. The control groups were included in order to evaluate the potential effects that location within the greenhouse, where the rest of the treatments where distributed, might have. There were 12 experimental units per treatment, from which 6 plants were chosen at random for evaluation. A total of 96 plants were used for experimental evaluations.

An automated, single step drip irrigation system was used throughout the experiment. Steiner nutrient solution (25%) was used for irrigation. A volume of 0.5 L solution was applied two times a day per pot. The solution pH was adjusted to 5.5–6.5 with phosphoric acid. Throughout the cultivation cycle, the electrical conductivity (EC) of the nutrient solution was maintained at approximately 1.40 mS cm^{-1}. Preventative treatment for whitefly was also applied. Within the greenhouse, the minimum temperature reached 8.3 °C, while the average was 17.7 °C, and the high was 32.4 °C. The minimum relative humidity was 30.3%, while the average and high were 54.8% and 75.5%, respectively. The mean photosynthetically active radiation (PAR) was 432.3 µmol m^{-2} s^{-1} while the maximum was 864.7 µmol m^{-2} s^{-1}, as measured with a WatchDog 1000 Series Micro Station, Spectrum technologies Inc., Aurora, IL, USA).

2.3. Sampling

The lettuces were harvested at 75 d.a.t. The 6 randomly chosen plants from each treatment were measured and weighed for their evaluation of biomass, mineral content, and iodine content (see Scheme 1). From those same plants, small samples from the central part of the heads were collected for the biochemical analyses. These lettuce head samples were kept in deep freeze and lyophilized.



Scheme 1. Treatments and general variables evaluated.

2.4. Biomass Production and Yields

Lettuce heads and leaves were weighed on an precision balance (OHAUS). The values were recorded as the biomass fresh weight (FW). A small part from the central part of each head (~10 g) was taken and stored at −86 °C for later determination of antioxidant and enzymatic activity. The heads'

remaining fresh matter was placed in paper bags. The leaves were separated and also placed in paper bags. Both head stems and leaves were dried in dehydrating ovens for 72 h at 75 ± 5 °C. Afterwards, their dry weights (DW) were recorded. Finally, mixed samples were prepared by combining the dry leaves and heads from each replica for each treatment. The mixed samples were placed in hermetic plastic bags for mineral analysis.

2.5. Mineral Content

Determination

Mineral content was determined from mixed samples of leaves and heads from 6 randomly chosen plants per treatment. Total nitrogen (N) content was determined according to the Kjeldahl method [30]. A sample of dry matter (0.05 g) was weighed out and then acid digested in 4 mL of digest mix (1 L of concentrated sulfuric acid with 25 g of potassium sulfate, 10 g of mercury red oxide, and 25 mL of saturated copper sulfate solution). The acid digest was then distilled along with 25 mL 50% sodium hydroxide. From the resulting distillate, 30 mL were placed in a beaker containing 2.2% boric acid and 4 drops of bromocresol green and methyl red. The samples were titrated with 0.025 N sulfuric acid. N content was calculated from the volume of sulfuric acid consumed.

Phosphorus content was determined through spectrophotometry [30]. Readings were performed on a Genesys 10S ultraviolet–visible (UV–Vis) spectrophotometer (Thermo Scientific, Waltham, MA, USA) at 640 nm. The contents of K, Mg, Ca, Na, Mn, Zn, and Fe were determined by atomic absorption spectrometry following wet digestion [31]. One gram of dry matter was digested with nitric acid at 100 °C. Afterwards, the solution was filtered through Whatman, Buckinghamshire, United Kingdom (No. 42 ash-free) paper filters, diluted accordingly, and measured in a Varian AA-1275 flame atomic absorption spectroscope (Palo alto, CA, USA).

2.6. Iodine Content Determination

The alkaline ash technique [32,33] was used to determine iodine content. A sample of dried, ground leaves and heads (0.5 g) was weighed out. Six replicas per treatment were evaluated. The sample was placed in crucible of known, constant weight and 2 mL 2 mol dm^{-3} KOH and 1 mL 2 mol dm^{-3} KNO_3 were added. After adding the reagents, pre-digestion took place while incubating in a stove at 100 °C for 2 h. The crucibles were placed in a muffle furnace at 580 °C for 3 h. After cooling back down to room temperature, the ashes were transferred to a conical tube for extraction with 2 mL 2 mM KOH. The samples were centrifuged at 12,000 rpm for 15 min. Finally, 1 mL of supernatant was decanted, and the volume adjusted to 10 mL with 2 mol dm^{-3} KOH. Quantification was performed with an Agilent 725 ICP-OES (Inductively coupled plasma-optical emission spectrometry, Santa Clara, CA, USA).

2.7. Chlorophyll Content Determination

The contents of chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll were determined according to the technique described in Munira et al. [34]. A sample (1 g) of fresh plant material was mixed with 5 mL 90% acetone. A pinch of magnesium carbonate was added to protect and stabilize the chlorophylls. From the homogenized mixtures, 2 mL were taken, placed in a 2 mL tube, and centrifuged for 5 min. at 10,000 rpm and 4 °C. The supernatants were decanted and the absorbances of Chl a and Chl b were read at 663 nm and 645 nm, respectively. The total chlorophyll, Chl a, and Chl b content were expressed in mg g^{-1}, and were calculated according to the following formulas:

$$\text{Chlorophyll a } (\text{mg}\cdot\text{g}^{-1}) = 25.38 \times A_{663} + 3.64 \times A_{645} \quad (1)$$

$$\text{Chlorophyll b } (\text{mg}\cdot\text{g}^{-1}) = 30.38 \times A_{645} - 6.58 \times A_{663} \quad (2)$$

$$\text{Chlorophyll, total } (\text{mg}\cdot\text{g}^{-1}) = 18.8 \times A_{663} + 34.02 \times A_{645} \quad (3)$$

2.8. Biomolecule Extraction

Lyophilized leaves ground into fine powder with a pestle and mortar were used for biomolecule extraction. From each treatment, 200 mg samples of lyophilized leaves were placed in polypropylene microtubes. Polyvinylpyrrolidone (20 mg) and 1.5 mL 0.1 mol dm^{-3} phosphate buffer (pH 7–7.2) were added, the samples were sonicated for 5 min. and afterwards, centrifuged at 12,500 rpm for 10 min. at 4 °C. The supernatants were decanted and filtered through a nylon membrane. The filtrates were diluted 1:15 with phosphate buffer [35].

2.9. Total Protein (TP) Content Determination

The technique described by Bradford [36] was used for total protein content determination. From the biomolecule extracts, 100 µL of extract were taken, placed in a test tube, and 1 mL of Bradford (Hercules, CA, USA) reagent was added. The samples with Bradford reagent were left to incubate for 5 min. Afterwards, the absorbance was read at 595 nm in a Genesys 10S UV–Vis spectrophotometer (Thermo Scientific). The results were recorded, and concentrations were extrapolated from a calibration curve prepared with bovine serum albumin (BSA). The protein concentrations were reported as mg g^{-1}.

2.10. Free and Cell Wall-Bound Phenol Determination

Phenolic compound content was determined according to the technique described by Gurr et al. [37]. Extraction was performed on 6 dried samples from each treatment. Methanol (1 mL) was added to each sample, the samples were vortexed, then centrifuged at 13,500 rpm for 15 min. The supernatants were decanted into vials and stored for the determination of free phenols. The remaining pellets were incubated with 0.25 mL 2 mol dm^{-3} NaOH for 16 h at 70 °C. Then, 0.25 mL 2 N hydrochloric acid was added, and the samples were centrifuged at 13,500 rpm for 15 min. The resulting pellets were discarded, and the supernatants saved, as they were considered to contain the cell wall-bound phenols. To quantify the free and cell wall-bound phenols, 20 µL of the corresponding supernatant were taken and mixed with 980 µL distilled water, then 100 µL Folin–Ciocalteau reagent was added, and the mixture left to incubate for 5 min. Subsequently, 600 µL of sodium bicarbonate solution saturated with 0.1 mol dm^{-3} NaOH were added. The samples were left to incubate for 2.5 h. The sample absorbance was read at 725 nm and the results were expressed as mg of gallic acid equivalents per g^{-1} dry weight (DW).

2.11. Superoxide Dismutase (SOD) Determination

The biomolecule extract was assayed for superoxide dismutase (SOD) activity using the CAYMAN®SOD, assay kit (Ann Arbor, MI, USA). SOD activity can be quantified spectrophotometrically by measuring the oxidation of WST (water soluble tetrazolium salt) to WST-formazan by superoxide ions created by xanthine (X)/xanthine oxidase complexes. The inhibition of WST oxidation is attributed to the neutralization of superoxide radicals by SOD. The units of SOD activity were expressed in U/mL. The microwell assay plates were read at 450 nm in an Elx808 plate reader (BioTek, Winooski, VT, USA).

2.12. Catalase Activity Determination

Catalase activity was quantified by spectrophotometry. Two reaction times, T0 and T1, were recorded for this technique. The blank solution was prepared by mixing 0.1 mL biomolecule extract, 1 mL phosphate buffer (pH 7.2), and 0.4 mL of 5% H_2SO_4. For measuring the reactions at T0, 0.1 mL of biomolecule extract was mixed with 1 mL 100 mM H_2O_2 and immediately after, 0.5 mL 5% H_2SO_4. For the T1 samples, the same mix was prepared except that the 0.5 mL 5% H_2SO_4 was added after 1 min. The reaction between extract and peroxide occurred at 20 °C with constant agitation. Finally, the consumption of H_2O_2 was determined by reading the absorbance at 270 nm in a

UV–Vis spectrophotometer. The catalase activity units were expressed in mM H_2O_2 per total protein content [38].

2.13. Glutathione Peroxidase (GPX) Quantification

Quantification of glutathione peroxidase was performed using the method described by Xue et al. [39], using H_2O_2 as a substrate. Biomolecule extract (0.2 mL), 0.1 mol dm^{-3} reduced glutathione (0.4 mL), and 0.067 mol dm^{-3} Na_2HPO_4 (0.2 mL) were placed in a test tube. The mixture was heated in a 25 °C water bath for 5 min., then 1.3 mM H_2O_2 (0.2 mL) was added to start the catalytic reaction. The mixture was allowed to react for 10 min. and then was stopped with the addition of 1 mL 1% trichloroacetic acid. The mixture was left in an ice bath for 30 min. Afterwards, it was centrifuged at 3000 rpm for 10 min. A sample of supernatant (0.48 mL) was placed in a test tube, along with 2.2 mL of 0.32 mol dm^{-3} Na_2HPO_4 and 0.32 mL of 1 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). The absorbance of the reaction mixture was read at 412 nm in a UV–Vis spectrophotometer. The catalytic activity of glutathione peroxidase was expressed as mM glutathione per minute per total protein content.

