Centro de Investigación en Alimentación y Desarrollo, A.C.

EFECTO DE LAS ALTAS PRESIONES HIDROSTÁTICAS ASISTIDA CON ACETOGENINAS DE SEMILLA DE AGUACATE SOBRE LA CALIDAD NUTRICIONAL, MICROBIOLÓGICA Y FISICOQUÍMICA DEL PURÉ DE FRESA

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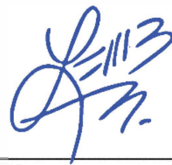

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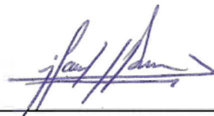

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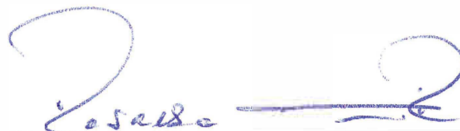

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RESUMEN

La contaminación de los alimentos por hongos y sus esporas es un problema importante entre los procesadores de frutas. La fresa y sus productos como el puré son muy susceptibles al desarrollo de hongos, principalmente por *Botrytis cinerea.* Además, durante su procesamiento puede sufrir pérdidas en sus compuestos bioactivos como son las vitaminas, compuestos fenólicos y antocianinas. Estas pérdidas son principalmente por las altas temperaturas del proceso de elaboración. Para tratar de combatir a *Botrytis cinerea* se utilizan conservadores sintéticos, incluido el sorbato de potasio (SP), y para conservar compuestos bioactivos en los productos de frutas. Se han investigado otras tecnologías de barrera como las altas presiones hidrostáticas (HHP), un proceso de pasteurización no térmico. Además, las acetogeninas de semilla de aguacate (ASA) se han estudiado por su actividad antibacteriana pero no por su efecto antifúngico. El presente estudio, evaluó el efecto de HHP (0, 400 y 600 MPa/5min) asistido con ASA (0, 661 y 7,500 µg/mL) sobre la calidad nutricional, microbiológica y fisicoquímica del puré de fresa. Se evaluó en primer término la concentración inhibitoria mínima (MIC) y el efecto antifúngico de las ASA utilizando el método de dilución en agar del extracto sobre del crecimiento del micelio y la germinación de conidias, se probó después de 96 y 5 h de incubación a 18 ± 1°C, respectivamente. Se utilizó un modelo cinético ajustado de Gompertz para determinar la fase de crecimiento en las que las ASA afectaron el crecimiento micelial y la germinación conidial. Las ASA (7,500 mg/L) afectaron directamente la fase estacionaria del hongo en desarrollo sin diferencias significativas ($p<0,05$) con un nivel de uso comercial de SP (1,000 mg/L). La concentración fungistática media (661 mg/L ASA) del crecimiento micelial redujo en 78% la germinación de conidias, mientras que su IC_{50} en esta etapa fue de 75.42 mg/L. La inoculación de 6.3 log de conidias de *Botrytis cinerea* en puré de fresas, disminuyó el color superficial del puré, reduciendo en un 25% la pelargonidina-3-glucósido, 38% el ácido elágico, 84% el ácido L-ascórbico y 38% el ácido ascórbico total. Por otra parte, utilizando HHP con ASA no se detectaron conidias viables de *Botrytis cinerea*; pero si aumentó el ácido elágico y la pelargonidina-3-glucósido; se mantuvo el ácido L-ascórbico, ácido ascórbico total, ácido p-hidroxibenzoico. Se fortalece el valor de las tecnologías no térmicas combinadas para la inhibición de hongos fitopatógenos y conservación de compuestos bioactivos en puré de fresas.

Palabras claves: Puré de fresas, acetogeninas de semilla de aguacate, sorbato de potasio, altas presiones hidrostáticas, compuestos bioactivos, *Botrytis cinerea.*

ABSTRACT

Contamination of foods by fungi and their spores is a major problem among fruit processors. Strawberries and their products such as puree are very susceptible to the development of fungi, mainly due to *Botrytis cinerea*. In addition, during its processing it can suffer losses in its bioactive compounds such as vitamins, phenolic compounds and anthocyanins. These losses are mainly due to the high temperatures of the manufacturing process. Synthetic preservatives, including potassium sorbate (SP), are used to try to combat *Botrytis cinerea* and to preserve bioactive compounds in fruit products. Other barrier technologies such as high hydrostatic pressures (HHP), a non-thermal pasteurization process, have been investigated. Furthermore, avocado seed acetogenins (ASA) have been studied for their antibacterial activity but not for their antifungal effect. The present study evaluated the effect of HHP (0, 400, and 600 MPa/5min) assisted with ASA (0, 661, and 7,500 µg/mL) on the nutritional, microbiological, and physicochemical quality of strawberry puree. The minimum inhibitory concentration (MIC) and the antifungal effect of the ASAs were first evaluated using the agar dilution method of the extract on the growth of the mycelium and the germination of conidia, it was tested after 96 and 5 h of incubation at 18 ± 1°C, respectively. A Gompertz adjusted kinetic model was used to determine the growth phase in which ASAs affected mycelial growth and conidial germination. The ASA (7,500 mg/L) directly affected the stationary phase of the developing fungus without significant differences ($p<0.05$) with a level of commercial use of SP (1,000 mg/L). The average fungistatic concentration (661 mg/L ASA) of the mycelial growth reduced the germination of conidia by 78%, while its IC_{50} at this stage was 75.42 mg/L. The inoculation of 6.3 log of *Botrytis cinerea* conidia in strawberry puree decreased the superficial color of the puree, reducing pelargonidin-3-glucoside by 25%, ellagic acid 38%, L-ascorbic acid 84% and 38% total ascorbic acid. On the other hand, using HHP with ASA, no viable conidia of *Botrytis cinerea* were detected; but it did increase ellagic acid and pelargonidin-3-glucoside; L-ascorbic acid, total ascorbic acid, p-hydroxybenzoic acid were maintained. The value of combined non-thermal technologies for the inhibition of phytopathogenic fungi and preservation of bioactive compounds in strawberry puree is strengthened.

Keywords: Strawberry puree, avocado seed acetogenins, potassium sorbate, high hydrostatic pressures, bioactive compounds, *Botrytis cinerea.*

1. SIPNOSIS

1.1. Justificación

Las fresas se consumen frescas o procesadas en varios alimentos/ingredientes tales como concentrado, jugo y puré; son reconocida por su alto valor nutritivo, ya que diferentes estudios reportan su aporte en compuestos bioactivos, tales como: fenoles, flavonoides, antocianinas y vitaminas (Marszałek *et al*., 2017; Teribia *et al*., 2021). Estos compuestos han demostrado ser preventivos contra enfermedades cardiovasculares, artritis, diabetes tipo 2, cáncer y otras enfermedades no transmisibles (Gao *et al*., 2016; Marszałek *et al*., 2017). La fresa se cultiva y se consume ampliamente en todo el mundo, uno de los productos elaborados a partir de la fresa es el puré. Para la industria alimentaria, este producto representa un ingrediente natural e interesante para preparaciones de conservas de frutas, helados, batidos y/o utilizado como ingrediente en repostería, entre otros; es ampliamente preferido por el color, aroma y el sabor que imparte (Teribia *et al*., 2021). Sin embargo, durante el proceso de elaboración del puré se utilizan altas temperaturas, las que pueden degradar los compuestos bioactivos generando una rápida degradación y afectación directamente de sus propiedades organolépticas y fisicoquímicas. No obstante, durante este procesamiento es deseable conservar el nivel más alto posible de compuestos bioactivos (Marszałek *et al*., 2017).

Por otro lado, la fresa y sus productos son muy susceptibles al desarrollo de hongos, principalmente por *Botrytis cinerea* Pers. Se trata de un hongo fitopatógeno, agente causal de la pudrición gris en fresas, una importante enfermedad con una gran relevancia económica y científica que causa pérdidas pre y poscosecha a nivel mundial (León *et al*., 2014). Provoca graves daños en el proceso productivo, al destruir del 60 al 70% de los frutos, constituyendo una de las principales pérdidas que ocurren en poscosecha con una incidencia máxima del 89% (Ugolini *et al*., 2014). En relación con los productos de fresas, como el puré, puede sufrir pérdidas de sus propiedades fisicoquímicas durante su procesamiento producidos principalmente por microorganismos y por el tipo de tratamiento de conservación aplicado (Chakraborty *et al*., 2014; Hurtado, 2017).

La contaminación de los alimentos con hongos y sus esporas es un problema importante entre los

procesadores de frutas, ya que se encuentra con frecuencia en jugos y concentrados de frutas, entre otros productos. La fresa debido a su alto contenido de azúcar y bajos niveles de pH es susceptible a la contaminación fúngica; las manchas marrones claras y las áreas húmedas en la superficie están asociadas con el deterioro microbiano, que luego se cubren con un polvo gris o masa algodonosa del micelio de hongos fitopatógenos. Esta contaminación da origen a sabores y olores desagradables generando pérdidas económicas considerables y problemas de seguridad alimentaria (Pinto *et al*., 2020; Pravallika y Chakraborty, 2022).

Una alternativa para reducir el efecto de altas temperaturas sobre la calidad nutricional de los alimentos y al mismo tiempo reducir la carga microbiana, es el uso de procesamiento por altas presiones hidrostáticas (HHP, por sus siglas en inglés), la cual prolonga la vida útil de los alimentos sólidos y líquidos. Destruye las células microbianas, las esporas y las enzimas mientras retiene el máximo de nutrientes. Evidencias científicas señalan que varios microorganismos presentan diferente sensibilidad y responden de manera diferente al procesamiento por HHP. El mecanismo de inactivación por HHP propuesto para hongos por es por la desnaturalización de las proteínas (Pravallika y Chakraborty, 2022).

Aunado a los tratamientos térmicos se utilizan aditivos como el sorbato de potasio; este aditivo se usa comúnmente y es el antimicrobiano más efectivo en la industria alimentaria (Mohammadzadeh *et al*., 2018). Además de inhibir el crecimiento de hongos, previene el deterioro y conserva la frescura de los productos a una concentración de 1,000 mg/kg (Quispe, 2010; Dehghan *et al*., 2018). Sin embargo, varios estudios han demostrado que una mayor ingesta de sorbato de potasio (> 25 mg/kg) puede provocar efectos citotóxicos y genotóxicos al producir compuestos mutagénicos, inducir aberraciones cromosómicas e intercambio de cromátidas hermanas (Dehghan *et al*., 2018). Los consumidores a menudo requieren alimentos y aditivos seguros, los que con su ingesta no afecte la salud y se aprovechen las propiedades nutricionales del alimento (Bonciu *et al*., 2018). Por lo anterior, actualmente existe una demanda creciente de consumidores e industrias por nuevos antimicrobianos de origen natural (Nazir *et al*., 2019).

El aguacate contiene derivados lipídicos con propiedades antimicrobianas llamadas acetogeninas. México es el centro de origen y distribución del aguacate y cuenta con gran variedad de “criollos”, los cuales se han diversificado a través del tiempo. La caracterización y evaluación de dichos recursos fitogenéticos permiten establecer su utilidad potencial. Existe un interés particular en los lípidos de la semilla de aguacate y sus derivados, ya que poseen una importante actividad

anticancerígena y capacidad antioxidante. La variedad nativa mexicana muestra un mayor contenido de aceite en la semilla (20 % a 30 %) (Lara *et al*., 2019; Lara *et al*., 2021).

Rodríguez *et al.* (2013), determinaron que existe una relación entre las acetogeninas presentes en el mesocarpio de la variedad 'Hass' y la actividad antioxidante del fruto. Estos compuestos pueden intervenir como antioxidantes lipofílicos en alimentos, al actuar como agentes estabilizantes de especies aniónicas y como agentes donadores de hidrógeno y se ha iniciado el interés por usarlos como sustitutos de los aditivos alimentarios sintéticos. Los estudios iniciales sobre su capacidad para inhibir microorganismo se realizaron utilizando extractos enriquecidos con acetogeninas purificadas en un entorno de laboratorio mediante aislamiento guiado por bioensayo (Rodríguez *et al*., 2019). Estudios previos también documentaron que las acetogeninas eran estables en condiciones de procesamiento de alimentos relevantes (temperatura, presión, pH) y eran capaces de controlar la germinación de conidias y el crecimiento micelial de *Botrytis cinerea* (Rodríguez *et al*., 2019; Echenique *et al*., 2021).

1.2. Antecedentes

1.2.1. Generalidades de la Fresa

La fresa es una inflorescencia de receptáculo carnoso, ovado de color rosa a rojo, suculento y fragante, de la planta de tallos rastreros, pertenece a la familia de las Rosáceas del género Fragaria. Los índices de calidad de la fresa están vinculados a su apariencia (frescas, limpias, sanas, color, tipo, forma, libre de defectos), firmeza, sabor, olor y valor nutricional (Cuadro 1) (NOM, 2002). El color ha sido siempre un gran reto en los alimentos después del procesamiento industrial y muchos parámetros están implicados en su estabilidad, tales como: pH, temperatura, luz, oxígeno, ácido L-ascórbico, reacciones enzimáticas y no enzimáticas. La estabilidad está influenciada por la auto asociación (condensación de antocianinas) y la copigmentación (interacción de antocianinas con polifenoles) (Bodelón *et al*., 2013).

Cuadro 1. Información nutricional de la fresa.

Fresa (*fragaria* × *ananassa*) – información nutricional (cantidad por 100 g)	
Energía (Kcal)	32
Carbohidratos (g)	7.68
- Azúcares (g)	4.89
- Fibra alimentaria (g)	2.00
Grasas (g)	0.3
- Saturadas (g)	0.015
- monoinsaturadas (g)	0.043
- poliinsaturadas (g)	0.155
Proteína (g)	0.67
Agua (g)	90.95
Retinol (vit. A) (μg)	1
- β-caroteno (μg)	7
Tiamina (vit. B1) (mg)	0.024
Riboflavina (vit. B2) (mg)	0.022
Niacina (vit. B3) (mg)	0.386
Ácido pantoténico (vit. B5) (mg)	0.125
Vitamina B6 (mg)	0.047
Ácido fólico (vit. B9) (μg)	24
Vitamina B12 (μg)	0.0
Vitamina C (μg)	58.8
Vitamina D (μg)	0.0
Vitamina E mg	0.29
Vitamina K (μg)	2.2
Calcio (mg)	16
Cobre (mg)	0.048
Hierro (mg)	0.41
Magnesio (mg)	13
Manganeso (mg)	0.386
Fósforo (mg)	24
Potasio (mg)	153
Selenio (μg)	0.4
Sodio (mg)	1
Zinc (mg)	0.14

Fuente: USDA (2016).

La aceptación y preferencia de la fresa y sus productos por parte de los consumidores se ven

influenciados por el color, un parámetro crítico de calidad ya que determina el precio del producto. Para investigar el color de forma sistemática es necesario medir el color (luminosidad, pureza, tonalidad) y la concentración de pigmento siendo la pelargonidina-3-glucósido y cianidina-3-glucósido las principales antocianinas responsables del atractivo color rojo en la fresa. Glucósidos de pelargonidina son responsables del color rojo brillante y los glucósidos de cianidina del rojo oscuro (Cao *et al*., 2011; Bodelón *et al*., 2013).

Las propiedades organolépticas y textura de los sub-productos de fresas dependen de la integridad estructural de la pared celular y la lámina central. Enzimas como pectinmetilesterasa y poligalacturonasa participan en la degradación de pectina y otros polímeros de la pared celular, generando un producto con viscosidad reducida y propiedades organolépticas indeseables (Marszałek *et al*., 2017). Se ha reportado en el puré de fresas la acción de especies reactivas del oxígeno (EROs), que se acumulan durante el estrés oxidativo causado por diversos factores. Estas EROs están involucradas en diferentes cambios de calidad en la fresa y sus productos, así mismo, se les ha asociado con diversas patologías como el cáncer, diabetes, enfermedades cardiovasculares y enfermedades crónico-degenerativas (Reyes, 2016).

Los procesos industriales comunes para la producción de puré de fresa consisten en diferentes pasos: (i) lavado y clasificación de la fruta, (ii) trituración de la fruta, (iii) tamizado como paso opcional (iv) un tiempo de espera en el que el puré se almacena en tanques de compensación durante 3 a 5 h antes de la pasteurización y finalmente, (v) pasteurización para garantizar la seguridad microbiana y la inactivación de las enzimas que degradan la calidad del puré. Los cambios de color que ocurren en el proceso de fabricación del puré de fresa son el resultado de una combinación de desarrollo de color marrón y decoloración del color rojo debido a la oxidación y/o degradación de las antocianinas (Teribia *et al*., 2021).

1.2.2. Compuestos Fenólicos

Las fresas y sus productos son ricas fuentes de compuestos fenólicos (Cuadro 2), los cuales pertenecen a un amplio y heterogéneo grupo de componentes químicos que poseen uno o más anillos aromáticos con un sistema aromático conjugado y uno o más grupos hidroxilo. Tienden a

donar un electrón o un átomo de hidrógeno a un radical libre y convertirlo en una molécula inofensiva (Skrovankova *et al*., 2015). Cuando las defensas del sistema antioxidante natural (enzimáticas y no enzimáticas) se ven superadas por una generación excesiva de especies reactivas de oxígeno o prooxidantes, se instala el estrés oxidativo, lo que provoca daños en las macromoléculas celulares y extracelulares. El número de grupos hidroxilo y su posición en relación con el grupo funcional carboxilo influye en la actividad antioxidante de los compuestos fenólicos (Vuolo *et al*., 2019).

Los polifenoles normalmente se generan como resultado de derivados glicosilados en plantas, muchos de los compuestos fenólicos son solubles en agua o en disolventes orgánicos. La modificación del pH puede influir en las reacciones químicas de los compuestos fenólicos, como en las antocianinas; un pH bajo (2.5) es mejor para la conservación de polifenoles en los productos de fresa durante el almacenamiento (Oliveira *et al*., 2015). Estos compuestos, individualmente o combinados, son los principales responsables de los beneficios para la salud por sus propiedades antioxidantes (Skrovankova *et al*., 2015).

