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Facultad de Química

“EVALUACIÓN DE LA CAPACIDAD ANTIOXIDANTE Y CARACTERIZACIÓN DEL ACEITE ESENCIAL DE PIMIENTA DE JAMAICA (PIMENTA DIOICA L. MERRILL) EXTRAÍDO MEDIANTE FLUIDOS SUPERCRÍTICOS ASISTIDO POR CAMPOS ELÉCTRICOS PULSADOS”

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RESUMEN

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El presente trabajo de investigación tuvo como objetivo extraer el aceite esencial de pimienta de Jamaica (*Pimenta dioica* L. Merrill) empleando la extracción con dióxido de carbono supercrítico (SC-CO_2), asistido por campos eléctricos pulsados (PEF), y posteriormente evaluar la capacidad antioxidante del aceite extraído. La motivación fue desarrollar una metodología eficiente, económicamente viable y ambientalmente sostenible para la extracción de compuestos naturales, que ofreciera ventajas potenciales sobre los métodos de extracción convencionales.

El objetivo principal fue mejorar el rendimiento y la eficiencia de la extracción de aceite esencial de pimienta de Jamaica utilizando SC-CO_2 mediante la incorporación de un paso de pretratamiento con PEF en las bayas de pimienta de Jamaica antes del proceso de extracción. Se llevaron a cabo pruebas experimentales en las que las bayas de pimienta de Jamaica se sometieron a pulsos de PEF de intensidades de campo eléctrico variables (0.1, 1.0 y 3.0 kV/cm) durante una duración de 1.0 segundo antes de someterse a una extracción de SC-CO_2.

Los resultados experimentales demostraron que la técnica de extracción combinada de SC-CO_2, precedida por el pretratamiento con PEF, mostró un mejor rendimiento y eficiencia en comparación con la utilización exclusiva de SC-CO_2. En particular, el rendimiento de aceite esencial aumentó de 0.894 g cuando se emplea SC-CO_2 solo a 1.396 g cuando se combina SC-CO_2 con 50 pulsos de PEF. Además, el contenido del compuesto principal, eugenol, aumentó del 52% al 64% mediante el proceso combinado. Se evalúo el contenido de fenoles totales en el que se obtuvo 4.92 mg equivalentes de ácido gálico por gramo de peso seco de pimienta de Jamaica.

En resumen, la aplicación del pretratamiento PEF facilitó la obtención de mayores rendimientos de aceite esencial de pimienta de Jamaica a presiones relativamente más bajas durante el proceso de extracción SC-CO2, demostrando así la mayor eficiencia y viabilidad

de esta metodología de extracción combinada. La investigación desarrolló con éxito un método que puede tener ventajas en términos de impacto ambiental, viabilidad económica y eficiencia de producción para extraer compuestos naturales de la pimienta de Jamaica.

ABSTRACT

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The objective of this research work was to extract the essential oil of allspice (Pimenta dioica L. Merrill) using extraction with supercritical carbon dioxide (SC-CO_2), assisted by pulsed electric fields (PEF), and subsequently evaluate the antioxidant capacity of the extracted oil. The motivation was to develop an efficient, economically viable and environmentally sustainable methodology for the extraction of natural compounds, offering potential advantages over conventional extraction methods.

The main objective was to improve the yield and efficiency of allspice essential oil extraction using SC-CO_2 by incorporating a PEF pretreatment step into allspice berries before the extraction process. Experimental tests were carried out in which allspice berries were subjected to PEF pulses of varying electric field strengths (0.1, 1.0 and 3.0 kV/cm) for a duration of 1.0 second before being subjected to an extraction of SC-CO_2.

The experimental results demonstrated that the combined SC-CO_2 extraction technique, preceded by pretreatment with PEF, showed better performance and efficiency compared to the exclusive use of SC-CO_2. In particular, the essential oil yield increased from 0.894 g when SC-CO_2 alone was used to 1.396 g when SC-CO_2 was combined with 50 pulses of PEF. Furthermore, the content of the main compound, eugenol, increased from 52% to 64% by the combined process. The total phenol content was evaluated, obtaining 4.92 mg equivalents of gallic acid per gram of dry weight of allspice.

In summary, the application of PEF pretreatment facilitated obtaining higher yields of allspice essential oil at relatively lower pressures during the SC-CO_2 extraction process, thus demonstrating the higher efficiency and feasibility of this combined extraction methodology. The research successfully developed a method that may have advantages in terms of environmental impact, economic viability, and production efficiency to extract natural compounds from allspice.

INTRODUCCIÓN

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En los últimos años, ha habido una demanda creciente de alimentos naturales, mínimamente procesados, con características de frescura y una vida útil prolongada. Esta tendencia ha impulsado la búsqueda de técnicas innovadoras de conservación de alimentos que puedan mantener la calidad nutricional y las propiedades sensoriales de los productos alimenticios. Uno de esos enfoques implica el uso de tecnologías no térmicas, que han surgido como alternativas prometedoras a los métodos tradicionales de procesamiento térmico.

Entre estas tecnologías no térmicas, la aplicación de fluidos supercríticos y campos eléctricos pulsados ha atraído atención en la industria alimentaria. La extracción con fluidos supercríticos (SFE) es una tecnología ecológica que utiliza fluidos en estado supercrítico, como el dióxido de carbono empleado como solvente para la extracción de compuestos bioactivos de fuentes naturales. Esta técnica ofrece varias ventajas sobre los métodos convencionales de extracción con disolventes, incluida una mayor selectividad, un menor impacto ambiental y la capacidad de preservar la integridad de compuestos térmicamente sensibles.

Por otro lado, la tecnología de campo eléctrico pulsado (PEF, por sus siglas en inglés) implica la aplicación de pulsos eléctricos cortos de alto voltaje a materiales alimentarios líquidos o semisólidos colocados entre dos electrodos. Este proceso no térmico puede provocar modificaciones estructurales en las membranas celulares, facilitando la extracción de compuestos valiosos y minimizando la degradación de componentes sensibles al calor.

La pimienta de Jamaica, también conocida como pimienta jamaicana o pimiento, es una especia valiosa originaria de México y Centroamérica, reconocida por su sabor y aroma únicos. El aceite esencial de pimienta de Jamaica es particularmente rico en eugenol, un compuesto fenólico con potentes propiedades antioxidantes y antimicrobianas. Los antioxidantes desempeñan un papel crucial en la conservación de los alimentos al prevenir o retrasar las reacciones de oxidación que pueden provocar pérdida de nutrientes, sabores desagradables y una vida útil más corta. Aprovechando los efectos sinérgicos de los fluidos supercríticos y los campos eléctricos pulsados, este proyecto tiene como objetivo desarrollar un método eficiente y respetuoso con el medio ambiente para extraer aceite esencial de pimienta de Jamaica preservando al mismo tiempo su potencial antioxidante.

La capacidad antioxidante del aceite esencial extraído será evaluada mediante la determinación del contenido fenólico total, este análisis proporcionará información sobre las posibles aplicaciones del aceite esencial extraído como antioxidante natural en productos alimenticios, nutracéuticos u otros sectores industriales.

En general, este proyecto de investigación representa un esfuerzo multidisciplinario para abordar la creciente demanda de tecnologías de conservación de alimentos sostenibles e innovadoras, al mismo tiempo que valoriza la rica biodiversidad de México y promueve el uso de antioxidantes naturales de fuentes vegetales autóctonas.

CONTRIBUCIÓN SOCIAL

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El proyecto de investigación propuesto tiene como objetivo extraer aceite esencial de pimienta de Jamaica (Pimenta dioica L. Merrill), un valioso cultivo originario de México y Centroamérica, utilizando un método de extracción innovador que involucra dióxido de carbono supercrítico asistido por campos eléctricos pulsados. Posteriormente al proceso de extracción, el proyecto se centra en evaluar la capacidad antioxidante del aceite esencial obtenido valorando su contenido fenólico total. La importancia de este proyecto radica en su enfoque multifacético para abordar diversas necesidades sociales y contribuir al desarrollo sostenible. En primer lugar, el método de extracción emplea fluidos supercríticos y campos eléctricos pulsados, que son técnicas respetuosas con el medio ambiente que no dependen de disolventes orgánicos agresivos ni de altas temperaturas. Esto se alinea con la creciente demanda de prácticas ecológicas y sostenibles en las industrias alimentaria y nutracéutica. A nivel mundial, contribuye al cumplimiento de uno de los Objetivos de Desarrollo Sostenible (ODS) planteados por los líderes mundiales: Salud y Bienestar; a nivel nacional, forma parte del Programa Nacional Estratégico (PRONACE)-Salud del Consejo Nacional de Humanidades, Ciencias y Tecnologías en México (CONAHCyT). Este avance en la ciencia y la tecnología de los alimentos puede tener implicaciones de gran alcance para la industria alimentaria, permitiendo la producción de productos alimenticios de alta calidad, seguros y nutritivos. Además, el proyecto destaca el potencial de la biodiversidad mexicana al utilizar un cultivo de origen local y culturalmente significativo: la pimienta de Jamaica. Al valorizar este recurso natural, el proyecto fomenta el uso sustentable y la apreciación de la rica biodiversidad de México, lo que puede generar oportunidades económicas y potencial creación de empleo en áreas rurales donde se cultiva la pimienta de Jamaica.

El enfoque en la extracción de antioxidantes naturales del aceite esencial de pimienta de Jamaica aborda la creciente demanda de los consumidores de productos alimenticios naturales, saludables y mínimamente procesados. Los antioxidantes desempeñan un papel crucial en la conservación de los alimentos al extender la vida útil y mantener la calidad nutricional, reduciendo así el desperdicio de alimentos y garantizando la seguridad alimentaria. Los compuestos extraídos podrían utilizarse potencialmente como conservantes alimentarios naturales o nutracéuticos, contribuyendo al desarrollo de productos alimentarios más saludables y sostenibles.

El énfasis del proyecto en métodos de extracción sustentables, la valorización de la biodiversidad mexicana, la promoción de antioxidantes naturales, el avance de la ciencia y la tecnología de los alimentos y las oportunidades económicas potenciales lo posicionan como un esfuerzo multifacético con importantes recompensas sociales. Al abordar estos diversos aspectos, el proyecto tiene el potencial de contribuir al desarrollo sostenible, promover opciones de alimentos saludables y apoyar a las comunidades locales, al tiempo que amplía los límites del conocimiento científico y las innovaciones tecnológicas en el sector alimentario.

ANTECEDENTES

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PIMIENTA DE JAMAICA

La Pimienta de Jamaica (*Pimenta dioica* L. *Merrill* o Pimiento officinalis) pertenece a la familia de las Mirtáceas y es conocida en inglés como allspice o pimento, en francés, jamaique o toute-epice, en portugués como pimenta da Jamaica y en español como pimienta gorda[1-2]. La pimienta gorda es una baya casi esférica que tiene menos de un centímetro de diámetro, de color café rojizo y un poco rugoso al tacto. La especia es el fruto inmaduro y seco que crece en algunas regiones de Veracruz, Puebla, Tabasco, Oaxaca y Chiapas[3]. México ocupa el segundo lugar como productor de pimienta gorda en América Latina[4].

ACEITE ESENCIAL DE PIMIENTA DE JAMAICA

Tradicionalmente, los frutos inmaduros y secos se consumen como especias y comúnmente son utilizados para aromatizar los alimentos, pero el aceite esencial de este fruto y los extractos acuosos de las hojas del árbol pimentero han sido utilizados como carminativos, hipoglucemiantes, estimulantes, antimicrobianos, acaricidas y preparaciones farmacéuticas fungicidas[5-11]. Los frutos tienen un fuerte olor fragante, ya que contienen de 2 a 5% de aceite esencial, cuyo contenido principal es eugenol (65-85%).

Por otra parte, desde que la humanidad ha optado por consumir productos naturales, el aceite esencial de pimienta se utiliza masivamente en alimentos, productos farmacéuticos y en la industria de perfumería[12]. Debido a su alto contenido de eugenol, metil-eugenol, mirceno y cariofileno[10,13]. Se denomina aceite esencial al conjunto sustancias líquidas, aromáticas y volátiles situadas en cualquier parte del vegetal, conformadas por un grupo heterogéneo de sustancias orgánicas. El aceite de pimienta ha sido estudiado en diferentes aplicaciones. El potencial antioxidante del aceite de pimienta fue evaluado por muchos investigadores y todos ellos han descubierto que este aceite esencial tiene buena actividad antioxidante y puede ser utilizada como un antioxidante natural[11,12,15,16].En aplicaciones más específicas el aceite esencial de pimienta se utiliza como un anti-inflamatorio[16,17].Algunas aplicaciones extraordinarias de los extractos de las hojas y bayas de pimienta han sido reportadas en los campos de la medicina y de los materiales[18, 19].

MÉTODOS DE EXTRACCIÓN DE ACEITES ESENCIALES

La investigación que se ha llevado a cabo en las últimas décadas ha demostrado el papel que desempeñan ciertos componentes químicos nutricionales en la prevención y tratamiento de muchas enfermedades. Esta situación ha provocado un cambio del simple concepto de alimento como fuente de nutrientes, a uno más integral que traduce la potencialidad que los alimentos pueden tener, no solo de nutrir, sino, también de prevenir y curar enfermedades.

Debido a este cambio, surgen los muy populares alimentos funcionales que se definen como cualquier alimento en forma natural o procesada, que además de sus componentes adicionales que favorecen la salud, la capacidad física y el estado mental de una persona.

Estos compuestos o ingredientes que dan al alimento la característica de alimento funcional son llamados ingredientes funcionales y pueden encontrarse de manera natural en el alimento o ser agregados como un aditivo, pero ya sea para incrementar su cantidad o adicionar un compuesto nuevo, siempre se prefiere que estos aditivos sean de un origen natural, ya que, junto con la búsqueda de alimentos más saludables, el consumidor busca siempre los productos más naturales posibles.

Para la industria, esta situación representa una oportunidad de crear nuevas líneas de productos, con valor agregado y gran receptividad por parte de los consumidores, surgiendo así un nuevo campo de investigación en donde especialistas en nutrición y tecnología de alimentos trabajen activamente en formular nuevos productos que brinden las características que el consumidor está buscando.

Dentro de esta línea de investigación, se ha desarrollado el estudio de nuevos métodos de obtención de aditivos que generen alimentos funcionales y que a la vez sean de origen natural. Dentro de los compuestos funcionales más estudiados, se encuentran aquellos que se relacionan con la prevención o cura de enfermedades crónicas, principalmente los que tienen función antioxidante. Por lo que muchos estudios de extracción de compuestos tienen como objetivo la obtención de estos antioxidantes por algún método que asegure la pureza y su efectividad.

Los métodos tradicionales de extracción de ingredientes funcionales utilizan cantidades altas de solventes tóxicos y tienen poca selectividad, lo cual no permite obtener productos naturales que los consumidores están buscando hoy, por lo que la extracción por medio de fluidos supercríticos es una alternativa muy interesante para realizar la obtención y purificación de este tipo de aditivos.

La extracción es una operación de separación por transferencia de masa en la que se ponen en contacto dos fases inmiscibles o parcialmente miscibles, en continuo o discontinuo, con objeto de transferir uno o varios componentes de una fase a la otra de diferente composición[20].

Los aceites esenciales contienen compuestos que han mostrado una amplia actividad biológica la cual resulta ser útil en el tratamiento para prevenir enfermedades relacionadas con una formación excesiva de radicales de oxígeno. Por tanto, la bioactividad de estos compuestos depende en gran medida de las condiciones de extracción como el tipo de disolvente, la temperatura y el proceso de extracción en sí. Por esta razón es muy importante seleccionar la combinación de adecuada de estas variables para mejorar la extracción y funcionalidad del aceite.

EXTRACCIÓN CON FLUIDOS SUPERCRÍTICOS (ESC)

Como consecuencia del desarrollo de nuevos procesos de separación que optimicen los costos energéticos asociados a los procesos de extracción de esencias y aceites esenciales, además de la reducción de riesgos ambientales, la industria química demanda el uso de solventes más eficaces, fácilmente recuperables y el aprovechamiento integral de materia prima de alta calidad y de subproductos. Razón por la cual la técnica de Extracción con Fluidos Supercríticos o Extracción Supercrítica (SFE por sus siglas en inglés)[6], es adecuada para este fin, debido a que se basa principalmente en la diferencia de solubilidades del agente de extracción respecto a los componentes de interés, usando como agente separador un fluido supercrítico. Los fluidos supercríticos tienen características de extracción ajustables debido a su densidad, que puede ser controlada por cambios en la temperatura y la presión[21].