2.14. Glutathione (GSH) Quantification

Glutathione (GSH) content was quantified according to the spectrophotometric technique established by Xue et al. [39], utilizing the reaction with 5,5′-DTNB. Biomolecule extract (0.48 mL), 0.067 mol dm^{-3} Na_2HPO_4 (2.2 mL), and 1 mM DTNB dye (0.32 mL) were placed in a test tube and mixed. The absorbance of the samples was read at 412 nm in a UV–Vis spectrophotometer. Values were reported as mg L^{-1}.

2.15. Antioxidant Capacity Determination by ABTS $^{\bullet+}$

Total antioxidant content was determined using the CAYMAN®Antioxidant assay kit. The assay determines antioxidant content based on a sample's ability to inhibit the oxidation of $ABTS^{\bullet+}$ (2,2′-azino-di-[3-ethylbenzthiazoline sulphonate]). The quantity of ABTS radicals produced was determined by reading the microwell plate at 405 nm. The reported values were expressed in mM.

2.16. Statistical Analyses

A completely randomized experimental design was used, with 6 repetitions per treatment. A single pot with one plant was considered an experimental unit. The collected data was subjected to analysis of variance (ANOVA) and to separation of means using Fisher's least significant difference (LSD, $p = 0.05$) tests, using the 2018 InfoStat statistical analysis software package (InfoStat Group, Córdoba, Argentina).

3. Results

3.1. Biomass Production and Yields

According to the ANOVA presented in Table 1, the concentration of iodine (C) only had no impact on the leaf dry weight (LDW) of lettuce. On average, there were positive effects on biomass at iodine concentrations of 5 mg kg^{-1} observed. No interactions between C and the presence of Cs or the different iodine species were observed. Also, there were no treatments that affected all the biomass variables evaluated. For example, some treatments, such as Cs-KIO_3 (5 mg kg^{-1}), significantly increased leaf dry weight (LDW) while others (doubly applied Cs-KI, 25 mg kg^{-1}) significantly decreased the lettuce head fresh weight (HFW). It was only for the leaf fresh weight (LFW) that an interaction between iodine and Cs could be observed, as that value increased following application of Cs-KIO_3 (5 mg kg^{-1}). The two factors together appeared to play an important role in biomass increase. However, in general, for the evaluated biomass parameters, the source of iodine was an important determining factor, since there were significant differences associated with this variable, except for the head dry weight parameter.

Table 1. Comparison of lettuce biomass means.

Treatments	Concentration (mg I kg^{-1} Substrate)	g $plant^{-1}$ (n = 6)			
		LFW	HFW	LDW	HDW
CsC	0	212.00 b–d	667.63 ab	17.62 c–e	28.48 a–d
Cs-KIO_3	5	294.32 a	695.52 a	24.27 a	34.05 a
Cs-KIO_3	25	264.95 ab	634.78 ab	22.50 ab	33.80 a
Cs-KIO_3 SDA	25	235.85 a–c	582.67 ab	19.58 a–c	29.28 a–c
AC	0	146.72 e–f	534.50 ab	14.63 de	22.37 d–e
KIO_3	5	201.42 c–f	579.57 ab	18.50 b–d	19.13 ef
KIO_3	25	201.17 c–f	595.68 ab	16.97 c–e	24.65 b–e
KIO_3 SDA	25	178.15 c–f	499.05 b	16.65 c–e	19.95 ef
CsC	0	196.97 c–f	596.07 ab	17.57 c–e	24.20 b–e
Cs-KI	5	190.12 c–f	577.57 ab	16.47 c–e	24.92 b–e
Cs-KI	25	155.77 d–f	526.18 ab	13.67 e	22.70 c–e
Cs-KI SDA	25	143.04 f	280.17 c	14.73 de	15.03 f
AC	0	194.55 c–f	499.48 b	17.37 c–e	25.87 b–e
KI	5	214.85 b–d	587.67 ab	19.13 b–d	29.57 ab
KI	25	205.10 b–e	581.43 ab	18.90 b–d	27.48 a–d
KI SDA	25	177.78 c–f	495.32 b	17.43 c–e	24.95 b–e
Concentration (C)		0.0311	0.0077	ns	0.0198
Chitosan (Cs)		0.0494	ns	ns	ns
Chemical species (CSp)		0.0041	0.0116	0.0229	ns
C × Cs		ns	ns	ns	ns
C × CSp		ns	ns	ns	ns
Cs × CSp		<0.0001	ns	0.0001	<0.0001
C × Cs × CSp		ns	ns	ns	ns

Means with the same letter are statistically identical (least significant difference (LSD), $p \leq 0.05$); sample size, 6 (n = 6), ns, not significant. SDA, split dose application; LFW, leaf fresh weight; HFW, head fresh weight; LDW, leaf dry weight; HDW, head dry weight.

3.2. Mineral Content

3.2.1. Macronutrient Content

Neither iodine source, be it I^- or IO_3^-, generally appeared to have antagonistic effects on the macroelements evaluated. The iodine species used, their concentrations, and the presence of Cs polymer had effects on various elements, when compared to other treatments, although there were hardly any differences between treatments and their respective controls with and without Cs (Table 2). Only magnesium concentrations increased significantly after application of 5 mg I kg^{-1} Cs-KI. There were significant differences among the Cs controls, although this was probably due to their location within the greenhouse. This is why the increases in Ca and Na after application of Cs-KI (5 mg kg^{-1}) cannot be considered significant, because even though their closest, neighboring Cs controls had lower contents of those elements, comparison with the other chitosan controls showed no differences. The interaction between the effects of concentration and iodine species were statistically significant for the concentrations of almost all the elements evaluated, except for K. On average, application of IO_3^- salts led to greater accumulation of macroelements than their I^- counterparts. However, these differences were not significant with respect to the controls.

3.2.2. Micronutrient Content

The source of iodine (I^- or IO_3^-) had a significant effect on the concentrations of microelements in lettuce. As observed with the macroelements, there were no antagonistic effects between the application of iodine and the concentrations of microelements (Table 3). Apart from that, there was a significant increase in Mn when Cs-KI is applied (single application, 25 mg I kg^{-1}). On the other hand, it appears that the interaction between iodine concentrations and Cs did not exert any significant effects on microelement concentrations.

Table 2. Macroelement contents in mixed samples of lettuce leaves and heads.

Treatments	Concentration (mg I kg^{-1} Substrate)	% Dry Weight (n = 6)					
		N	P	K	Ca	Mg	Na
CsC	0	0.82 b	0.43 a	13.27 a	4.20 ab	5.78 b	13.97 a
Cs-KIO_3	5	1.29 ab	0.40 a	12.01 ab	2.25 cd	3.91 c	7.57 b
Cs-KIO_3	25	1.48 a	0.24 b–e	10.81 a–c	2.08 c–e	3.62 cd	3.91 bc
Cs-KIO_3 SDA	25	1.40 ab	0.25 b–d	8.06 b–d	1.00 d–f	2.20 de	3.24 c
AC	0	1.33 ab	0.26 b–d	8.15 b–d	1.71 c–f	1.91 e	3.16 c
KIO_3	5	1.21 ab	0.20 c–g	7.73 b–d	1.87c–f	2.25 c–e	3.41 c
KIO_3	25	1.55 a	0.22 b–f	8.06 b–d	2.95 bc	3.08 c–e	6.03 bc
KIO_3 SDA	25	1.36 ab	0.29 b	6.61 cd	1.50 d–f	2.70 c–e	4.32 bc
CsC	0	1.21 ab	0.20 c–g	6.78 cd	1.41 d–f	2.33 c–e	3.78 bc
Cs-KI	5	1.43 ab	0.28 bc	7.48 b–d	5.24 a	7.90 a	14.26 a
Cs-KI	25	1.19 ab	0.24 b–d	6.91 cd	1.29 d–f	2.33 c–e	3.25 c
Cs-KI SDA	25	1.01 ab	0.12 g	5.49 d	1.21 d–f	2.20 de	3.29 c
AC	0	1.13 ab	0.15 e–g	5.53 d	0.75 ef	2.04 de	2.62 c
KI	5	1.18 ab	0.13 g	8.02 b–d	0.96 d–f	2.37 c–e	5.41 bc
KI	25	0.93 ab	0.14 fg	6.15 cd	1.08 d–f	1.91 e	2.95 c
KI SDA	25	1.07 ab	0.19 d–g	4.16 d	0.67 f	1.54 e	2.53 c
Concentration (C)		ns	0.0001	0.0004	<0.0001	<0.0001	ns
Chitosan (Cs)		ns	<0.0001	<0.0001	<0.0001	<0.0001	0.0107
Chemical Species (CSp)		0.0147	<0.0001	<0.0001	<0.0001	0.0368	ns
C × Cs		ns	<0.0001	ns	<0.0001	<0.0001	ns
C × CSp		0.006	<0.0001	ns	<0.0001	<0.0001	0.0061
Cs × CSp		ns	ns	0.0049	0.0002	ns	ns
C × Cs × CSp		ns	0.0006	ns	<0.0001	<0.0001	ns

Means with the same letter are statistically identical (LSD, $p \leq 0.05$); sample size, 6 (n = 6), ns, not significant.

Table 3. Microelement content in mixed samples of lettuce leaves and heads.

Treatments	Concentration (mg I kg^{-1} Substrate)	mg kg^{-1} Dry Weight (n = 6)			
		Zn	Fe	Mn	Cu
CsC	0	49.24 a	166.17 a–c	86.49 de	3.82 ab
Cs-KIO_3	5	45.89 ab	156.47 a–d	79.15 e	4.16 a
Cs-KIO_3	25	37.77 b	157.72 a–d	88.35 de	3.83 ab
Cs-KIO_3 SDA	25	40.05 ab	158.38 a–d	90.40 c–e	3.99 a
AC	0	39.24 ab	171.09 ab	97.76 b–e	2.99 ab
KIO_3	5	41.25 ab	181.96 a	96.31 b–e	2.99 ab
KIO_3	25	42.56 ab	132.33 b–e	106.89 b–e	3.49 ab
KIO_3 SDA	25	42.72 ab	143.96 a–e	125.02 a–c	3.66 ab
CsC	0	39.56 ab	133.65 b–d	98.74 b–e	2.83 ab
Cs-KI	5	39.91 ab	133.55 b–e	131.72 ab	3.66 ab
Cs-KI	25	41.75 ab	137.41 b–e	150.55 a	3.16 ab
Cs-KI SDA	25	41.89 ab	138.14 b–e	116.86 a–d	3.49 ab
AC	0	36.09 b	120.26 de	85.00 de	3.00 ab
KI	5	37.59 b	100.78 e	107.77 b–e	2.99 ab
KI	25	37.91 ab	125.86 c–e	120.70 a–d	3.33 ab
KI SDA	25	36.56 b	129.45 b–e	108.19 b–e	2.49 b
Concentration (C)		ns	ns	0.0001	ns
Chitosan (Cs)		0.0177	0.0333	ns	0.0012
Chemical Species (CSp)		0.0036	<0.0001	<0.0001	0.0013
C × Cs		ns	ns	ns	ns
C × CSp		ns	0.0051	0.0003	ns
Cs × CSp		ns	ns	<0.0001	ns
C × Cs × CSp		0.0492	0.0211	ns	ns

Means with the same letter are statistically identical (LSD, $p \leq 0.05$); sample size, 6 (n = 6), ns, not significant.