Los compuestos fenólicos como el ácido elágico y el ácido p-hidroxibenzoico son metabolitos secundarios presentes en las plantas con un papel importante en la calidad sensorial y nutricional de frutas, hortalizas y otras plantas, también se han identificado como los principales antioxidantes de las frutas. Estos compuestos actúan como antioxidantes en el cuerpo humano y realizan funciones antimutagénicas, anticancerígenas, antihipertensivas y antiinflamatorias (Parra *et al*., 2020; Guevara *et al*., 2022).

Su estructura comprende un anillo aromático, que contiene uno o más sustituyentes hidroxilo. Pueden variar desde simples moléculas fenólicas hasta compuestos altamente polimerizados. La mayoría de los compuestos fenólicos ocurren naturalmente como conjugados con monosacáridos y polisacáridos, asociados con uno o más grupos fenólicos. Además, también se pueden vincular a ésteres y ésteres metilados. Debido a su diversidad estructural, existe una amplia gama de compuestos fenólicos que se encuentran en la naturaleza. Actualmente se conocen más de 8000 estructuras de compuestos fenólicos. Las clases de compuestos fenólicos más importantes que se encuentran en la dieta humana son los ácidos fenólicos, los flavonoides y los taninos. Químicamente, los ácidos fenólicos tienen al menos un anillo aromático donde al menos un hidrógeno está sustituido por un grupo hidroxilo (Vuolo *et al*., 2019).

Cuadro 2. Composición de polifenoles en fresas.

PRINCIPALES COMPUESTO ENÓLICOS	GRUPOS	COMPUESTOS
	Antocianinas	Cianidina- 3 – glucósido
		Cianidina - 3 - rutinosido
	Glucósidos de cianidina	Cianidina - 3 - galactósido
		Cianidina - 3 - malonilglucósido
		Cianidina - 3 - malonilglucósil - 5- glucósido
	Glucósidos de pelargonidina	Pelargonidina - 3 – glucósido
		Pelargonidina - 3,5 - diglucósido
		Pelargonidina - 3 - rutinosido
		Pelargonidina - 3 - galactósido
		Pelargonidina - 3 - arabinósido
		Pelargonidina - 3 - malonilglucósido
		Pelargonidina - 3 - malilglucósido
		Pelargonidina - 3 - acetilglucósido
		Pelargonidina - 3 - disacárido
	Glucósidos de peonidina	Peonidina - 3 – glucósido
	Flavonoides	Quercetina - 3 – glucurónido
	Glucósidos de quercetina	Quercetina - 3 - malonilglucósido
		Quercetina - 3 - rutinoside = rutina
		Quercetina - 3 – glucósido
	Glucósidos de kaempferol	Kaempferol - 3 – glucósido
		Kaempferol - glucurónido
		Kaempferol - 3 - malonilglucósido
		Kaempferol - cumaroil - glucósido
Ácidos fenólicos	**Flavanoles**	Proantocianidina B1 (EC-4,8-C)
		Trímero de proantocianidina (EC-4,8-EC-4,8-C)
		Proantocianidina B3 (C-4,8-C)
		(+) – Catequina
	Ácidos hidroxicinámicos	P-cumaroil hexosa
		Ácido p-cumárico
		Ácido cafeico
Taninos hidrolizables	Elagitaninos	Bis - hexahidroxidifenilo - glucosa
		Galloil - hexahidroxidifenilo - glucosa
		hexahidroxidifenilo - galoil - glucosa
		Galloil - bis - hexahidroxidifenilo - glucosa
		Dímero de galioil-bis- hexahidroxidifenilo
		Sanguiin H-6
		Metil- ácido elágico -pentosa conjugados
		Ácido elágico pentosodico
		Ácido elágico
		Ácido gálico

Factores a depender: Cultivo, genotipo y variedad. Localización creciente; técnica de cultivo (orgánico o convencional); condiciones de cultivo (Invernadero, plástico, campo abierto, túnel, luz).; temporada de crecimiento, maduración, procesamiento y almacenamiento (tiempo, temperatura). Fuente: Skrovankova *et al*., (2015).

Por su parte, las antocianinas se derivan de los flavonoles que tienen un grupo hidroxilo en la posición 3. Además, tienen dos dobles enlaces (uno entre el átomo de oxígeno y el carbono y otro entre el carbono 3 y 4), y falta una cetona de oxígeno en posición 4; por lo tanto, su estructura se describe básicamente como un ion flavilio (Albuquerque *et al*., 2021). Las antocianinas son compuestos solubles en agua que dan color a los tejidos de las plantas con colores que van desde el rojo, púrpura y azul dependiendo del pH vacuolar y su composición estructural. Son derivados glicosilados del catión 3,5,7,4′-tetrahidroxiflavilio y existen diferentes sustituyentes en el anillo B para formar distintas clases de antocianinas. Entre ellas se identifica seis clases que se incluyen con frecuencia; pelargonidina, cianidina, delfinidina, peonidina, petunidina y malvidina. El compuesto más importante, la pelargonidina 3-glucósido, es la antocianina responsable del color rojo de la fruta del género *Fragaria* (He *et al*., 2018; Parra *et al*., 2020).

Las principales antocianinas de la fresa se han identificado como glucósidos de pelargonidina y cianidina o sus formas aciladas. La expresión génica y la activación de las enzimas de biosíntesis de antocianinas están influenciadas por tres factores principales: (i) prácticas culturales, (ii) condiciones climáticas y, (iii) etapas fenológicas (Parra *et al*., 2020).

Numerosos estudios han demostrado los efectos positivos de HHP sobre la estabilidad de los ingredientes bioactivos, en comparación con el procesamiento térmico, y se confirmó el aumento de la bioaccesibilidad de compuestos nutricionales seleccionados. HHP puede afectar las estructuras de los tejidos vegetales y aumentar la capacidad de extracción de los ingredientes bioactivos, al mismo tiempo que tiene un efecto positivo en su estabilidad inicial. Además, este tratamiento puede alterar la estructura de los ingredientes, tanto los bioactivos como los capaces de unirlos (como la pectina), haciéndolos más disponibles (Trych *et al*., 2020). Por lo tanto, HHP puede ser un método útil de conservación en el diseño de alimentos funcionales con altos valores nutricionales. De acuerdo con lo señalado por Trych *et al*., (2020), hay un número limitado de estudios sobre la influencia de HHP en la bioaccesibilidad de las antocianinas, compuestos fenólicos y vitamina C.

1.2.3. Vitamina C

Entre los compuestos bioactivos de la fresa, se encuentra la vitamina C o ácido ascórbico es una

vitamina hidrosoluble que actúa como un potente reductor (antioxidante) donando átomos de hidrógeno a otros agentes, que son así reducidos, pasando entonces esta vitamina a su forma oxidada (ácido L-dehidroascorbico). Constituye una de las principales defensas neutralizando radicales libres orgánicos altamente dañinos, formados por la acción de EROs, que se acumulan durante el estrés oxidativo. Estas EROs están involucradas en diversas patologías como el cáncer, diabetes, enfermedades cardiovasculares y enfermedades crónico-degenerativas (Loja *et al*., 2016; Marszalek *et al*., 2017).

La mayoría de los animales son capaces de sintetizar la vitamina C en su organismo, en cambio, los humanos no tienen la capacidad de generar su propio ácido ascórbico; para conseguirlo, el hombre debe obtenerla a través de la dieta (Loja *et al*., 2016). Sin embargo, la vitamina C se caracteriza por ser una de las vitaminas más termosensibles durante los tratamientos térmicos, razón por la cual es usada como indicador de degradación térmica de nutrientes durante el procesamiento de los alimentos (Mendoza *et al*., 2016). La pérdida de ácido ascórbico en frutos como naranja, piña y pimiento morrón, contaminados por hongos como *Sclerotium rolfsii*, ha sido reportada como una alteración en la fisiología de los frutos por patogenia, provocando reacciones de oxidación en las que actúa como antioxidante (Frans *et al*., 2021).

El ácido L-ascórbico es el principal antioxidante que actúa en la reacción oxidativa mediante la formación de productos no oxidantes como el dehidroascorbato y el ácido 2,3 dicetogulónico (Frans *et al*., 2021, Javanmardi *et al*., 2023). Además, se ha reportado que patógenos como: *Rhizopus stolonifer, Aspergillus flavus, Penicillium digitatum, Curvularia lunata* y *Fusarium moniliforme* producen toxinas que disminuyen la concentración de ácido ascórbico total (Sawant y Gawai, 2011). En otros estudios se observó que, durante el tiempo de espera entre la molienda y la pasteurización, las antocianinas y el color se mantuvieron sin cambios, pero el AA se oxidó a DHAA (Teribia *et al*., 2021).

1.2.4. Hongos Fitopatógenos: *Botrytis cinerea*

Su ciclo de vida comienza en la generación, por parte del micelio, de esporas asexuales o conidias que se dispersan mediante corrientes aéreas o por otros vectores, y si se dan las condiciones

idóneas, las conidias germinarán produciendo un tubo germinal, provocando el desarrollo de una infección primaria, que rápidamente se propagará colapsando tejidos y así apareciendo los primeros síntomas de podredumbre, la cual vendrá acompañada por la formación de nuevos conidióforos y conidias, que serán de nuevo dispersadas (Nakajima y Akutsu 2014).Las esporas son estructuras latentes altamente resistentes formadas a partir de células vegetativas que son diferentes de sus contrapartes de células vegetativas en composición química, estructura morfológica y fisiológica, así como los hongos (Keynan, 1969).

Las esporas se desarrollan cuando los nutrientes y la humedad son limitados, lo que les permite sobrevivir durante períodos prolongados en diversas condiciones ambientales extremas. Una espora puede romper su letargo a través de la germinación inducida por agentes nutrientes y no nutrientes y volver a crecer cuando se restablecen las condiciones favorables (por ejemplo; agua, temperatura y nutrientes). También se sabe que el calentamiento subletal de las esporas estimula la germinación de las esporas. Ciertas bacterias, hongos y levaduras pueden producir esporas, aunque las esporas producidas por hongos son biológicamente diferentes de las producidas por bacterias. Aparte de su función de supervivencia, la mayoría de los hongos producen esporas como parte de sus ciclos reproductivos (Silva, 2019).

Los alimentos con alto contenido de ácido o acidificados son propensos a la contaminación por hongos y formadores de esporas de levadura. El crecimiento de hongos podría resultar en un aumento del pH de los alimentos por encima de 4.6, aumentando así el potencial de crecimiento y riesgo (Silva, 2019). Para el control de la contaminación por hongos y levaduras en alimentos, se han empleado diversos conservadores, entre los cuales se encuentra el sorbato de potasio. El mecanismo de inhibición del sorbato de potasio en hongos está asociado a los dobles enlaces del sorbato de potasio, que interfieren con la actividad catalítica de las enzimas responsables del crecimiento microbiano (Lück y Jager, 2000). La Administración de Alimentos y Medicamentos (FDA, por sus siglas en inglés) había ordenado una regulación para el límite de microorganismos en las frutas y sus productos, es decir, una reducción de > 5 log o presencia de < 1 log CFU/g o mL en muestras procesadas se toma como un nivel aceptable (Yuan *et al*., 2018).

Por otra parte, se ha reportado que *Botrytis cinerea* induce un choque oxidativo en las células vegetales durante las primeras etapas del proceso de infección, acumulando peróxido de hidrógeno en el tubo germinativo, así como peroxirredoxina y catalasa; enzimas que regulan los procesos para mantener altos niveles de peróxido de hidrógeno en el tubo germinativo durante la germinación.

Song *et al*., (2022) mencionaron que las esporas en germinación son más susceptibles a diversos factores estresantes que sus formas latentes. En este sentido, inducir la germinación de esporas antes del paso HHP puede ser una estrategia potencial para erradicar varias esporas, mientras que a presiones más altas (>400 MPa), la germinación de esporas puede inducirse abriendo canales que permitan la pérdida de ácido dipicolínico, reduciendo su resistencia al calor. Por lo tanto, se necesita proporcionar más información científica sobre los estudios en puré de fresas contaminados con hongos ya que a la fecha son escasos.

1.2.5. Tecnologías no Convencionales para el Control de Hongos: Altas Presiones Hidrostáticas

Diferentes estudios proponen alternativas a los tratamientos térmicos desarrollando varias estrategias para minimizar la contaminación de los alimentos inactivando esporas de hongos, como son las tecnologías de procesamiento de alimentos no térmicos (Pinto *et al*. 2020). Entre estas tecnologías se recomienda el uso de altas presiones hidrostáticas; es una tecnología no térmica que consiste en aplicar altas presiones en lapsos de tiempos cortos, simulando presiones similares a las profundidades del mar. Esto con la finalidad de reducir la carga microbiana inicial del producto y mantener las propiedades fisicoquímicas y nutricionales de la materia prima, utilizando presiones que van de 100 a 2000 Mpa (Hygreeva *et al*., 2016; Marszałek *et al*., 2017).

Para asegurar productos inocuos de fitopatógenos como *Botrytis cinerea* la industria alimentaria, utiliza tratamientos térmicos y algunos aditivos, se han propuesto las HHP como una alternativa técnica de pasteurización no térmica para alimentos (Wang *et al*., 2017). Se ha reportado que el procesamiento con HHP inactiva las formas vegetativas de bacterias patógenas y de deterioro, hongos y levaduras (Silva, 2020), preservando el valor nutricional de los compuestos bioactivos (Marszałek *et al*., 2017). La HHP a nivel industrial generalmente opera a presiones de 400–600 MPa, para tratar alimentos líquidos y sólidos utilizando tiempos de procesamiento entre 5 y 10 minutos. Según el principio de Le Chatelier, la presión hidrostática reduce el volumen del material presurizado homogéneamente. Como los enlaces covalentes no se rompen por la presión, los componentes alimenticios originales permanecen sin cambios con el proceso (Silva, 2019).

El objetivo principal de HHP es la pasteurización de los alimentos a través de la inactivación de microorganismos patógenos y de deterioro (generalmente por 5 o 6 reducciones de registro) seguido de almacenamiento y distribución en frío. La vida útil de los alimentos con procesos de HHP está influenciada principalmente por el nivel de presión, el tiempo de mantenimiento, las condiciones de almacenamiento y otros factores, como las características de empaque. Estos alimentos generalmente tienen una vida útil de 3 a 10 veces que los de los alimentos no tratados (Hiperbaric, 2015).

Se ha observado que las presiones comerciales por sí solas resultan en una inactivación menor de esporas fúngicas y bacterianas en productos frutales, lo que limita la vida útil (Silva, 2019). El procesamiento de alta presión y alta temperatura (HPHT, por sus siglas en inglés), también conocido como procesamiento térmico asistido por presión (PATP, por sus siglas en inglés), permite a las HHP lograr una inactivación eficiente de esporas (Luu-Thi *et al*., 2014). Silva *et al*. (2020) reportaron sobre la resistencia a la presión de las esporas de los hongos *Byssochlamys nivea* y *Aspergillus fischeri* inoculados en puré de fresa y sometidos a altas presiones hidrostáticas (600 MPa) combinadas con alta temperatura (75°C) durante 15 min; Los tratamientos dan como resultado reducciones de registro de 1.7 y 1.4 en la germinación de esporas de *B. nivea* después de 12 semanas en agar papa dextrosa. Por lo tanto, los desafíos técnicos de las tecnologías de HHP están relacionados con la inactivación de esporas resistentes, ya que las unidades HHP a escala industrial pueden funcionar a presiones máximas de 650 MPa con o sin temperatura combinada a 50°C (Sarker *et al*., 2015).

Villa *et al*. (2012) demostraron la inactivación de las conidias de *Botrytis cinerea* aplicando tratamiento térmico a 42°C por 45 min. Sin embargo, el uso de estos tratamientos térmicos, afecta directamente los compuestos bioactivos y por consecuencia las características organolépticas y nutricionales del puré de fresas (Marszalek *et al*., 2017). Por lo tanto, las altas presiones pueden interactuar con la pared celular y la membrana de los conidias, provocando una posible fuga de los constituyentes plasmáticos como lo menciona Balasubramaniam *et al*. (2016). Además, González *et al*., (2015) identificaron un precursor mitocondrial (peroxirredoxina-5), dos dismutasas peroximales y dos superóxidos dismutasas en células latentes. En este contexto, Van Leeuwen *et al*. (2013) sugieren que la resistencia de las conidias fúngicas al estrés puede desarrollarse fuertemente durante las primeras etapas de la germinación, lo cual puede afectar el proceso de desarrollo como lo explica Choquer *et al*., (2007). Dado que las esporas germinantes son más

susceptibles a varios factores estresantes que sus formas latentes, la implementación de la germinación de esporas antes del paso HHP puede ser una estrategia potencial para erradicar varias esporas (Kwon *et al*., 2022).

1.2.6. Conservadores Naturales

Aunado a los tratamientos térmicos se utilizan aditivos como el sorbato de potasio; este aditivo se usa comúnmente y es el antimicrobiano más efectivo en la industria alimentaria (Mohammadzadeh *et al*., 2018). Además de inhibir el crecimiento de hongos, previene el deterioro y conserva la frescura de los productos a una concentración de 1,000 mg/kg (Quispe, 2010; Dehghan *et al*., 2018). Sin embargo, varios estudios han demostrado que una mayor ingesta de sorbato de potasio (> 25 mg/kg) puede provocar efectos citotóxicos y genotóxicos al producir compuestos mutagénicos, inducir aberraciones cromosómicas e intercambio de cromátidas hermanas (Dehghan *et al*., 2018). Los consumidores a menudo requieren alimentos y aditivos seguros, los que con su ingesta no afecten la salud y se aprovechen las propiedades nutricionales del alimento. Por lo anterior, actualmente existe una demanda creciente de consumidores e industrias por nuevos antimicrobianos de origen natural (Nazir *et al*., 2017).

Una alternativa para lograrlo es mediante el uso de extractos de plantas y compuestos bioactivos naturales con propiedades antifúngicas, entre otros enfoques (Redondo *et al*., 2020). En este sentido se han estudiado las semillas y cáscaras de aguacate, las cuales tienen un contenido de compuestos bioactivos equivalente o incluso mayor que en la pulpa, encontrándose 9 moléculas lipídicas biosintetizadas por la polimerización de subunidades acetilo y propionilo con propiedades antimicrobianas y conservante (Rodríguez-Sánchez *et al*., 2019). La persenona-C, la persenona-A y la persenona-B contenidas en las semillas de aguacate inhiben la germinación de endosporas de *Clostridium sporogenes* y el crecimiento de células vegetativas (Rodríguez *et al*., 2013).