La SFE permite llevar a cabo la extracción de ingredientes activos a partir de hierbas y especias con una mejor reproducción del sabor y fragancia en comparación con los procesos convencionales. Se evita la degradación térmica y la descomposición de compuestos lábiles, debido a que el proceso opera a temperaturas reducidas, y en ausencia de luz y oxígeno, lo que previene reacciones oxidativas. Este último punto es de especial interés para la extracción de antioxidantes, ya que garantiza la conservación de las propiedades biológicas de los extractos obtenidos

Se entiende por fluido supercrítico a toda aquella sustancia llevada a condiciones operativas de presión y temperatura por encima de su punto crítico[22]. Un fluido supercrítico es un estado de la materia que presenta propiedades de los gases y de los líquidos, es decir, al igual que un gas, no es compresible, tiene valores similares en sus propiedades de transporte, viscosidad y difusividad, pero en su densidad es parecida a la de los líquidos, por tanto, se asemejan en su poder disolvente. En otras palabras, el estado supercrítico es una forma de la materia en la que los estados líquido y gaseoso son indistinguibles entre sí. En la Figura 1, se muestra un diagrama de fases para una sustancia pura.

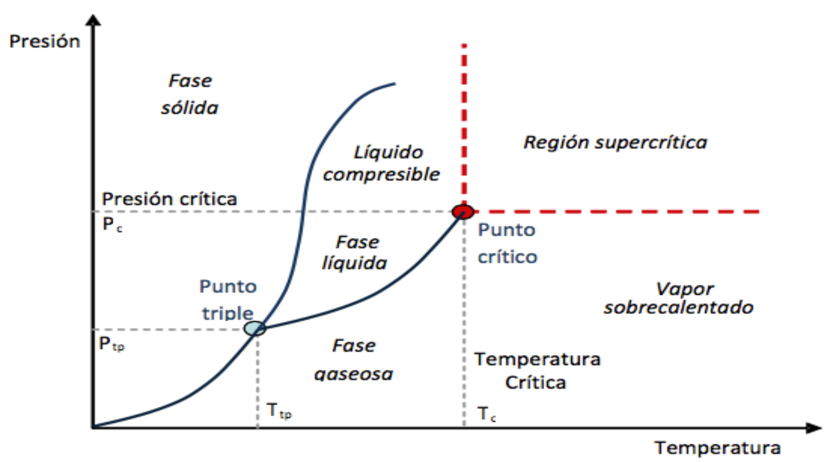


Figura 1. Diagrama de fases de una sustancia pura en función de presión y temperatura

El dióxido de carbono es el más apropiado para usarse como solvente en la extracción con fluidos supercríticos. Sus condiciones críticas son 31.1ºC y 73.8 bar las cuales lo hacen muy atractivo para la extracción de compuestos termolábiles[23]. Adicionalmente es un solvente inerte, no inflamable, no explosivo, sin olor ni color y no costoso[24]. Su alta densidad hace que la tasa de extracción sea mayor que la de métodos convencionales como la extracción por arrastre de vapor o hidroextracción[25].

CAMPOS ELECTRICOS PULSADOS (PEF)

El desarrollo de la tecnología de los campos eléctricos pulsados (PEF) para aplicaciones de procesamiento no térmico de alimentos ha sido uno de los principales campos de investigación en los últimos años.

Esta tecnología consiste en la aplicación de pulsos eléctricos de alta intensidad a un material colocado en una cámara de tratamiento confinada entre 2 electrodos, en la cual la duración de los pulsos generalmente es de ms o µs[26].

Diversas investigaciones en el sector alimenticio han demostrado varias aplicaciones de los PEF como un tratamiento para mejorar la extracción intracelular de metabolitos[27,28], mejorar la eficiencia de secado[29], modificar la actividad enzimática[30,31], preservar ingredientes alimenticios[32] y producir metabolitos secundarios induciendo reacciones de estrés en sistemas de plantas[33].

Para la extracción de antocianinas, los PEF sirven como un pretratamiento al material vegetal del cual se obtienen dichos componentes, generando un fenómeno intracelular llamado electroporación[34,35].

La electroporación es un proceso que consiste en la ampliación o ruptura de los poros de una membrana vegetal debido a la aplicación de un potencial eléctrico de alta intensidad[36] (Figura 2). Este proceso hace que los materiales celulares dentro de la membrana queden más expuestos a los disolventes utilizados en la extracción haciendo que se transporten más fácilmente[37].

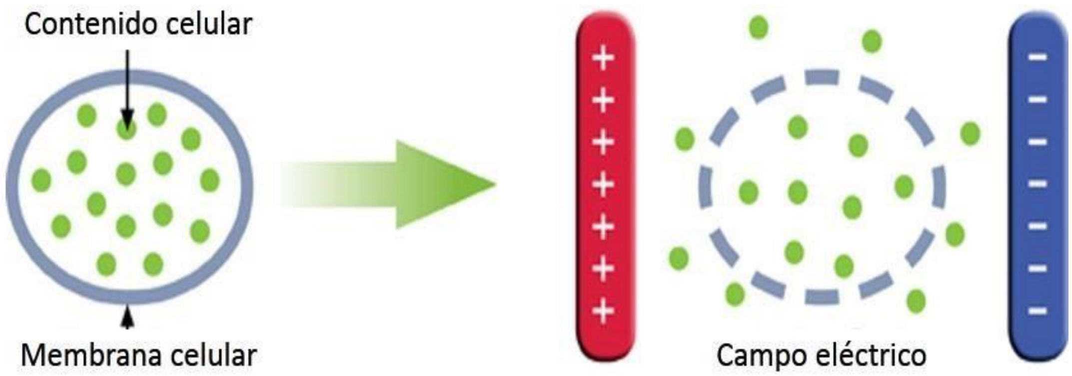


Figura 2. Fenómeno de electroporación

La cantidad de potencial eléctrico aplicado depende del uso de los PEF, todo esto basado en investigaciones que se han realizado en años recientes.

ENERGÍA EN EL PROCESO DE PEF

La cantidad de energía utilizada es un factor importante por considerar desde el diseño hasta la operación de un proceso, en el caso del pretratamiento con campos eléctricos pulsados esta no es la excepción debido a que de esto dependerá su eficiencia de acuerdo al tipo y a la cantidad de muestras a tratar, así como de los tiempos de procesamiento que estas requieran[38].

De acuerdo a Jaeger et al. en el proceso de PEF, la energía eléctrica se aplica a los medios de tratamiento y la cantidad de energía suministrada por pulso único (energía de pulso) se puede calcular en función del voltaje y la corriente eléctrica[39].

$$W_{Pulse} = \int U(t) \cdot I(t) \cdot dt \qquad (1)$$

Don W_{Pulse} (J) es la cantidad de energía por pulso, I (A) es la intensidad de corriente.

Dependiendo del número de pulsos aplicados a una cantidad de producto, se puede calcular el aporte de energía específica total de acuerdo al siguiente modelo:

$$W_{Spec} = \frac{f}{\dot{m}} \cdot W_{Pulse} \qquad (2)$$

donde W_{Spec} (J / mol) es la energía total aplicada, f (s^{-1}) es la frecuencia, m (kg / h) es el flujo másico y W_{Pulse} (J) es la energía aplicada por pulso eléctrico.

La energía especifica total suministrada se considera como un parámetro clave para describir la intensidad del tratamiento ya que permite una estimación del aumento de temperatura que ocurre durante este proceso[40].

AVANCES RECIENTES EN TECNOLOGÍAS DE EXTRACCIÓN

En los últimos años, se han realizado numerosas investigaciones enfocadas en el desarrollo de tecnologías innovadoras para la extracción de compuestos bioactivos de fuentes naturales, como plantas, frutas y subproductos agroindustriales. Dos de las técnicas más prometedoras que han surgido son la extracción con fluidos supercríticos y el tratamiento con campos eléctricos pulsados.

La extracción con fluidos supercríticos, particularmente con dióxido de carbono supercrítico (SC-CO_2), ha demostrado ser una alternativa eficiente y respetuosa con el medio ambiente para la obtención de diversos compuestos bioactivos. Numerosos estudios han explorado el uso de SC-CO_2 para la extracción de aceites esenciales, compuestos fenólicos, carotenoides, entre otros, a partir de diferentes matrices vegetales.

Por ejemplo, investigadores han utilizado SC-CO_2 para extraer el aceite esencial de romero, obteniendo rendimientos superiores en comparación con métodos convencionales, además de un perfil de compuestos más rico en antioxidantes[41]. Asimismo, se ha logrado extraer compuestos fenólicos de residuos agroindustriales, como la cáscara de uva, utilizando SC-CO_2[42].

Por otra parte, la tecnología de campos eléctricos pulsados (PEF) ha ganado atención como un pretratamiento no térmico para facilitar la extracción de compuestos bioactivos. Esta técnica consiste en aplicar pulsos eléctricos de alta intensidad y corta duración al material vegetal, lo que induce la formación de poros en las membranas celulares, liberando así los compuestos intracelulares.

Diversos estudios han demostrado el potencial del tratamiento con PEF para mejorar la extracción de compuestos antioxidantes de diferentes fuentes naturales. Por ejemplo, Corrales et al. (2008) que aplicaron PEF a subproductos de la uva y observaron un aumento significativo en la actividad antioxidante de los extractos obtenidos. Además, Parniakov et al. (2015) lograron incrementar la extracción de carotenoides de zanahorias utilizando PEF como pretratamiento.

Si bien ambas tecnologías, fluidos supercríticos y PEF, han demostrado su efectividad de manera individual para la extracción de compuestos bioactivos, también se ha explorado la combinación de estas técnicas con el fin de aprovechar sus efectos sinérgicos y obtener mayores rendimientos y eficiencias de extracción.

En los últimos años se realizó un estudio que informa la primera combinación de varios pasos de tratamiento con campo eléctrico pulsado (PEF) y extracción con fluido supercrítico (SFE) con CO_2 para la extracción de compuestos bioactivos de orujo de uva agotado (EGM)[45]. Donde sus principales resultados fue un aumento de la capacidad antioxidante total (TAC) de EGM aumentó hasta un 68% después del tratamiento con PEF (3 kV/cm, 100 kJ/kg, 2 Hz, 100 ms) en comparación con el remojo convencional. En los extractos se identificaron varios polifenoles, incluidos kaempferol, luteolina, escutelarina y resveratrol, junto con otras estructuras glicosiladas, mediante cromatografía líquida junto con análisis de espectrometría de masas. La combinación de PEF y SFE con CO_2 demostró ser un enfoque eficaz de varios pasos para extraer compuestos lipídicos y glicosilados bioactivos de EGM. El pretratamiento con PEF mejoró la extracción de antioxidantes y carbohidratos, mientras que el proceso SFE posterior aumentó aún más la recuperación de compuestos valiosos. El estudio demostró el potencial de esta tecnología combinada para la valorización de subproductos agroindustriales como el EGM.

Numerosos estudios recientes se han centrado en optimizar los parámetros de operación, como la intensidad del campo eléctrico, el número y duración de los pulsos PEF, así como las condiciones de presión y temperatura en la extracción con SC-CO_2. También se han explorado diferentes configuraciones del equipo y escalas de operación, con miras a una posible implementación industrial de esta tecnología combinada.

En resumen, la integración de técnicas como la extracción con SC-CO_2 y el pretratamiento con PEF representa una alternativa prometedora para la obtención eficiente y sostenible de compuestos naturales de interés desde fuentes vegetales. Estos avances tecnológicos ofrecen oportunidades para el desarrollo de productos innovadores en diversas industrias, al tiempo que promueven prácticas más respetuosas con el medio ambiente.

ACTIVIDAD ANTIOXIDANTE EN ACEITES ESENCIALES

Los aceites esenciales son mezclas liquidas de compuestos volátiles obtenidos de plantas aromáticas. Muchos aceites esenciales tienen propiedades, y el uso de aceites esenciales como antioxidantes naturales es un campo de interés creciente debido a que algunos antioxidantes sintéticos como el BHA y BHT se sospecha son potencialmente dañinos para la salud humana.

Adicionar aceites esenciales a productos comestibles, ya sea por mezcla directa o en envases activos o revestimientos comestibles, pueden por tanto representar una alternativa válida para prevenir auto oxidación y prolongar la vida útil. Sin embargo, la evaluación del rendimiento antioxidante de los aceites esenciales es un tema crucial porque muchas pruebas de uso común son inapropiadas y dan resultados contradictorios que pueden incurrir a errores a futuras investigaciones. Cientos de compuestos con puntos de ebullición relativamente bajos se han identificado en los aceites esenciales y la gran diversidad química de sus componentes influye en la estabilidad oxidativa de los aceites esenciales. Estos atributos se deben a la capacidad inherente de algunos de sus componentes particularmente fenoles, para detener o retrasar la oxidación aeróbica de materia orgánica

ANTIOXIDANTES

Desde el punto de vista biológico los antioxidantes son compuestos capaces de oponerse a la oxidación y aquellas moléculas capaces de retrasar o inhibir la oxidación de un sustrato. Un radical libre es una especie química con uno o más electrones desapareados en sus orbitales de valencia. Debido a su configuración electrónica, son inestables y extremadamente reactivos, tendiendo a captar un electrón disponible de alguna molécula o átomo, a fin de alcanzar su estabilidad electrónica. La molécula que cede el electrón se convierte a su vez en un radical libre, iniciándose así una reacción en cadena. Los antioxidantes detienen el efecto de los radicales libres.

Existen factores exógenos y endógenos al organismo humano que estimulan o detienen la generación de radicales libres. Entre los estímulos exógenos que incrementan la generación de especies oxidantes se destacan diversos tipos de radiación, contaminantes ambientales, la metabolización de fármacos, el humo del tabaco, la acción de células del sistema inmunológico, o dietas deficientes en antioxidantes.

La concentración de los antioxidantes presentes de manera natural en los alimentos disminuye en gran medida por efecto del procesamiento. Por ello, generalmente es necesario suplementarlas en los procesos de transformación. Los antioxidantes más utilizados en la actualidad en la industria alimentaria son sintéticos, tales como el butilhidroxitolueno (BHT), butilhidroxianisol (BHA), terbutilhidroxiquinona (TBHQ) y propilgalato (PG), a los que se les ha asociado con una dudosa seguridad en su uso.

Estudios anteriores sobre la composición química del aceite esencial de pimienta de Jamaica han identificado dos componentes principales: eugenol (60-95 %) y metil eugenol (10 %).

DETERMINACIÓN DE ACTIVIDAD ANTIOXIDANTE

Los aceites esenciales son extractos de plantas altamente concentrados que contienen una mezcla compleja de compuestos aromáticos volátiles. Entre estos compuestos, los compuestos fenólicos son particularmente importantes debido a sus potentes propiedades antioxidantes. Se sabe que los compuestos fenólicos poseen la capacidad de neutralizar los radicales libres, que son moléculas altamente reactivas que pueden causar estrés oxidativo y daño a las células, las proteínas y el ADN.

La determinación del contenido total de fenoles (TPC) en los aceites esenciales es crucial porque proporciona un indicador valioso de su potencial capacidad antioxidante. Cuanto mayor sea el TPC, mayor será la actividad antioxidante potencial del aceite esencial. Los antioxidantes desempeñan un papel vital en diversas industrias, incluidas la alimentaria, la cosmética y la farmacéutica, ya que ayudan a prevenir el deterioro oxidativo y prolongar la vida útil de los productos.

Al evaluar el TPC, los investigadores y fabricantes pueden evaluar los posibles efectos beneficiosos de los aceites esenciales en diversas aplicaciones. Por ejemplo, los aceites esenciales con alto TPC pueden ser útiles como conservantes naturales en productos alimenticios, ya que pueden inhibir la oxidación y el deterioro de los lípidos. En la industria cosmética, se pueden incorporar aceites esenciales con potentes propiedades antioxidantes a las formulaciones para proteger contra el estrés oxidativo y el envejecimiento prematuro causado por los radicales libres.

Además, el análisis TPC puede ayudar en el control de calidad y la estandarización de los aceites esenciales, asegurando la coherencia en sus propiedades antioxidantes y su eficacia. También contribuye a la identificación de fuentes potenciales de potentes antioxidantes a partir de materiales vegetales, lo que puede conducir al desarrollo de productos nuevos y mejorados.

JUSTIFICACIÓN

JUSTIFICACIÓN

La búsqueda de antioxidantes naturales ha dado lugar a un gran número de estudios sobre el potencial antioxidante de los aceites esenciales presentes en una inmensa variedad de plantas, semillas y frutos. El cuerpo humano está sometido bajo constante estrés oxidativo, esto se debe a que todos los organismos aerobios requieren oxígeno para la producción eficiente de energía, sin embargo, durante la respiración ocurren procesos oxidativos que implican trasferencia de electrones y liberación de radicales libres.