3.2.3. Iodine and Minerals Correlation Analysis

A general analysis of correlation between iodine and each mineral element was carried out (Table 4). Of the elements evaluated, manganese, potassium, and calcium demonstrated significant correlations. Manganese had a positive correlation while for the other two, the correlation was negative. Regardless, the correlation values of each element with iodine are low. In that case, biofortification with iodine is not expected to interfere with the assimilation or accumulation of other mineral elements. There are various scales used to score Spearman correlation coefficients [40]. For this research, scale 2 was chosen. That scale indicates that a Spearman coefficient greater than |0.5| corresponds to a moderately strong correlation.

Table 4. Correlation analysis of iodine and minerals.

Treatments	Variable 1	Variable 2	Spearman	*p*-value
General treatment analysis	Iodine	Zinc	−0.04	0.7056
		Manganese	0.4	0.0001
		Iron	−0.06	0.5316
		Copper	−0.03	0.7894
		Sodium	−0.16	0.1275
		Potassium	−0.31	0.0018
		Nitrogen	0.05	0.6013
		Magnesium	−0.18	0.0827
		Phosphorus	−0.17	0.0951
		Calcium	−0.22	0.0301

Values of $\alpha \leq 0.05$ are significant. Spearman correlation coefficients greater than +0.5 indicate positive, moderately strong correlations or less than −0.5 indicate negative, moderately strong correlations, according to a previously described scale [40].

3.3. Iodine Content in Lettuce

The highest concentrations of iodine were seen in lettuce subjected to the SDA treatments (25 mg I kg^{-1} KI or KIO_3), regardless of Cs (Figure 1). SDA of KI and Cs-KI increased lettuce iodine concentrations to levels 77.9- and 63.7-times, respectively, above the corresponding controls. Similarly, SDA of KIO_3 and Cs-KIO_3 increased concentrations by factors of 97.4 and 36.4, respectively. The highest recorded concentration was 103.16 mg I kg^{-1} dry weight (DW), following SDA of KIO_3 (25 mg I kg^{-1}). Apart from those results, single applications of Cs- KIO_3 and KIO_3 (25 mg I kg^{-1}) also led to improvements in iodine concentrations (17.4- and 18.2-times above control concentrations, respectively). Single applications of KI and Cs-KI (25 mg I kg^{-1}) increased iodine concentrations by factors of 14.4 and 24.4, respectively. However, these improvements were not significant. According to the analysis of variance the iodine species did not have a significant impact on the results, rather the concentrations applied, and the presence of chitosan were the statistically important factors. The only interaction with significant differences that the iodine species factor had was with the chitosan factor (Figure 1).

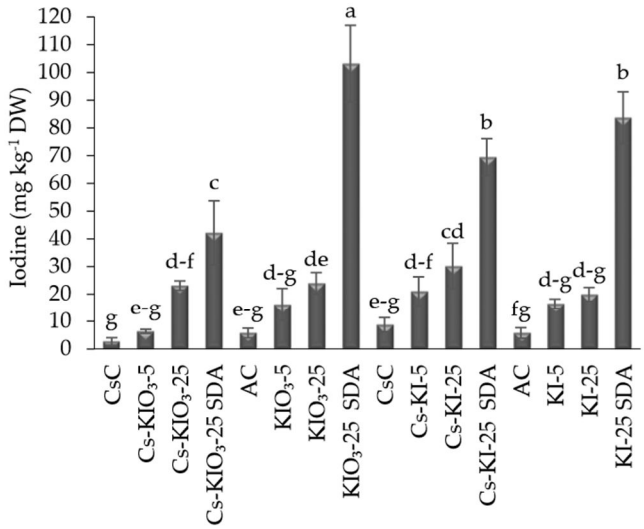


Figure 1. Iodine concentrations in mixed samples of lettuce leaves and heads following iodine salt treatments with and without chitosan. Means with the same letter are statistically identical (LSD, $p \leq 0.05$); the bars represent the standard error (n = 6).

3.4. Cell Wall-Bound and Free Phenol Content

Only KIO_3 at high concentrations (25 mg I kg^{-1}), both singly and doubly applied, led to increases in wall-bound phenols compared to the absolute controls (Figure 2). None of the factors alone had significant effects on phenol concentrations. However, the interaction between the iodine species and chitosan factors led to significant differences between treatments, as did the interaction between all three experimental factors. Conversely, there was neither a positive nor negative effect on the concentration of free phenols. Overall, the presence or absence of Cs, along with either iodine salt, had little to no effect on phenol content.

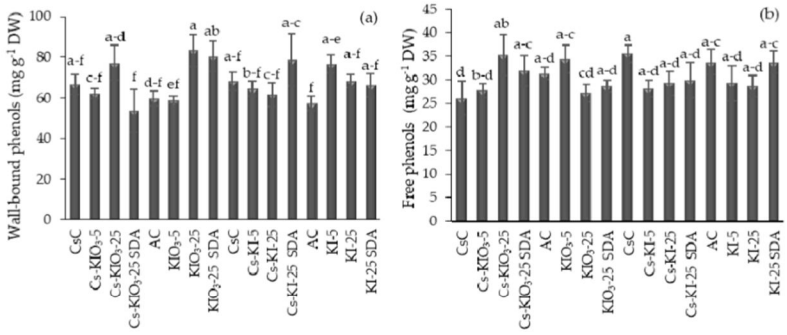


Figure 2. (**a**) Content of cell wall-bound phenols. (**b**) Content of free phenols measured in dry lettuce samples. Means with the same letter are statistically identical (LSD, $p \leq 0.05$); the bars represent the standard error (n = 6).

3.5. Chlorophyll Content

According to the analysis of variance, only the interaction between chitosan and the different iodine species had an effect on Chl a and total chlorophyll (Ct). However, with respect to the controls, there were no significant differences (Table 5). The Chl b concentration did not change after any treatment. In general, the application of I^- or IO_3^- did not exert significant changes in chlorophyll content, either with or without chitosan.

Table 5. Content of chlorophyll a, chlorophyll b, and total chlorophyll.

Treatments	Concentration (mg I kg^{-1} Substrate)	mg g^{-1} Dry Weight (n = 6)		
		Chl a	Chl b	Ct
CsC	0	0.10 b	0.05 b	0.15 c
Cs-KIO_3	5	0.11 b	0.09 ab	0.19 bc
Cs-KIO_3	25	0.10 b	0.09 ab	0.19 bc
Cs-KIO_3 SDA	25	0.19 ab	0.12 ab	0.31 a–c
AC	0	0.18 ab	0.18 ab	0.36 a–c
KIO_3	5	0.28 a	0.19 ab	0.47 a–c
KIO_3	25	0.18 ab	0.33 a	0.51 a–c
KIO_3 SDA	25	0.32 a	0.24 ab	0.56 ab
CsC	0	0.17 ab	0.12 ab	0.30 a–c
Cs-KI	5	0.25 ab	0.12 ab	0.36 a–c
Cs-KI	25	0.28 a	0.12 ab	0.39 a–c
Cs-KI SDA	25	0.28 a	0.31 ab	0.59 a
AC	0	0.17 ab	0.17 ab	0.35 a–c
KI	5	0.11 b	0.08 ab	0.19 bc
KI	25	0.18 ab	0.09 ab	0.27 a–c
KI SDA	25	0.30 a	0.12 ab	0.42 a–c
Concentration (C)		0.038	ns	ns
Chitosan (Cs)		ns	ns	ns
Chemical Species (CSp)		ns	ns	ns
C × Cs		ns	ns	ns
C × CSp		ns	ns	ns
Cs × CSp		0.0035	ns	0.0089
C × Cs × CSp		ns	ns	ns

Means with the same letter are statistically identical (LSD, $p \leq 0.05$); sample size, 6 (n = 6), ns, not significant. Chl a, chlorophyll a; Chl b, chlorophyll b; Ct, chlorophyll total.

3.6. Enzymatic Activity, Antioxidants, and Proteins

No experimental treatment produced significant differences, compared to the chitosan and absolute controls, for superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPX), nor total antioxidant activity (Figure 3). It appears that iodine treatments did not directly either contribute to an increase or decrease in catalytic and antioxidant activity, presumably because there was no plant stress generated. However, total protein content (TP) increased considerably after KI treatments, both at low (5 mg I kg^{-1}) and high (25 mg I kg^{-1}) doses applied either once or as split dose.