Se ha demostrado que la germinación de endosporas de *C. sporogenes* se inhibe por completo con aceite de semilla de aguacate semi-comercial enriquecido en acetogeninas (Pacheco *et al*., 2017). Las acetogeninas contienen poderosas moléculas bioactivas que están presentes exclusivamente en las semillas de aguacate, como AcO-aguacateína y persenona A, los principales compuestos con

dobles enlaces, donde un terminal metileno confiere un mayor potencial a estas acetogeninas derivadas de un terminal acetileno aumentando la actividad antimicrobiana (Pacheco *et al*., 2017; Salinas *et al*., 2017; Villarreal *et al*., 2019; Rodríguez *et al*., 2019). Las acetogeninas pueden estar afectando la morfología de las conidias, generando cambios en el pH, y afectando su integridad y actividad metabólica, como lo menciona Villareal *et al*. (2019).

Salinas *et al*. (2017) demostraron que la actividad de las acetogeninas era comparable a la del producto sintético lipofílico (Mirenat®). Este producto se encuentra entre los productos formulados a base de lauroil etil arginato (LAE®), destinado a la conservación de alimentos con alta actividad antimicrobiana contra mohos y levaduras a muy bajas dosis. Su mecanismo de acción es sobre la membrana citoplasmática de los microorganismos, alterando sus procesos metabólicos e inhibiendo su ciclo normal sin producir lisis celular.

Echenique *et al*. (2021), demostraron el efecto antifúngico de las acetogeninas de semilla de aguacate sobre el hongo *Botrytis cinerea*, las cuales mostraron un efecto fungistático contra el hongo reduciendo su crecimiento micelial en condiciones *in vitro* y afectó principalmente su fase de crecimiento estacionario, el porcentaje de inhibición de la germinación de conidias fue muy similar al encontrado con sorbato de potasio. Se hipotetiza que la naturaleza lipofílica de las acetogeninas les permite penetrar la membrana celular de las conidias debido a sus dobles enlaces, similares al sorbato de potasio, que interfieren en la actividad catalítica de las enzimas. responsable del crecimiento, atribuyéndole su efecto fungistático. Además, los radicales libres podrían estabilizarse mediante la donación de un electrón por molécula de hidrógeno, lo que aumentaría la permeabilidad de la membrana y culminaría en la lisis celular. Por lo tanto, este aceite de semilla de aguacate podría ser una alternativa natural en el control de *Botrytis cinerea.* Sin embargo, se requieren más estudios para probar su efecto antifúngico y evaluar su uso como conservador en una matriz alimentaria como el puré de fresas.

Por la importancia de los compuestos bioactivos en los productos de fresa y la necesidad de estudiar y proponer alternativas al control de *Botrytis cinerea*; principal hongo que ataca estos productos con altas pérdidas pre y poscosecha, se planteó este estudio con la hipótesis y objetivos descritos a continuación.

1.3. Hipótesis

Las altas presiones hidrostáticas asistida con acetogeninas de semilla de aguacate conservan la calidad nutricional (compuestos fenólicos, antocianinas y vitamina C) y las propiedades fisicoquímicas (sólidos solubles totales, potencial de hidrógeno, acidez titulable, actividad acuosa y color), reduciendo la viabilidad de las conidias de *Botrytis cinerea* en puré de fresas.

1.4. Objetivo General

Evaluar el efecto de las altas presiones hidrostáticas asistida con acetogeninas de semilla de aguacate sobre la calidad nutricional, microbiológica y fisicoquímica del puré de fresa.

1.5. Objetivos Específicos

- Aislar e identificar el hongo *Botrytis cinerea* de la fresa.
- Determinar las concentraciones mínimas y máximas de las acetogeninas de semillas de aguacate *in vitro* para el control de *Botrytis cinerea.*
- Determinar cuantitativamente por cromatografía líquida de ultra rendimiento, acoplado a un detector con arreglo de diodos los compuestos bioactivos del pure de fresas antes y después de ser inoculados con *Botrytis cinerea* y luego de ser sometidos a altas presiones hidrostáticas asistidas con acetogeninas de semillas de aguacate.
- Evaluar la calidad microbiológica del puré de fresas antes y después de ser inoculadas con *Botrytis cinerea* y luego de ser sometido a altas presiones hidrostáticas asistidas con acetogeninas de semillas de aguacate.
- Evaluar las características fisicoquímicas del puré de fresas antes y después de ser inoculadas con *Botrytis cinerea* y luego de ser sometido a altas presiones hidrostáticas

asistidas con acetogeninas de semillas de aguacate.

1.6. Sección Integradora

El desarrollo de los estudios realizados para cumplir los objetivos de esta tesis y dar respuesta a la hipótesis se describe en dos capítulos, que, junto con la sinopsis, conclusiones generales y recomendaciones, conforman el trabajo de tesis. En el primer capítulo "Antifungal effect of acetogenins from avocado (*Persea americana* mill.) seed against the fungus *Botrytis cinerea"* fue realizado con el objetivo de investigar el efecto antifúngico de un extracto de semillas de aguacate crudo enriquecido con acetogeninas contra el crecimiento micelial y la germinación conidial de *B. cinerea in vitro.* Se obtuvo la concentración mínima y máxima de acetogeninas a utilizar, y se demostró el efecto que ejercen sobre el crecimiento micelial y la germinación de conidias *in vitro*. Por lo tanto, las acetogeninas de semillas de aguacate podrían ser una posible alternativa como agente fungistático natural contra *Botrytis cinerea* en productos alimenticios procesados como el puré de fresa.

En el segundo capítulo se presenta el trabajo titulado "*Botrytis cinerea* induced phytonutrient degradation of strawberry puree: effects of combined preservation approaches with high hydrostatic pressure (HHP) and natural antifungal avocado lipids", en el cual se realizó con el objetivo de investigar el efecto del procesamiento de las altas presiones hidrostáticas y las acetogeninas de semillas de aguacate en las propiedades nutricionales, microbiológicas y fisicoquímicas de puré de fresa inoculado con 6.3 log de conidias de *Botrytis cinerea*. Se obtuvo que los tratamientos con bajo nivel de acetogeninas de semillas de aguacate conservaron más el ácido ascórbico total y el ácido L-ascórbico, mientras que los niveles más altos de ácido L-dehidroascórbico se observaron usando altas presiones hidrostáticas y sorbato de potasio; los tratamientos de altas presiones hidrostáticas combinados con acetogeninas de semilla de aguacate también aumentaron el contenido de ácido elágico y conservaron más el contenido del ácido p-hidroxibenzoico y pelargonidina-3-glucósido. Además, no se detectaron conidias viables de *Botrytis cinerea*, proporcionando mayor evidencia científica sobre las tecnologías no térmicas combinadas para su inhibición.

2. ANTIFUNGAL EFFECT OF ACETOGENINS FROM AVOCADO (*PERSEA AMERICANA* MILL.) SEED AGAINST THE FUNGUS *BOTRYTIS CINEREA*

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Antifungal effect of acetogenins from avocado (*Persea americana* Mill.) seed against the fungus *Botrytis cinerea*

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strawberry purée

Abstract

Botrytis cinerea (grey mould) is the causal agent of grey rot in strawberries. Worldwide, it causes substantial pre- and postharvest losses (40 - 60%), attacking over 1,400 crops. To combat this phytopathogenic fungus, synthetic preservatives including potassium sorbate (PS) are used, and other barrier technologies have been investigated. Avocado seed acetogenins (ASAs) have been studied for their antibacterial activity but not for their antifungal effect. The effect of ASAs against mycelial growth and conidia of *B. cinerea in vitro* is unknown. Therefore, the aim of the present work was to investigate the antifungal effect of a crude avocado seed extract enriched with acetogenins (Avosafe®) against mycelial growth and conidial germination of *B. cinerea in vitro*. Twelve Avosafe® treatments were tested using an agar extract dilution method. The minimum inhibitory concentration (MIC) and the antifungal effect of Avosafe® on mycelial growth and conidial germination were tested after 96 and 5 h of incubation at 18 ± 1°C, respectively. An adjusted kinetic Gompertz model was used to determine the growth phase in which Avosafe® affected mycelial growth and conidial germination. Avosafe® at a concentration of 7,500 mg/L directly affected the stationary phase of the developing fungus with no significant differences ($p > 0.05$) with typical usage level of PS (1,000 mg/L). The mean fungistatic concentration of mycelial growth (661 mg/L) was associated with a 78% reduction in the percentage of conidial germination, whereas its IC_{50} at this stage was 75.42 mg/L. Therefore, Avosafe® could be a possible alternative as natural fungistatic agent against *B. cinerea* in processed food products such as strawberry purée.

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Introduction

Botrytis cinerea Pers., also known as grey mould, is the causal agent of grey rot in strawberries, an important disease causing pre- and postharvest losses worldwide (León *et al.*, 2014). It causes serious damage to the production process, by destroying 60 to 70% of the fruits (Sánchez, 2014). Infections of the flowers and fruits are caused mainly by aerial conidia which develop in mature fruits postharvest, and could overwinter in dead plant tissues and debris (Braun and Sutton, 1988; Ilhan and Karabulut, 2013). The main losses occur postharvest with a maximum incidence of 89% (Ugolini *et al.*, 2014).

Presently, there is a growing consumer and industrial demand for novel antimicrobials of natural origins (Nazir *et al.*, 2017). Potassium sorbate (PS) is commonly used as a synthetic food additive, and is the most effective antimicrobial in the food industry (Mohammadzadeh *et al.*, 2018). It inhibits mould growth, prevents spoilage, and preserves the freshness of the products at a concentration of 1,000 mg/kg (Quispe, 2010; Dehghan et al., 2018). However, various studies have shown that increased PS intake (> 25 mg/kg) may lead to cytotoxic and genotoxic effects by producing mutagenic compounds, and inducing chromosomal aberrations, sister chromatid exchange, and DNA breakage (Dehghan *et al.*, 2018).

Consumers often require safe foods and additives, which can impact their nutrition intake and health (Bonciu, 2018). This can be achieved by using plant extracts and natural bioactive compounds with antifungal properties, among other approaches (Redondo-Blanco *et al.*, 2020). In this context, avocado seeds and peels have an equivalent or even greater content of bioactive compounds than the pulp (Rodríguez-Sánchez *et al.*, 2019). This food waste

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represents a potential source of molecules with applications in the food, pharmaceutical, and cosmetic industries (Wang *et al.*, 2010).

Previous reports have shown that the crude extracts from different avocado tissues exhibit antimicrobial activity against yeast (Rodríguez *et al.*, 2011), fungal spores (Leite *et al.*, 2009), fungal vegetative cells (Domergue *et al.*, 2000), and bacterial vegetative cells (Lu *et al.*, 2012). Persenone-C, persenone-A, and persenone-B contained in avocado seeds inhibit *Clostridium sporogenes* endospore germination and vegetative cell growth (Rodríguez *et al.*, 2013). *C. sporogenes* endospore germination has been shown to be completely inhibited by semi commercial avocado seed oil enriched in acetogenins (Pacheco *et al.*, 2017).

So far, 9 lipid molecules have been identified in Avosafe® and was detected by HPLC-PDA/ELSD to be of food grade and contain a total acetogenin concentration of 94.74 (w/w) of its organic solids; these acetogenins are biosynthesised by the polymerisation of acetyl and propionyl subunits including AcO-avocadyne (17%), avocadenyne (4%), avocadene (25%), AcO-avocadiene (2%), persediene (2%), persenone C (4%), persenone A (23%), persin (14%), persenone B (9%), and other constituents (5.23%) (Rodríguez-Sánchez *et al.*, 2019). Avosafe® inhibits Gram-positive bacteria and has higher efficacy toward spore-forming bacteria (*C. sporogenes, C. perfringens, Bacillus subtilis*, and *Alicyclobacillus acidocaldarius*) because of its double bonds and aromatic rings, which are inhibitory properties against Gram-positive bacteria (Villarreal-Lara *et al.*, 2019). To our knowledge, this is the first study that shows the control of *Listeria monocytogenes* by avocado acetogenins in a refrigerated food matrix, strengthening their potential use as a natural antimicrobial food additive (Villarreal-Lara *et al.*, 2019). However, there are no reports on the effects of Avosafe® on conidial germination of *Botrytis cinerea* with known chemical identity. Therefore, the aim of this work was to test a crude avocado seed extract enriched in acetogenins (Avosafe®) against mycelial growth and conidial germination of *Botrytis cinerea* by agar extract dilution assay.

Materials and methods

Crude avocado seed extract preparation

The Monterrey Institute of Technology and Higher Education in Monterrey, Mexico donated semi commercial avocado seed oil rich in acetogenins (Avosafe®) for this experiment. From this product, a working solution was prepared with 0.4% propylene glycol (PG) (v/v) (Sigma-Aldrich, USA). Sterile distilled water and avocado seed extract were subsequently added with 0.033% Tween 20 (v/v) (CTR, México). Different concentrations of Avosafe® (T_1 = 50, T_2 = 100, T_3 = 250, T_4 = 500, T_5 = 1,000, T_6 = 2,000, T_7 = 2,750, T_8 = 5,000, T_9 = 7,500, T_{10} = 10,000, T_{11} = 12,000, and T_{12} = 661 mg/L) were prepared from the working solution, and they were added to a culture medium of potato dextrose agar (PDA) (CTR, México) (Moreno *et al.*, 2012; Pacheco *et al.*, 2017). In addition to these 11 treatments, two controls were included: a positive control (PC) with 1,000 mg/L PS, and a negative control (NC), which only had PDA and/or Czapek medium. The mean fungistatic concentration (661 mg/L) was calculated by plotting the inhibitor concentration against the percentage of activity using the linear ($y = mx + n$) or parabolic ($y = ax^2 + bx + c$) equation in this activity. Since $y = 50$, x was converted to IC_{50} value using the Microsoft Excel functions. After calculation, the IC_{50} was included as a new treatment (T_{12}) to determine its activity against conidial germination.

Isolation and identification of Botrytis cinerea

Botrytis spp. were isolated from strawberries showing symptoms of grey mould. The fruits were obtained from a local market in Hermosillo, Sonora, Mexico. The pathogens were isolated and identified according to Ugolini *et al.* (2014) and Moreno *et al.* (2012). Isolates with high similarity of morphological and taxonomic characteristics to *B. cinerea* as reported by Pescador (2010) were selected. The selected isolate was sub-cultured onto PDA for isolation and purification (Moreno *et al.*, 2011). Additionally, strawberries at a commercial maturity no. 6 (NOM, 2002) were artificially inoculated with the isolate to verify its pathogenicity (based on Koch's postulates) by the agar extract dilution method.

Preparation of bioactive compounds

The evaluation of the antifungal activity was carried out using the technique of extract dilution in agar as proposed by Moreno *et al.* (2011), which consisted of making a homogeneous mixture of the extracts. Sterile Avosafe® concentrations (50 - 12,000 mg/L) were put into 125 mL flasks, and sterile PG (0.4%) and Tween 20 (0.03%) were added as vehicles. Consecutively, sterile PDA was added (40 - 50°C), and 20 mL of the solutions was poured into Petri dishes in triplicate, which were later

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(ANOVA). When significant differences were detected, they were compared using Tukey-Kramer's multiple means comparison methods. The statistical package Statistical 2010 was used to determine the kinetic parameters. The analyses were performed in triplicate, with a confidence level of 95%, using the statistical package of NCSS 2007/GESS 2006.

Results and discussion

During the isolation of *B. cinerea*, a white cottony mass was observed macroscopically, which later changed to olive brown or diffused grey, producing discoidal black sclerotia. Microscopically, flat ovoid conidia, uni-, and multinucleated with a size of approximately 8 to 10 µm representing colourless septate mycelia, which developed conidiophores with a size of 15 - 25 µm were observed. These are the typical *B. cinerea* macro- and micromorphology as reported by Pescador (2010) and Apolonio-Rodríguez *et al.* (2017). Following Koch's postulates and the experimental principles proposed by Fredricks and Relman (1996), it was observed that the artificially inoculated strawberries presented typical symptoms of *B. cinerea* disease Pescador (2010) and Apolonio-Rodríguez *et al.* (2017). Therefore, the *B. cinerea* was isolated, and a monosporic culture was obtained. The isolated *B. cinerea* was re-inoculated onto healthy strawberries to observe the development of the disease, and since a similar disease was observed, the *B. cinerea* was re-isolated, and a monosporic culture identical to the initial culture was re-obtained.