El estudio de la capacidad antioxidante de aceites esenciales extraídas de especias mexicanas abre puertas a nuevas investigaciones sobre los posibles beneficios de estas especias en la salud humana. La pimienta de Jamaica es una especia ampliamente utilizada en México por sus propiedades culinarias y medicinales. el aceite esencial de pimienta se utiliza masivamente en alimentos, productos farmacéuticos y en la industria de perfumería. Debido a su alto contenido de compuestos fenólicos que le refieren una buena actividad antioxidante teniendo efecto contra enfermedades crónico-degenerativas. Algunos de los métodos convencionales de extracción utilizados para la obtención del aceite limitan el contenido de fenólicos en la matriz final. Sin embargo, pocos estudios existen dirigidos hacia la optimización de tecnologías extractivas para sustancias antioxidantes naturales y más aún, enfocadas al aprovechamiento de especias mexicanas. La temperatura, el tamaño de partícula y el tiempo de extracción se perfilan como los factores más importantes que influencian la eficiencia de extracción en términos de la calidad y rendimiento del producto a obtener. Esta eficiencia también se ve significativamente afectada por la composición del solvente.

El aporte científico y la novedad de este trabajo de investigación radica en el desarrollo y aplicación de un método de extracción combinado utilizando dióxido de carbono supercrítico y campos eléctricos pulsados (PEF) para la extracción de aceite esencial de pimienta de Jamaica. Este enfoque representa un avance significativo en el campo de la extracción de productos naturales. Este método tiene el potencial de superar las limitaciones de las técnicas de extracción convencionales, como el uso de disolventes peligrosos o altas temperaturas, que pueden degradar la calidad de los compuestos extraídos.

El presente trabajo presenta una evaluación de la capacidad antioxidante del aceite esencial de Pimienta de Jamaica obtenido por extracción supercrítica asistida con campos eléctricos pulsados con el objetivo de comparar la calidad del extracto obtenido mediante la presencia de sus compuestos antioxidantes.

HIPÓTESIS

HIPÓTESIS

El rendimiento de la extracción y la cantidad de compuestos antioxidantes presentes en el extracto será mayor con la utilización de la tecnología fluidos supercríticos asistida por campos eléctricos pulsados a la alternativa sin pretratamiento.

OBJETIVOS

OBJETIVOS

OBJETIVO GENERAL

Extraer el aceite esencial de pimienta de Jamaica por el método de fluidos supercríticos asistido con campos eléctricos pulsados, caracterizar y evaluar posteriormente la capacidad antioxidante.

OBJETIVOS ESPECÍFICOS

Extraer el aceite esencial de pimienta de Jamaica por el método de fluidos supercríticos asistido con campos eléctricos pulsados.

Determinar el rendimiento de extracción.

Caracterizar los componentes químicos del aceite esencial por CG-EM del aceite esencial obtenido por fluidos supercríticos asistido con campos eléctricos pulsados.

Determinar las propiedades termodinámicas del proceso de extracción por medio de fluidos supercríticos asistido por campos eléctricos pulsados.

Evaluar la actividad antioxidante del aceite esencial de Pimienta de Jamaica obtenido mediante extracción supercrítica asistida con campos eléctricos pulsados.

Evaluar la actividad antioxidante por el contenido de fenoles totales.

MATERIALES Y MÉTODOS

MATERIALES Y MÉTODOS

MATERIALES

Para la extracción del aceite esencial se utilizaron las bayas de pimienta cosechadas en el Rancho "El Pimiento", ubicado en La Mesa, Puebla, México. Los frutos de pimienta fueron secados previamente. Para posteriormente ser triturados y tamizados con una malla 20. Se utilizó dióxido de carbono (99.99% de pureza) adquiridos de la compañía INFRA (México) S.A. de C.V., Toluca, Estado de México.

CAMPOS ELÉCTRICOS PULSADOS (PEF)

La pimienta seca y molida fue procesada por lotes de 100 g en un equipo PEF, el cual consta de un generador de pulsos (Makita EG4550A) que a su vez está conectado a un osciloscopio (Owon Sds1022). La cámara de tratamiento está conectada al generador mediante 2 electrodos de acero inoxidable. El control de las condiciones de operación se realizó a través de un equipo de cómputo, el cual está en contacto con el osciloscopio y el generador de pulsos. La Figura 3 muestra la representación del proceso.

A cada muestra se le aplicaron pulsos durante 1s con potenciales eléctricos de 0.1, 1.0 y 3.0 kV / cm

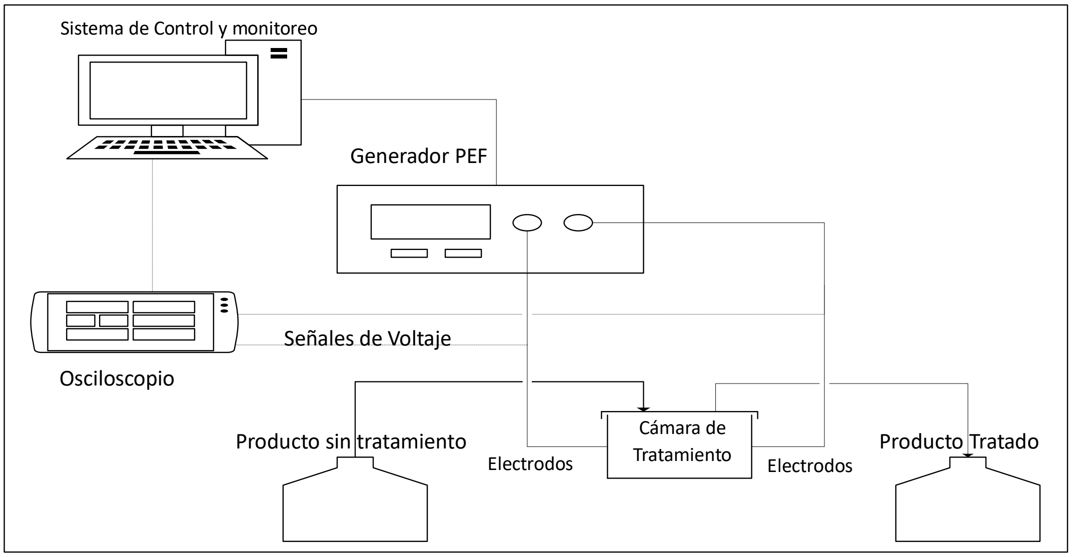


Figura 3. Pretratamiento con campos eléctricos pulsados (PEF)

EXTRACCIÓN CON DIÓXIDO DE CARBONO SUPERCRÍTICO

En la celda de extracción se colocaron 100 g de pimienta seca y molida. La celda fue conectada a una línea de tuberías y se dejó dentro de la cámara de acondicionamiento. Se hizo pasar CO_2 contenido en el tanque a través de las líneas para desplazar el aire en las tuberías y en la celda de extracción. El CO_2 fue comprimido utilizando una bomba de alta presión. Posteriormente se hizo pasar el CO_2 comprimido a la celda de extracción hasta que se alcance la presión experimental deseada, la cual se obtuvo a través de un sensor (Honeywell GM) y medidor de presión. La cámara de acondicionamiento cuenta con 2 fuentes de calor con las cuales se alcanzó la temperatura deseada, y se registró con un medidor de temperatura (Honeywell DC). Cuando la presión y la temperatura se mantienen estables, se deja en contacto la muestra seca con el CO_2 dentro de la celda durante 6 h. Una vez transcurrido el tiempo de contacto, se liberó el CO_2 de la celda abriendo una válvula de calefacción micrométrica. En esta etapa del proceso el aceite extraído fue arrastrado a través del CO_2 y se recolectó en una celda de recuperación. El extracto obtenido fue pesado en una balanza analítica (Mettler Toledo AB204S) para su cuantificación.

Siguiendo la metodología descrita en este apartado, se realizaron distintos experimentos a 100 bar de presión y 45 ºC de temperatura, considerando que estas son condiciones supercríticas del CO_2, ya que se encuentran por arriba de su punto crítico (73.8 Bar y 31.1ºC).

La Figura 4 muestra la representación esquemática del equipo utilizado para este proceso.

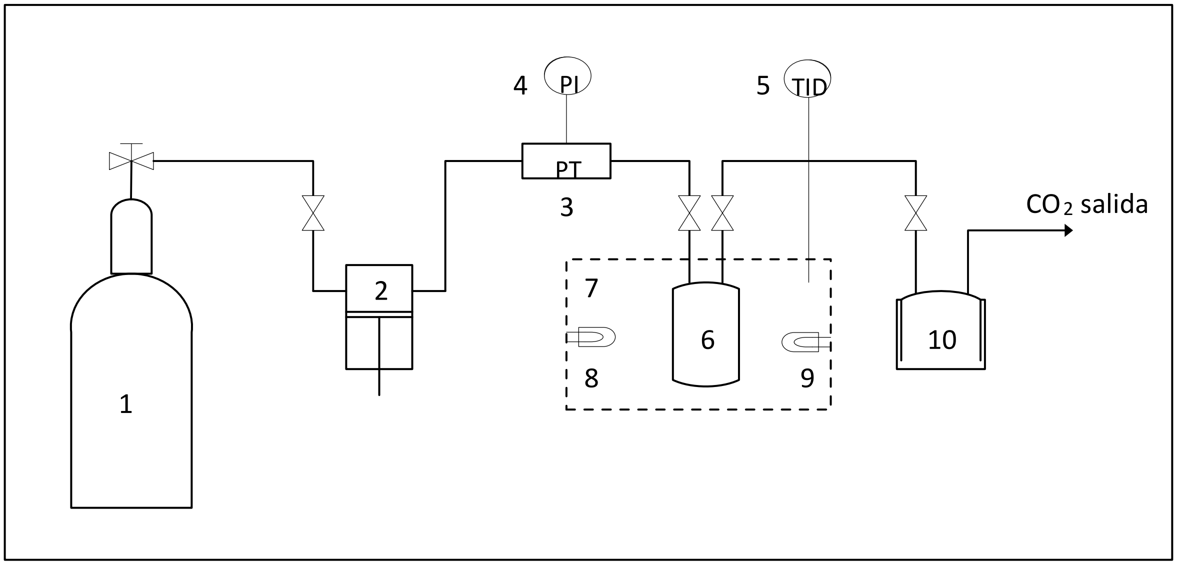


Figura 4. Proceso de extracción con CO_2 supercrítico. (1) Tanque de CO_2, (2) Bomba de alta presión, (3) Sensor de presión, (4) Medidor de presión, (5) Medidor de temperatura, (6) Celda de extracción, (7) Cámara de acondicionamiento, (8,9) Fuentes de calor, (10) Celda de recuperación

PROPIEDADES TERMODINÁMICAS Y COEFICIENTE DE REPARTO

La evaluación de los parámetros termodinámicos del proceso de extracción podría determinar la viabilidad del proceso. Los valores negativos de la energía libre (ΔG^0) indicarían que el proceso es espontáneo, endotérmico o exotérmico dependiendo del valor de la entalpía (ΔH^0) y la reversibilidad del proceso podría ser descrito por el valor de la entropía (ΔS^0) Los parámetros termodinámicos se evalúan con las siguientes ecuaciones:

$$\Delta G^0 = -RT\ln K_D \quad (3)$$

Donde K_D es el coeficiente de distribución o de reparto, ΔG^0 ($J \cdot mol^{-1}$) es la energía libre de extracción, T (K) es la temperatura absoluta y R es la constante universal de los gases.

La K_D pueden ser expresadas en términos de ΔH^0 ($J \cdot mol^{-1}$) y ΔS^0 ($J \cdot mol^{-1} K^{-1}$) como una función de la temperatura:

$$\boldsymbol{lnK_D = \frac{\Delta H^0}{R}\left(\frac{1}{T}\right) + \frac{\Delta S^0}{R}} \quad (4)$$

Los valores de ΔH^0 y ΔS^0 pueden ser calculados a partir de la pendiente y de la intersección de la ordenada de lnK_D vs. 1/ T.

Coeficiente de reparto o de distribución

En un sistema de extracción sólido – líquido, el coeficiente de distribución (K_D) se manifiesta por:

$$K_D = \frac{m_{oe}}{m_{or}} \quad (5)$$

donde m_{oe} es la masa de aceite esencial en el CO_2-SC, m_{or} es la masa del resto de aceite esencial en el material molido. Este modelo supone que la cinética de la fase de extracción inicial y posterior para la división líquido-sólido es rápida y por lo tanto no afecta significativamente a la tasa de extracción. Si el tamaño de las partículas sólidas es uniforme y no se produce ningún cambio, el peso del aceite en cada unidad de masa de fluido extraído que queda en el resto del material durante un período determinado puede ser calculado sobre la base de los valores de K_D. Con el fin de obtener *mor*, primeramente, es necesario calcular la cantidad máxima de aceite esencial en el material molido, en base seca, según el método normalizado de la Norma Mexicana (NMX-FF-063-1987).

CARACTERIZACIÓN DEL ACEITE ESENCIAL

La concentración de diversos componentes principales del aceite esencial se determinará mediante el método de Cromatografía de gases acoplado a un espectrómetro de masas (CG-

EM). Los componentes del aceite se identificarán por comparación de sus espectros de masas con los de la base de datos del sistema.

DETERMINACIÓN DEL CONTENIDO DE FENOLES TOTALES

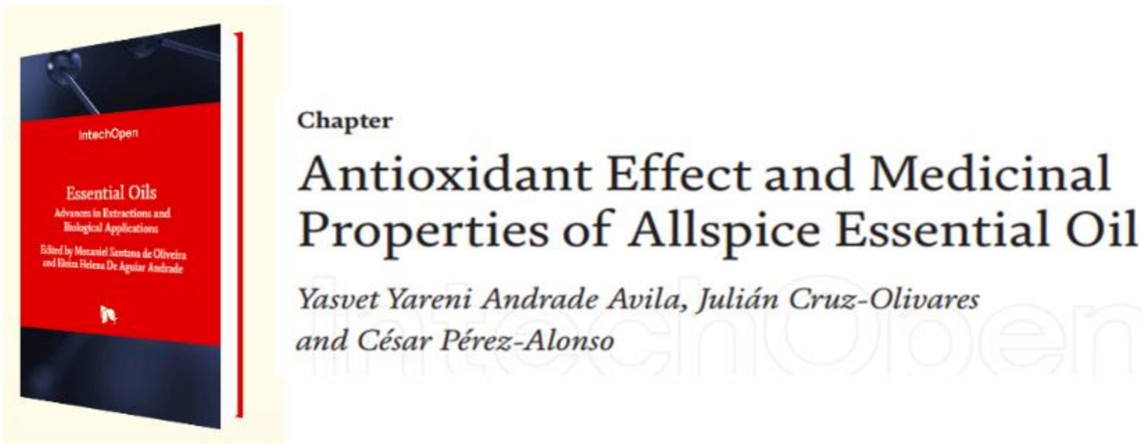
El contenido de TPC se determinó mediante el reactivo de Folin-Ciocâlteu y siguiendo la metodología descrita por Guadarrama-Lezama et al., 2012[46]. Para la preparación de la solución del reactivo de Folin-Ciocâlteu, se mezclaron 0.5 ml de reactivo de Folin con 4.5 ml de agua desionizada, dando como resultado una solución diluida. En cada determinación, el aceite esencial de pimienta de Jamaica se diluyó en etanol en una proporción de 1:5 (v/v). De esta mezcla, se tomaron 0.1 ml y se mezclaron con 0.75 ml de la solución diluida del reactivo de Folin. La solución se dejó incubar durante 5 min y se agregaron 0.75 ml de una solución de bicarbonato de sodio (60 g L-1), se agitó (200 rpm) y se dejó reposar durante 90 min. Luego la mezcla se filtró utilizando una membrana de 0.45 µm (Syringe Filter Corning, Alemania). Se determinó la absorbancia de la solución a 750 nm. Los datos se ajustaron a una curva de calibración de ácido gálico (AG), obtenida con la metodología descrita por Singleton et al 1999.; Huang et al.2005. El contenido de TPC se expresó en mg de GA por gramo de seco. Las determinaciones se hicieron por triplicado.