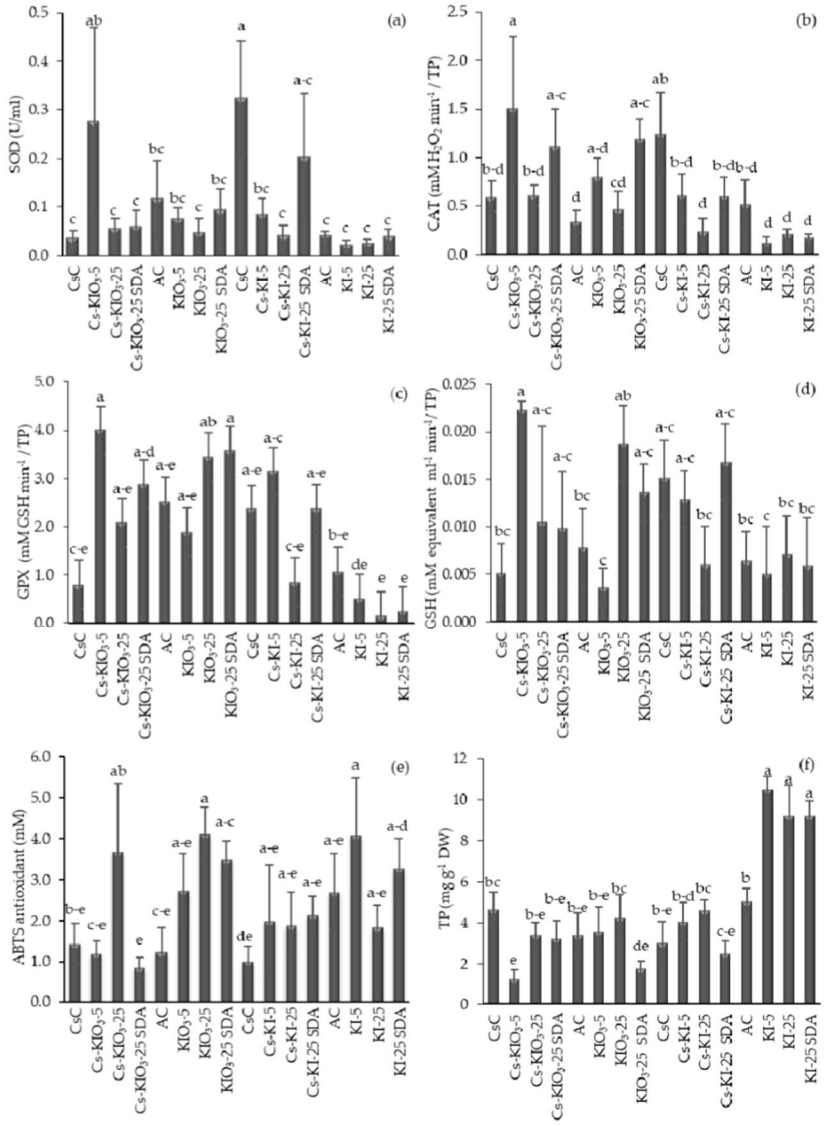


Figure 3. Changes in enzymatic, antioxidant, and protein content of lettuce after treatment with iodine and chitosan. (**a**) Superoxide dismutase, SOD; (**b**) catalase, CAT; (**c**) glutathione peroxidase, GPX; (**d**) reduced glutathione, GSH; (**e**) total antioxidant content; (**f**) total protein, TP. Means with the same letter are statistically identical (LSD, $p \leq 0.05$); sample size, 6 (n = 6); the bars represent the standard error. Sample size was 6 (n = 6) for all measurements except SOD (n = 5).

Despite the fact that there are differences in several enzymes in one factor or between factors detected in ANOVA, there is no enzyme activity that has been increased by any treatment.

4. Discussion

Four possible outcomes for iodine biofortification have been described: (1) it has a wholly negative impact on plant growth and other physiological and biochemical variables; (2) it has no effect on plants; (3) it has a wholly positive impact on plant growth and other physiological and biochemical variables; or (4) it has mixed positive and negative effects on plants [9,41–43]. With the exception of three specific treatment combinations, there were no changes in lettuce biomass. Something similar happened with the concentrations of mineral nutrients, which potentially indicates an ideal scenario where increases in iodine content do not antagonize the accumulation of other nutrients such as iron and zinc. Biofortification with iodine was achieved and we found greater concentrations after iodine treatments applied as two doses. The working hypothesis was that the presence of chitosan would augment the absorption of iodine in lettuce plants, but the results did not show any appreciable changes for treatments that included chitosan. There have only been two similar studies that investigate the uses of chitosan biopolymers with iodine, applied as thin films. One involved the application of Cs-KI films on tomato plants and found that it did not affect the antioxidant activity of the fruit [42]. The other applied edible films of Cs-KIO_3 to hot pepper and found that the iodine ion does not leach away due to the strong ionic interactions between the chitosan cationic groups and IO_3^- [7].

4.1. Biomass Production and Yields

With the exception of three treatment combinations, no impact on plant growth was found after iodine application. There were increases in LFW and LDW biomass following a single application of Cs-KIO_3, which were consistent with results from previous lettuce biofortification studies using KIO_3 [10,15,44]. Conversely, there were decreases in HFW and HDW biomass after SDA of Cs-KI, potentially due to the second application direct to the substrate at 15 d.a.t. and subsequent rapid absorption by the plant roots. In addition, there is a high correlation between LFW and LDW (R = 0.9).

The I^- anion is known to have greater solubility in soil and better absorption by roots than its IO_3^- counterpart [44]. Reports of greater plant sensitivity to high doses of KI than to KIO_3 applied to the substrate have been published [15,45]. At low doses (0.013–0.129 mg L^{-1}), there are no toxic effects reported for either iodine species [45]. In hydroponic systems, lettuce has demonstrated greater sensitivity to I^-. Phytotoxic effects from excessive iodine accumulation were seen at I^- doses of 80 µM, while IO_3^- did not affect biomass at doses up to 240 µM [10].

Considering those previous reports, the iodine concentrations utilized in the present work were well within the ranges used for lettuce cultivation. The best iodine species for biofortification is reportedly IO_3^- [12,13,43]. Our study found that Cs-KIO_3 complexes (at 5 and 25 mg I kg^{-1}) applied to the substrate led to biomass increases. Compared to KI, that iodine salt is less phytotoxic. Additionally, appropriate doses of chitosan have been found to stimulate plant growth, development, and yields [24,46]

The largest improvements to LFW, LDW, and HDW were seen after single applications of Cs-KIO_3 (at 5 and 25 mg I kg^{-1}), rather than either Cs-KI or KIO_3. Based on those results, we believe that the complex of Cs and IO_3^- favor improvements in biomass, as there are also other studies that show that Cs and IO_3^- alone have positive effects on plant biomass [9,22,47]. Cs by itself is a good source of C, H, O and N for plants, it also works as a chelating agent for metals, which favors chelated elements that are available in assimilable forms for plants, and it has also been related for exercising its function as an elicitor, these and other Cs functions can contribute to plant growth [23,47]. On the other hand, IO_3^- is less phytotoxic than I^-, as it must first be reduced to I^- before it can be assimilated. Perhaps this step is a checkpoint for the plant's signaling processes. IO_3^- could also be acting as an elicitor. On the other hand, I^- is rapidly assimilated by the plant, probably leading to greater accumulation in the chloroplasts while exerting its phytotoxic effects, which have been noted as chlorosis or biomass

reductions [10,15]. However, the mechanisms of iodide and iodate root absorption have not yet been fully elucidated and little is known for sure about the subject [48,49].

4.2. Mineral Content

There were significant changes between treatments in the concentrations of N, P, K, Ca, Na, Cu, Fe and Zn. However, those changes were within the ranges seen for the absolute and chitosan controls and no other antagonistic effects between iodine and the mentioned minerals could be verified. Magnesium and manganese concentrations went up after single applications of Cs-KI (5 and 25 mg I kg^{-1}, respectively). In this study, we find significant positive correlations ($R \geq 0.8$) between Ca-Mg, Mg-Na and Ca-Na.

Soil application of fertilizers containing KI (0.5, 1.0, and 2.0 kg I ha^{-1}) reportedly led to increases in Ca, Mg, Mn, and Cd levels in lettuce [11]. Similarly, levels of Mg, Mn, and Cu in tomato leaves and stems increased with application of KI solutions (4–100 mg I kg^{-1}), although iron levels fell [50]. The authors attribute those results to the changes in redox equilibrium causes by iodine during cultivation. Increases in Mn and Cu concentrations in prickly pear cactus have been seen following KI (10^{-4} mol dm^{-3}) application, as well as P and Mg increases after KIO_3 (10^{-4} mol dm^{-3}) application via fertigation [51]. Conversely, negative correlations have been seen for K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Cd and Pb content after fertilization with either KI or KIO_3 [14]. In the present study, there were no negative correlations seen in the evaluated minerals after I^- or IO_3^- application, either with or without chitosan.

4.3. Iodine Content in Lettuce

Even though iodine is not considered an essential nutrient for plants, several investigations have shown that the right doses of iodine can improve yields and that it is translocated to diverse tissues within the plant. Maximum concentrations of iodine were seen following application of KIO_3 (25 mg I kg^{-1}) as a split dose. Possibly, iodine was rapidly absorbed by the roots due to the direct application to the substrate. Some authors have suggested that biofortification is most successfully with IO_3^- as it does not have phytotoxic effects and the biomass either increases or is not negatively affected [12,47,52].

Regarding the original question of whether Cs-KI or Cs-KIO_3 complexes would increase iodine absorption, neither complex had an outstanding impact on iodine content when compared to the treatments without chitosan. For example, for I^-, the plant response was similar after single application treatments with and without Cs. The greatest concentrations were after SDA of KI treatments, even those changes were not significant. In contrast, KIO_3 and its Cs complexed form induced significant differences in iodine concentrations after two applications. Of the two forms, the KIO_3 alone led to a greater increase in iodine concentration. There are two potential explanations for these results. One, in order to be absorbed IO_3^- must first be reduced to I^-, a process that is carried out by iodate reductase enzymes [53]. So, when IO_3^- is complexed with Cs, the reduction process takes longer than when the free salt is applied and thus it becomes bioavailable slower. Two, the Cs-KIO_3 may become fixed in the ground, not volatilizing, which means crops with short cultivation cycles, like lettuce, would be unable to fully take advantage of its presence. Both I^- and IO_3^- applied to soil are converted to organic iodine after only 14 days, and in that form become fixed in the ground and less readily soluble in water [54]. This could explain why there was no significant difference between I^- and IO_3^- applied in a single treatment, with and without Cs, at either concentration (5 or 25 mg I kg^{-1}).

Several authors have reported high concentrations of iodine after biofortification with KIO_3 [15,55]. Conversely, iodine content in lettuce after biofortification with high doses of iodide during two trials (summer and winter) was up to five times higher than with iodate. The iodine concentrations of inner and outer leaves reached 653 and 764 μg I kg^{-1}, respectively [45]. Doses of iodine below 2.5 mg I kg^{-1} have no effect on biomass [55,56], while at times doses between 5 and 25 mg I kg^{-1} applied to soil have been found to not affect yields [57,58]. Those previously reported results are consistent with the

findings of the present study, with the exception of the SDA of Cs-KI (25 mg I kg^{-1}) which ended up reducing biomass.