Antifungal test of mycelial growth

During the mycelial growth of *B. cinerea*, all the treatments (T_1 - T_{11}) showed significant differences ($p < 0.05$) when compared with PS (Figure 1). It was observed that the Avosafe® treatment had the same fungistatic but not fungicidal effect as PS, which is a preservative used to extend the shelf life of processed products, and inhibited the growth, development, and pathogenicity of *B. cinerea*. The highest percentages of inhibition occurred with T_9, T_{10}, T_{11}, and PS (73%). However, T_{11} (70%) did not present significant differences from T_{10} (67%), and the inhibition percentages of T_9 and T_{10} (65%, respectively) were similar ($p > 0.05$), thus this concentration was chosen as the MIC to determine the average IC_{50}. Since T_{11} showed a greater percentage of inhibition than T_9, the kinetic parameters of mycelial growth were estimated to determine which of the treatments exerted the greatest fungistatic effect (Table 1) based on the four observed variables. Focusing on T_9 inhibition in contrast to T_{11}, T_{10}, and PS, they did not present significant differences during the lag phase (λ); therefore, *B. cinerea* managed to adapt to the culture medium after 46 h as opposed to the negative control that managed to adapt to the culture medium in 26 h. T_9 was more effective when using the lowest concentration of PS. However, for the maximum mycelial growth rate (V_{max}), T_9 did not present significant differences from T_{10} and T_{11}, but T_{11} did not present significant differences from PS, and was associated with a reduction in the maximum growth rate of *B. cinerea* from 1.70 to 1.32 mm/h, in contrast with the NC, which presented a maximum growth rate of 3.38 mm/h, and was significantly different from the other treatments ($p < 0.05$). The growth of V_{max} and the regeneration time of *B. cinerea* were closely related since a period of time would be taken by *B. cinerea* to continue its development. The T_9, T_{10}, and T_{11} were not significantly different; the PS delays the regeneration time (TG) by 52 min, thus it can continue its growth in contrast with the NC, where *B. cinerea* only had to wait for 21 min to continue its development. During the maximum growth of *B. cinerea* in stationary (A) phase, the T_9 stationary phase did not show significant differences with that of T_{10}, T_{11}, and PS, where *B. cinerea* only reached 27 to 30 mm in the culture medium; in contrast, when there was no preservative in the medium (NC), it achieved up to 94 mm ($p < 0.05$). This variable is very important because at this stage, the metabolic state together with the adaptation period of *B. cinerea* in the lag phase showed an increase in the size of hyphae, greater protein content, DNA, and biomass (Kavanagh, 2018). In addition, Estrada and Ramírez (2019) reported that during this phase, nutrients are depleted, and biomass growth occurs; the mycelium enters a phase of dormancy or cell death, where modified hyphae create conidiophores and the formation of conidia, enzymes, and other metabolites such as mycotoxins begins. Since T_9 did not show significant differences from PS in these two phases (λ and A), we chose this concentration as the MIC. Avosafe® was shown in a study by Salinas *et al.* (2017) to be comparable to other lipophilic synthetic products such as Mirenat®.

Figure 2 shows the kinetics of mycelial growth of *B. cinerea* in the presence of 7,500 mg/L Avosafe® (T_9); this phase (A) occurred after 60 h of incubation at 18 ± 1°C, an inversely proportional sigmoid curve representing the lag phase, exponential phase, and stationary phase of *B. cinerea*. T_9 increased 3.80 log with respect to 2.84

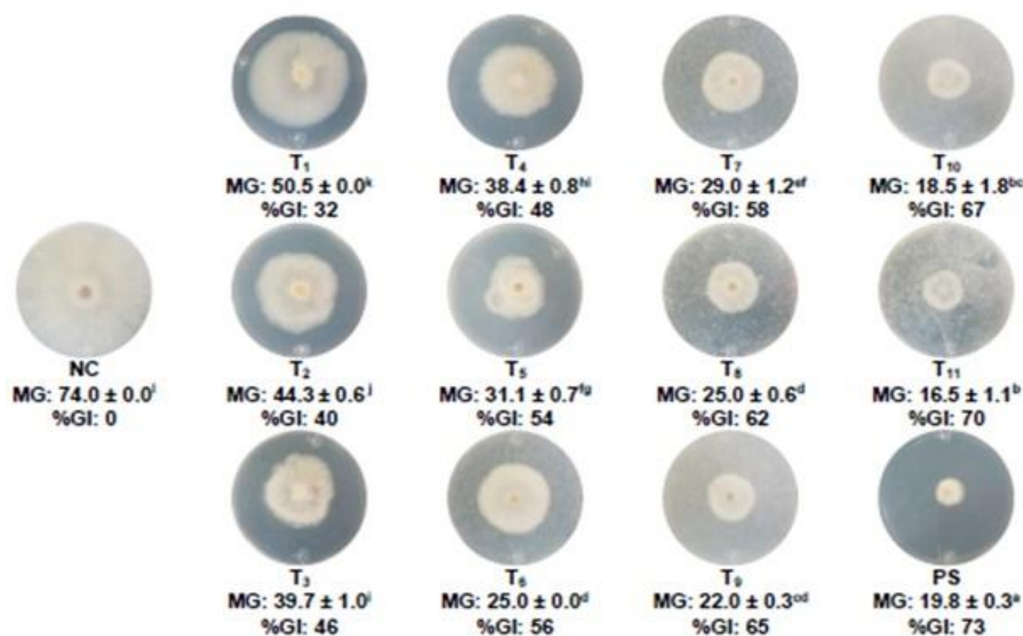

Figure 1. Growth inhibition percentage of *Botrytis cinerea* on potato dextrose agar following 96 h incubation at 18 ± 1°C. MG = mycelial growth (mm); %GI = percentage of growth inhibition; NC = negative control; and PS = potassium sorbate. Values are mean ± standard deviation of triplicates (n = 3). Means followed by different lowercase superscripts are significantly different ($p < 0.05$).

Table 1. Estimated kinetic parameters of the mycelial growth of *Botrytis cinerea* on potato dextrose agar following 96 h of incubation at 18 ± 1°C using the modified Gompertz model.

Treatment	ASA (mg/L)	λ (h)	V_{max} (mm/h)	A (mm)	GT (h)	R^2 (%)
PS	1000	51.1 ± 1.4^{f}	1.3 ± 0.0^{a}	27.0 ± 3.4^{a}	0.5 ± 0.0^{g}	99.9
T$_{11}$	12000	47.2 ± 1.1^{ef}	1.5 ± 0.0^{abc}	25.9 ± 1.6^{a}	0.5 ± 0.0^{def}	99.9
T$_{10}$	10000	46.1 ± 0.5^{ef}	1.6 ± 0.2^{bc}	27.0 ± 1.2^{a}	0.4 ± 0.0^{cdef}	99.6
T$_9$	7500	45.6 ± 1.8^{ef}	1.7 ± 0.2^{bcd}	30.2 ± 1.2^{a}	0.4 ± 0.0^{cde}	99.8
T$_8$	5000	42.3 ± 0.9^{de}	1.5 ± 0.0^{abc}	37.4 ± 0.7^{b}	0.5 ± 0.0^{defg}	99.8
T$_7$	2750	37.1 ± 2.0^{cd}	1.5 ± 0.0^{abc}	46.4 ± 2.4^{c}	0.5 ± 0.0^{efg}	99.8
T$_6$	2000	32.7 ± 2.8^{bc}	1.5 ± 0.1^{ab}	47.7 ± 1.4^{c}	0.5 ± 0.0^{fg}	99.8
T$_5$	1000	32.3 ± 1.1^{bc}	1.6 ± 0.1^{bc}	44.9 ± 0.7^{c}	0.4 ± 0.0^{cdef}	99.6
T$_4$	500	32.4 ± 2.0^{bc}	1.7 ± 0.0^{bcd}	58.1 ± 1.5^{d}	0.4 ± 0.0^{cde}	99.9
T$_3$	250	30.8 ± 2.9^{ab}	1.7 ± 0.1^{cd}	57.8 ± 2.9^{d}	0.4 ± 0.0^{cd}	99.8
T$_2$	100	30.8 ± 2.8^{ab}	1.9 ± 0.0^{d}	68.2 ± 3.9^{e}	0.4 ± 0.0^{bc}	99.7
T$_1$	50	28.9 ± 2.2^{ab}	2.2 ± 0.1^{e}	67.0 ± 3.5^{e}	0.3 ± 0.0^{b}	99.6
NC	0	25.98 ± 0.57^{a}	3.38 ± 0.02^{f}	93.77 ± 0.43^{f}	0.21 ± 0.00^{a}	99.57

ASA = avocado seed acetogenin; ʎ = lag phase of the fungus (h); Vmax = maximum growth rate (mm/h); A = maximum growth of the fungus in the stationary phase (mm); GT = generation time; PS = potassium sorbate; and NC = negative control (potato dextrose agar). Values are mean ± standard deviation of triplicates (n = 3). Means followed by different lowercase superscripts are significantly different ($p < 0.05$).

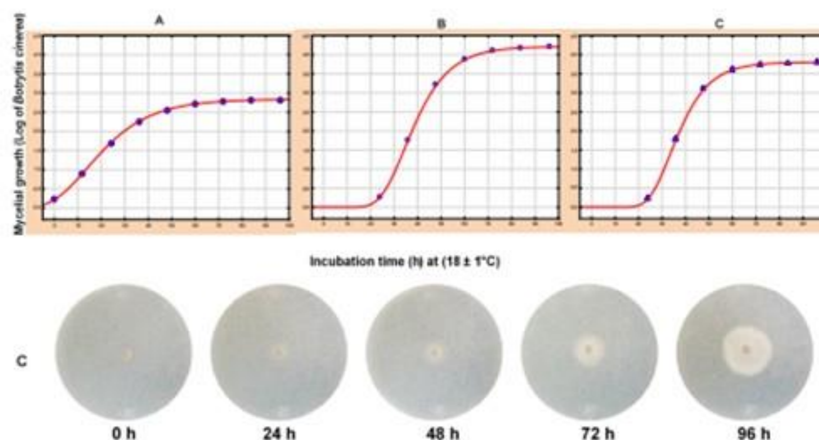

Figure 2. Kinetics of mycelial growth of *Botrytis cinerea* on potato dextrose agar following 96 h of incubation at 18 ± 1°C; (A) negative control, potato dextrose agar; (B) positive control, potassium sorbate; and (C) is T9 = 7,500 mg/L Avosafe®.

log of NC, and 4.21 log of PS, achieving a reduction of 0.96 log during this phase. Avosafe® showed a similar inhibition of *Listeria monocytogenes* by decreasing the initial concentration (1 log) to undetectable levels within 22.3 h after inoculation, and maintaining undetectable counts until the end of the experimental period (Salinas *et al.*, 2017). *Listeria monocytogenes* possesses sex and vegetative cells that are even more resistant than *B. cinerea* cell wall. Millán *et al.* (2001) and Quispe (2010) showed that PS stops microbial growth by inhibiting the dehydrogenases involved in the oxidation of fatty acids, causing the accumulation of B-unsaturated fatty acids that are intermediate products in lipid metabolism. Another mechanism of action is associated with the double bonds of PS, which interfere with the catalytic activity of the enzymes responsible for microbial growth, thus becoming an effective fungicide (Lück and Jager, 2000). Avosafe® contains potent bioactive molecules that are exclusively present in avocado seeds; AcO-avocadene and persenone A are the major compounds with double bonds, where a terminal methylene confers greater potency to avocado lipid derivatives than a terminal acetylene group (AcO-avocadenyne). This group possesses a terminal acetylene that increases the antimicrobial activity four-fold as compared to AcO-avocadene, which has a terminal methylene, an acetyl moiety, and multiple unsaturated bonds (two to three) in the aliphatic chain. Some persenones, including the most potent antilisterial acetogenins (persenone C and A), also feature a *trans*-enone group (Pacheco *et al.*, 2017; Salinas *et al.*, 2017; Villarreal-Lara *et al.*, 2019; Rodríguez-Sánchez *et al.*, 2019).

Characteristic charge-remote fragmentation is a phenomenon of unsaturated fatty acids, and the presence of abundant fragments corresponded to the cleavage of the carbon-carbon single bonds adjacent to every existing unsaturated bond on its side of the unsaturation (vinylic cleavage, C=C-) for spectra of AcO-avocadiene B. This results reflected the occurrence of vinylic cleavage, which confirmed the presence of two double bonds (C_{12} - C_{13} and C_{16} - C_{17}) for AcO-avocadyne and AcO-avocadene, reflecting a type of cleavage (acetylenic C-C- and vinylic cleavage, respectively). The fragment generated from a carbon-carbon single bond suggested allylic carbon-carbon cleavage (C=C-C-) on the side of the unsaturated bond (Salinas *et al.*, 2017; Rodríguez-Sánchez *et al.*, 2019).

Salinas *et al.* (2017) showed that the activity of Avosafe® was comparable to that of the lipophilic synthetic product (Mirenat®); this product is within the formulated products based on LAE® (ethyl lauroyl arginate), intended for food preservation with high antimicrobial activity against all kinds of moulds and yeasts at very low doses. Its mechanism of action is on the cytoplasmic membrane of microorganisms, altering their metabolic processes, and inhibiting their normal cycle without producing cell lysis. Therefore, T_9 was the best treatment for the following tests.

Antifungal test of conidial germination

T_9 was used as the minimum fungistatic concentration (MFC), and T_{12} was used as the IC_{50} (661 mg/L) of mycelial growth to determine the effect on conidial germination. The conidia content in Czapek agar between T_9 and T_{12} was not different

Table 2. Estimated kinetic parameters of percentage, inhibition, and germination of conidia of *Botrytis cinerea* on Czapek agar following 5 h incubation at 18 ± 1°C.

Treatment	ASA (mg/L)	%G	%I	S_{max} (%)	S_0 (%)	K (1/h)	R^2
PS	**1000**	10^c	90^c	13.43 ± 2.12^a	0.01 ± 0.01^a	1.69 ± 0.31^b	98.6
T_9	**7500**	18^b	82^b	20.97 ± 2.03^b	0.19 ± 0.11^a	1.29 ± 0.19^a	97.8
T_{12}	**661**	22^b	78^b	23.84 ± 1.85^b	0.29 ± 0.16^a	1.32 ± 0.20^a	97.6
NC	**0**	94^a	6^a	98.61 ± 2.37^c	0.77 ± 0.23^b	1.60 ± 0.11^{ab}	99.5

ASA = avocado seed acetogenins; %G = percentage of germination; %I = percentage of inhibition; S_{max} = maximum percentage of germinated conidia when $t \to \infty$ (%); S_0 = initial percentage of germinated conidia (%); K = germination speed (1/h); PS = potassium sorbate; and NC = negative control (potato dextrose agar). Values are mean ± standard deviation of triplicates (n = 3). Means followed by different lowercase superscripts are significantly different ($p < 0.05$).

($p > 0.05$) (Table 2), where conidial germination was inhibited by 82 and 78%, respectively. The conidia were observed under a digital microscope for 5 h (Figure 2), and germination was considered when the germ tube of the conidia was more than half of the conidial length. There were no differences in the MFC and IC_{50} of mycelial growth, achieving up to 82% inhibition in conidia, and obtaining an IC_{50} of 75.42 mg/L for conidial germination; while PS inhibited 90% of conidia, presenting differences ($p < 0.05$) with other treatments. The initial percentage of germinated conidia (S_0) did not show significant differences with PS and other treatments. When the germination speed (K) started, there were significant differences between PS and other treatments; therefore, Avosafe® retains even more of the germination speed than PS, similar to Leite *et al.* (2009), who studied *Colletotrichum gloeosporioides*, and showed a higher percentage of inhibition over the germination of conidia, and to González *et al.* (2015), who identified a peroxiredoxin-5 mitochondrial precursor, two peroximal dismutases, and two superoxide dismutases in dormant cells. This suggests that the stress resistance of conidia may develop strongly during the early stages of germination (Van Leeuwen *et al.*, 2013). In addition, a related phenomenon that may affect this process was as explained by Choquer *et al.* (2007). *Botrytis cinerea* induces oxidative shock to plant cells during early stages of the infection process, accumulating hydrogen peroxide in the germ tube, which could also explain how peroxiredoxin and catalase enzymes are downregulated during the germination process to maintain high levels of hydrogen peroxide in the germ tube during germination. During this phase (S_0), it was suggested that stress resistance of the conidia develops in the first stages of germination if these enzymes are affected; no significant difference ($p > 0.05$) was found among T_9, T_{12}, and P_S for S_0. It also presented the highest percentage of inhibition for germinated conidia; the S_{max} for the maximum percentage of x-time germinated conidia, PS showed greater inhibition, and was significantly different from T_9 and T_{12}.

Avosafe® stopped the start of germination speed (K), thus affecting some signalling pathways responsible for the germination process of *B. cinerea* conidia; germination induction by rich media is weakly dependent on BMP1 (gene essential for pathogenicity). Induction by carbon sources requires BCG3 (mutant), cAMP, and BMP1 (host surface recognition and penetration ability of germinated conidia). Additionally, induction by contact with hydrophobic surfaces depends on BMP1 (Doehlemann *et al.*, 2006), so Avosafe® may affect this gene. Therefore, Avosafe® could cause a delay of up to 0.30 min represented by an R^2 of 97.6% (Table 2), which predicts a good adjustment of the kinetic model used.

According to these results, it can be hypothesised that the lipophilic nature of acetogenins may easily allow Avosafe® to penetrate the cell membrane of conidia because of its double bonds, similar to PS, which interfere with the catalytic activity of the enzymes responsible for growth, attributing to its fungistatic effect. This stabilises free radicals through the donation of an electron by hydrogen molecule, thus resulting in an increase in the membrane permeability and then in cell lysis (Figure 3); structurally, a second OH- group at C-4 in the aliphatic chain of AcO-avocadene, as compared to a *trans*-enone group in persenones, could confer the former a higher polarity against *B. cinerea*, showing that Avosafe® had good bioactivity (Villarreal-Lara *et al.*, 2019). Furthermore, PS mechanisms of growth inhibition on fungi are due to the presence of a carboxyl group (-COOH) and the number of carbon atoms in their structure

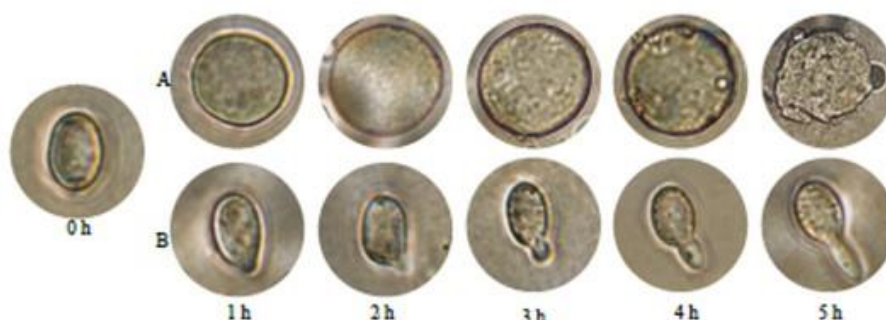

Figure 3. Conidia of *Botrytis cinerea* (40× magnification) on Czapek agar following 5 h incubation at 18 ± 1°C; (A) (treatments of T_8 and T_{12}); and (B) (without treatments)

(Mohammadzadeh *et al.*, 2018). These results agree with those determined for endospores of *Clostridium sporogenes* (Doyle *et al.*, 2001) and vegetative cells of *L. monocytogenes* (Salinas *et al.*, 2017). *Botrytis cinerea* could be affected by AcO-avocadenyne, AcO-avocadene, and persenones. Pacheco *et al.* (2017) showed its antimicrobial activity against *C. sporogenes* endospore germination with high inhibitory zones.