RESULTADOS Y DISCUSIÓN

RESULTADOS Y DISCUSIÓN

CAPÍTULO DE LIBRO 1

Antioxidant effect and medicinal properties of allspice essential oil



ANTIOXIDANT EFFECT AND MEDICINAL PROPERTIES OF ALLSPICE ESSENTIAL OIL

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Keywords: Pimenta dioica, Essential oil, Eugenol, Antioxidant effect, Chemical composition

Abstract

Pimenta dioica L. Merrill. Myrtaceae family, known for its berries called pimenta or allspice, is one of the oldest spices in the world, widely used for its culinary and medicinal qualities. The main commercial product obtained from this spice is its essential oil, the reason for the interest in essential oil is based on the versatility of its use in different industrial areas (food, cosmetics, perfumery and pharmaceuticals) due to its harmless and beneficial effects for

health. In addition, it contains compounds that have shown broad biological activity, which turns out to be useful in the treatment of diseases related to the excessive formation of oxygen radicals. As a result, the extraction process and operating conditions have a significant impact on the bioactivity of these molecules. As a consequence, selecting the correct mix of variables to improve oil extraction and functionality is essential. The most of study on this essential oil is being focused on resolving these issues, as well as purification and identification. This chapter will cover the methods for obtaining P. dioica essential oil, as well as the chemical profile of the oil and its biological properties, which include its effects on humans, plants, animals, insects, and microorganisms.

1. Introduction

Allspice (Pimenta dioica L. Merrill or Pepper officinalis) belongs to the Myrtaceae family native to the West Indies and Central America[1]. In Mexico it is found in the wild and is cultivated towards the east and southeast[2]. It is a small tree, it is up to 6-12 m tal[3] with small, whitish flowers with a peculiar aroma, its dry, almost spherical, reddish-brown berries are the commercial pepper spice, known in Mexico as pimienta gorda. and in English as "allspice" for its flavors that resemble a mixture of cinnamon, cloves and nutmeg[4]. This spice is known for its antioxidant qualities, which are attributed to the presence of bioactive components, most especially polyphenolic compounds[5]. P. dioica is one of the most important spices as a source of essential oils high in eugenol, a phenolic compound having antibacterial and antioxidant properties against a variety of pathogens. P. dioica produced in Central America is sent to the international markets because its use in the local market is minimal. Its manufacture and drying, on the other hand, are entirely traditional[6].

Allspice contains its oils both in its leaves and in the berry itself[7], with fairly variable returns (1.5–4.5%)[8]. According to reports, the oil content varies depending on where it originates located[8]. González and Pino [10]and Shaik et al. [11] they also discovered that environmental parameters, harvesting procedures, drying, and the age of the trees all influence the chemical composition of the oil.

It is important to mention that the oil obtained from the leaf is a brownish yellow liquid with a dry, woody, warm and spicy aromatic smell, while the oil extracted from the berry is yellow in color with a warm spicy sweet smell and a note of sweet and fresh output and placed in the spicy sweet and warm group[12].

Allspice essential oil is utilized in the food sector, specifically in the meat and tanner industries, and also in perfumery and cosmetic products[13]. In addition, it has been useful for the treatment of gastrointestinal disorders, for cramps, flatulence, indigestion and nausea. Likewise, it has managed to help in cases of depression, nervous exhaustion, tension, neuralgia and stress, it is also used as a natural repellent[14]. Anesthetic, analgesic, antibacterial, antioxidant, antiseptic, acaricide, carminative, muscle relaxant, rubefacient, stimulant, and tonic are some of the medicinal effects of this essential oil[15].

The versatility of essential oils' use in different industrial areas (pharmaceuticals, food, cosmetics) has sparked interest in recent years, not only because of the possibility of obtaining aromatic compounds, but also because of their use as antioxidants, food preservatives, and medicines, as well as their use as crop and plant protectants, incorporating them into the packaging material of the products[16].

2. Essential oil extraction

Steam distillation, hydrodistillation, and the use of organic solvents are the most common extraction procedures. To produce the essential oil, steam distillation uses saturated steam at atmospheric pressure. When the steam breaks the cells of the plant walls, the water generates steam, and the essence is freed, the extraction is complete[17-18]. They allow the process to be favorable for the creation of alcohols and acids when the esters disintegrate by employing high temperatures and the presence of water, resulting in a decrease in the extraction of the oil, which is one of the limitations of distillation by steam entrainment[19].

In recent years, several novel techniques for extracting essential oils have been developed, including ultrasound-assisted extraction, microwave-assisted extraction, and extraction using supercritical fluids, with the goal of reducing extraction time, reducing solvent consumption, increasing extraction yield, and improving the quality of the extracts[20]. Traditional organic solvent extraction, while easy, has drawbacks such as expensive prices, is not environmentally friendly, and is non-selective, requiring post-treatment processes for product purification. Non-recyclable organic solvent disposal can also be hazardous to human health and the environment.

On the other hand, at the laboratory and pilot scale, supercritical fluid extraction of flavonoid compounds presents a viable alternative for a more efficient and environmentally friendly extraction process. The volatile concentrate obtained from allspice by supercritical fluids was compared to the oil obtained by the hydrodistillation method by Marongiu [21], with the primary differences being the amount of eugenol, 77.9% against 45.4 %. It was also demonstrated that by employing supercritical CO2, the extract has an additional benefit in that it is free of hydrocarbons, which can conceal or degrade the oil's natural aroma.

Other studies compared the effects of microwave energy supply and hydrodistillation radiation time (MHD) on the performance and composition of allspice essential oil[22]. While there were no significant differences in the yields (2.68% versus 3.25%) and chemical composition of essential oils obtained by HD and MHD, the advantage was obtained in the reduction of the extraction cost in terms of time and energy.

3. Allspice essential oil chemical profile

Polyphenols, lignins, and terpenoids are the most prevalent components found in allspice essential oil currently[23]. The basic component of the oil is eugenol, finding that the oil content obtained from the leaves (65-96%) is somewhat higher than that of the berry oil[14]. Table 1 shows the chemical composition of the essential oil of Pimenta dioica obtained by using gas chromatography coupled to mass spectrometry (GC-MS) analysis technique, as well as data from the literature obtained from various researchers denoting the main compounds present in the essential oil, according to the extraction method, geographical origin, and plant part used in the extraction. Essential oils are complicated combinations with a high number of elements, and their physicochemical qualities are controlled by factors such as harvest time, soil type, and fruit storage conditions and time[24]. The quality of Jamaican berries is greater to that of other islands, and they are preferred for commerce. Allspice's oil content and flavor deteriorate when it is stored for an extended period of time[1].

Table 1. Chemical composition of the essential oil of Pimenta dioica

Country of Origin	Component of the plant	Year	Method of extraction	Main constituents (%Area)	Reference
Antilles	Leaves	2007	Commercial	Eugenol (47.78 %) Myrcene (26.76%)	[15]
Australia	Leaves	2005	SCD	Eugenol (77.9%) β-caryophyllene (5.1%)	[25]
	Leaves	2005	HD	Eugenol (45.4%) β-caryophyllene (8.9%)	[25]
Brazil	Fruit	2011	HD	Eugenol (76.98%) β-pinene (6.52%) 5-indanol(5.88%) limonene (4.09%)	[24]
	Leaves	2014	HD	Eugenol (60.8%) Myrcene (19.3%) limonene (6.48%)	[26]
	Fruit	2020	HD	Eugenol (76.88%) β-Pinene (6.52%)	[27]

China	Fruit	2013	HD	Eugenol (28.84%) Methyl eugenol (43.01%)	[22]
Cuba	Leaves	1997	HD	Eugenol (28.04%) 1,8-cineole (14.5%) α-humulene(10.12%) γ-cadinene (11.12%)	[28]
	Leaves	1997	SCD	Eugenol (93.87%) thymol (1.82%)	[28]
	Leaves	1997	SE	Eugenol (91.68%) thymol (2.72%)	[28]
	Leaves	2003	HD	Eugenol (34.14%) 1,8-cineole (14.69%) α-humulene (10.12%)	[29]
Guatemala	Leaves	2020	HD	Eugenol (71.4%) Myrcene (10.0%)	[30]
	Fruit	2020	HD	Eugenol (65.9%) Myrcene (10.1%)	[30]
India	Fruit	2013	HD	Eugenol (68.4%) chavicol (10.4%) methyl eugenol (6.1%)	[31]
	Fruit	2015	Commercial	Eugenol (35.42%) methyl eugenol (28.02%)	[32]

				β-caryophyllene (8.66%) β-Myrcene (8.55%)	
Jamaica	Leaves	1991	SD	Eugenol (66.38% – 79.24%)	[33]
	Leaves	2007	Commercial	Eugenol (76.02%) methyl eugenol (7.14%) β-caryophyllene (6.47%)	[34]
	Leaves	2007	HD	Eugenol (79.81-83.68)	[35]
	Berries	2007	Commercial	Eugenol (86.44%) β-caryophyllene (7.70%) Methyl eugenol (3.87%)	[36]
	Leaves	2009	Commercial	Eugenol (76.0%)	[37]
	Berries	2016	SCD	Eugenol (63.94%) β-caryophyllene (4.65%)	[38]
	Berries	2016	HD	Eugenol (66.8%) β-caryophyllene (4.69%)	[38]
México	Berries	1997	SD	Methyl eugenol (48.3%) Myrcene (17.7%) Eugenol (17.3%)	[39]
	Berries	1997	HD	Methyl eugenol (62.7%) Myrcene (16.5%) eugenol (8.3%)	[39]

	Berries	1997	SCD	Methyl eugenol (67.9%) Eugenol (14.9%) Myrcene (6.0%)	[39]
	Berries	2011	SD	Methyl eugenol (62.7%) Eugenol (8.3%)	[40]
	Fruit	2011	HD	Methyl eugenol (48.7%) Myrcene (17.1%) Eugenol (16.3%)	[41]
	Leaves	2013	HD	Eugenol (94.86%) α-terpineol (2.45%)	[42]
	Berries	2018	HD	Methyl eugenol (65.14%) β-Myrcene (12.72%)	[43]
	Fruit	2020	HD	Eugenol (48.5%) Methyl eugenol (35.0%)	[44]
Sri Lanka	Leaves	2015	HD	Eugenol (85.33%) β-caryophylene (4.36%) Cineole (4.19%)	[45]
USA	Leaves	2012	HD	Eugenol (62.1%) Methyl eugenol (22.9%)	[46]

SD=Steam Distillation HD= Hydrodestillation SCD= Supercritical Carbon Dioxide SE= Solvent Extraction

Because of the extraction process used, the quantity and quality of compounds found varies. Essential oil composition has an important role in determining the spices pharmacological potential[16]. The essential oil of Pimenta dioica extracted using HD, SCD, SE, and SD have significant qualitative and quantitative changes in their chemical composition. Hydrodistillation was the most used procedure. Eugenol, methyl eugenol, and myrcene are the three main constituents of this oil.

4. Antioxidant effect

Spices and herbs are recognized as sources of natural antioxidants[47]. Some of the biological functions of essential oils are dependent on their antioxidant properties. These properties are attributable to some essential oil components' inherent potential to prevent or delay aerobic oxidation of organic matter. However, it's important to be cautious before thinking that essential oils' antioxidant properties are just a result of their chemical components. However, taking into account its composition can help to estimate its antioxidant capacity[48].

In terms of free radical scavenging activity against the radicals DPPH, ABTS, and superoxide anion, the composition and antioxidant activity of the essential oil obtained by hydrodistillation of the berries were studied[49]. A total of 45 components were discovered. Eugenol (74.71 %, 73.35 %) was the most common component found, followed by methyl eugenol (4.08 %, 9.54 %) and caryophyllene (4.08 %, 9.54 %). Antioxidant evaluation revealed that the oil had a high rate of radical scavenging. The total phenolic content, total reducing power, and metal chelating capacity were also calculated, and the metal chelating capabilities and reducing power were both found to be extremely high. The essential oil has a substantial antioxidant activity that is comparable to pure eugenol, according to the results.

Another study showed a positive correlation between the anticancer and antioxidant effects of allspice essential oil[43]. As a member of the Myrtaceae family, this oil has shown to have great cytotoxic effect against cancer cells. As a result, it might be considered a natural source of anticancer medicines. According to research, consuming foods containing synthetic antioxidants can result in health problems such as cancer owing to the accumulation of free radicals in the body. As a result, research has been done to return to using natural compounds as an alternative for synthetic substances and as a source of novel food preservatives. These essential oils with high inhibitory percentages can now be utilized to replace synthetic additives since they help to eliminate pollutants and chemical residues, which can cause issues and diseases[17].

Allspice is a powerful hydroxyl radical scavenger. The berries of P. dioica had a high level of antioxidant activity. and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities[50]. The capacity of P. dioica leaf essential oil to combat DPPH (2,2-diphenyl-1-picrylhydrazyl), hydroxyl (OH), and superoxide radicals was studied to determine its antioxidant characteristics[34]. The intrinsic characteristics of many of their bioactive components, particularly phenols, to block or delay oxidation, are responsible for the antioxidant potential of P. dioca essential oil.

Although not all phenolic molecules had antibacterial activity, antioxidant activity was significantly related to total phenol content. P. dioica leaf extracts include phenolic chemicals that can be employed as antioxidants in the food, cosmetics, and pharmaceutical industries[51].

Allspice essential oil showed a high concentration of antioxidants The antioxidant characteristics of the essential oil were compared to those of propyl gallate, a synthetic antioxidant, and it was discovered that the essential oil's free radical scavenging activity was dependent on the concentration and higher than that of propyl gallate[52]. Antioxidants were found in abundance in allspice essential oil. (i.e.> 75 mmol / 100 g) [53]. Applications in medicine have been reported due to the presence of antioxidant chemicals in P. dioica's essential oil.

5. Medicinal properties

The essential oil of allspice is a significant source of phytochemicals in medicine. Phytochemicals are a large group of plant-derived bioactives that may have disease-fighting properties[54]. Plants are one of the most important natural sources of secondary metabolites for medical purposes, due to their biological capacity to combat lethal or endemic diseases, as well as disorders that impact living beings.

Anticancer, antidermatophytic, anti-hemorrhagic, anti-inflammatory, antimicrobial, anti-mutagenic, antipyretic, central nervous system depressant, hypoglycemic, hypotensive, inhibitor of the enzyme histone acetyl transferase, and inhibitor of the enzyme histidine have all been discovered as pharmacological effects of allspice essential oil[55-58].

5.1 Nematicidal Activity

In other studies Park (2007) et al discovered allspice essential oil looks to be effective as a natural nematicide for B. xylophilus, but more research on systemic action, phytotoxicity, and formulation is needed to improve nematicidal potency and stability while reducing cost.

5.2 Antimicrobial activity

The presence of antioxidant properties and antimicrobial effects of allspice suggests that it can be used against human pathogenic bacteria and for the control of other diseases and the support of immunity for rejuvenation. The ability of allspice to alleviate bacterial infections and its use in traditional medicine in different parts of the world was observed. Due to its use it is possible that this plant has anti-QS properties[59]. Its important bacteriostatic and inhibitory properties of pathogenic and decomposition microorganisms against Bacillus subtilis, Clostridium botulinum, Escherichia coli, Listeria monocytogenes, Salmonella typhimurium and Staphylococcus aureus were also reported[60].

In another study, essential oil extracted from Pimenta dioica (Myrtaceae) was evaluated for its antimicrobial activities using a panel of gram-positive pathogens, gram-negative strains, and fungi[61]. Antimicrobial activity was measured by the minimum inhibitory concentration required to inhibit the growth of microorganisms. The cytotoxicity of the essential oil was tested ex vivo using the THP-1 macrophage cell model. The results showed that it had antimicrobial activity.

Allspice oil reduced xanthine oxidase activity, resulting in a decrease in superoxide radical formation. Both the synthesis of conjugated dienes and the development of secondary products from lipid peroxidation were effectively inhibited by allspice oil. Infections caused by Klebsiella, Pseudomonas, A. niger, A. flavus, and T. versicolor can be treated with P.offcinalis as an alternative to synthetic medications. According to the literature, depending on the chemical composition of the allspice oil[62]. Escherichia coli, Salmonella enterica, and Listeria monocytogenes have all been observed to be suppressed by allspice[63].

The antibacterial activity of allspice essential oil was tested using the agar diffusion method against three microorganism strains. B. cereus, S. typhimurium, and S. aureus were found to be inhibited by it. B. cereus was found to be the microbe most vulnerable to the presence of oil in the microdilution. The predominant component of P. dioica was eugenol, which had an abundance proportion of 94.86% as determined by GC-MS[42].

5.3 Anticancer activity

Cancer is a worldwide health issue. In breast (MCF-7), hepatocellular (HepG-2), colon (HCT-116), prostate (PC-3) and cervical cancer cell lines, allspice essential oil was examined for cytotoxicity. The MTT assay was used on HeLa cells. The essential oil had cytotoxic action against the cell lines that were examined[43]. The results showed that the essential oil of Mexican allspice has cytotoxic activity (IC_{50} <15 µg/mL) against the cancer cell lines examined.