4.4. Phenol Content

This study found an increase in cell wall-bound phenols after application of IO_3^- (25 mg I kg^{-1}), either in single dose or SDA, when compared to the absolute controls. The lack of changes in phenol concentrations may indicate that no oxidative stress was induced in the plants following treatment, except for the case of the treatments mentioned. In those cases, the action of cell wall-bound phenols could have potentially impeded iodine oxidation, which could be why no increases in free phenols were seen. Substrate and foliar applications of I^- or IO_3^-, either at 1 µM daily or 100 µM every two weeks, were not found to significantly affect phenol concentrations in tomato seedlings (var. Rio Grande) [35]. Similarly, in chili pepper covered with edible films of Cs-IO_3, no changes in total phenol content were seen for either the controls or the treated peppers [7]. On the other hand, tomatoes coated with chitosan-iodine films demonstrated greater antioxidant activity and total phenol content than uncoated fruit [42].

4.5. Chlorophyll Content

Little is known about the mechanisms of iodine toxicity or transport within plants. What is known, however, is that I^- toxicity is a consequence of intracellular oxidation or electron loss turning it into molecular iodine, which can bind to cellular components including chlorophyll [58]. In this study, the applied doses of iodide and iodate did not cause changes in chlorophyll content compared to the controls. Total chlorophyll has a high correlation with Chl a and Chl b and likewise Chl a and Chl b ($R \geq 0.7$). Similarly, other authors have reported insignificant changes in the contents of photosynthetic pigments [59,60]. Chitosan, on the other hand, applied in concentrations of 0.10%, 0.15%, 0.20%, and 0.30% has been observed to increase the index of chlorophyll content in lettuce leaves from 29.8 to 34.4, 35.5, 37.5, and 41.4, respectively [24]. Iodide does not inhibit chlorophyll synthesis, but it is known to reduce photosynthetic activity [59]. Nonetheless, chloroplasts are known major sites of ROS production [60]. It would appear that at the doses used in this study, iodide and iodate did not significantly affect the chlorophyll content of lettuce.

4.6. Total Protein Content

Treatments with KI were the only treatments that led to increases in total protein content. Those increases may be due to the fact that KI is more readily assimilated, potentially leading to the production of secondary metabolites and the concomitant production of the proteins necessary for that metabolism. Moreover, the amount of total proteins has a highly significant negative correlation ($R \geq 0.8$) with the enzymatic activity of GSH and GPX. Previous works have also observed significant increases in total protein content, although those treatments used IO_3^-, with maximum protein concentrations reached at doses of 80 µM iodine [61].

4.7. Enzymatic and Antioxidant Activity

There were no significant differences observed in enzymatic activity after any treatments. Once again, it was demonstrated that the applied doses of iodine did not induce a defensive response in lettuce. There are high positive correlations ($R \geq 0.5$) between enzymatic activities of GSH-GPX, CAT-GPX, and CAT-GSH that we believe have to do with maintaining a balance at the cellular level. There are no high and significant correlations between enzyme and antioxidant activities, with the exception of SOD and wall-linked phenols where there is a low but significant correlation. The latter we can attribute it to the fact that SOD heads the first line of defense of enzymatic activity and its correlation with phenols linked to the wall, we perceive it as a redox or neutralization mechanism against high concentration IO_3^- salts. Conversely, some authors have reported increases in enzymatic activity following biofortification with iodine. During soilless cultivation of lettuce, SOD was reduced

after application of I^- at 20, 40, and 80 μM, while IO_3^- led to its increase in doses greater than 40 μM. Ascorbate peroxidase (APX) activity increased with IO_3^- application at any dose, while CAT activity also increased with IO_3^- as well as I^- application, reaching its maximum value at IO_3^- doses ≥80 μM [43]. Similarly, hydroponic lettuce grown under salt stress and biofortified with IO_3^- had increased activities of SOD, APX, Dehydroascorbate reductase (DHAR), and Glutathione reductase (GR) [12]. Soybeans grown under Cd^{2+} stress and biofortified with IO_3^- at various concentrations (20, 40, and 80 μM) demonstrated increased SOD, APX, and GR activity [62]. Tomato biofortified with I^- or IO_3^-, applied to soil or foliage, either daily (1 μM) or biweekly (100 μM), had reduced SOD activity following the biweekly I^- treatments and the daily, foliar treatments of either iodine salt. However, the daily application of foliar I^- led to increases in the concentrations of non-enzymatic antioxidants, namely ascorbate (22%) and glutathione (85%) [35].

Plants have evolved several antioxidant systems to help them avoid damage from oxidative stress. One of those systems involves the enzymes SOD, CAT, and APX, acting in that order. SOD reduces O_2^- to H_2O_2, which in turn is a substrate for CAT, APX, and other enzymes [60]. Iodide is considered an inorganic antioxidant that reacts with ROS (ozone, singlet oxygen, and superoxide) at rates 12 to 500 times those of ascorbate and glutathione [63]. In aquatic plants of the genus *Laminaria*, the enzyme haloperoxidase catalyzes the oxidation of iodide to hypoiodous acid and molecular iodine in the presence of H_2O_2 [64].

The results of the present work differed from those previously reported, although that could be attributed to a number of factors. For example, in this study, cultivation was substrate-based while previous reports have used hydroponic systems [43]. Additionally, the accumulation of iodine and other biomolecules can depend on factors such as the dose and species of iodine applied, the plant species, and the type of application, be it foliar or directly to the substrate, all of which can lead to the differential accumulation of iodine [15].

5. Conclusions

The biofortification of lettuce using complexes of chitosan with KI or KIO_3 (Cs-KI and Cs-KIO_3) did not induce negative effects on the mineral content, chlorophyll content, enzymatic activity, antioxidant content, nor the phenol content of lettuce. Moreover, Cs-KIO_3, either at 5 mg I kg^{-1} or 25 mg I kg^{-1}, applied prior to transplanting increased plant biomass and led to iodine levels of 6.4 and 23.1 mg I kg^{-1} dry weight, respectively. Plant biomass was reduced after split-dose application (SDA) of Cs-KI before and after transplanting. The manner of treatment application as a split dose before and after transplant was a determining factor for greater iodine accumulation. Application before and after transplanting of KIO_3 led to the highest iodine concentrations (103.2 mg I kg^{-1} dry weight), although biomass was considerably reduced compared to single applications of Cs-KIO_3 at either concentration (5 or 25 mg I kg^{-1}). Although Cs-KI and Cs-KIO_3 did not increase iodine absorption compared to the free salts, in some cases they caused increases in biomass while maintaining levels of iodine accumulation in lettuce leaves. As far as we know, there are no similar reports on crop biofortification with chitosan-iodine complexes applied to either soil or substrate. Further biofortification studies with chitosan-iodine complexes in crop species other than lettuce are required in order to evaluate the effects they may have when treatment times are longer.

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Artículo

COMPORTAMIENTO EN POSCOSECHA DE FRUTOS DE TOMATE BIOFORTIFICADOS CON YODO Y COMPLEJOS DE QUITOSÁN-YODO

Comportamiento en poscosecha de frutos de tomate biofortificados con yodo y complejos de quitosán-yodo

Postharvest behavior of tomato fruits biofortified with iodine and a complex of chitosan-iodine

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RESUMEN

La biofortificación de los cultivos con yodo permite obtener plantas que proporcionan este nutriente esencial a la población. El objetivo fue evaluar el impacto del yodo en el comportamiento del fruto en poscosecha. Se cosecharon frutos maduros del tercer racimo de plantas de tomate biofortificados con KI, KIO_3 y complejos de quitosán-KI y quitosán-KIO_3 (5 y 25 mg I kg^{-1} de sustrato) aplicados antes del trasplante. Para los tratamientos testigo se usó agua y quitosán. Los frutos se mantuvieron a temperatura ambiente (promedio de 18.9 ºC), durante 12 días. Cada tres días se determinó: peso fresco, peso seco, diámetro polar y ecuatorial, contenido de yodo, sólidos solubles totales, firmeza, pH, conductividad, pérdida de peso y número de frutos. El contenido de yodo total en los frutos máximo fue de 54 mg kg^{-1} PS en quitosán-KI de 25 mg kg^{-1} y a los 12 días de poscosecha, se obtuvo 46.4 mg kg^{-1} PS con quitosán-KIO_3. Se concluyó que la biofortificación con sales de yodo y/o complejos quitosán-yodo no modifica la mayoría de las variables poscosecha evaluadas durante 12 días, excepto por el contenido de yodo, diámetro polar y número de frutos que disminuyó con quitosán-KI de 5 mg I kg^{-1}.

Palabras clave: Biopolímeros, nutrición vegetal, poscosecha, yodo.

ABSTRACT

The biofortification of crops with iodine makes it possible to obtain plants that provide this essential nutrient to the population. The objective was to evaluate the impact of iodine on postharvest fruit behavior. In this study, red-colored fruits of the third bunch were harvested from tomato plants biofortified with KI, KIO_3, and complexes of chitosan-KI and chitosan-KIO_3 (5 and 25 mg I kg^{-1} substrate) applied before transplantation. Water and chitosan iodine-free were used as control treatments. The fruits kept at room temperature (average 18.9 ºC) and monitored for 12 days. Every three days it was determined: fresh weight, dry weight, polar and equatorial diameter, iodine concentration in fruits total soluble solids, firmness, pH conductivity weight loss and fruits number, and were determined. The maximum average values of iodine in fruits were 54 mg kg^{-1} DW in Cs-KI of 25 mg kg^{-1} and at 12 days post-harvest, 46.4 mg kg^{-1} PS was obtained with Cs-KIO_3. It was concluded that biofortification with iodine salts and / or complexes does not modify most of the variables evaluated during 12 days, except for the iodine content, polar diameter and number of fruits decreased with chitosan-KI of 5 mg I kg^{-1}.

Keywords: Biopolymers, plant nutrition, postharvest, iodine.

INTRODUCCIÓN

El yodo es un elemento esencial para el ser humano, lo adquirimos de diferentes fuentes de alimentos, en la fortificación de sal de mesa o suplementos alimenticios. El requerimiento diario para el consumo de yodo , para niños o infantes de 0 a 59 meses es de 90 µg, para niños de 6 a 12 años 120 µg, en adultos y mayores de 12 años 150 µg y para embarazadas y mujeres en lactancia 250 µg (WHO, 2007) . El yodo se requiere para un buen funcionamiento y síntesis de hormonas tiroideas. Los desórdenes por deficiencia de yodo (IDD) pueden provocar: hipotiroidismo, cretinismo, bocio, deterioro cognitivo y psicomotor (Zimmermann, 2019). Alrededor de 1570 millones de personas a nivel mundial pueden tener riesgo de deficiencia de yodo (Gizak *et al.,* 2017). La yodación universal de la sal de mesa se inició en el año de 1920 en Suiza y partes de Italia, desde entonces se aplica a nivel mundial (Fuge and Johnson, 2015). El consumo de la sal de mesa fortificada ha logrado disminuir a nivel mundial el déficit del yodo. Sin embargo, una desventaja de las sales inorgánicas de yodo es que presentan pérdidas por volatilización del 20% por cocción y preparación de alimentos (Longvah *et al*., 2012). Además, la sal de mesa, contiene sodio que puede provocar problemas de presión sanguínea alta, por esta razón la WHO acordó reducir su consumo (WHO, 2019). Una alternativa a esto es la biofortificación de cultivos con yodo del cual se han reportado efectos en biomasa, actividad antioxidante y contenido de yodo en partes comestibles de frutos o vegetales (Kiferle *et al*., 2013; Medrano-Macías *et al*., 2016).