Conclusions

Avosafe® showed a fungistatic effect against *B. cinerea* which reduced its mycelial growth under *in vitro* conditions, and mainly affected its stationary growth phase. The inhibition percentage of conidial germination was very similar to that found with potassium sorbate. Therefore, Avosafe® could be a natural alternative in the control of *B. cinerea*. However, further studies are needed to test other inhibition techniques and evaluate the use of Avosafe® as a preservative in a food matrix.

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3. *BOTRYTIS CINEREA* INDUCED PHYTONUTRIENT DEGRADATION OF STRAWBERRY PUREE: EFFECTS OF COMBINED PRESERVATION APPROACHES WITH HIGH HYDROSTATIC PRESSURE AND SYNTHETIC OR NATURAL ANTIFUNGAL ADDITIVE

1 ***Botrytis cinerea* induced phytonutrient degradation of strawberry puree: effects of**
2 **combined preservation approaches with high hydrostatic pressure and synthetic or natural**
3 **antifungal additives**
4
5 **Degradación de fitonutrientes del puré de fresa inducida por *Botrytis cinerea*: efectos de**
6 **enfoques combinados de conservación con alta presión hidrostática y aditivos antifúngicos**
7 **sintéticos o naturales**
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23
24
25 **Abstract**
26 Strawberry products can develop fungi, mainly *Botrytis cinerea*, which results in relevant losses.
27 Thermal preservation results in vitamin losses. The present study evaluated the effects of high
28 hydrostatic pressure (HHP, 0, 400, and 600 MPa/5min) processing with potassium sorbate (PS,
29 1000 μm/mL) or avocado seed acetogenins (ASA 0, 661, and 7,500 μg/mL), on the nutritional,
30 microbiological, and physicochemical properties of strawberry puree inoculated with *Botrytis*
31 *cinerea* conidia. Inoculation resulted in significant losses of L-ascorbic acid (84%) and total
32 ascorbic acid (38%). Mash color was reduced and correlated to declines in pelargonidin-3-
33 glucoside (25%) and ellagic acid (38%) contents. HHP processing prevented further degradation
34 treatments resulted in higher retention of vitamin C and physicochemical properties. HHP-ASA
35 treatments conserved the highest total ascorbic acid, whereas highest L-dehydroascorbic acid levels
36 were observed using HHP-PS. HHP-ASA and HHP-SP treatments increased ellagic acid and
37 pelargonidin-3-glucoside contents, while non-viable conidia were detected; strengthening the value
38 of combined non-thermal technologies for its inhibition.
39
40 **Keywords:** Strawberry puree, avocado seed acetogenins, potassium sorbate, high hydrostatic
41 pressure, bioactive compounds, *Botrytis cinerea.*
42
43

Resumen

Los productos de fresa pueden desarrollar hongos, principalmente *Botrytis cinerea* ocasionando pérdidas importantes. La conservación térmica provoca pérdidas de vitaminas. Se evaluó el efecto del procesamiento a alta presión hidrostática (HHP, 0, 400 y 600 MPa/5min) con sorbato de potasio (HHP-PS, 1000 µm/mL) o acetogeninas de semilla de aguacate (HHP-ASA 0, 661 y 7.500 µg/mL), sobre las propiedades nutricionales, microbiológicas y fisicoquímicas del puré de fresa inoculado con conidios de *Botrytis cinerea*. La inoculación resultó en pérdidas significativas de ácido L-ascórbico (84%) y ácido ascórbico total (38%). El color del pure se redujo y se correlacionó con disminuciones en los contenidos de pelargonidin-3-glucósido (25%) y ácido elágico (38%). El procesamiento HHP evitó una mayor degradación con mayor retención de vitamina C y compuestos bioactivos. Los tratamientos con HHP-ASA conservaron el ácido ascórbico total más alto, mientras que los niveles más altos de ácido L-dehidroascórbico se observaron con HHP-PS. Los tratamientos con HHP-ASA y HHP-SP aumentaron los contenidos de ácido elágico y pelargonidin-3-glucósido, mientras que se detectaron conidios no viables; reforzando el valor de las tecnologías no térmicas combinadas para su inhibición.

Keywords: Pure de fresa, acetogeninas de semillas de aguacate, sorbato de potasio, altas presiones hidrostáticas, compuestos bioactivos, *Botrytis cinerea.*

Practical Application

This project focuses on the phytonutrient stability of strawberry puree in the presence of 6.3 logs of *Botrytis cinerea* conidia. High hydrostatic pressure (HHP) processing and two antifungal food additives (potassium sorbate and avocado seed acetogenins (ASA) were explored as combined preservation approaches. Fungi-induced oxidative stress generated the highest phytonutrient losses. HHP processing combined antifungal additives treatments resulted in slight amelioration of further phytonutrient losses; including higher retention of vitamin C, ellagic acid, pelargonidin-3-glucoside. Non-viable *Botrytis cinerea* conidia were observed the lower pressure treatments with food additives, strengthening the value of combined non-thermal technologies for fungi inhibition and further reduction of detrimental nutritional impact.

91 **Introduction**

92 Strawberries are eaten fresh or processed into various foods/ingredients such as concentrate, juice,
93 and puree. They are recognized for their high content of bioactive compounds such as vitamin C
94 and phenolic compounds such as flavonoids and anthocyanins (Teribia et al., 2021). For the food
95 industry, strawberry puree represents an interesting natural ingredient for preparations of desserts,
96 ice creams, and milkshakes, among others, highlighting its color, smell, and flavor (Teribia et al.,
97 2021). Thermally processed strawberry puree results in the degradation of its bioactive compounds
98 such as ascorbic acid, p-hydroxybenzoic acid, ellagic acid, and pelargonidin-3-O-glucoside (Gao
99 et al., 2016; Marszałek et al., 2017). However, thermal pasteurization is commonly applied to
100 achieve microbial safety and the inactivation of undesirable enzymes. A color change is also a
101 processing effect resulting from a combination of brown color development and red color
102 discoloration due to anthocyanin degradation (Skrede et al., 1992). Strawberry products are very
103 susceptible to the development of fungi, mainly *Botrytis cinerea* Pers (León et al., 2014,
104 Chakraborty et al., 2014; Hurtado, 2017), which is a phytopathogenic fungus that causes gray rot,
105 a disease with great economic and scientific relevance since it can be responsible for up to 89% of
106 postharvest losses of the fruit (Ugolini et al., 2014). Food contamination with fungi and their spores
107 is a major problem in processed fruits due to their high sugar content and low pH levels; light
108 brown spots and damp strawberry surface areas are associated with spoilage, which later gets
109 covered with a gray powder or mycelium, resulting in off-flavors and smells (Pravallika and
110 Chakraborty, 2022). Consumer rejection due to the loss of sensory acceptability generates
111 considerable economic losses and food security problems (Pinto et al., 2020). Villa et al. (2012)
112 demonstrated the inactivation of *Botrytis cinerea* conidia by applying heat treatment at 42°C for
113 45 min. However, the use of these heat treatments directly affects the bioactive compounds and
114 consequently the organoleptic and nutritional characteristics of strawberry puree (Marszalek et al.,

115 2017). High-pressure processing (HHP) has been proposed as an alternative non-thermal
116 pasteurization technique for foods (Wang et al., 2017). HHP processing has been reported to
117 inactivate vegetative forms of pathogenic and spoilage bacteria, yeasts, and molds (Silva, 2020),
118 preserving bioactive compounds of nutritional value (Marszałek et al., 2017). However,
119 commercial pressures alone have been observed to result in minor inactivation of fungal and
120 bacterial spores in fruit products, which limits shelf-life (Sarkeret al., 2015, Silva, 2019). High-
121 pressure, high-temperature processing (HPHT), also known as pressure-assisted thermal
122 processing (PATP), allows HHP to achieve efficient spore inactivation (Luu-Thi et al., 2014). Silva
123 et al. (2020) reported on the pressure resistance of *Byssochlamys nivea* and *Aspergillus fischeri*
124 fungi spores inoculated into strawberry puree and subjected to HHP (600 MPa) combined with
125 high temperatures (75°C) for 15 min; treatments resulted in 1.7 and 1.4 log reductions in the
126 germination of *B. nivea* spores after 12 weeks in potato dextrose agar. Therefore, technical
127 challenges remain for HHP technologies for the inactivation of resistant spores, as industrial-scale
128 HHP units can operate at maximum pressures of 650 MPa with or without combined temperatures
129 up to 50 °C (Sarker et al., 2015). In addition to heat treatments, additives such as potassium sorbate
130 (PS) are used as preservatives (Mohammadzadeh et al., 2018). PS inhibits fungi growth and in
131 fruit, preserves prevent product deterioration at 1 g/kg (Quispe, 2010; Dehghan et al., 2018).
132 However, consumers are moving away from synthetic additives such as potassium sorbate;
133 although approved as a safe food additive, at higher intakes (> 25 mg/kg) has been reported to
134 cause cytotoxic and genotoxic effects by producing mutagenic compounds, inducing chromosomal
135 aberrations and sister chromatid exchange (Dehghan et al., 2018). For the above, consumers and
136 industries have a growing demand for new antimicrobials of natural origin (Nazir et al., 2019). In
137 this sense, avocado seeds have been studied, which have a content of bioactive compounds
138 equivalent to or even higher than its pulp, finding nine lipid molecules, biosynthesized by the

139 polymerization of acetyl and propionyl subunits with antimicrobial and preservative properties
140 (Rodríguez-Sánchez et al., 2019). Prior literature indicates that persenone-A, persenone-B, and
141 persenone-C contained in avocado seeds inhibit the germination of *Clostridium sporogenes*
142 endospores and the growth of vegetative cells (Rodríguez et al., 2013). It has also been shown that
143 the germination of endospores of *C. sporogenes* was inhibited with semi-commercial avocado seed
144 oil enriched in acetogenins (Pacheco et al., 2017). In previous works, it was also documented that
145 avocado seed oil was effective as a natural alternative for the control of *B. cinerea* (Echenique et
146 al., 2021). Therefore, the objective of this work was to evaluated the effects of HHP (0, 400, and
147 600 MPa/5min) processing with potassium sorbate (PS, 1000 μm/mL) and avocado seed
148 acetogenins (ASA 0, 661, and 7,500 μg/mL), on the nutritional, microbiological, and
149 physicochemical properties of strawberry puree inoculated with *Botrytis cinerea* conidia.

150 **Materials and methods**

151 The experimental design included two studies. In the first study the effect of *Botrytis cinerea*
152 inoculation on the phytonutrient stability of strawberry puree was analyzed. The second study
153 focused on exploring the effects of HHP processing of strawberry puree inoculated with *Botrytis*
154 *cinerea* conidia and added with ASA and potassium sorbate as combined preservation approaches.

155 ***1.1. Strawberry puree preparation***

156 Strawberries (*Fragaria* x *ananassa*) Festival variety was obtained by a donation from the company
157 ALPEFRESH (Michoacán, México) through the HEB supermarket (HEB S.A. de C.V., Monterrey,
158 NL México), with a ripeness index #06 according to NMX-FF-062-2002 (Official Mexican
159 Standard, 2002a). They were transported to the fruit and vegetable processing and engineering
160 laboratory of the Technologic de Monterrey, FEMSA Biotechnology Center. Fresh strawberries
161 free of mechanical injury and microorganisms were selected. Cleaning and disinfection with 150
162 mg/L NaClO for 2 min were performed followed by four more washes with distilled water.

163 Strawberry crowns were removed, and the puree was prepared in an industrial blender (Torrey LM-
164 12, TORREY, Nuevo Leon, México). Strawberry puree was homogenized and stored at 4°C for
165 immediate preparation of treatments.

166 ***1.2. Treatments***

167 *1.1.1. Study I- Botrytis cinerea inoculation*

168 Two treatments were prepared including, strawberry puree without inoculum (control), and
169 strawberry puree with 6.3 logs *Botrytis cinerea* conidia/mL. Subsequently, the treatments were
170 vacuum packed (3.2 s/2 psi and 90% humidity; Evd-20 Torrey) in triplicate into nylon-polyethylene
171 stomacher bags (3mm standard barrier, 10x12", oxygen transmission rate: 63 cm3/m2, for 24 ha at
172 23°C dry; moisture vapor transmission rate 4.8 g/m2, for 24 ha at 38°C and 90% relative humidity,
173 Unline, Monterrey, Mexico) and stored at 4°C until subsequent microbiological analyses. Samples
174 for physicochemical evaluations were stored at -80°C.

175 *1.1.2. Study II- Botrytis cinerea inoculation prior to high hydrostatic pressure (HHP)*
176 *processing*

177 Three different treatments were prepared, including strawberry puree added with 6.3 log
178 conidia/mL + 661 µg/mL of avocado seed acetogenins, strawberry puree + 6.3 log conidia/mL +
179 7,500 µg/mL of avocado seed acetogenins, and strawberry puree + 6.3 log of conidia/mL + 1 g/Kg
180 of potassium sorbate. Subsequently, the treatments were vacuum packed and stored as described
181 in study I and treatment with HHP as described in the following section.

182 ***1.3. High hydrostatic pressure (HHP) processing***

183 Experiments were carried out in a high-pressure processing equipment (2L, Avure Technologies,
184 Middletown, OH, USA) using water as pressurizing medium. Equipment was operated at pressures
185 between 400 and 600 MPa for 5 min, recorded water temperature (after reaching the pressure level)
186 ranged from 18 to 32°C due to adiabatic heating during the holding time. Compression rates were

187 within 420-630 MPa/min; the decompression was almost instantaneous. The bags were introduced
188 into the high-pressure equipment, processed, and immediately stored at 4°C. Triplicate HHP
189 processing treatments were performed, and untreated control treatments were also submerged in
190 the equipment chamber for 5 min, after processing, all treatments were stored immediately at -
191 80°C until their subsequent analysis. Treatments from studies I and II were analyzed according to
192 the techniques described below.

193 ***1.4. Physicochemical analysis***

194 Physicochemical measurements of strawberry puree included pH, titratable acidity, total soluble
195 solids, water activity and color, according to AOAC (2002) methodologies. Titratable acidity was
196 determined using titration equipment (METTLER TOLEDO-DL21), soluble solids using a
197 refractometer (PR-32 ATAGO), water activity using a hydrometer (HP23-AW, ROTRONIC®,
198 Switzerland). Instrumental color was determined with a Konica Minolta™ CM Series 600 d
199 colorimeter (Osaka, Japan), using system scale CIELAB with a standard illuminant D65 and a
200 viewing angle of 10, the instrument was calibrated with a white mosaic as a color standard, where
201 L* represents the values from 0 to 100 meaning shades from black (0) to white (100). Instrumental
202 color was expressed in polar coordinates as Chroma $[(a^*2 + b^*2)^{1/2}]$ (C*) and hue or hue angle
203 [arctan (b*/a*) x 180/π] (*h**) . Instrumental color measurements were determined ten times.

204 ***1.5. Analysis of bioactive compounds***

205 ***1.1.3.*** *Determination of vitamin C*

206 L-ascorbic acid, L-dehydroascorbic acid and total ascorbic acid were extracted according to
207 Marszaleck et al., (2017) with slight modifications. Treatments (2g) were titrated with 0.01%
208 metaphosphoric acid (HPO3) (10mL), shaken manually and sonicated (Branson Ultrasonics™
209 S800) for 10 min/40Hzh at 25°C in the dark with N_2 in the headspace. Samples were then
210 centrifuged at 3700 x g for 10 min at 4°C and the supernatant was recovered. Sep-pack C18

211 columns were conditioned and the eluents from the treatments was recovered. Aliquots were then
212 taken for L-ascorbic acid and additional aliquots for L-dehydroascorbic acid, to which
213 Dithiothreitol (8mM) was added and left to settle for 1h. All treatments were filtered using PVDF
214 syringe filters with 0.22 µm membranes (Millex®GV, Millipore, Billerica, MA, USA), placed in
215 amber vials and N_2 was flushed into the headspace. L-ascorbic acid, L-dehydroascorbic acid and
216 total ascorbic acid were quantified in a UPLC unit (Acquity, Waters, Milford, MA. USA) coupled
217 to a diode array detector (DAD) and quantified at a wavelength of 245 nm (λ: 210-400nm).
218 Chromatographic separation was achieved using a Nova Pack C18 column (4µm, 3.9x150mm)
219 maintained at 25°C. The mobile phase consisted of 0.01% HPO3 using the isocratic method and a
220 flow rate of 0.7 mL/min, a volume of 20µL of the treatments was injected at 10°C for 10 min. L-
221 ascorbic acid quantification was performed with calibration curves of commercial L-ascorbic acid
222 standards, and DL-dithiothreitol (Sigma Aldrich, USA) to determine total ascorbic acid.