5.4 Antifungal activity

The antifungal efficacy of Pimenta dioica leaf essential oil against toxin-producing Aspergillus flavus was investigated in one study. Antifungal activity of P. dioica leaf EO was shown on A. flavus in vitro experiments (IISRaf1). These tests revealed that this EO could be used as a food additive because of its antifungal properties and capacity to decrease ergosterol formation, which would extend the storage life of post-harvest ítems[64].

Allspice oil was found to have a superior antifungal impact against Fusarium oxysporum, Fusarium verticillioides, Penicillium expansum, Penicillium brevicompactum, Aspergillus flavus, and Aspergillus fumigatus. As a result, its efficacy is comparable to that of synthetic fungicides often used to treat severe human mycoses. The MIC values of P. dioica, which were detected against all pathogens tested, are very remarkable[65].

The fungal activity and chemical composition of the essential oil obtained from the fruits of Pimenta dioica in the mycelial development of Fusarium oxysporum f. sp. lycopersici, Fusarium oxysporum f. sp. passiflorae, Fusarium subglutinans f. sp. ananas, Fusarium oxysporum f. sp. vasinfectum The oil contained 76.88 % eugenol and suppressed fungal mycelial development by up to 97.78 % in an average of 7.2 days, according to the findings. As a result, the oil could be used as a natural fungicide[27].

Aspergillus niger, Candida blanki, Candida tropicalis, Candida cylindracea, Saccharomyces cerevisiae, and Candida albicans were found to have strong inhibitory activity, while Candida glabrata, Candida krusei, Candida albicans, and Candida albicans were found to have moderate inhibitory activity. With an activity index of 1.20 to 2.80, all of the test fungi were suppressed. This suggests that ketoconazole has a stronger antifungal effect against Candida albicans, Candida glabrata, Candida tropicalis, Candida cylindracea, Candida albicans, and Aspergillus niger[66].

It has also recently become a research hub for the development of novel insecticides for ecologically friendly plants. Its insecticidal action has been demonstrated in numerous studies, and it can be utilized as a natural repellant[67].

5.5 Antidiabetic effect

Allspice berry extract was reported to inhibit protein glycation, indicating its potential to be used as an effective antidiabetic agent[68]. Studies have shown that individual flavonoids inhibit glycation by 50%.

5.6 Acaricidal effect

Essential oil derived from P. dioica berries were found to be highly harmful to R. microplus 10-day-old larvae in this investigation. As a result, the findings point to a viable new technique that could be utilized as an alternative to synthetic acaricides for tick management. The main components, methyl eugenol (62.7 percent) and eugenol (62.7 %), could be responsible for the acaricidal activity (8.3 %)[40].

The active components of allspice essential oil were used in one investigation to cause mortality and limit the development of B. microplus to a level comparable to commercial acaricides. The phenylpropanoid molecules responsible for this activity, eugenol and methyl eugenol, could be studied for use as Acarina chemosterilants and as templates for the synthesis of further acaricides. All extracts, commercial acaricides, and methyl eugenol were found to be less effective in suppressing oviposition and causing tick mortality than berry essential oil. Eugenol, a component contained in more than 65 percent of the oil composition, is responsible for the effectiveness of berry essential oil[69].

6. Conclusions.

Over the years, researchers have studied the enormous range of biological activities of allspice essential oil and its potential applications. Pimenta dioica essential oil contains a large number of medicinal compounds. Currently, the need to extract compounds of interest from plant materials drives the continuous search for economically and ecologically viable extraction technologies. We have given a quick rundown of the medicinal characteristics of allspice essential oil, with a focus on the chemical components that have biological activity.

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ARTÍCULO DE INVESTIGACIÓN 1

Supercritical extraction of essential oil of allspice (*pimenta dioica* l. Merrill) with pulsed electric fields pretreatment

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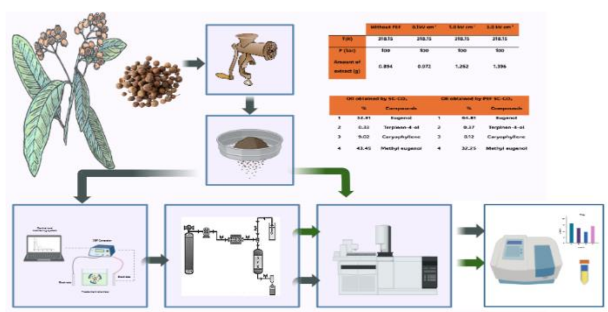
Graphical abstract



The study aimed to improve the yield and efficiency of supercritical CO_2 extraction using pulsed electric field (PEF) pretreatment on allspice berries. Pretreatment with PEF increased the essential oil yield from 0.894 g to 1.396 g and the eugenol content from 52% to 64%.

Abstract

The objective of this work was to improve the yield and efficiency of the allspice essential oil extraction process using supercritical CO_2 (SC-CO_2) and a pretreatment with pulsed electric fields (PEF). For this purpose, allspice berries were exposed to PEF pulses of an electric field intensity of 0.1, 1.0 and 3.0 kV cm^{-1} for 1.0 s before supercritical extraction. The main compounds in the extracted allspice essential oil were eugenol and methyl eugenol, as determined by GC-MS analysis. The maximum content of phenolic compounds determined by the Folin-Ciocâlteu method was 4.92 mg gallic acid equivalents per gram of allspice dry weight. The essential oil yield increased from 0.894 g using SFE alone to 1.396 g using SFE combined with 50 pulses of PEF. The amount of eugenol increased from 52% to 64% with the combined process that included pretreatment with PEF. The results showed that the use of PEF treatment before SC-CO_2 extraction of essential oils improved the efficacy and feasibility of this extraction method. The application of PEF pretreatment allows higher yields of essential oils to be obtained at relatively lower pressures during SC-CO_2 extraction.

Keywords: Antioxidant activity, Supercritical carbon dioxide, Phenolic compounds, Eugenol

1. INTRODUCTION

Allspice (*Pimenta dioica* L. Merrill) is an aromatic spice obtained from the unripe, dried berries of the Pimenta dioica tree[1]. It has been used as culinarily and medicinally sources for centuries in various cultures. The characteristic spicy aroma and flavor of allspice are mainly due to its rich content of volatile essential oils, particularly eugenol, methyl eugenol, myrcene, and caryophyllene[2,3]. Eugenol made up the highest proportion (>50%) of compounds, playing a key role in the antioxidant, anti-inflammatory, and antimicrobial activities[4–8]. Trace bioflavonoids, sesquiterpenoids, and vitamin E homologues further add to the therapeutic value[9]. Antibacterial, antifungal, insecticidal, analgesic, antioxidant, anticancer, gastroprotective, and antidiabetic qualities are just a few of the many health advantages of allspice essential oil [1,6,10–12]. This composition and bioactivity profile contribute to the use of allspice essential oil in food preservation, pharmaceuticals, cosmetics, and complementary medicine. Conventional methods of extraction of phenolic compounds from allspice fruits are time and energy consuming. Conventional methods of extraction of phenolic compounds from allspice fruits are time and energy consuming[13]. In recent years, interest has increased in the use of unconventional extraction techniques, such as pulsed electric field (PEF) technology, for the extraction of bioactive compounds from foods. PEF is a non-thermal technique that uses short duration high voltage pulses to induce electroporation the cell membrane[14]. This technology has recently attracted attention as an eco-friendly pre-extraction technique to increase the extraction yield of beneficial phytochemicals from plant materials. It works by delivering short pulses of intense electric fields that temporarily pierce the membranes of plant cells through a process known as electroporation. This increase in porosity favors better diffusion and recovery of the bioactive compounds present inside the cell and in the cell wall matrix. Essentially, this technology creates transient pores in plant tissues, facilitating the improved extraction of valuable compounds like metabolites, antioxidants, and oils through environmentally friendly, non-destructive means[15]. When integrated with traditional extraction techniques, this pretreatment step can substantially boost the overall extraction efficiency and bioactive compound yield in a sustainable way [16–18] . The potential of this method to enhance the eco-friendly recovery of phytochemicals has spurred extensive research into optimizing process parameters and combining it with other green technologies [19–21]. The effects of process parameters such as field strength, treatment time and pulse characteristics have been discussed on a wide range of plant sources[16]. The functionalities center on facilitated solvent diffusion, cell rupture and enzymatic reactions activating mechanisms for better bioactive component availability[22]. The allspice essential oil extraction process has been experimented with a variety of methods, but the integration of SC-CO_2 extraction with PEF offers an innovative and efficient approach.

This case focuses specifically on the extraction of the essential oil of allspice, a plant revered for its versatile therapeutic and aromatic properties. Considering the extensive uses and high market demand for allspice essential oil, creating extraction methods that are both highyielding and sustainable is extremely valuable. Developing approaches that efficiently extract this botanical oil while minimizing environmental impacts should be a priority.

Supercritical CO_2 extraction utilizes carbon dioxide under carefully controlled temperatures and pressures to reach a supercritical state. This technique has gained attention for its non-toxic properties, minimal environmental footprint, and capacity to produce high-value bioactive materials. SC-CO_2 extraction employs carbon dioxide in its supercritical state, which exhibits unique diffusive properties between typical liquid and gaseous phases. This allows the SC-CO_2 to thoroughly permeate plant matrices without compromising or degrading heat-sensitive compounds. Meanwhile, pulsed electric field processing utilizes short, high-voltage electric pulses to gently permeabilize plant cell structures. This facilitates diffusion across membranes and release of valuable phytochemicals residing within cells. Using PEF as a pretreatment can thus enhance the efficiency and yield of subsequent SC-CO_2 extraction. Overall, both methods embody principles of green chemistry and sustainable extraction, avoiding excessive heating and harsh chemicals. The targeted permeability enhancements induced by PEF complement the penetrating yet mild characteristics of supercritical fluids. Combining these two environmentally friendly approaches leverages their complementary strengths to optimize recovery of plant-derived chemicals.

SC-CO_2 extraction takes advantage of the unique properties of supercritical fluids, especially SC-CO_2, to extract essential oils[23]. In its supercritical state, CO_2 combines the properties of the gas and liquid phases, allowing it to completely penetrate plant material and dissolve essential oils efficiently. Supercritical fluid extraction with CO_2 promises high yields while maintaining oil quality and eliminating solvent residue contamination. As a preprocessing step, applying PEF to the plant material can further enhance the extraction of essential oils. Exposing cells to short bursts of strong electric fields causes electroporation, that is, the formation of pores in cell membranes. This permeation improves diffusion across the membranes, granting access to internal components like essential oil droplets. The combined approach leverages the ability of supercritical CO_2 to dissolve out target compounds, while PEF acts to weaken cell structures and increase release. Using these two green technologies in tandem can potentially achieve very effective essential oil extraction with low environmental impact.

This research aims to investigate the yield and efficiency of using supercritical fluids and pulsed electric fields in combination, for the extraction of essential oil from allspice berries. The study seeks to provide a comprehensive comparative analysis of these green technologies against traditional extraction techniques, focusing on extraction performance, energy consumption and quality of the extracted oil.

2. MATERIALS AND METHODS

2.1 Materials

For the extraction process of the essential oil, all spice berries harvested at the "El Pimiento" Ranch, located in La Mesa, Puebla, Mexico, were used. The allspice fruits were previously dried. To later be crushed and sieved with a 20 mesh. Carbon dioxide will be used (99.99% purity) acquired from the company INFRA (México) S. A. de C.V., Toluca, State of Mexico. Folin-Ciocâlteu reagent and Gallic acid were acquired from Sigma-Aldrich, Naucalpan, State of Mexico, Mexico. Ethanol and sodium bicarbonate are analytical grade and were acquired from J. T. Baker, Ecatepec, State of Mexico.

2.2 Pretreatment with pulsed electric fields (PEF)

The ground and dried allspice was processed in 100 g batches using PEF equipment, which is comprised of an oscilloscope (Owon Sds1022) and a pulse generator (Makita EG4550A). Two stainless steel electrodes connect the generator to the treatment chamber. A computer system connected to both the oscilloscope and pulse generator was utilized to monitor and regulate the pulsed electric field operational parameters. This enabled precise control over the electric pulses applied during the pretreatment. Electrical potentials of 0.1, 1.0, and 3.0 kV cm^{-1} were applied to every sample for a duration of one second. The experimental procedure used for the pretreatment is depicted in Figure 1.

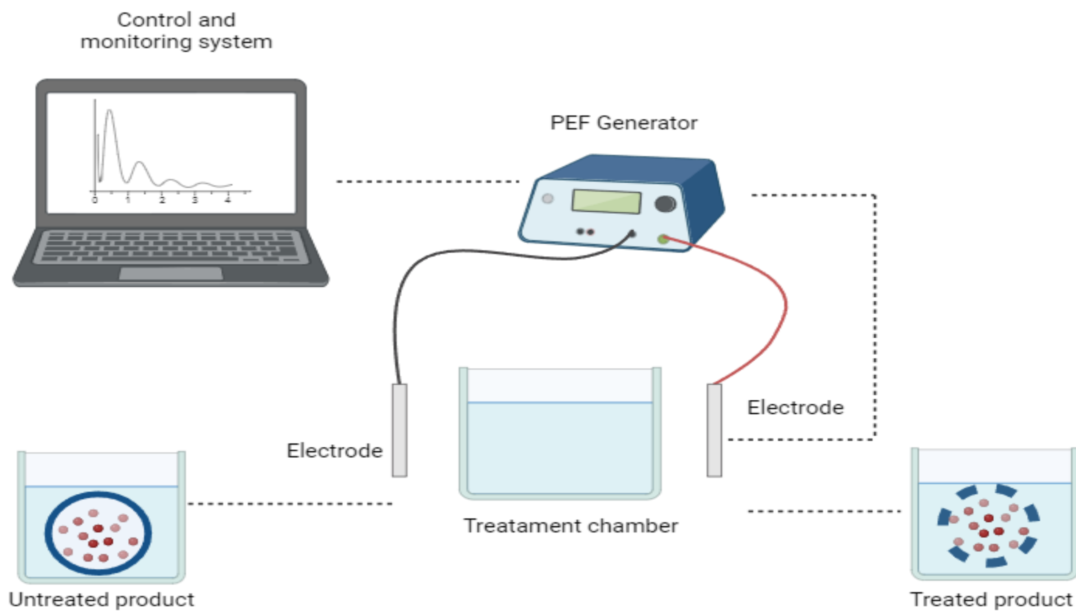


Figure 1. Schematic representation of the equipment used for pretreatment of samples with pulsed electric fields (PEF)

2.3 Extraction with supercritical carbon dioxide

Each of the PEF-treated allspice samples was exposed to the extraction process with supercritical CO_2 using the equipment shown in Figure 2, using the procedure described below: 100 g of ground allspice pretreated with PEF was placed inside the high-pressure extraction cell (5). By manipulating valves 2 and 4, opening and closing alternately, CO_2 was introduced through the manual pressurization pump (3), keeping valve (7) completely closed. This operation lasted at least 90 minutes until the pressure inside the cell reached 100 bar. At the same time, the heating system was activated to maintain the thermal equilibrium of the system at 45°C, during a contact time of the allspice sample with the supercritical CO_2 of 6 hours. Under these conditions, the mass transfer process was carried out, that is; supercritical CO_2 diffused into the ground allspice particles and selectively dissolved the biocompounds which were extracted as allspice essential oil. The mixture of supercritical CO_2 with the dissolved essential oil was slowly discharged into a recovery cell where the CO_2 was separated. This controlled operation was carried out by manipulating the micrometric valve (11), which must be kept covered with a heating tape to prevent cooling and crystallization of the extract due to the sudden change in pressure experienced in the expansion process. Finally, the amount of allspice oil collected in the recovery cell is measured using an analytical balance.