Una alternativa, para disminuir los efectos de volatilización y aumentar disponibilidad del yodo, es la biofortificación con quitosán-yodo.

El quitosán (Cs) es un biopolímero policatiónico de enlaces poli D-glucosamina que se obtiene de la desacetilación de la quitina. Es biodegradable y tiene una amplia gama de aplicaciones en la industria, medicina, agricultura (Rinaudo, 2006). La ventaja de la biofortificación con los complejos de quitosán-yodo (Cs-I) es que el I forma interacciones electrostáticos débiles permitiendo mayor disponibilidad y menor volatilización en comparación con las sales inorgánicas (Moulay, 2013).

A la fecha es nula la información del comportamiento poscosecha en frutos de tomate biofortificados con I y Cs-I.

El objetivo fue evaluar los efectos de Cs-I aplicados directamente en sustrato sobre el comportamiento poscosecha de frutos de tomate. La hipótesis planteada es que los complejos Cs-I, además de ejercer un efecto en la acumulación de yodo en frutos, modifican positivamente el comportamiento poscosecha de los frutos.

MATERIALES Y MÉTODOS

Preparación de los complejos Cs-KI, Cs-KIO_3 y sales de yodo.

Los complejos de Cs-KI y Cs-KIO_3 fueron preparados en el Centro de Investigación en Química Aplicada (CIQA) con un quitosán (Cs) de peso molecular de 200,000 g/mol y grado de desacetilación del 98%. Para su preparación se utilizó una solución de Cs al 1% en ácido acético (AcOH) al 1%. El Cs se agregó lentamente hasta su completa disolución, con agitación de 300 rpm a una temperatura de 60-65 °C. La solución resultante se filtró y se aforó a 1 L.

Para los complejos de Cs-KI y Cs-KIO_3, se prepararon soluciones de yoduro de potasio (KI) 0.1 M y yodato de potasio (KIO_3) 0.1 M disueltas y aforadas en Cs al 1% (p/v), para obtener una relación molar de [Cs/I] = 5. Los complejos tenían 1.06 mg I-mL^{-1}.

Para la preparación de KI y KIO_3, se prepararon soluciones de 0.025 M en 1 L de agua desionizada. Cada solución contenía 3.17 mg I mL^{-1}.

Material vegetal y tratamientos aplicados.

El experimento se inició con la siembra en Junio del 2018, en un invernadero del departamento de Horticultura de la Universidad Autónoma Agraria Antonio Narro en Saltillo Coahuila, México. Se utilizaron semillas de *Lycopersicon esculentum* híbrido "El cid F1", se sembraron en charolas de poliestireno de 200 cavidades con sustrato de peat moss y perlita en proporción 1:1 *v/v*. El trasplante se hizo en Julio del 2018 en macetas de polietileno de color negro con una mezcla de peat moss y perlita 1:1 *v/v*. Las concentraciones de yodo utilizadas fueron: 0, 5 y 25 mg kg^{-1} aplicados antes del trasplante.

Tratamientos aplicados.

Para cada tratamiento, el volumen total del sustrato se dividió en tres partes. Los tratamientos fueron divididos en dos parte iguales con sales de yodo y los complejos Cs-KI y Cs-KIO_3. La primer parte de cada tratamiento se aplicó sobre el primer tercio del sustrato colocado en la maceta, se añadió otro tercio del volumen del sustrato y se aplicó la parte restante del tratamiento. Finalmente se llenó la maceta con el volumen restante de

sustrato y se trasplantó. Suponemos que esta técnica permite que las raíces de las plantas, conforme crezcan, puedan tener disponible el I^- y IO_3^-.

Para los testigos absolutos (TA) se añadió agua. En los testigos de Cs (TCs) se aplicó un volumen total 140.7 ml de la solución de Cs al 1%. Al final cada tratamiento tenía el mismo volumen de Cs.

Para KIO_3 y KI de 5 mg I kg^{-1} de sustrato, se aplicaron en total 9.5 mL por maceta de las soluciones de 0.025 M. Para KIO_3 y KI de 25 mg I kg^{-1} se aplicaron en total 47.3 mL por maceta.

En Cs-KI y Cs-KIO_3 de 5 mg I kg^{-1} de sustrato se aplicaron en total 28.1 mL de las soluciones de 0.1 M, añadimos a la par un volumen total de 112.56 ml de solución de Cs al 1% (para tener el mismo volumen de Cs que se empleó en TCs). En Cs-KI y Cs-KIO_3 de 25 mg I kg^{-1} se aplicó un volumen total de 140.7 ml de solución al 0.1 M.

Se contó con 10 unidades experimentales por tratamiento, se eligieron 6 plantas al azar de cada tratamiento. Se tomaron muestreos de frutos maduros del tercer racimo de cada planta.

Se utilizó un sistema de riego por goteo de un solo paso automatizado. Para el riego se preparó solución nutritiva Steiner al 25%, 50% y 75% de acuerdo a los requerimientos de cada etapa fenológica: vegetativa, floración y llenado de fruto. El pH fue ajustado entre 5.5 y 6.5 con ácido fosfórico. Se mantuvo una conductividad eléctrica en solución nutritiva entre 1.4 y 2.4 mS cm^{-1} dependiendo de la etapa.

Muestreo.

El muestreo se hizo en plantas con frutos maduros del tercer racimo a los 101 ddt, los cuales fueron cosechados de acuerdo a la clasificación de color (6) (USDA, 1991), que señala que un fruto "Rojo", significa que más del 90% de la superficie de la muestra en conjunto presenta color rojo. Los frutos cosechados se etiquetaron, registraron y fueron colocados en el laboratorio a temperatura ambiente, para ser evaluados a lo largo de 12 días. Se registraron temperaturas entre 17.7 °C Min y 20.1°C Máx.

Variables evaluadas.

En cada fruto se evaluaron las siguientes variables: Peso fresco de fruto (PFF), peso seco de frutos (PSF), contenido de yodo, solidos solubles totales (SST), firmeza, diámetro polar (DP), diámetro ecuatorial (DE), pH y conductividad eléctrica (CE). Cada una de las variables se evaluó cada tres días: día de inicio (D0), a los tres días (D3), seis días (D6), nueve días (D9), doce días (D12).

Peso fresco y seco de frutos. Se pesó y registro de manera individual el PFF y PSF (g fruto^{-1}). Para el peso seco, las muestras fueron colocadas en una estufa a 70°C, hasta su pérdida de humedad. Todas las muestras fueron molidas hasta obtener un polvo fino se etiquetaron y se guardaron en bolsas herméticas. Se usó una balanza granataria marca OHAUS.

Diámetro polar y ecuatorial. El diámetro polar está definido como la distancia entre el ápice del tomate y el extremo del tallo. Diámetro ecuatorial, se midió de manera

perpendicular al diámetro polar, tomando como base el eje central de los frutos. La medición se hizo usando vernier digital, (marca STEREN) los resultados se expresaron en milímetros (mm).

Contenido de yodo. Se siguió la técnica de cenizas alcalinas (Cortés *et al*., 2016). Se pesaron 0.5 g de frutos secos. Las muestras se colocaron en crisoles, se agregaron 2 ml de KOH 2 M y 1 ml de KNO_3 2 M. Una vez agregados los reactivos a las muestras, se llevaron a pre-digestión en estufa a 100°C por 2 h, bajo una campana de extracción. Los crisoles se colocaron en una mufla a una temperatura de 580°C y se mantuvo a esa temperatura por 3 horas, se dejaron enfriar a temperatura ambiente y las cenizas se pasaron a un tubo cónico para ser extraídas con 2 mL de KOH a 2 mM. Las muestras se centrifugaron a 12000 rpm por 15 min. Finalmente 1 mL del sobrenadante se decantó, se aforo a 10 mL con KOH a 2M y se filtró. La cuantificación se hizo en el ICP-OES Agilent 725. La concentración de yodo se expresó en mg kg^{-1} Peso seco (PS).

Solidos solubles totales. Se extrajo el jugo de cada fruto, se colocó de una a dos gotas sobre el lector óptico del refractómetro (marca Atago).

pH y Conductividad eléctrica. Se realizó un corte en el eje central de cada fruto y se insertó de manera manual un pH metro digital (marca HANNA) que determina pH y conductividad eléctrica.

Firmeza. Se evaluó mediante la resistencia a la penetración y fue determinada con un penetrómetro digital compacto (marca HUMBOLDT). Las mediciones se realizaron en la parte central de cada fruto, los resultados se registraron en kilogramos (kg).

Pérdida de peso. La pérdida de peso se evaluó a partir de la diferencia entre el PFF en el tiempo (t) con respecto al siguiente tiempo de muestreo (t+1) y así sucesivamente (D0-D3, D3-D6, D6-D9, D9-12).

Número de frutos. Se hizo un conteo del número de frutos en el tercer racimo por planta de cada uno de los tratamientos, con la finalidad de evaluar el efecto de los tratamientos sobre esta variable

Análisis experimental

Los tratamientos se evaluaron bajo un diseño completamente al azar. Debido a que no contamos con el mismo número de muestras en cada tratamiento, se realizó un análisis de varianza no paramétrico por Kruskal Wallis con un valor de p <0.05. Usando un paquete de análisis estadístico software InfoStat.

RESULTADOS Y DISCUSIÓN

En la actualidad no hay información de biofortificación con yodo en forma de sales y complejos Cs-I en poscosecha de tomate. Hay estudios con aplicaciones de Cs-I en forma de películas comestibles en ají picante y tomate indicando una buena vida de anaquel (Limchoowong *et al*., 2018, 2016).

El tomate es un cultivo objetivo para la biofortificación con yodo, por su eficiencia agronómica de acumular yodo (Caffagni *et al*., 2011).

Peso fresco y seco de frutos.

Las variables de PFF y PSF muestran diferencias significativas entre sí pero no contra los testigos (Tabla 1). Por lo tanto los tratamientos con sales de yodo y Cs-I no afecta en el peso de frutos. Esto coincide con lo reportado por Kiferle *et al.,*(2013), quienes mencionan que al biofortificar con I y IO_3^- no tuvo impacto en el peso seco de frutos de tomate.