223 ***1.1.4.*** *Determination of phenolic compounds*

224 Strawberry phenolic compounds including p-hydroxybenzoic acid and ellagic acid were extracted
225 according to Irakli et al., (2012) and Marszałek et al., (2017) with slight modifications. Samples of
226 puree were weighed (5 g) and mixed with MeOH:H2O:HCl (15 mL, 80:19.9:0.1), shaken and
227 sonicated (Branson Ultrasonics™ S800) for 10 min/40 Hzh/25° C in the dark with N_2 in the
228 headspace. Samples were centrifuged at 3700 x g for 5 min at 4°C and the supernatant was
229 recovered, filtered through Whatman #01 paper, evaporated under vacuum at 200 rpm/30 min/35
230 °C obtaining 20 mL. Samples were adjusted to 25 mL with 0.1% H2O:PO4 (v/v) and the treatments
231 were kept at -20°C until later use. 1 mL of the extract was weighed and added to 39 mL of
232 MeOH:HCl:H2O (56:25(6M):19), then it was hydrolyzed at 120°C / 90 min, allowed to cool to
233 room temperature and made alkaline with NaOH (10M) at pH 3.5. An SP-C18 MeOH:H2O
234 (50:50(pH2)) column was conditioned twice, ending with MeOH; subsequently, 5 mL of the extract

235 was added to the SP-C18 and the eluent was recovered, 5 mL of H2O (pH 3.5) was added to the
236 same SP-C18 and it was homogenized with the eluent. To recover the phenolic acids, the above
237 SP-C18 was eluted with 10 mL of MeOH:H2O:HCl (80:19.9:0.1) and the eluent was recovered.
238 The treatments were filtered using PVDF syringe filters with 0.22 µm pores and made up to 25 mL
239 with MeOH:H2O:HCl (90:9.9:0.1) and stored at -20°C. To determine the content of p-
240 hydroxybenzoic acid and ellagic acid, the equipment and column mentioned in step 2.5.1. were
241 used, at a wavelength of 270nm (260-280nm). The mobile phases consisted of A:
242 MeOH:H2O:CH_3-CO2H (10:88:2; v/v/v) and B: MeOH:H2O:CH_3-CO2H (90:8:2; v/v/v) . The
243 solvent flow rate was 0.7 mL/min, using gradients of 100% A (0-2 min); 100 to 75% A (2-8 min);
244 75 to 60% A (8-22 min); 60 to 55% A (22-23 min); from 55 to 100% A (23-28 min). p-
245 hydroxybenzoic acid (y=223774x-29819; R^2=0.9989) and ellagic acid (y=313720x-122397;
246 R^2=0.9984) were quantified using commercial standard calibration curves obtained from Sigma
247 Aldrich. The standards were diluted in MeOH:H2O:HCl (50:49:1) and expressed in mg/100 g of
248 fresh weight.

249 ***1.1.5.*** *Determination of anthocyanins*

250 Pelargonidin-3-O-glucoside quantification was measured according to Marszałek et al., (2017)
251 with slight modifications. Extracts (10 mL) from the previous step (2.5.2. determination of phenolic
252 compounds) were filtered on a previously conditioned SP-C18 column, the eluent was discarded
253 and the SP-C18 fraction was eluted with 5 mL of MeOH:H2O:HCl (75:24.9:0.1), the eluent was
254 recovered and filtered through PTFE with 0.22 µm membranes and stored at -20°C. Quantification
255 of pelargonidin-3-O-glucoside, the equipment, column, flow rate and mobile phase mentioned in
256 the previous step were used, at a wavelength of 520 nm. Gradients from 100% A (0-2 min), from
257 100 to 63% A (2-6 min), from 63 to 62% A (6-9 min) were used; 62 to 61% A (9-10 min); 61 to
258 50% A (0-11 min); from 50 to 100% A (11-18 min). Pelargonidin-3-O-glucoside was quantified

259 using calibration curves from a commercial standard.

260 ***1.6. Microbiological analysis***

261 Raw material analysis: To validate disinfection of the strawberries, the total aerobic and *Botrytis*
262 *cinerea* conidia were counted following the procedure described by NOM-213-SSA1-2002
263 (Official Mexican Standard, 2002b) and by the FDA-BAM, (2022). Microbial determinations were
264 also performed for all study I and study II treatments.

265 ***1.7. Statistical analysis***

266 A completely randomized design with 3 x 4 factorial arrangement was used to analyze the effect
267 of high hydrostatic pressures assisted with ASA and PS on the physicochemical quality, bioactive
268 compounds, and microbiology of strawberry puree. High pressure treatments were applied at three
269 levels (0, 400 and 600 MPa/5min) and antifungal food additives at four levels including two ASA
270 concentrations (661 and 7,500 µg/mL), one concentration of PS (1,000 µg/mL) and a control
271 without preservative (0 µg/mL). Significant mean differences were determined by the Tukey-
272 Kramer multiple comparison method at a confidence level of 95%, all treatments were applied in
273 triplicate. Analysis of variance was conducted in the statistical package NCSS 2007/GESS 2006.

274 **Results and discussion**

275 ***1.8. Study I: Physicochemical quality of strawberry puree without and with Botrytis cinerea***
276 ***inoculum***

277 An increase in pH and soluble solids was observed when the phytopathogenic fungus was
278 inoculated in the strawberry puree, presenting significant differences ($p<0.05$) (Table 1); while the
279 titratable acidity and aqueous activity decreased. Regarding the surface color, it was observed that
280 the luminosity did not present differences when *Botrytis cinerea* was inoculated. However, the C*
281 and *hue* decreased, becoming a less bright red color and a less intense tone (opaque), which can be
282 perceived by the human eye, considering it unpleasant. In the contamination of food by fungi,

283 deterioration occurs, which can be perceived in the quality parameters; in the case of juices, it can
284 be identified by turbidity; in foods such as purees, superficial films are very common. This is a
285 symptom of contamination by bacteria. In the case of contamination by fungi, it is possible to see
286 the mycelium (a cottony mass of the characteristic color of the phytopathogenic fungus that is
287 developing) on the strawberry puree, which causes a color change, bad flavors, etc. (Pravallika and
288 Chakraborty, 2022). In red peppers contaminated with *Fusarium* spp, these changes are observed
289 in the decrease in the *hue* angle (Frans et al., 2021). In the case of strawberry puree, these changes
290 may start with color change based on C* and *h**, pH, and soluble solids. It is reported that in plant-
291 pathogen interactions there is an increase in ROS, known as oxidative burst upon pathogen
292 infections plays a signaling role, leading to enhanced disease resistance in plants (Wang et al.,
293 2019), however, the excessive ROS accumulation in the infection sites causes damage to cellular
294 membranes through lipid peroxidation (Chen et al., 2019), and by another hand could cause color
295 changes. The instability of the cell wall could cause the extraction of compounds which could
296 increase the soluble solids and affect the pH.

297 ***1.9. Study I: Stability of bioactive compounds in strawberry puree without and with Botrytis***
298 ***cinerea inoculum***

299 *1.1.6. Vitamin C*

300 In the mash inoculated with *Botrytis cinerea* conidia, there were significant changes ($p<0.05$)
301 compared to the control (Figure 1A). L-ascorbic acid and total ascorbic acid are reduced by 84%
302 and 38%, respectively; for its part, L-dehydroascorbic acid, the oxidized form of the vitamin,
303 increased by 89% (Figure 1A and Supplementary Table 1). The loss of ascorbic acid in other fruits
304 such as oranges, pineapple, and bell peppers, contaminated by fungi such as *Sclerotium rolfsii,* has
305 been reported by other authors (Frans et al., 2021). Potential plant metabolism mechanisms include
306 as an alteration in the physiology of the fruits due to pathogenesis, causing oxidation reactions in

307 which it acts as an antioxidant. L-ascorbic acid is the main antioxidant that acts in the oxidative
308 reaction through the formation of non-oxidizing products such as dehydro-ascorbate and 2,3
309 diketogulonic acid (Frans et al., 2021, Javanmardi et al., 2023). In addition, pathogens such as:
310 *Rhizopus stolonifer, Aspergillus flavus, Penicillium digitatum, Curvularia lunata, and Fusarium*
311 *moniliforme* has been reported that these pathogens to produce toxins that decrease the
312 concentration of total ascorbic acid (Sawant and Gawai, 2011). In other studies, it was observed
313 that during the waiting time between grinding and pasteurization, anthocyanins and color remained
314 unchanged, but AA was oxidized to DHAA (Teribia et al., 2021). In the present work, the
315 conversion appeared exacerbated by the fungi, leaving only 15% of the original L-AA levels in a
316 reduced form (Figure 1A).

317 *1.1.7. Phenolic compounds*

318 In the inoculated puree, the concentration of p-hydroxybenzoic acid (Figure 1B) did not present
319 significant differences ($p<0.05$) with respect to the control, however, a slight increase (7%) was
320 observed. On the contrary, ellagic acid was degraded by 38% ($p<0.05$) (Figure 1B and
321 Supplementary Table 1). Other authors have reported the reduction of phenolic compounds in
322 whole strawberries contaminated by *Botrytis cinerea* and attributed it to oxidation reactions
323 (Javanmardi et al., 2023). As previously discussed, plant-pathogen interactions usually coincide
324 with the accumulation of reactive oxygen species (ROS); the infection also damages cell
325 membranes through lipid peroxidation. Additionally, faced with the proliferation of ROS in
326 infected plants, their response is to increase antioxidant enzymatic activities to eliminate ROS
327 under stressful conditions (Chen et al., 2019; Ahammed et al., 2020). Furthermore, the damaged
328 tissue manufactures various secondary metabolites to enhance resistance to biotic and abiotic
329 stressors. Flavonoids, phenols, and lignin are the major groups of secondary metabolites that play
330 an important role in plant defense against pathogens (El-Sharkawy et al., 2018; Wang et al., 2019).

331 *1.1.8. Anthocyanins content*

332 Pelargonidin-3-glucoside is one of the anthocyanins responsible for strawberry color. In the
333 presence of *Botrytis conidia*, it was degraded by 25% (Figure 1C and Supplementary Table 1). The
334 instrumental color changes of C* and h° parameters (Table 1) were correlated with pelargonidin-
335 3-glucoside losses. Degradation was possibly attributed to oxidative reactions (Wang et al., 2019).
336 Industrialization steps include washing and classifying the fruit, crushing the fruit, and sieving as
337 optional. Although holding times can vary, the mash can be stored for 3–5 h before the final
338 pasteurization. Based on the present results, in addition to the undesirable color changes caused by
339 anthocyanin degradation (Marszalek et al., 2017; Pinto et al., 2020; Teribia et al., 2021), fungi
340 contamination can cause extreme phytonutrient degradation during industrialization.

341 ***1.10. Study II: Microbiological and physicochemical quality of strawberry puree inoculated***
342 ***with Botrytis cinerea conidia, as affected by high hydrostatic pressure and antifungal***
343 ***food additives.***

344 *1.1.9. Microbial counts*

345 Figure 4A shows that colony-forming units (CFUs) of aerobic bacteria in unprocessed purees (0
346 MPa) were below 100 CFU/mL for all treatments, including unprocessed purees, indicating that
347 product complied with good manufacturing standards by NOM-130 (Official Mexican Standard,
348 2002b). HHP processing resulted in complete reductions of aerobic counts to non-detectable levels
349 in all puree samples, with or without antifungal food additives (Figure 4A). In contrast, microbial
350 determinations for the fungi indicated that no *Botrytis cinerea* conidia were detected in the purees
351 treated with 600 MPa/5 min for either of the treatments (Figure 4B). However, at lower pressures
352 (400 MPa, conidia remained viable in the treatments without preservatives and in the low ASA
353 concentration treatments (661 ug/mL); but not were non-viable in the other two treatments
354 subjected to the same HHP level. Indicating that both potassium sorbate and higher ASA level

355 (7500 ug/mL) assisted the 400 MPa/5 min high-pressure treatment in the inactivation of conidia.
356 Acetogenins inactivation mechanism was not the focus of the present work, however Domergue et
357 al., (2000) studied antifungal properties of two acetogenin molecules (persin and persenone A),
358 also present in the ASA extract evaluated in the present work. The authors concluded that
359 concluded that these compounds have antifungal activity, since they inhibit the germination and
360 elongation of the germ tube of the phytopathogenic fungus *Colletotrichum gloeosporioides*
361 affecting the morphology of the conidia. The ASA generating changes in the pH, and affecting the
362 integrity and metabolic activity of vegetative spores in bacteria as mentioned by Villareal et al.
363 (2019). Salinas et al. (2017) reported on the antibacterial properties of acetogenins and observed
364 that their action was comparable to that of the lipophilic synthetic product lauroyl ethyl arginate
365 (LAE). The antimicrobial spectrum of LAE has been reported to inhibit molds and yeasts at lower
366 doses, and that LAE have the had notable ability to damage the structure of fungal and bacterial
367 cells; loss of intracellular protein and nucleic acid, in addition to reduced the membrane potential,
368 and the depolarization ratios, caused a rough surface, irregular cellular organelles, protoplast
369 shrinkage, intracytoplasmic coagulation and empty cavities (Xu et al., 2018). According to prior
370 works, the mechanism of action of ASA involves cytoplasmic membranes of microorganisms,
371 altering their metabolic processes and inhibiting their normal cycle producing cell lysis (Salinas et
372 al., 2017). As previously mentioned, potassium sorbate also assisted pressure (400 MPa) in the
373 inactivation of *Botrytis conidia* (Figure 4B). The antifungal effect of the additive has been
374 attributed to its double bonds, which interfere with the catalytic activity of key enzymes needed for
375 microbial growth (Lück and Jager, 2000). Acetogenins mechanisms of action are less known, but
376 ASA extracts also contain double bonds in their aliphatic chain; also, trans-enone groups, and other
377 chemicals features such as a terminal acetylene group that have been reported to potentiate
378 antibacterial activity (Pacheco et al., 2017; Salinas et al., 2017; Villarreal-Lara et al., 2019;

379 Rodríguez-Sánchez et al. al., 2019). Although more testing is required, it can be hypothesized that
380 the lipophilic nature of acetogenins allows them to penetrate the cell membrane of conidia and
381 exert interferences with the catalytic activity of enzymes. Potassium sorbate has been reported to
382 affect enzymes responsible for growth, attributing its fungistatic effect (Quispe et al., 2010;
383 Dehghan et al., 2018). Another hypothetical mechanism involves the stabilization of free radicals
384 by the fungi metabolism that results in increased membrane permeability and cell lysis (Echenique
385 et al., 2021). High pressures also may directly impact the cell wall and membrane of the conidia,
386 causing a possible leakage of the plasmatic constituents, as described by Balasubramaniam et al.
387 (2016). Song et al. (2022) mentioned that in the case germinating bacterial spores are more
388 susceptible to stressors than their dormant forms. In this sense, implementing spore germination
389 before the HHP step can potentially eradicate several spores at higher pressures (>400 MPa), spore
390 germination can be induced by opening channels that allow the loss of dipicolinic acid. In this
391 context, Van Leeuwen et al. (2013) suggested that fungal conidia's stress resistance can thrive
392 during germination's early stages. This related phenomenon can affect the development process, as
393 explained by Choquer et al. (2007). In addition, González et al. (2015) identified a mitochondrial
394 precursor (peroxiredoxin-5), two proximal dismutase, and two superoxide dismutase enzymes in
395 latent cells of *Botrytis cinerea* during germination of conidia, which is considered that the conidia
396 will be viable; *Botrytis cinerea* induces an oxidative shock in plant cells during the early stages of
397 the infection process, accumulating hydrogen peroxide in the germ tube, as well as peroxiredoxin
398 and catalase, these enzymes regulate processes to maintain high levels of hydrogen peroxide in the
399 germ tube during germination. These effects may be related to water activity, where it was observed
400 that when using preservatives and low levels of pressure, they maintained the water activity without
401 presenting significant differences ($p<0.05$) (Table 2) compared to the treatment without
402 preservatives and pressure. However, when the pressure increases with low concentrations of

403 acetogenins and potassium sorbate, the availability of aqueous activity decreases, presenting
404 significant differences.

405 ***1.11. Study II: Physicochemical parameters of strawberry puree inoculated with Botrytis***
406 ***cinerea conidia, as affected by high hydrostatic pressure and antifungal food additives***

407 It was observed that the soluble solids were not affected by ASA addition. On the contrary, the
408 addition of potassium sorbate resulted in a slight decline in soluble solids ($p<0.05$) (Table 2). As
409 in study I, the presence of *Botrytis* conidia caused color changes in all treatments ($p<0.05$),
410 specifically in the h° and C* parameters (Table 2). ASA addition increased h* values, which were
411 higher with both high-pressure treatments (400 and 600 MPa/5 min). However, colorimetric
412 parameters of potassium sorbate treatments were not affected by HHP treatments (Table 2).
413 Hurtado et al. (2017) reported that treatment to low pressure (350 MPa/10°C/5 min) was slightly
414 less effective than the thermic process (85°C/7 min) in inactivating microbes (mesophilic and
415 psychrophilic bacteria, coliforms, yeasts, and molds) treated red-fruit smoothies. That data on
416 antioxidant status, color parameters, browning index, transmittance, turbidity, and viscosity
417 confirmed that HPP red-fruit smoothies had a higher oxidization tendency and presented
418 undesirable changes in colorimetric parameters.

419 *1.1.10. Vitamin C*

420 As previously discussed, inoculation with *Botrytis* had a significant impact on the vitamin C
421 profiles of the strawberry puree; L-ascorbic was no longer the main form of the vitamin (Figure
422 2A). The oxidized form (L-DHA) was the main vitamin form (Figure 2B). Starting in that highly
423 oxidized scenario, some of the treatment combinations of high pressure and antifungal additives
424 were able to inactivate conidia and prevent further vitamin degradation. Processing resulted in
425 further L-AA losses, ranging from 7.7 to 100%. The highest L-AA retention was achieved in the
426 no-preservative and HHP-ASA (661 μg/mL) treatments pressurized at 600 MPa/min ($p<0.05$)

427 (Figure 2A). HHP at 400 MPa/min resulted in L-AA declines and conversion into L-DHA. The
428 HHP-ASA treatments at 7500 µg/mL did not prevent further vitamin degradation. As for L-DHA
429 levels (Figure 2B), the use of potassium sorbate resulted in significantly higher concentrations that
430 corresponded with the L-AA interconversion ($p<0.05$). The overall vitamin C balance summarized
431 as TAA (Figure 2C) indicated that HHP-PS treatments conserved higher levels ($p<0.05$) and
432 contained no viable conidia (Figure 4B). The no-preservative and HHP-ASA (661 µg/mL)
433 treatments also contained higher TAA, however, those treatments contained viable conidia (Figure
434 4B) that could result in further phytonutrient losses during the product shelf-life. Results were like
435 those reported by Hurtado et al. (2019) for smoothie blends containing strawberries and low
436 ascorbic acid contents; also, vitamin C profiles indicated that the predominant form was L-DHA.
437 *Botrytis cinerea* conidia growth has been reported to produce the enzyme ascorbate oxidase, which
438 catalyzes the conversion of L-AA into DHA (González et al., 2014; González et al., 2015); a
439 possible explanation for the intense oxidation observed in the present work.