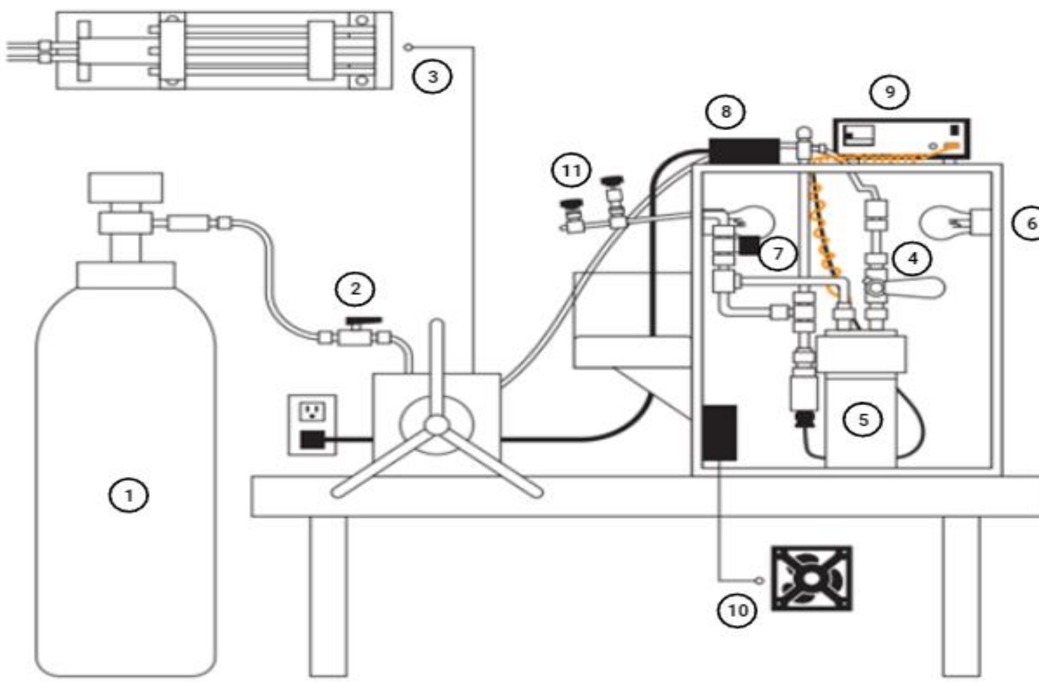


Figure 2. Diagram of supercritical extraction experimental equipment. CO_2 tank (1), Shut-off valves (2,4,7), Manual pressurization pump (3), High pressure extraction cell (5), Heat sources 200W (6), Pressure controller-indicator (Sensotec LA/7093-03 pressure transducer) (8), Temperature controller-indicator (PID+25A SSR+J Sensor) (9), Fan (10) and CO_2 outlet micrometric valve (11).

2.4 Characterization of Allspice Essential Oil

The extracted allspice oil was analyzed in a gas chromatograph (Agilent technologies-7890B) coupled to an autosampler (7683B) and a mass selective detector (5977A). Chromatographic separation was done on an HP5-MS column (30 m × 0.25 mm × 0.25 µm). The injection volume was 1 µL. The samples were injected in splitless mode at 280 °C. The oven conditions were initial temperature at 60 ºC for 1 min to increase 6 ºC min^{-1} to 270 ºC, which was maintained for 1 min. The column flow was 1 mL/min of ultrapure helium (99.99%). The transfer line was maintained at 300 °C throughout the analysis. The temperatures of the quadrupole and ionization source were 150 ºC and 230 ºC, respectively. Data acquisition was done in electron impact mode in a range of m/z 50 – m/z 550 in SCAN mode. Compounds were identified by comparison of their mass spectra with those of the NIST 2011 library and/or comparison with standard compounds.

2.5 Determination of total phenolic compounds by the Folin-Ciocâlteu method

The content of TPC was determined using the Folin-Ciocâlteu reagent and following the methodology described in previous works[24]. For the preparation of the Folin-Ciocâlteu reagent solution, 0.5 mL of Folin's reagent was mixed with 4.5 mL of deionized water, resulting in a diluted solution. In each determination, the allspice essential oil was diluted in ethanol in a 1: 5 (v/v) ratio. From this mixture, 0.1mL was taken and mixed with 0.75 mL of the Folin reagent diluted solution. The solution was allowed to be incubated by 5 min, and 0.75 mL of a sodium bicarbonate solution (60 g L^{-1}) was added, stirred (200 rpm), and allowed to stand for 90 min. Then the mixture was leaked using a 0.45 µm membrane (Syringe Filter Corning, Germany). The absorbance of the solution at 750 nm was determined. The data were adjusted to a gallic acid (GA) calibration curve, obtained with the methodology described by Singleton et al.[25]; Huang et al.,[26]. The TPC content was expressed in mg of GA per gram of dry. The determinations were made in triplicate.

3. RESULTS AND DISCUSSION

3.1 Effect of PEF on yield extraction

As can be seen in the results shown in Table 1, regarding the performance of the extraction process, the effect of pretreatment of the samples with PEF is positive. In this case an increase of 56.2% was achieved when the sample was pretreated with the highest PEF value compared to the sample without PEF pretreatment.

Table 1. Amount of extract obtained with SC-CO_2 and PEF SC-CO_2 treatments.

	Without PEF	0.1kV cm^{-1}	1.0 kV cm^{-1}	3.0 kV cm^{-1}
T(K)	318.15	318.15	318.15	318.15
P (bar)	100	100	100	100
Amount of extract (g)	0.894	0.972	1.262	1.396
Yield increase (%)	0	8.7	41.2	56.2

Although the effect of PEF on yield is increasing, in the case of ground allspice with 12% humidity, it is not possible to apply PEF greater than 3.0 kV cm^{-1} because it causes combustion of the sample. Therefore, applying 50 pulses of 3.0 kV cm^{-1} for 1.0 s are the best conditions for pretreatment of allspice samples with PEF. Particularly, for every 100 g of allspice, 0.894 g and 1.396 g of extract were obtained in allspice samples with and without pretreatment with PEF, respectively. It is worth saying that the extraction process with supercritical CO_2 was carried out under the same pressure and temperature conditions (100 bar, 45°C) in both cases. In this sense, the difference in performance is exclusively due to the effect of pretreatment of the sample with PEF. This aligning with observations from previous studies by other researchers that utilized similar PEF assisted extraction approaches, for example, in the extraction process of anthocyanins obtained from strawberries[27]. Pretreatment with PEF can alter the cellular structure of ground pepper, facilitating the release of the essential oil. This alteration increases the accessibility of the target molecules to the supercritical fluid during the extraction process, leading to more efficient extraction based on the electroporation phenomenon generated by PEF[22]. By increasing the permeability of cell membranes by creating temporary pores in the cell walls, this improved permeability allows the supercritical fluid to penetrate the plant material more effectively, promoting essential oil extraction according to what was reported.

3.2 Effect of PEF on the composition of the allspice essential oil

The use of PEF as a pretreatment before SC-CO_2 extraction of essential oil from allspice berries not only proved to be efficient in terms of process performance, but also in terms of the composition of the extract. It is clear that PEF treatment significantly increased the yield of phenolic compounds from allspice fruits. Furthermore, PEF pretreatment can influence the chemical composition of this essential oil through several mechanisms. Applying short bursts of high-voltage electrical pulses to plant tissues before extraction can cause changes in cellular structure, permeability, and biochemical processes within the plant material.

Table 2. Composition of essential oils obtained from SC-CO_2 and PEF SC-CO_2

Oil obtained by SC-CO_2			Oil obtained by PEF SC-CO_2		
	%	Compounds		%	Compounds
1	52.91	Eugenol	1	64.81	Eugenol
2	0.33	Terpinen-4-ol	2	0.37	Terpinen-4-ol
3	9.02	Caryophyllene	3	0.12	Caryophyllene
4	43.45	Methyl eugenol	4	32.25	Methyl eugenol

The Table 2 shows the chemical composition for the sample without PEF pretreatment and the sample treated at 3.0 kV cm^{-1} with PEF pretreatment. When compared to extraction without pretreatment, the eugenol content of the extracts from pulsed electric field pretreatment of allspice was significantly higher. The target intracellular compounds' selectivity and extractability may have been considerably affected by the PEF pretreatment and them the effects of permeabilization, which caused a notable recovery of eugenol in the extracts.

The analysis of the chemical composition revealed eugenol and methyl eugenol as the major constituents, which play an important role in the distinctive spicy aroma of allspice essential oil. The proportion of the main bioactive component eugenol also increased from 52% with solely SC-CO_2 extraction to 64% with PEF-assisted SC-CO_2 extraction.

This increase in eugenol content after PEF can be attributed to the fact that eugenol in its free form, as opposed to bound as glycosides, is likely more electrophilic due to its allylic hydroxyl group. The electrophilicity may allow the eugenol to be more easily oxidized or released from intracellular compartments when the electric pulses disrupt cell structures[28,29]. PEF likely causes oxidation or breakdown of subcellular compartments such as vacuoles housing reserves of bound eugenol glycosides[30]. The intense electric pulses induce brief reactive conditions that preferentially liberate the more electrophilic free form of eugenol compared to glycosylated forms. Furthermore, research suggests that enzymatic activity of β-glucosidase is enhanced by PEF[31,32], which caused hydrolysis of eugenol glycosides, liberating more free eugenol components that were extracted by the SC-CO_2. On the other hand, the amount of methyl eugenol experienced a slight decrease from 43% to 32% after applying pulsed electric field pretreatment before supercritical CO_2 extraction. A plausible reason for this observation could be that methyl eugenol possesses a weaker electrophilic character compared to eugenol, rendering it less prone to oxidation or hydrolysis processes when the cell membranes are disrupted by the electric pulses.

3.3 Effect of PEF on antioxidant activity

The Figure 3 provides a visual representation of the total phenolic compounds (TPC) values for each sample, allowing for easy comparison and identification of any potential variations or trends among the samples.

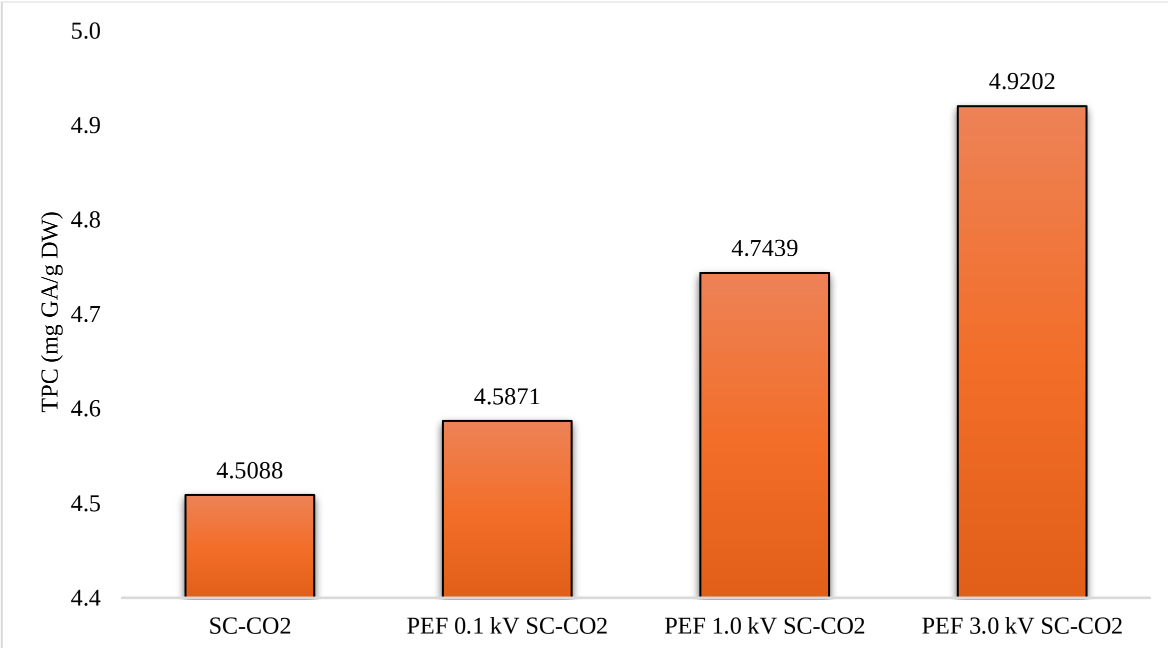


Figure 3. Total phenolic compounds for the Folin-Ciocâlteu method

The calculated TPC value for the allspice essential oil sample in this case was almost 5.0 mg gallic acid equivalents per gram of dry weight (mg GAE gDW^{-1}). This value can be considered relatively low compared to the TPC values reported for allspice essential oils in some literature studies. For instance, a study by Jirovetz et al.[33] reported a TPC value of 37.9 mg GAE g^{-1} for an allspice essential oil sample obtained by hydrodistillation. Another study by Rao et al.[9] found TPC values ranging from 20.7 to 26.9 mg GAE g^{-1} for allspice essential oils extracted by different methods (hydrodistillation, solvent extraction, and microwave-assisted extraction). Morsy reported the TPC value for the Mexican allspice (Pimenta dioica) berries essential oil obtained by hydrodistillation. The TPC was determined using the Folin-Ciocâlteu assay and expressed as gallic acid equivalents (GAE). The TPC value reported by Morsy for the allspice essential oil was 37.9 ± 0.7 mg GAE g^{-1} of essential oil.

The data showed represents the total phenol content extracted from samples using supercritical carbon dioxide, with varying pretreatments conditions with PEF of different intensities. The sample pretreated with 0.1 kV PEF, showed a modest 1.7% increase in phenol content compared to the sample without pretreatment. This suggests low-intensity PEF had a small effect on enhancing extraction efficiency. The sample, pretreated with 1.0 kV PEF exhibited a 5.2% increase compared to the sample without pretreatment. The higher PEF intensity likely caused greater cell wall disruption, facilitating better extraction by supercritical carbon dioxide. The sample pretreated with 3.0 kV PEF, had the highest phenol content, with a 9.1% increase over the sample without pretreatment. Overall, the data indicates a progressive increase in total phenol content with higher PEF pretreatment intensities before supercritical carbon dioxide extraction. The higher the PEF intensity, the more effective the pretreatment in facilitating extraction, potentially due to increased cell wall disruption and improved accessibility of phenolic compounds. This trend suggests that optimizing PEF pretreatment conditions could enhance the extraction efficiency of phenolic compounds using supercritical carbon dioxide as the solvent.

The significant differences in TPC values between the current result and those reported in the literature could be attributed to several factors: 1) The extraction method used to obtain the essential oil can greatly influence the yield and composition of phenolic compounds, with techniques like hydrodistillation or solvent extraction potentially extracting higher amounts of phenolic compounds compared to the method used in this analysis. 2) The phenolic content in allspice can vary depending on factors such as the plant variety, growing conditions, geographical origin, and maturity stage of the plant material. 3) The specific analytical method used for TPC determination, including the reagents, solvents, and measurement conditions, can affect the results. 4) The sample preparation steps, such as dilutions, filtration, or any pretreatment procedures, can impact the recovery and quantification of phenolic compounds. 5) The way the results are expressed (e.g., mg GAE g^{-1}, mg GAE mL^{-1}, or mg GAE $100g^{-1}$) can also influence the numerical values reported in different studies.

The drying process is crucial in preserving the quality and composition of plant materials, including allspice berries, before extracting essential oils or other valuable compounds. Inappropriate drying conditions can lead to the degradation or loss of phenolic compounds, subsequently affecting the TPC in the extracted essential oil. According to Hossain et al.[34], the drying temperature and duration play a significant role in preserving the phenolic content of plant materials. High temperatures and prolonged drying times can cause thermal degradation and oxidation of phenolic compounds, resulting in lower TPC values in the extracted essential oils. Orphanides et al.[35] reported that the drying method itself can influence the TPC in plant materials. They found that oven-drying at high temperatures (above 60°C) led to a significant reduction in TPC compared to air-drying or lyophilization (freeze-drying) methods.

It is important to note that the TPC assay provides an estimate of the total phenolic content but does not distinguish between individual phenolic compounds present in the sample. Therefore, the composition and relative proportions of specific phenolic compounds may vary among different allspice essential oil samples, contributing to the observed differences in TPC values[25].

The application of short external electric fields increased accumulation and extraction of the key bioactive eugenol while lowering levels of methyl eugenol. This demonstrates that PEF conditions can be optimized not only to improve the essential oil yield, but to enrich target compounds of interest, adding further value to implementing a PEF-assisted SC-CO_2 extraction process. Further studies are warranted to explore the mechanisms behind the release, hydrolysis and solubilization of phenylpropenes like eugenol.

These findings can be attributed to the enhanced disruption and permeability of the allspice cells and intracellular structures caused by the external electric fields applied by PEF. This subsequently facilitated an increased diffusion and solubilization of the target essential oil components into the supercritical CO_2 used in the extraction step.

Overall, PEF pretreatment allowed supercritical extraction to be conducted at milder temperatures and pressures while achieving significantly higher yields. This demonstrates that PEF-assisted SC-CO_2 extraction is a promising green, efficient technique for allspice essential oil production with potential for scaling up to industrial implementation. Further techno-economic analysis is recommended to evaluate the feasibility for commercial adoption.