Diámetro polar y ecuatorial

El DP y DE son una forma de evaluar el crecimiento de los frutos y medir si alguno de los tratamientos influye en esto.

El DE no presentó diferencias de los tratamientos de yodo y/o Cs-I en comparación con los testigos (Tabla 1). En cambio, el tratamiento de KI de 25 mg I kg^{-1} incrementó el valor de DP (Figura 1).

Contenido de yodo

La evaluación de contenido de yodo total (Figura 2), demuestra que los complejos de Cs-KI de 25 mg I kg^{-1}, incrementaron la biodisponibilidad del yodo y su acumulación en frutos con respecto a los testigos. Sin embargo, en el análisis a través del tiempo del período de poscosecha, se observaron diferencias significativas, pero ningún tratamiento expresa un incremento con respecto a los testigos. Creemos que esta desigualdad entre los

dos análisis se debe al tamaño de muestra, que fue mayor en el contenido de yodo total por el análisis en conjunto de todos los tiempos de muestreos.

Con respecto a la disponibilidad del yodo a través del tiempo, la concentración de yodo máxima disponible por un periodo de hasta 12 días de anaquel fue de 46.37 mg I kg^{-1} PS con Cs-KIO_3 de 5 mg de I kg^{-1} (Tabla 2). Esto es equivalente a 253.5 µg I en PFF, lo cual estaría cubriendo 101 % de la ingesta diaria recomendada de yodo para mujeres embarazadas o en lactancia (WHO, 2007). A pesar de que el análisis no marca diferencias en el día 12, podemos resaltar que para Cs-KIO_3 de 5 mg, el contenido de yodo marca una diferencia de 15 veces mayor con respecto al promedio de los testigos, además, el número de frutos no se vio afectado. En contenido de yodo total, la máxima fue de 54 mg I kg^{-1} PS con Cs-KI de 25 mg I kg^{-1}, equivalente a 2.70 µg I g^{-1} en PFF con disminución de 19 frutos en contraste con TCs. Se ha visto que aplicaciones de KI puede incrementar el contenido de yodo y causar disminución de biomasa (Blasco *et al*., 2008; Kopeć *et al*., 2015). Caffagni *et al.*, (2011) reportaron en frutos de tomate que llegaban a absorber 3,900 µg / 100 g aplicando I^- y disminución de biomasa al aplicar 43 mg I $maceta^{-1}$. En un estudio de recubrimiento de fruto de tomate cherry con Cs-KI, aumentó la vida de anaquel y el contenido de yodo fue 0,4 µg I g^{-1} PFF, menor en comparación con nuestro estudio (Limchoowong *et al*., 2016).

Existen interacciones fuertes entre el yodato y el grupo amino del Cs y que por lo tanto provoca que no se lixivie (Limchoowong *et al*., 2018). Además, los complejos Cs-I se encuentran formando interacciones electrostáticas débiles que permiten mayor disponibilidad y menor volatilización (Moulay, 2013).

Solidos solubles totales, pH, conductividad y firmeza.

La degradación de los polisacáridos de las membranas nos permite evaluar, el efecto en el contenido de SST y de forma indirecta evalúa la vida de anaquel y calidad de frutos.

Las variables de SST, pH, conductividad y firmeza, no se vieron afectados por la aplicación de los distintos tratamientos (Tabla 3). Un indicador de buena vida de anaquel es la firmeza, sin embargo no observamos cambios.

Pérdida de peso y número de frutos.

La pérdida de peso mide el comportamiento y calidad en vida de anaquel de frutos. No observamos diferencias al evaluar la pérdida de peso total y a través del tiempo (Figura 3). Se sabe que aplicaciones de Cs o Cs-KIO_3 disminuye la pérdida de peso, esto se atribuye al efecto del Cs aplicado directamente en frutos de ají picante, que además aumenta el valor nutricional y calidad durante el almacenaje (Limchoowong *et al*., 2018). A pesar de que no observamos cambios en pérdida de peso, el número de frutos es 16.5 y 14 veces mayor en TCs y Cs-KIO_3 de 5 mg I kg^{-1} en contraste con TA. Por otra parte, el número de frutos disminuyo considerablemente con Cs-KI de 5 mg I kg^{-1} con una diferencia de 34.5 frutos con respecto a TCs (Figura 4). De igual manera, se ha reportado en lechuga biofortificada con Cs-KI una disminución en biomasa (Dávila *et al*., 2020). Algunos estudios indican que el KI, puede causar fitotoxicidad o disminución en biomasa (Blasco *et al*., 2008; García Osuna *et al*., 2014; Lawson *et al*., 2015). Posiblemente debido a que el KI es absorbido por la planta fácilmente en contraste con IO_3^-, que para ser absorbido tiene que ser reducido por acción de enzimas yodato reductasas (Medrano-Macías *et al*., 2016).

CONCLUSIONES

La biofortificación con sales de yodo y/o complejos no modifica la mayoría de las variables evaluadas durante 12 días, excepto por el contenido de yodo, diámetro polar y número de frutos. Los complejos de Cs-KIO_3 permiten una mayor disponibilidad y acumulación del yodo a través del tiempo en frutos de tomate. Con este complejo la diferencia en el número de frutos es 14 veces mayor con respecto a TA y la ingesta de yodo con periodo de hasta 12 día de poscosecha es de 253.5 µg g^{-1} PFF recomendamos la biofortificación con Cs-KIO_3 de 5 mg I kg^{-1} de sustrato. Además, se recomienda el consumo de tomate antes de los 12 días, puesto que la concentración de yodo disminuye conforme los días de poscosecha aumentan.

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TABLAS Y FIGURAS

Tabla 1. Valores promedio de peso fresco, peso seco, diámetro polar, diámetro ecuatorial evaluado en frutos de tomate biofortificados con yodo y Cs-I.

Table 1. Average values of fresh weight, dry weight, polar diameter, equatorial diameter evaluated in tomato fruits biofortified with iodine and Cs-I.

Tratamientos	Concentración de I kg^{-1} sustrato	PFF (g)	PSF (g)	DE (mm)	DP (mm)
TCs	0 mg I kg^{-1}	121.91 ± 4.25 ab	5.31 ± 0.24 ab	55.46 ± 0.70 a	67.14 ± 0.84 ab
TA	0 mg I kg^{-1}	137.33 ± 5.85 bc	6.21 ± 0.34 b-d	58.23 ± 1.04 bc	70.08 ± 1.05 b-d
Cs-KIO_3	5 mg I kg^{-1}	113.26 ± 5.97 a	4.51 ± 0.31 a	55.02 ± 0.99 a	65.76 ± 1.31 a
Cs-KIO_3	25 mg I kg^{-1}	143.21 ± 5.44 c	6.46 ± 0.34 cd	58.84 ± 0.90 bc	71.17 ± 1.24 c-e
KIO_3	5 mg I kg^{-1}	140.04 ± 7.53 c	6.38 ± 0.43 cd	58.05 ± 1.35 a-c	70.79 ± 1.83 c-e
KIO_4	25 mg I kg^{-1}	132.48 ± 6.46 a-c	5.16 ± 0.40 ab	57.61 ± 1.22 a-c	68.55 ± 1.74 a-c
Cs-KI	5 mg I kg^{-1}	133.17 ± 8.99 a-c	6.69 ± 0.40 b-d	59.17 ± 1.54 bc	73.1 ± 1.12 de
Cs-KI	25 mg I kg^{-1}	118.39 ± 6.54 ab	5.45 ± 0.43 a-c	54.83 ± 1.23 a	67.24 ± 1.39 a-c
KI	5 mg I kg^{-1}	143.98 ± 13.69 bc	7.01 ± 1.31 b-d	56.59 ± 1.06 ab	70.23 ± 1.25 b-e
KI	25 mg I kg^{-1}	139.69 ± 9.55 c	6.79 ± 0.40 d	59.80 ± 0.10 c	73.58 ± 0.97- e
p Value		0.0026	0.0003	0.0016	0.0001

Valores de media con ± error estándar. Valores con la misma letra son estadísticamente similares (Kruskal wallis, p <0.05).Mean values with ± standard error. Values with the same letter are statistically similar (Kruskal wallis, p <0.05).

Figura 1. Diámetro polar de frutos de tomate biofortificados yodo y Cs-I.

Figure 1. Polar diameter in tomato fruits biofortified with iodine and Cs-I.

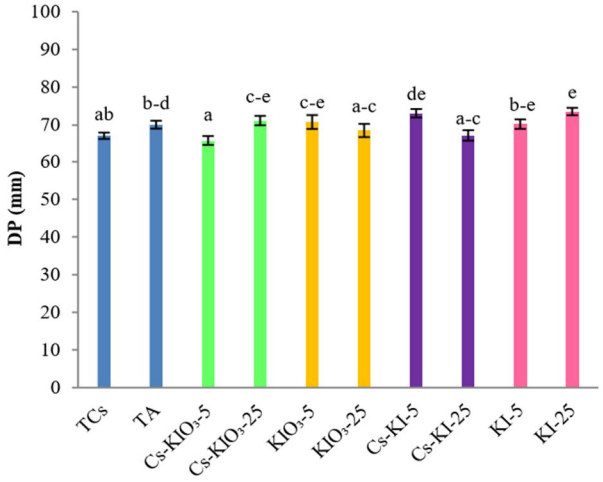


Figura 2. Contenido de yodo total en poscosecha de frutos de tomate biofortificados con yodo y Cs-I.

Figure 2. Post-harvest total iodine content of tomato fruits biofortified with iodine and Cs-I.

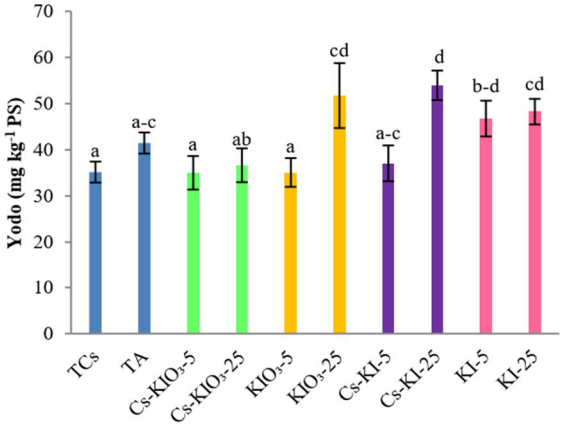


Tabla 2. Concentración de yodo en frutos evaluados a través de 12 días de poscosecha.