440 *1.1.11. Phenolic compounds*

441 p-hydroxybenzoic acid concentrations were the highest in all treatments prior to the application of
442 HHP treatments (Figure 3A) ($p<0.05$). The treatment without preservative and the HHP-ASA (661
443 µg/mL) purees processed at 400 MPa contained the highest p-hydroxybenzoic acid concentrations
444 ($p<0.05$), they were also the HHP treatments with viable conidia (Figure 4B). It is possible that the
445 viable fungi may have been an additional stressor in those treatments, which resulted in higher
446 concentrations of that specific phenolic compound. Ellagic acid (Figure 3B) levels, appeared more
447 affected by the pressure levels, particularly in treatments without preservative and with potassium
448 sorbate that were treated at 600 MPa/5 min ($p<0.05$). As reported by Navarro et al. (2022) various
449 fruit and vegetable products treated with HHP have shown higher phenolic contents than their
450 untreated counterparts. Results that have been associated with higher extractability from cellular

451 tissues, and to de novo biosynthesis. HHP treatments ranging from 500 to 600 MPa have been
452 associated with cell wall disruption facilitating the release of phenolic compounds from cell
453 compartments. Whereas HPP treatments ranging from 15 to 100 MPa during 10–20 min at room
454 temperature have resulted in the biosynthesis of phenolic compounds with increments up to 155%
455 (Navarroet al., 2022). In addition, biotic stress is caused by the action of fungi that attack plants by
456 secreting enzymes to break down tissues and may also result in the release of phenolic compounds
457 (Vidal, 2010). Ortega et al. (2013) suggested that pressure as an abiotic stressor can lead to cell
458 wall fracture or deformation, causing cell wall loosening by crosslinking or depolymerizing its
459 components, plant cells can sense the mechanical perturbation at their cell surfaces, and they
460 respond (Liang, 2018). Stress responses have been associated to the production of ROS, including
461 H_2O_2, which later acts, controls, and initiates enzymatic responses to repair the damaged cell wall
462 via stress-responsive gene, oxidative burst linked with cell wall reinforcement, biosynthesis of
463 phenolics, among others; the release of H_2O_2 is carried out in minutes, acting as the elicitor in the
464 biosynthesis, the production of H_2O_2 at the cellular level acts as Ca^{+2} signaling, activates kinases,
465 hormonal signaling, and regulates gene expression (Ślesak et al., 2007). H_2O_2, as a signaling
466 molecule activating metabolic pathways, leads to increased phenylalanine ammonium lyase
467 activity, which, as previously mentioned, synthesizes simple phenols derived from cinnamic acid
468 (Gordo, 2018; Jacobo et al., 2021). It has been reported that in HHP-treated carrots, after one day
469 of storage, the total phenolic content increased by 69.1% at 60 MPa and 154.9% at 100 MPa
470 (Yasunaga et al., 2018). Bioactive compounds, such as phenolics, are contained in specific
471 organelles in the cell, which can vary depending on each type of product and variety (Gómez et al.,
472 2020). The increment in phenolic compounds observed after HHP is not always attributed to
473 biosynthesis, the release of phenolics by extraction from specific organelles could be responsible
474 for the increments. The better extractability of phenolic at pressure levels above 100 MPa has been

475 related to cell membrane disruption and release of bound phenolics, resulting in higher
476 extractability and an improvement in bioaccessibility, making it different from biosynthesis, which
477 is suggested to be a dual stress-response mechanism related to ATP, ROS, and the activation-
478 deactivation of enzymes (Navarro-Baez et al., 2022). In addition to this, the activities of PPO and
479 POD can increase in HHP treatments so that the alterations or decrease in the concentration could
480 be related to oxidative stress (Zhang et al., 2021), as well as by the polymerization induced by high-
481 pressure enzymatic reactions (Szczepańska et al., 2020).

482 *1.1.12. Anthocyanins content*

483 Pelargonidin-3-glucoside contents were affected by the different treatments studied in the present
484 work. As previously described inoculation resulted in degradation of 25% of the initial
485 concentrations present in the strawberry puree, which had an impact in the red color of the product.
486 Starting in that highly oxidative environment it was observed that the application of HHP resulted
487 in higher concentrations in all treatments regardless the type of antifungal additive and
488 concentration. But with two exceptions to that generalization, the HHP samples treated at 400
489 MPa/5 min without preservative and with ASA (600 µg/mL) resulted in the degradation of
490 pelargonidin-3-glucoside (-12 and -21%, respectively). Both treatments contained viable conidia
491 (Figure 4B), confirming its relevant role in the degradation of strawberry anthocyanins and its
492 negative impacts on nutritional and color characteristics of the fruit. In plants, anthocyanins are in
493 vacuoles, and HHP processing has reported to affect the vacuole membrane. In prior works, HHP
494 made the extraction of anthocyanins from strawberry pulps more accessible (Cao et al., 2011).
495 Some studies have shown that HPPs can affect the microstructure of food plant tissues, increasing
496 the extractability of bioactive compounds (Vázquez-Gutiérrez et al., 2013; Xi & Luo, 2016;
497 Pimenta et al., 2020; Szczepańska et al., 2020). Rodríguez-Roque et al. (2015) observed that HHP
498 increased the bioaccessibility of phenolic compounds from fruit juices and related beverages.

499 Additionally, PPO and POD activities have been reported to increase in HHP treatments, therefore
500 oxidative stress induced biosynthesis can also take place in the plant tissues (Zhang et al., 2021,
501 Szczepańska et al., 2020).

502 **Conclusion**

503 In this study, fungi-induced oxidative stress generated the highest phytonutrient losses (L-AA was
504 reduced by 84% , total vitamin C by 38%, ellagic acid 38%, and pelargonidin-3-glucoside content
505 by 25%). The highest pressures (600 MPa for 3 min) resulted in complete conidia inactivation,
506 with or without antifungal additives. For the lower pressure treatments (400 MPa for 3 min), the
507 presence of ASA (661 μg/mL) and potassium sorbate assisted pressure and achieved non-viable
508 *Botrytis cinerea* conidia inactivation and ASA increased p-hydroxybenzoic acid, ellagic acid and
509 pelargonidin-3-glucoside retention and maintained the L-ascorbic acid. However, further studies
510 are required.

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515 **Disclosure Statement.**

516 Some of the potential commercial uses of acetogenins in the food, pharmaceutical, and personal
517 care industries are protected under granted patents and patent applications including
518 US2018013671A1, EP2851062B1, US 10582707B2 and CA2807779. The authors declare that the
519 research was conducted in the absence of any commercial or financial relationships that could be
520 construed as a potential conflict of interest.

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798 **Table 1.** Effect of *Botrytis cinerea* conidia inoculated in strawberry puree on physicochemical
799 analyses.

800 **Cuadro 1.** Efecto de conidias de *Botrytis cinerea* inoculadas en puré de fresas sobre los análisis
801 fisicoquímicos.

Parameters	Without inoculum	With inoculum

physicochemical		(0 Log conidia/mL)	(6.3 Log conidia/mL)
pH (H+)		3.1^{b} ± 0.00[1]	3.2^{a} ± 0.06
Total soluble solids (°Brix)		8.97^{b} ± 0.15	10.87^{a} ± 0.06
Titratable acidity (g citric acid /100mL)		0.72^{a} ± 0.001	0.64^{b} ± 0.000
Water activity (Aw)		0.95^{a} ± 0.003	0.94^{b} ± 0.002
	L	29.83^{a} ± 2.03	30.91^{a} ± 1.19
Color	*C*	32.17^{a} ± 0.36	24.08^{b} ± 2.26
	h°	34.98^{a} ± 0.55	29.48^{b} ± 0.83

[1]Values are means ± standard deviation of triplicates (n=3). Means followed by different lowercase superscripts are significantly different (Tukey-Kramer multiple comparison test. $p<0.05$).

[1]Los valores son medias ± desviación estándar de triplicados (n=3). Las medias seguidas de diferentes superíndices en minúsculas son significativamente diferentes (prueba de comparación múltiple de Tukey-Kramer. p<0,05).

Table 2. Effect of high hydrostatic pressures in the presence of potassium sorbate or avocado seed acetogenins (ASA) in strawberry puree inoculated with *Botrytis cinerea* conidia (6.3 log conidia/mL) on physicochemical analyses.

Cuadro 2. Efecto de altas presiones hidrostáticas en presencia de sorbato de potasio o acetogeninas de semilla de aguacate (ASA) en puré de fresas inoculado con conidias de *Botrytis cinerea* (6.3 log conidias/mL) en análisis fisicoquímicos.

Parameters physicochemical		Pressure (MPa/5min)	Without preservative (0 µg/mL)	Potassium sorbate (1000 µg/mL)	ASA (661 µg/mL)	ASA (7500 µg/mL)
pH (H+)		0	3.23^{a}±0.06[1]	3.23^{a}±0.06	3.23^{a}±0.06	3.23^{a}±0.06
		400	3.23^{a}±0.06	3.30^{a}±0.00	3.27^{a}±0.06	3.23^{a}±0.06
		600	3.37^{a}±0.06	3.33^{a}±0.06	3.23^{a}±0.06	3.27^{a}±0.06
Total soluble solids (°Brix)		0	10.87^{a}±0.06	9.37^{d}±0.06	10.53^{b}±0.15	10.57^{b}±0.12
		400	10.47^{b}±0.06	9.50^{d}±0.10	10.50^{b}±0.10	10.70^{ab}±0.10
		600	10.10^{c}±0.10	9.33^{d}±0.06	10.47^{b}±0.06	10.87^{a}±0.06
Titratable acidity (g de citric acid / 100mL)		0	0.640^{b}±0.000	0.641^{b}±0.000	0.648^{b}±0.006	0.629^{b}±0.001
		400	0.642^{b}±0.002	0.648^{b}±0.001	0.631^{b}±0.001	0.621^{b}±0.001
		600	0.637^{b}±0.011	0.646^{b}±0.005	0.724^{a}±0.044	0.648^{b}±0.006
Water activity (Aw)		0	0.938^{abc}±0.002	0.940^{ab}±0.003	0.947^{a}±0.006	0.943^{ab}±0.003
		400	0.929^{c}±0.004	0.929^{c}±0.002	0.937^{abc}±0.003	0.939^{abc}±0.004
		600	0.900^{e}±0.006	0.933^{bc}±0.005	0.92^{d}±0.003	0.937^{abc}±0.003
Surface color	*L*	0	30.91^{bcdef}±1.19	31.80^{abc}±1.22	31.10^{bcdef}±1.07	34.15^{ab}±0.45
	C		24.08^{bcd}±2.26	26.32^{ab}±1.22	25.60^{abc}±1.23	27.25^{ab}±0.40
	h°		29.48^{de}±0.83	28.20^{fg}±0.28	30.04^{cd}±0.14	31.44^{ab}±0.06
	L	400	27.78^{def}±1.71	27.23^{ef}±0.69	31.53^{bcde}±0.34	36.95^{a}±1.78
	C		26.19^{ab}±2.93	21.45^{cd}±0.56	27.01^{ab}±0.13	29.74^{a}±1.25
	h°		29.74^{cde}±0.19	27.32^{g}±0.12	30.58^{bc}±0.10	31.76^{a}±0.27
	L	600	29.48^{cdef}±1.01	26.99^{f}±0.34	22.35^{g}±2.63	32.61^{abc}±2.71
	C		25.81^{ab}±0.58	25.07^{bc}±0.26	20.31^{d}±1.41	25.71^{ab}±1.78
	h°		29.67^{cde}±0.16	28.99^{ef}±0.35	29.93^{cde}±0.57	31.12^{ab}±0.05

[1]Values are means ± standard deviation of triplicates (n=3). Means followed by different lowercase superscripts are significantly different (Tukey-Kramer multiple comparison test. $p<0.05$).

[1]Los valores son medias ± desviación estándar de triplicados (n=3). Las medias seguidas de diferentes superíndices en minúsculas son significativamente diferentes (prueba de comparación múltiple de Tukey-Kramer. p<0,05).

Supplementary Table 1. Percent relative losses or increments of strawberry puree phytonutrients as affected by the inoculation of *Botrytis cinerea* conidia.

Tabla complementaria 1. Porcentaje relativo de pérdidas o incrementos de fitonutrientes del puré de fresas afectados por la inoculación de conidias de *Botrytis cinerea.*

Bioactive compounds	Concentration without inoculum	Concentration with inoculum	Degradation or Increase[1]

	(0 log conidia/mL)	(6.3 log conidia/mL)	(%)
L-ascorbic acid (mg 100 g^{-1} strawberry puree)	36.93 ± 0.16[2]	5.98 ± 0.41	**-83.82**
L-dehydroascorbic acid (mg 100 g^{-1} strawberry puree)	1.92 ± 0.11	18.18 ± 0.51	**+846.18**
Total ascorbic (mg 100 g^{-1} strawberry puree)	38.85 ± 0.16	24.15 ± 0.30	**-37.83**
p-Hydroxybenzoic acid (mg 100 g^{-1} strawberry puree)	5.62 ± 0.17	6.01 ± 0.05	**+6.94**
Ellagic acid (mg 100 g^{-1} strawberry puree)	23.99 ± 0.04	14.95 ± 0.06	**-37.69**
Pelargonidin-3-glucoside (mg 100 g^{-1} strawberry puree)	16.69 ± 0.07	12.50 ± 0.05	**-25.13**

837 [1]Percent relative change in concentration calculated in reference to the fresh strawberry puree
838 concentrations without inoculation of *Botrytis cinerea* conidia. [2]Values are means ± standard
839 deviation of triplicates (n=3).
840 [1]Cambio relativo porcentual en la concentración calculado con referencia a las concentraciones de
841 puré de fresas fresca sin inoculación de conidias de *Botrytis cinerea*. [2]Los valores son medias ±
842 desviación estándar de triplicados (n=3).
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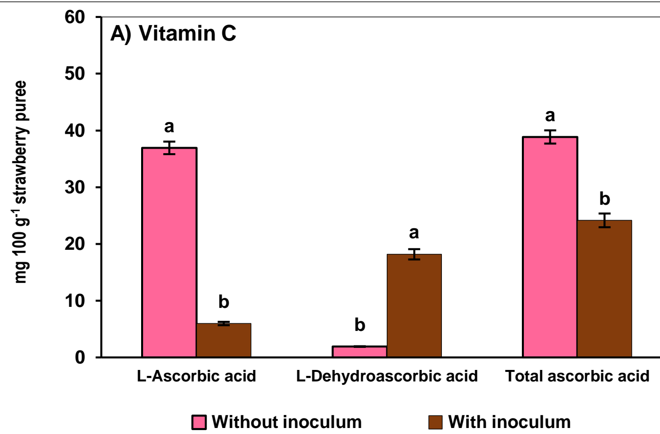
846 **Supplementary Table 2.** Relative losses or increments of strawberry puree phytonutrients as affected by high hydrostatic pressure
847 processing, antifungal food additives and the presence of *Botrytis cinerea* conidia (6.3 Log conidia/mL).
848 **Tabla complementaria 2.** Pérdidas o incrementos relativos de fitonutrientes del puré de fresas afectados por el procesamiento de alta
849 presión hidrostática, los aditivos alimentarios antifúngicos y la presencia de conidias de *Botrytis cinerea* (6,3 log de conidias/mL).

		Without Preservatives (0 µg/mL)		**Potassium Sorbate** (1000 µg/mL)		**Avocado seed acetogenins** (661 µg/mL)		**Avocado seed acetogenins** (7500 µg/mL)	
Bioactive Compounds	**Pressure** (MPa/5min)	**Degradation or increase[1]** (%)	**Total degradation or increase[2]** (%)	**Degradation or Increase** (%)	**Total degradation or Increase** (%)	**Degradation or Increase** (%)	**Total degradation or Increase** (%)	**Degradation or Increase** (%)	**Total degradation or Increase** (%)
L-ascorbic acid	0		-83.82		-94.49		-84.51		-87.60
(mg 100 g^{-1} strawberry	400	-24.48	-87.78	-44.84	-96.96	-52.91	-92.71	-100.00	-100.00
puree)	600	-7.70	-85.06	+13.26	-93.75	-12.47	-86.44	-100.00	-100.00
L-dehydroascorbic acid	0		+846.18		+1207.96		+905.18		+775.91
(mg 100 g^{-1} strawberry	400	+1.17	+857.29	-15.47	+1005.64	-1.67	+888.35	+15.79	+914.20
puree)	600	-24.26	+616.62	-22.47	+914.03	-29.24	+611.24	-1.55	+762.37
Total ascorbic	0		-37.83		-30.08		-35.58		-44.90
(mg 100 g^{-1} strawberry	400	-5.18	-41.05	-17.68	-42.44	-13.40	-44.21	-8.98	-49.85
puree)	600	-20.16	-50.37	-19.80	-43.93	-25.40	-51.94	-22.61	-57.36
p-Hydroxybenzoic acid	0		+6.94		+26.45		+21.35		-33.16
(mg 100 g^{-1} strawberry	400	-14.92	-9.02	-59.85	-49.23	-0.54	+20.70	-47.20	-64.71
puree)	600	-16.36	-10.56	-84.62	-80.55	-75.71	-70.52	-53.95	-69.22
Ellagic acid	0		-37.69		-42.26		-48.24		-41.09
(mg 100 g^{-1} strawberry	400	-6.24	-41.58	+2.50	-40.82	+11.38	-42.35	-7.22	-45.35
puree)	600	+23.57	-23.01	+18.98	-31.30	+18.30	-38.76	-8.49	-46.10
Pelargonidin-3-glucoside	0		-25.13		-31.30		-21.38		-22.86
(mg 100 g^{-1} strawberry	400	-11.71	-33.90	+27.35	-12.51	-20.55	-37.53	+2.61	-20.84
puree)	600	+22.97	-7.94	+16.40	-20.04	+4.32	-17.98	-6.32	-27.73

850 [1]Percent relative concentration as affected by HHP processing and type of preservative, calculated in reference to the unprocessed
851 strawberry puree concentrations without high pressure treatment (0 pressure treatments for each type of antifungal additive). [2]Total
852 percent relative changes in phytonutrient concentrations calculated in reference to the fresh strawberry puree concentrations without
853 inoculation of *Botrytis cinerea* conidia. ND=not detected.