4. CONCLUSIONS

The research conclusively validates pulsed electric field (PEF) technology as a green and highly effective method for extracting phenolic compounds from allspice fruits. Notably, the combined SC-CO_2 extraction and PEF approach emerges as a truly sustainable, zero-waste process. This innovative synergy harnesses solvent-free SC-CO_2 extraction and PEF ability to enhance mass transfer through electroporation, a potent union of green principles with ultra-short electrical pulses, no hazardous solvents, and reduced extraction times. The optimized PEF protocol with 3.0 kV cm^{-1} electric field strength, 50 pulses of 1-second duration increased essential oil yields from allspice when combined with subsequent SC-CO_2 extraction at 100 bar and 45 °C, compared to SC-CO_2 extraction alone under the same conditions. While parameter optimization is crucial for maximizing yields, the fundamental sustainability of this integrated PEF assisted SC-CO_2 approach is indisputable. Phenolic compounds are known to be sensitive to various processing conditions, including drying. The drying process might have caused degradation or loss of these compounds, leading to a lower total phenol content in the analyzed sample. When interpreting results related to phenolic content or other phytochemicals, it is essential to consider the processing steps the plant material has undergone, as these can significantly influence the final composition. Drying is a common processing method used for preserving and storing plant materials, but it can also lead to losses or alterations in certain phytochemicals, depending on the specific conditions employed (temperature, duration, method, etc.). As demand for eco-friendly processes intensifies, this convergence positions itself as a frontrunner sustainable extraction technology. Its innate greenness, solvent waste elimination, and industrial scalability potential render PEF assisted SC-CO_2 a gamechanger, poised to spearhead environmentally conscious manufacturing of high-value plant extracts aligning with green chemistry principles.

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CAPÍTULO DE LIBRO 2

Extraction of biocomposites using green technologies: extraction of allspice essential oil using supercritical co_2 and pulsed electric fields

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ABSTRACT

As awareness of health benefits grows, consumer demand for functional foods and nutraceuticals increases, largely due to their preference for promoting health. Consequently, attention is increasingly focused on natural bioactive compounds or biocomposites. Furthermore, the extraction of these biocompounds offers attractive industrial and technological prospects. There are a variety of traditional and advanced methodologies for the extraction of these vital molecules. Current conventional methods, including liquid-liquid and solid phase extraction, frequently require the use of hazardous solvents and are energy-intensive and take a long time to complete. Not only does this put considerable environmental pressure on resources, but the high temperatures often employed in such methods can also degrade the quality and potency of the extracted compounds. However, emerging green extraction techniques, such as supercritical fluid extraction, microwave-assisted extraction, pulsed electric fields, and ultrasonic-assisted extraction, are gaining ground due to their economic efficiency and environmental sensitivity. They also align with the growing demand for energy-efficient methods to recover important chemicals. Further enhancing their appeal, these cutting-edge techniques produce significantly less hazardous waste and pollutants.

Keywords

Allspice, Pulsed electric fields, Phenolic compounds, Antioxidant activity, Conventional and green technologies.

INTRODUCTION

Extraction, as a unitary separation operation, is one of the most energy-demanding technologies, surpassed only by distillation, drying and evaporation[36]. Understanding extraction procedures requires considering a number of essential elements, including the target compounds, the extraction solvent, the solute's characteristics (particularly its chemical properties), and the solvent's properties. The extraction of valuable bioactive compounds from natural products is an important means of producing value-added products, which is considered to have a beneficial effect on health[37]. Bioactive compounds are present in fruits, nuts, roots, vegetables, herbs and spices. Due to the importance of bioactive compounds, the manufacturing sector seeks innovative eco technologies to minimize the loss of bioactive compounds. These compounds have antioxidant, anticancer, anti-inflammatory, antidiabetic, antilipidemic and antidepressant properties.[38–40]. In the wake of environmental degradation and growing concerns about sustainable development, green technologies have emerged as critical instruments for shaping the future of industrial processes. The extraction of biocomposites has traditionally relied on solvent-based methods that often involve potentially harmful chemicals and excessive energy consumption. As society moves towards cleaner and more environmentally friendly methodologies, there is an inevitable need to revamp these conventional techniques, one such promising avenue using green technologies. Common extraction methods such as maceration, hydrodistillation, Soxhlet extraction, and others generally involve large amounts of solvent, are time-consuming, and can cause degradation of some of the desired compounds. That is why these methods are considered non-ecological. For this reason, there is a tendency to explore novel and ecological methods for the extraction of bioactive compounds. The objective of exploring new extraction techniques is to shorten extraction time, reduce energy, consumption and negative environmental impact, increase safety, as well as improve innovation and competitiveness.

In this way, microwave technology, pulsed electric fields, supercritical fluids or ultrasound have recently been proposed to shorten the processing time, increase the recovery yield and improve the functionality of the extracts[41]. The primary goals of the extraction process connect to one or more key attributes:

- High efficiency: The compounds of interest are thoroughly or nearly thoroughly collected.
- High selectivity/purity: The final extract has minimal amounts of interfering or unwanted extracted substances.
- High sensitivity: The final extract enables various quantification methods that generate proper calibration curves.
- Low detection/quantification limits: Extract components can be spotted/quantified at low concentrations since low background levels are achieved in the analytical framework.

These properties differ in terms of importance depending on the scale of the process. For example, the most important characteristics for analytical-scale separations are selectivity, sensitivity, and detection limit, but for industrial-scale semi-preparative and preparative separations, the key properties are yield and purity[42]

Both traditional and new extraction technologies and their combinations are also the focus of this section. These novel extraction techniques are used to obtain highest yields faster, increase quality, and account for environmental factors. Currently, several researchers are focusing on combined application of these cutting-edge extraction technologies. These processes could be an essential step for the production and long-standing utilization of bioactive substances from medicinal plants.

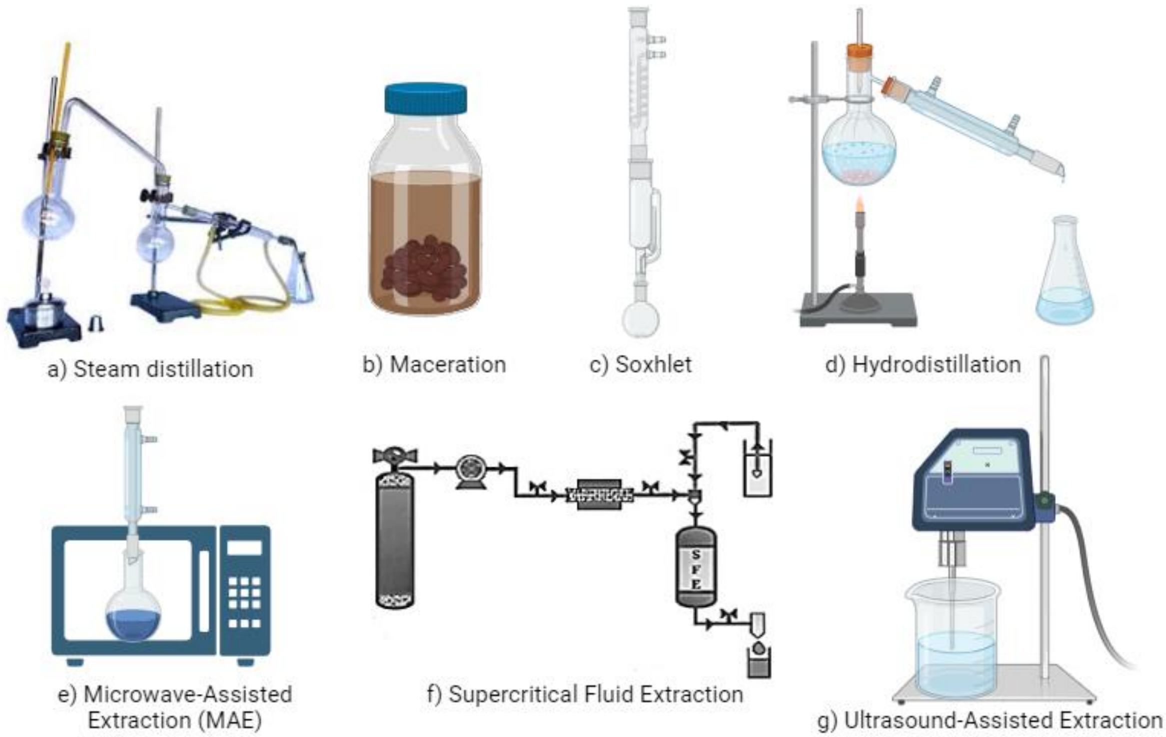

Figure 1. Examples of traditional and novel technologies in the extraction of natural compounds

Biocompounds (or bioactive compounds) are natural molecules found in plants, microorganisms and animals that may have biological activity and potential health benefits for humans. Over the years, various extraction techniques, both traditional and green, have been developed to obtain these biocompounds from natural sources. Some examples of traditional and novel extraction techniques are shown in Figure 1, such as maceration, Soxhlet, steam distillation, hydrodistillation, supercritical fluid extraction, ultrasound-assisted extraction, and microwave-assisted extraction.

The choice of extraction technique often depends on the type of biocomposite, the matrix in which it is found, and the desired purity. Green extraction techniques are gaining ground due to their reduced environmental impact and often greater efficiency.

1. Traditional technologies

Traditional methods for biocomposite extraction revolve around mechanical and chemical processing principles. These include solvent extraction, precipitation, milling, and various forms of heat treatment, each with their own unique advantages and nuances. While these methodologies have been instrumental in the early development of the biocomposites industry, they also have their limitations, particularly with respect to efficiency, environmental impact, and sometimes the integrity of the extracted compound. However, conventional methods have been the cornerstone of our current understanding and utilization of these biobased materials[43]. It is its reliability and simplicity that has led to its widespread use and deep knowledge, facilitating the subsequent development of more advanced and environmentally friendly extraction techniques.

1.1 Maceration

The method includes placing plant materials, usually in powder or coarse form, in a covered vessel with a specific solvent. The mixture is permitted to stand at room temperature (±25°C) for at least 72 hours with constant agitation. The process is intended to soften and rupture the plant cell wall to discharge the soluble bioactive metabolites. After 3 days, the entire mixture is pressed and filtered using filter paper. In this technique, heat is transferred by convection and conduction and the solvent chosen determines the type of phytochemical extracted from the samples. This method is recommended to retain the characteristic essence of extracts of some valuable herbs having very sensitive, heat-labile and volatile compounds[44]. It is utilized for making traditional food items with precise flavors and organoleptic properties. These authors performed lycopene extraction from tomato peels using refined olive oil as a solvent, attaining an overall yield of 99.3%. Lycopene is a red carotenoid, abundantly found in tomato peels and has excellent antioxidant properties[45]. Like other carotenoids, lycopene is an oil-soluble pigment, therefore, employing vegetable oils as solvents to extract this compound can be an outstanding alternative to replace organic solvents since it can generate a contaminant-free extract, eliminates the extra cost of evaporation and delays the oxidation and degradation rates of lycopene. Although the lycopene efficiency was not satisfactory, using this solvent displayed higher lycopene yields and it was also possible to attain these yields faster and at lower temperatures.

1.2 Steam distillation

Steam distillation involves passing dry steam through plant material, where the volatile compounds in the steam get vaporized, condensed, and collected in containers. It has been utilized for extracting essential oils for many years. It is carried out when the plant material can withstand direct steam. In this method, the plant material is held on a perforated screen or rack placed some distance above the base of the still, using low-pressure steam that replaces the volatile compounds in the intact plant material[46]. The distillation of the leaves, stem and whole plant of lemongrass was obtained by water vapor distillation. The results showed that the essential oil distilled from the lemongrass stem has greater antioxidant activity with 72.72% inhibition than the oil acquired through hydrodistillation with 70.11% inhibition, which can be used as a natural antioxidant in chicken meat.

1.3 Hydrodistillation

Hydrodistillation is another traditional technique that utilizes water or steam to extract bioactive compounds, primarily essential oils. This method is commonly performed using a setup called the Clevenger apparatus where the hydrated sample is heated to vaporize the volatile parts. In this process, two layers (aqueous and oil-rich) are formed, and the oil can be further separated via separatory funnels. Economically, this technique does not need organic solvents, making it a favorable option when extraction cost is crucial[47]. Hydrodistillation is used to extract volatile compounds from foods/plants by employing distilled water. This extraction of volatile organic compounds by azeotropic distillation and non-volatile organic compounds by boiling water takes 6 to 8 hours. It involves three processes: penetration of water into solutes (hydrodiffusion), hydrolysis and some degradation of thermolabile compounds due to high temperature. High temperatures during extraction can degrade compounds, limiting this technique's use. Plant extracts obtained are volatile and easily dissolve or discharge their active components. This extraction method is ideal for obtaining thermostable and water-soluble components from raw plant materials. The extraction yield and chemical makeup of the essential oil samples were compared by hydrodistillation and soxhlet method. Forty-one chemical compounds were identified in the soxhlet essential oil components, while forty were found in the oil sample extracted with hydrodistillation. The oils obtained by both methods are quantitatively similar but qualitatively differ. Hydrodistillation generated more oxygenated compounds contributing to the oil's aroma.

1.4 Solvent extraction (Soxhlet)

Soxhlet extraction is the most popular method since, besides being easy to perform and cost-effective, it achieves remarkable yields with many matrices due to the continuous leaching of the target compounds by reflux of the condensed condenser in successive washing cycles. Thanks to repetitive sample-solvent contacts, the Soxhlet design can achieve quality extractions in relatively short times and lower solvent volumes than other classical options, although large quantities are wasted compared to advanced alternatives currently in use. Additionally, the high solvent/sample ratios involved can cause environmental problems and high temperatures, generally close to the boiling point of the solvent, can impact the recovery of heat-sensitive native species. Furthermore, the extensive use of hazardous solvents necessitates post-extraction evaporation/concentration cleaning phases to remove solvent traces, which complicates workflows and makes them costlier.

Soxhlet extraction is one of the traditional techniques that has been widely utilized for extracting bioactive compounds from numerous natural sources. For several decades, this method has been consistently functional in various analytical processes related to the extraction of bioactive compounds. One of the main advantages of Soxhlet extraction is that compounds with medium to low solubility can be extracted with this technique. To obtain satisfactory performance, as well as to avoid losing volatile compounds, the appropriate solvent selection is imperative. Furthermore, the solvent type used suggests the polarity of the extracted compounds. The extraction period is extremely long, resulting in some thermolabile compounds getting destroyed. With Soxhlet extraction, a small dried sample is placed in an extractor and repeatedly brought into direct contact with a suitable solvent (water, petroleum ether, hexane) until extraction completes[48]. Its higher solvent consumption, energy requirement and prolonged extraction time are major issues. Rodríguez-Cabo *et al*., 2018 studied the Soxhlet ethanolic extraction of Vitis vinifera canes, evaluating the suitability of alcoholic distillates from wine production waste as a green solvent[49], finding that with this solvent it was possible to obtain catechin, a valued polyphenol, achieving yields equivalent to those of purified solvents and/or ethanol-water mixtures.

2. New technologies

In response to growing environmental concerns and the push toward sustainable industrial practices, the advent of green biocomposite extraction technologies marks an important step forward. These innovative methodologies underscore the commitment to minimizing environmental impact, preserving the integrity of extracted compounds, and improving process efficiency. Green technologies take advantage of advances in science and engineering to optimize the extraction process, while mitigating the environmental footprint. Techniques such as supercritical fluid extraction, microwave-assisted extraction, enzymatic methods and pulsed electric field extraction have been identified as key players in this new wave of eco-innovation. These methods share a common goal: optimizing the extraction process while maintaining a careful balance with environmental sustainability. Its objective is to reduce energy consumption and dangerous solvents, limit the generation of waste and ensure greater performance of high-quality compounds. At the same time, green technologies often offer the additional advantage of preserving the structural and functional integrity of biocomposites, enhancing their potential applications across industries.

The dawn of green technologies signifies a promising shift in the biocomposites industry, heralding an era of environmentally conscious progress and innovation. As these methods continue to evolve and mature, they are poised to redefine the landscape of biocomposite extraction and utilization, pushing the boundaries of what is possible and reaffirming our commitment to sustainability. Below is a mention of some of these technologies.

2.1 Extraction with supercritical fluids

Extraction with supercritical fluids employing carbon dioxide is a promising alternative for obtaining value-added products since a mild temperature is utilized that enables extracting thermally labile or easily oxidizable compounds[50]. In supercritical fluid, the physicochemical properties of a given fluid, such as density, diffusivity, dielectric constant and viscosity, can be easily regulated by altering pressure or temperature without ever going across phase boundaries. Supercritical fluids have liquid-like densities, a higher diffusion coefficient and low surface tension, which promotes the penetration of the supercritical solvent into the porous structure of the solid matrix to discharge the solute. The critical point of carbon dioxide (CO_2) is 31.06°C and 7.386 MPa. CO_2 is the preferred solvent for supercritical fluid extraction because it is generally recognized as safe (GRAS), non-toxic, non-flammable, economical and its critical temperature and pressure are relatively low, which prevents thermal degradation of the extracted food components. Phan *et al,* 2020 extracted polyphenols from *Vietnamese C. fragrans* leaves by supercritical carbon dioxide (SC-CO_2) extraction employing ethanol as a co-solvent[51]. The findings showed that supercritical carbon dioxide is suitable for industrial production of polyphenols with high antioxidant activity. Therefore, it could be considered an upcoming potential extraction method that could replace conventional techniques.