Table 2. Iodine concentration in tomato fruits evaluated through 12 days of post-harvest.

Tratamientos	Concentración de I kg^{-1} sustrato	Yodo (mg I kg^{-1})				
		Día de Inicio	Día 3	Día 6	Día 9	Día 12
TCs	0 mg kg^{-1}	28.0 a	50.65 a	34.99 ab	29.62 a	29.96 a
TA	0 mg kg^{-1}	37.04 ab	47.05 a	47.50 a-c	37.89 a	32.16 a
Cs-KIO_3	5 mg kg^{-1}	20.58 a	50.45 a	21.80 a	41.8 a	46.37 a
Cs-KIO_3	25 mg kg^{-1}	22.61 a	53.68 a	36.32 a-c	34.13 a	33.3 a
KIO_3	5 mg kg^{-1}	30.33 ab	42.33 a	43.94 a-c	27.6 a	25.63 a
KIO_3	25 mg kg^{-1}	48.06 ab	61.34 a	74.42 c	37.54 a	24.45 a
Cs-KI	5 mg kg^{-1}	31.56 ab	42.78 a	46.30 a-c	--	--
Cs-KI	25 mg kg^{-1}	51.15 b	52.44 a	60.27 c	40.48 a	--
KI	5 mg kg^{-1}	34.25 ab	56.04 a	54.94 bc	38.48 a	--
KI	25 mg kg^{-1}	56.20 b	45.59 a	53.94 bc	32.07 a	34.29 a
p Value		0.0028	0.0696	0.0014	0.7837	0.8454

Valores de media. Valores con la misma letra son estadísticamente similares (Kruskal wallis, p <0.05). líneas punteadas (--), corresponden a los tratamientos con menor número de frutos que por lo tanto, no fue posible evaluar esos días.

Mean values . Values with the same letter are statistically similar (Kruskal wallis, p <0.05). Dotted lines (-), correspond to the treatments with the least number of fruits, therefore it was not possible to evaluate these days.

Tabla 3. Evaluación de parámetros de calidad en poscosecha.

Table 3. Evaluation of quality parameters in post-harvest.

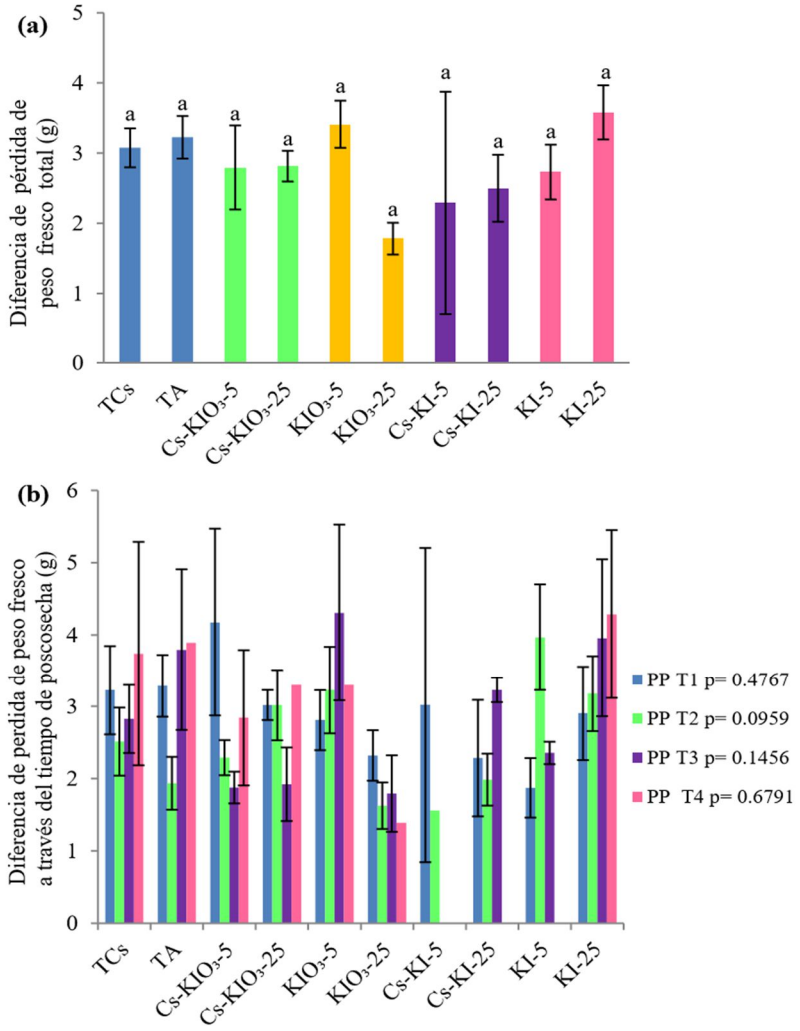
Tratamientos	Concentración de mg I kg^{-1} sustrato	SST	pH	Conductividad (mS/cm)	Firmeza (kg)
TCs	0 mg I kg^{-1}	4.51 ± 0.09 a	4.58 ± 0.05 a	1.54 ± 0.11 a	1.59 ± 0.08 a
TA	0 mg I kg^{-1}	4.61 ± 0.09 a	4.54 ± 0.08 a	1.48 ± 0.13 a	2.06 ± 0.10 b
Cs-KIO_3	5 mg I kg^{-1}	4.38 ± 0.09 a	4.60 ± 0.05 a	1.44 ± 0.13 a	1.68 ± 0.12 a
Cs-KIO_3	25 mg I kg^{-1}	4.61 ± 0.11 a	4.56 ± 0.06 a	1.57 ± 0.14 a	1.62 ± 0.09 a
KIO_3	5 mg I kg^{-1}	4.62 ± 0.13 a	4.64 ± 0.08 a	1.67 ± 0.12 a	1.99 ± 0.15 ab
KIO_3	25 mg I kg^{-1}	4.52 ± 0.13 a	4.55 ± 0.03 a	1.55 ± 0.23 a	1.59 ± 0.13 a
Cs-KI	5 mg I kg^{-1}	4.51 ± 0.14 a	4.58 ± 0.05 a	1.22 ± 0.14 a	1.84 ± 0.21 ab
Cs-KI	25 mg I kg^{-1}	4.95 ± 0.21 a	4.56 ± 0.04 a	1.52 ± 0.15 a	2.16 ± 0.30 ab
KI	5 mg I kg^{-1}	4.23 ± 0.10 a	4.59 ± 0.04 a	1.36 ± 0.12 a	1.66 ± 0.17 a
KI	25 mg I kg^{-1}	4.69 ± 0.07 a	4.62 ± 0.05 a	1.59 ± 0.13 a	1.94 ± 0.43 ab
p Value		0.3724	0.9559	0.6295	0.0084

Valores de media con ± error estándar. Valores con la misma letra son estadísticamente similares (Kruskal wallis, p <0.05).

Mean values with ± standard error. Values with the same letter are statistically similar (Kruskal wallis, p <0.05).

Figura 3. Pérdida de peso total y a través del tiempo de poscosecha.

Figure 3. Total Weight loss and through post-harvest time.

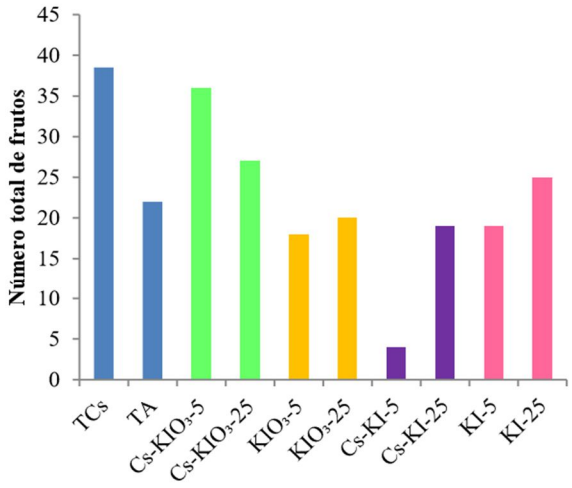


Diferencias de pérdida de peso fresco: (a) Pérdida de peso fresco total; (b) Pérdida de peso a través del tiempo. Leyenda: D0-D3 (PP T1); D3-D6 (PP T2), D6-D9 (PP T3), D9-D12 (PP T4). Algunas barras carecen de error estándar a causa del pequeño tamaño de muestra.

Differences from fresh weight loss: (a) Total fresh weight loss; (b) Weight loss over time. Legend: D0-D3 (PP T1); D3-D6 (PP T2), D6-D9 (PP T3), D9-D12 (PP T4). Some bars lack standard error due to small sample size

Figura 4. Número total de frutos.

Figure 4. Total number of fruits.



CONCLUSIONES GENERALES

En lechuga, la biofortificación con complejos Cs-KI y Cs-KIO_3, no afectó el contenido mineral, actividad enzimática y contenido de antioxidantes, siendo esto un factor importante, puesto que el enfoque primordial es el contenido de yodo.

Las dos formas de aplicación evaluadas en los tratamientos en cultivo de lechuga (una sola dosis antes del trasplante y en dosis divididas- al inicio y después del trasplante), nos permitieron evaluar el impacto sobre la biomasa, mostrando una disminución con Cs-KI en dosis divididas y el impacto en el contenido de yodo siendo mayor con dosis divididas de KIO_3, aunque la biomasa disminuyó considerablemente en contraste con Cs-KIO_3 de una sola dosis. A pesar de que los complejos de quitosán-yodo, no incrementaron la absorción de yodo comparado con las sales, se recomienda su uso en especial de los complejos de Cs-KIO_3 ya que aumentaron la biomasa y se logró una acumulación de yodo en hojas de lechuga.

Se encontró disminución en el número de frutos de tomate con el tratamiento Cs-KI de 5 mg I kg^{-1} de sustrato. En cambio, con el tratamiento de Cs-KIO_3 de 5 mg I kg^{-1} de sustrato se obtuvo aumento en el número de frutos en comparación con el testigo absoluto. En el estudio de poscosecha de tomate no se encontraron efectos adversos al biofortificar con sales o complejos de yodo. Los complejos de Cs-KIO_3 de 5 mg I kg^{-1} de sustrato mostraron un ligero incremento en disponibilidad y acumulación del yodo a lo largo de 12 días. Por lo tanto, se recomienda el uso de este complejo Cs-KIO_3 aplicado al sustrato.

En general en ambos estudios vemos que la biofortificación con Cs-KIO_3 no afectó el rendimiento y se logró la acumulación de yodo en las partes comestibles. Por otra parte, disminuyó la biomasa total de la lechuga y el número de frutos en tomate con los complejos de Cs-KI.