854 [1]Concentración relativa porcentual afectada por el procesamiento HHP y el tipo de conservante, calculada en referencia a las
855 concentraciones de puré de fresas sin procesar sin tratamiento de alta presión (0 tratamientos de presión para cada tipo de aditivo
856 antifúngico). [2]Cambios relativos porcentuales totales en las concentraciones de fitonutrientes calculados con referencia a las
857 concentraciones de puré de fresas fresca sin inoculación de conidias de *Botrytis cinerea*. ND=no detectado.



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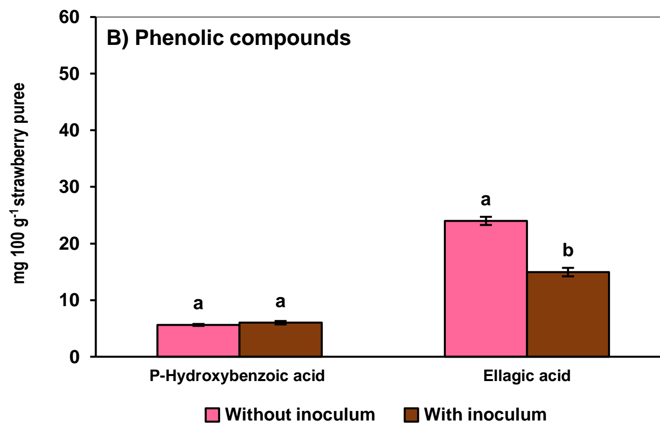

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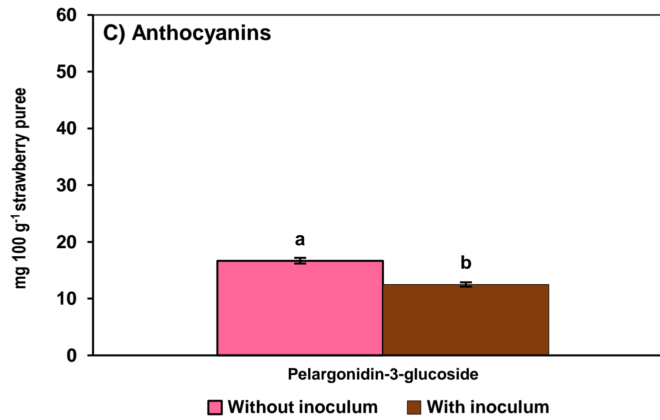

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861 **Figure 1.** Effects of *Botrytis cinerea* conidia inoculated into strawberry puree on the stability of
862 bioactive compounds. **A)** L-ascorbic acid, L-dehydroascorbic acid and total ascorbic acid; **B)** p-
863 hydroxybenzoic acid and ellagic acid; **C)** pelargonidin-3-glucoside. Error bars (‡) represent the
864 standard deviations of means (n=3). Different lowercase letters above the graph bars indicate
865 statistical significance (Tukey-Kramer multiple comparison test, $p<0.05$).

866 **Figura 1.** Efectos de conidias de *Botrytis cinerea* inoculadas en puré de fresas sobre la estabilidad
867 de compuestos bioactivos. **A)** ácido L-ascórbico, ácido L-dehidroascórbico y ácido ascórbico total;
868 **B)** ácido p-hidroxibenzoico y ácido elágico; **C)** pelargonidin-3-glucósido. Las barras de error (‡)
869 representan las desviaciones estándar de las medias (n=3). Diferentes letras minúsculas sobre las
870 barras del gráfico indican significancia estadística (prueba de comparación múltiple de Tukey-
871 Kramer, $p<0,05$).
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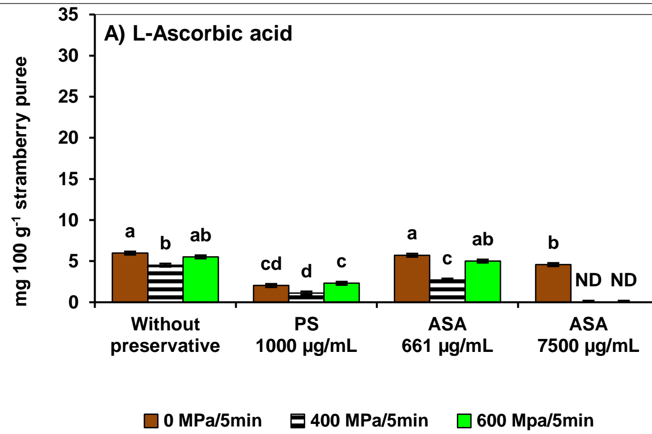

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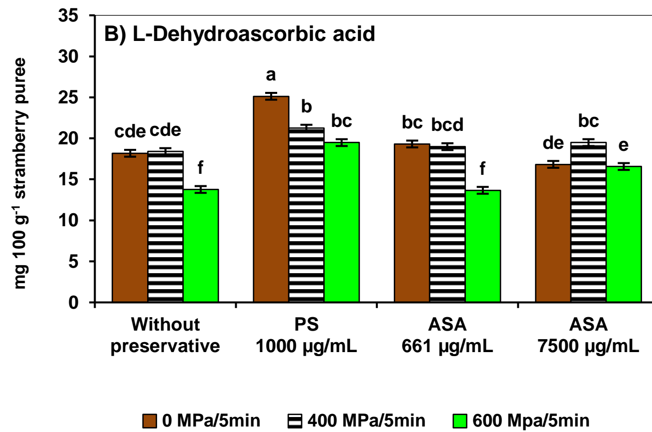

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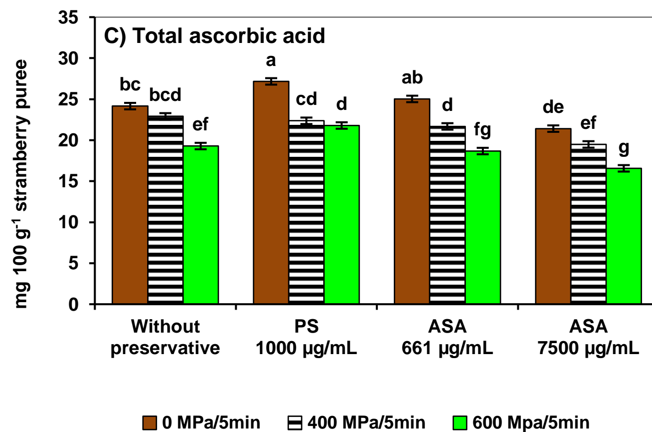

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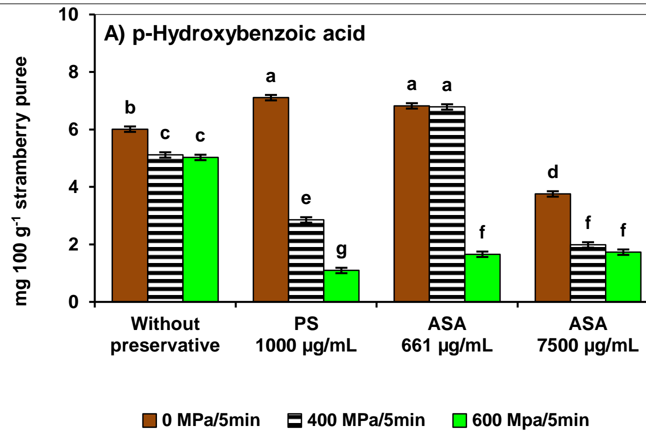
893 **Figure 2.** Stability of vitamin C contents in strawberry puree inoculated with conidia of *Botrytis*
894 *cinerea* as affected by high hydrostatic pressure processing in the presence of potassium sorbate
895 (PS) or avocado seed acetogenins (ASA). **A)** L-ascorbic acid; **B)** L-dehydroascorbic acid; **C)** total
896 ascorbic acid. Error bars (‡) represent the standard deviations of means (n=3). Different lowercase
897 letters above the graph bars indicate statistical significance (Tukey-Kramer multiple comparison
898 test, $p<0.05$). ND= not detected.

Figura 2. Estabilidad del contenido de vitamina C en puré de fresas inoculado con conidias de *Botrytis cinerea* afectadas por procesamiento a alta presión hidrostática en presencia de sorbato de potasio (PS) o acetogeninas de semilla de aguacate (ASA). **A)** ácido L-ascórbico; **B)** ácido L-dehidroascórbico; **C)** ácido ascórbico total. Las barras de error (‡) representan las desviaciones estándar de las medias (n=3). Las letras minúsculas diferentes sobre las barras del gráfico indican significancia estadística (prueba de comparación múltiple de Tukey-Kramer, p<0,05). ND= no detectado.



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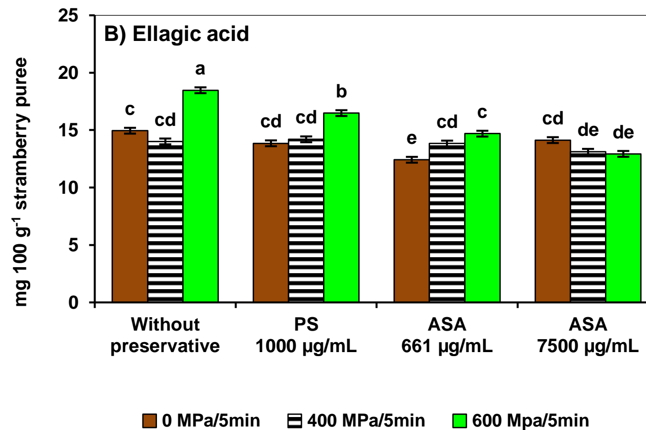

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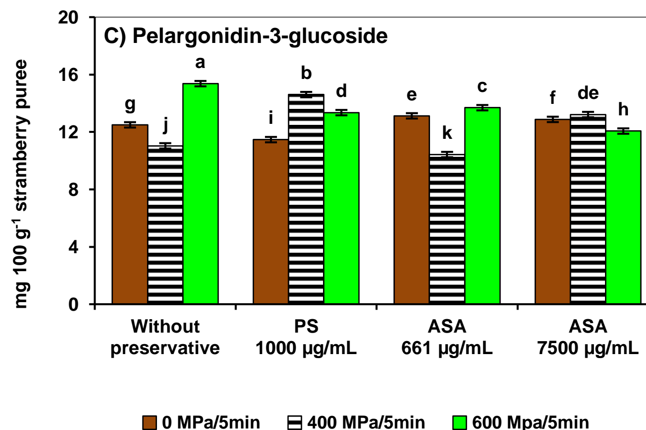

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926 **Figure 3.** Stability of phenolic compounds in strawberry puree inoculated with conidia of *Botrytis*
927 *cinerea* as affected by high hydrostatic pressure processing in the presence of potassium sorbate
928 (PS) or avocado seed acetogenins (ASA). **A)** p-hydroxybenzoic acid; **B)** ellagic acid; **C)**
929 pelargonidin-3-glucoside. Error bars (‡) represent the standard deviations of means (n=3).
930 Different lowercase letters above the graph bars indicate statistical significance (Tukey-Kramer
931 multiple comparison test, $p<0.05$).

932 **Figura 3.** Estabilidad de compuestos fenólicos en puré de fresas inoculado con conidias de *Botrytis*
933 *cinerea* afectadas por procesamiento a alta presión hidrostática en presencia de sorbato de potasio
934 (PS) o acetogeninas de semilla de aguacate (ASA). **A)** ácido p-hidroxibenzoico; **B)** ácido elágico;
935 **C)** pelargonidin-3-glucósido. Las barras de error (‡) representan las desviaciones estándar de las
936 medias (n=3). Las letras minúsculas diferentes sobre las barras del gráfico indican significancia
937 estadística (prueba de comparación múltiple de Tukey-Kramer, $p<0,05$).

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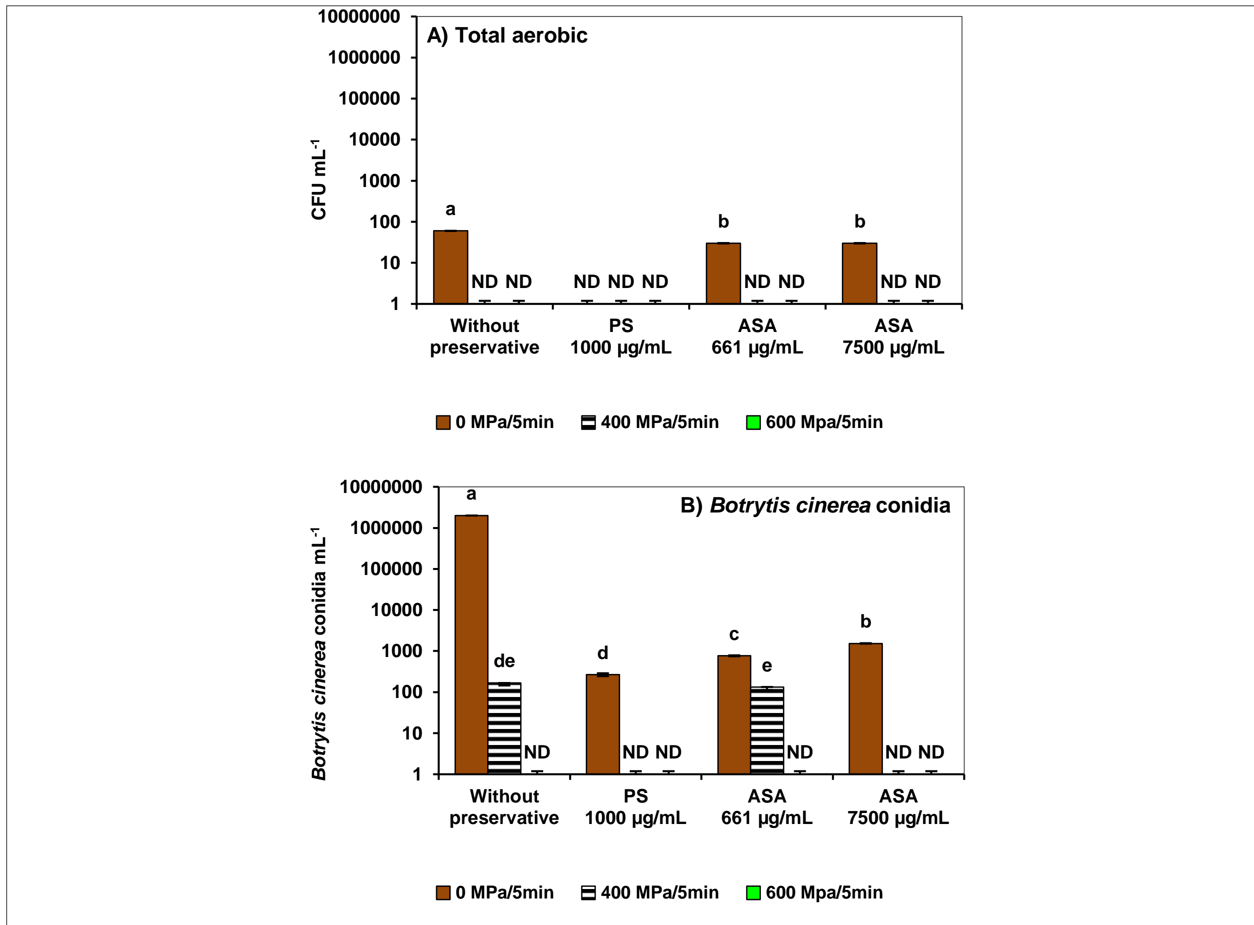

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958 **Figure 4.** Microbial counts in strawberry puree inoculated with conidia of *Botrytis cinerea* as
959 affected by high hydrostatic pressure processing in the presence of potassium sorbate (PS) or
960 avocado seed acetogenins (ASA). **A)** total aerobic on plate count agar; **B)** *Botrytis cinerea* conidia
961 on potato dextrose agar. Error bars (‡) represent the standard deviations of means (n=3). Different
962 lowercase letters above the graph bars indicate statistical significance (Tukey-Kramer multiple
963 comparison test, $p<0.05$). ND= not detected.

964 **Figura 4.** Recuentos microbianos en puré de fresas inoculado con conidias de *Botrytis cinerea*
965 afectados por procesamiento a alta presión hidrostática en presencia de sorbato de potasio (PS) o
966 acetogeninas de semilla de aguacate (ASA). A) aerobio total en agar de recuento en placa; B)
967 Conidias de *Botrytis cinerea* en agar patata dextrosa. Las barras de error (‡) representan las
968 desviaciones estándar de las medias (n=3). Las letras minúsculas diferentes sobre las barras del
969 gráfico indican significancia estadística (prueba de comparación múltiple de Tukey-Kramer,
970 $p<0,05$). ND= no detectado.

4. CONCLUSIÓN GENERAL

Durante esta investigación las acetogeninas de semilla de aguacate mostraron un efecto fungistático contra *Botrytis cinerea* reduciendo su crecimiento micelial en condiciones *in vitro* y afectó principalmente su fase de crecimiento estacionario. El porcentaje de inhibición de la germinación de conidias fue muy similar al encontrado en conservadores comerciales. Por lo tanto, las acetogeninas de semilla de aguacate fueron una alternativa natural para el control de *Botrytis cinerea*. *Botrytis cinerea* causó pérdidas en el ácido L-ascórbico y el color en puré de fresa sin ningún tratamiento aplicado. Al utilizar HHP asistidas con ASA, no se detectaron conidias viables de *Botrytis cinerea* y aumentaron los contenidos de compuestos bioactivos como: ácido elágico y pelargonidin-3-glucósido, manteniendo así el ácido p-hidroxibenzoico y el ácido ascórbico total. Lo que fortalece el valor de las tecnologías no térmicas combinadas para la inhibición de hongos fitopatógenos y conservación de compuestos bioactivos. Sin embargo, se requieren de más estudios.

5. RECOMENDACIONES

1. Desarrollar una nueva formulación para las acetogeninas de semilla de aguacate con mayor miscibilidad en productos procesados líquidos ácidos.
2. Realizar pruebas enzimáticas para determinar cómo influyen las enzimas POD, PPO y PME cuando se aplican altas presiones hidrostáticas y el conservador de acetogeninas de semilla de aguacate.
3. Evaluar otras metodologías de extracción de fenoles y flavonoides menos abrasivas.
4. Evaluar otras fases móviles para la determinación de antocianinas por UPLC.
5. Identificar el mecanismo de acción de las acetogeninas de semillas de aguacate *in vitro* e *in vivo* en hongos fitopatógenos.

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