2.2 Microwave

Microwave-assisted extraction technology effectively extracts compounds of interest from plant materials and is one of the preferred methods. This technology utilizes electromagnetic energy in the 300 MHz-300 GHz frequency range to facilitate the partitioning of solute from the sample to the solvent. Electromagnetic radiation is produced when perpendicular oscillation occurs between electric and magnetic fields. In this way, dipolar rotation of the molecules is prompted, which interrupts hydrogen bonds and therefore improves the migration of dissolved ions and promotes solvent penetration into the sample. It is widely utilized in extracting and utilizing natural compounds from plant materials and by-products owing to its advantages of rapid heating rate, reduced thermal gradient and higher extraction yield. In many cases, the extraction efficiency escalates for wet tissues. Matrices containing a large quantity of water that efficiently absorbs microwave energy undergo a swift rise in internal temperature, causing cell rupture and thus improving the extraction of intracellular compounds. Fine particles (100 µm–2 mm) also favor deeper microwave penetration, enhancing matrix-solvent interaction due to greater surface area. Simha *et al.* 2016 investigated the efficacy of microwave-assisted extraction to recover bioactive compounds from medicinal plants *Cymbopogon citratus* and *Adathoda vasica* of pharmaceutical significance, reporting similar yields although with a drastic reduction in extraction time (210 s) compared to the average 10 hours in the Soxhlet apparatus[52].

2.3 Ultrasound

Ultrasonic extraction (UE) relies on the propagation of mechanical waves that cause the formation of cavitation bubbles due to variations in temperature and pressure. This technology stands out as a sustainable alternative owing to its simplicity, low cost and greater efficiency compared to conventional methods like maceration. It is based on the principle of acoustic cavitation, which comprises the formation, growth and implosion of bubbles that damage the plant matrix cell walls and thus promote the release of bioactive compounds. Ultrasonic equipment delivers the waves via a bath or probe, differing in how it supplies the ultrasonic energy. The ultrasonic bath is the most widely used device, consisting of a stainless-steel tank that usually operates at 40 kHz frequencies. On the other hand, the probe-coupled ultrasound system typically operates at 20 kHz frequencies and provides 100 times the power of the ultrasonic bath. The ultrasonic probe is directly immersed in the sample container, commonly made of glass or stainless steel. Ultrasound can be implemented indirectly or directly during extraction through the bath and probe modes, respectively. Extraction parameters that significantly impact the ultrasonic process efficiency are particle size, solvent-to-feed ratio, extraction temperature, time and ultrasonic power or intensity. The power delivered by the probe is usually greater than bath systems since the power is supplied through the probe tip. This makes experiments with probe systems more reproducible than with bath equipment. However, probe systems are smaller, and generally, smaller amounts of plant material are treated. Xu, Li and Sun (2015) reported on the impact of ultrasound-assisted extraction of natural antioxidants from Eucommia Oliver using distilled water as solvent[53]. The use of ultrasound improved the effectiveness of traditional treatments, providing higher yields and selectivity of natural antioxidants.

2.4 Infrared

Combined infrared assisted extraction (IRAE) equipment consists of an IR lamp radiation source with power modes varying from 50 to 1000 W. The plant material is placed inside a container such as a round bottom flask of variable volume based on solid density. This flask is filled with water, ethanol or another solvent. Most IRAE tools have a condenser for solvent recovery. The flask is positioned near the lamp to expose the contents to IR radiation. IR radiation heats the solvent and plant material, enhancing flavonoid extraction, generally over several minutes. Cheaib *et al*, 2018 compared the efficiency of various technologies like ultrasound, microwave and infrared in terms of polyphenol yield and bioactivity from apricot pomace. IR was the most effective method with highest polyphenol yields and efficacy. The IR extract of apricot pomace displayed the highest inhibitory activity against all the studied gram-positive strains (methicillin-resistant *Staphylococcus aureus, Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermidis, Staphylococcus epidermidis*) and one gram-negative strain (*Escherichia coli*). Furthermore, IR extracts had the highest antiradical activity by far[54].

2.5 Pulsed electric fields

Pulsed electric field (PEF) extraction is a technique involving exposing a plant matrix to an electrical potential. PEF consists of treating the plant matrix for extracting phytochemicals. Electrical pulses are generated by a transformer, escalating voltages from 140 or 220 V to 1000 V or over 25000 V. A capacitor transforms this high voltage into a narrow pulse, achieved in a closed chamber with metal electrodes. There is no single weight, shape or particle size of solids. The PEF treatment chamber size depends on the solid volume. PEF has therefore been successfully utilized to treat whole fruits (or peels) such as oranges or grapes or slices and cut pieces differing in particle size. The electrode plate perimeter can vary from 10 to 100 cm, and the distance between electrodes can be 1 to 20 cm, although the chamber size largely depends on the plant matrix volume to be processed. The influence of pulsed electric fields (PEF) of different intensities on extracting total phenols from lemon peel waste by pressing was investigated. The pressing-assisted extraction effectiveness was evaluated by measuring total phenolic content (TPC), actual antioxidant capacity and concentrations of main lemon polyphenols. It was concluded that PEF provides a new methodology to improve polyphenol extraction with a non-thermal and eco-friendly technology, representing a method to increase economic benefits of industrial processes.

2.6 Ionic liquids

Ionic liquids are a unique class of salts with melting points below 100°C, allowing them to exist as liquids at room temperature. Their chemical structure consists of large asymmetric organic cations paired with organic or inorganic polyatomic anions. By selecting different cation-anion combinations, the physicochemical properties of ionic liquids like polarity, viscosity, and solubility can be tailored to target specific extraction applications. This tunability has enabled ionic liquids to emerge as designer solvents that can overcome limitations of conventional volatile organics. For example, Du et al. demonstrated that varying the anion and cation components of ionic liquids had a significant influence on the microwave-assisted extraction of polyphenols from medicinal plants. Their results highlight the potential of ionic liquids, when integrated with green extraction techniques like microwave processing, to effectively extract valuable phytochemicals from botanical matrices in a more sustainable manner than traditional organic solvents. Overall, the vast possible permutations of ionic liquids allow their solutions to be optimized as green extraction media for compounds across a diverse array of natural sources.

3. Advantages of green technologies

The extraction of bioactive phytochemicals from plant materials represents the initial, critical separation stage for recovering these high-value compounds. However, the intrinsic chemical properties of these metabolites often make them challenging extraction targets. Specifically, the poor solubility and matrix barrier imposed by rigid cell walls and membranes hinders diffusion of solvents into the plant matrix and extraction efficiency. Consequently, appropriate green extraction techniques must balance production metrics like yield and purity with environmental responsibility. In contrast to traditional methods that frequently rely on extensive solvent volumes, processing times, temperatures, and degradation of sensitive actives, advanced green approaches aid selective, efficacious extractions. By effectively deconstructing cell walls using non-thermal mechanisms and enhancing solvent penetration, these technologies greatly facilitate recovery of bioactive compounds in their native forms, retaining valued bioactivity. Key advantages of green extraction technologies include reduced energy and solvent use, shorter extraction times, minimal processing artefacts that necessitate purification, and importantly, high-quality extracts. As the natural products industry evolves, these sustainable techniques will likely form the cornerstone of next-generation biocomposite production, balancing both economic incentivization and environmental stewardship.

3.1 Lower energy consumption

Green extraction technologies frequently demonstrate superior efficiency at recovering bioactive compounds compared to conventional techniques. Although adopting these innovative methods may necessitate greater initial capital investment, their implementation can reduce overall operating expenses in the long term. Specifically, green technologies require fewer material inputs, conserve energy, and generate less waste byproducts that demand treatment. The heightened extraction efficiency simultaneously increases product yields and profitability. Therefore, the high performing and more sustainable nature of these advanced methods can offset their startup costs over time, leading to both environmental and economic incentives. While further lifecycle and cost-benefit analyses are warranted, the promise of improved productivity and energy profiles highlights green extraction's potential to lower expenditures and elevate returns across the industry.

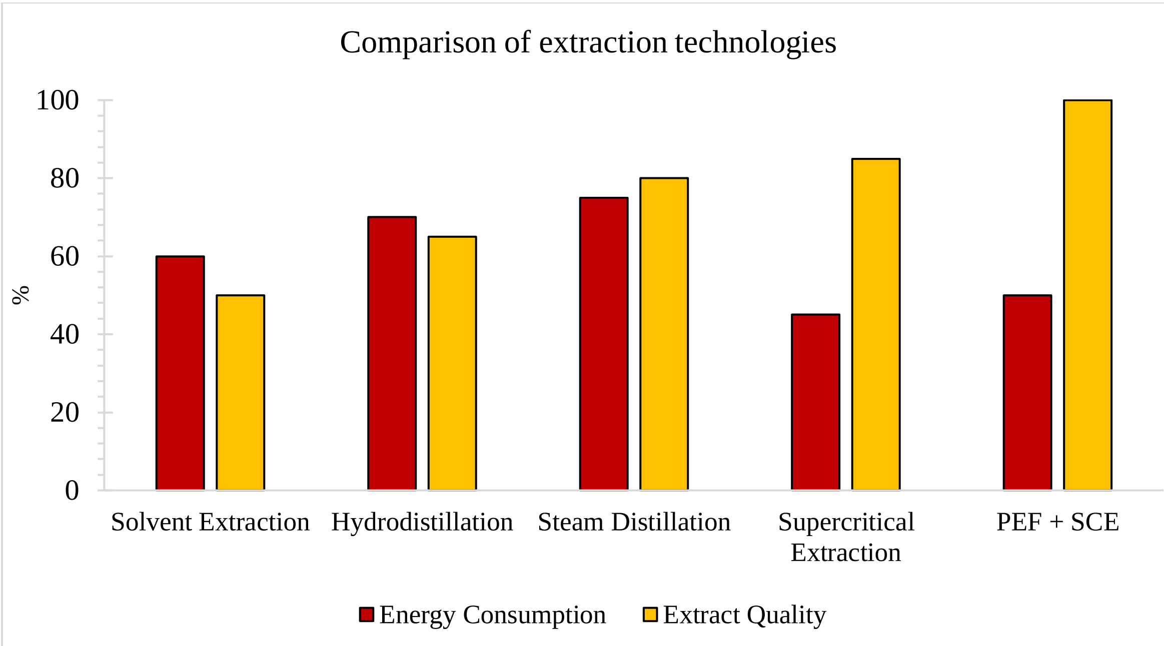

Figure 2. Comparison of energy consumption in extraction technologies

Energy consumption is reduced when using green extraction technologies such as ultrasound, pulsed electric fields (PEF), microwaves and supercritical fluids due to several factors internal to these methods such as improved mass transfer, when using this type of technologies promote efficient mass transfer. Ultrasound and PEF create physical alterations in the material, increasing the contact area between the solvent and the target compounds. In supercritical fluid extraction, the supercritical state improves solubility and mass transfer, allowing for faster extraction. Another factor is effective heating as microwaves and PEF provide rapid and selective heating to the sample itself, this avoids unnecessary heating of the surrounding environment, reducing energy waste.(Jha and sit, 2022). Additionally, green extraction technologies are generally faster than conventional methods. Shorter processing times mean less energy is required to maintain extraction conditions. Another factor is the use of low temperatures, as in the case of supercritical fluids and PEF can be used at lower temperatures compared to traditional extraction techniques, reducing the energy required for heating. Ultrasound, PEF and microwaves allow precise and controlled application of energy to the extraction process, minimizing energy loss to the environment. Figure 2 shows a comparison for different extraction methods in terms of their energy consumption and quality of the extract, where we find that combined green technologies turn out to have lower energy consumption and higher quality in the extract. In short, these technologies are not only more environmentally friendly but also economically advantageous due to their energy-saving features.

3.2 Shorter processing time

Using alternative extraction methods can reduce the duration of extraction processes to a few hours or even minutes in the case of ultrasound or microwaves. Shorter processing times are achieved in novel extraction technologies such as supercritical fluid, ultrasonic-assisted, infrared, and microwave due to various mechanisms and advantages associated with each of these methods. Supercritical fluids are often used as solvents in extraction processes. They offer high diffusivity and low viscosity, allowing for faster mass transfer, they can penetrate porous materials more efficiently, reducing the time needed for extraction, and their adjustable properties, such as pressure and temperature, allow optimization of extraction conditions. extraction for shorter processing times. In ultrasound-assisted extractions, ultrasound waves generate cavitation, the formation and collapse of small bubbles, which enhances the breakdown of cell walls and increases contact between the solvent and the material. Cavitation-induced microcurrent promotes faster diffusion of target compounds, leading to shorter extraction times. Ultrasound can create localized heating, which can speed up extraction by increasing the temperature in specific regions. In the case of infrared radiation, it directly heats the sample being extracted, which produces rapid and uniform heating. This direct and efficient heating can accelerate the extraction process, especially for heat-sensitive compounds. Infrared extraction minimizes the need for preheating and temperature balancing, saving time. Lastly for microwave use, provide volumetric and selective heating, focusing the energy on the sample rather than the surrounding environment, selective heating leads to a faster rise in temperature, promoting rapid extraction. Microwaves can reduce extraction time for a wide range of materials, from food products to organic compounds.

In summary, these green extraction technologies offer shorter processing times due to their ability to improve mass transfer and reduce the time required to extract target compounds. The specific mechanisms may vary, but all contribute to more efficient and environmentally friendly extraction processes.

3.3 Fewer product purification operations

Green technologies often result in products that require fewer purification steps. This is because these methods generally produce extracts relatively free of impurities, unlike some traditional methods that may introduce additional contaminants or alter the chemical profile of the extract, requiring further refinement.

3.4 Higher product quality

The quality of extracts is often judged based on their chemical composition and sensory attributes. Green extraction methods are known to produce high quality products due to the minimal processing involved. The more intense processing conditions of many traditional technologies, on the other hand, can lead to the degradation of certain sensitive compounds and consequently affect the quality of the resulting extract.

4. Case Study: Allspice Essential Oil Extraction

Allspice, scientifically known as Pimenta dioica, is a spice derived from the dried, unripe fruit of the allspice tree, native to tropical regions of America.[3]. In addition to its culinary importance, allspice is prized for its essential oil, which features a unique combination of eugenol, caryophyllene, and other valuable compounds with potential therapeutic properties.[1]. The extraction of this essential oil has seen various methods, but the integration of supercritical fluid extraction (SFE) with pulsed electric fields (PEF) offers an innovative and efficient approach.

Pimenta dioica (Allspice) fruits are rich in phenolic compounds, which have varied biological activities including antioxidant, anti-inflammatory and antimicrobial properties[6]. Traditional methods of extracting phenolic compounds from allspice fruits are time-consuming and energy intensive. Recently, there has been growing interest in employing unconventional extraction techniques like pulsed electric field (PEF) technology for obtaining bioactive compounds from foods[19]. PEF is a non-thermal approach utilizing short duration high voltage pulses to prompt electroporation in the cell membrane[21]. This leads to the discharge of intracellular constituents, comprising phenolic compounds, into the extraction medium.

This case focuses specifically on the extraction of the essential oil of allspice, a plant revered for its versatile therapeutic and aromatic properties. The essential oil derived from allspice finds wide use in sectors such as cosmetics, pharmaceuticals, food and aromatherapy.[5]. Given the wide applications and consequent demand for allspice essential oil, it is of utmost importance to develop efficient and environmentally friendly extraction techniques.

In the search for more sustainable solutions, supercritical carbon dioxide (SC-CO_2) extraction and pulsed electric fields (PEF) have emerged as possible alternatives to traditional extraction methods. Supercritical extraction uses carbon dioxide in a supercritical state, operating under controlled temperature and pressure conditions. This method is particularly praised for its non-toxic nature, low environmental impact, and ability to extract high-quality biocomposites.

Supercritical fluid extraction takes advantage of the unique properties of supercritical fluids, especially supercritical carbon dioxide (SC-CO_2), to extract essential oils[23]. In its supercritical state, CO_2 combines the properties of the gas and liquid phases, allowing it to completely penetrate plant material and dissolve essential oils efficiently. This method guarantees high performance, preserves oil quality and works without leaving solvent residues. PEF involves applying short bursts of high-voltage electrical pulses to plant material, leading to the formation of pores in cell membranes, a phenomenon called electroporation. This permeabilization of cell membranes facilitates the release of cellular contents, including essential oils.

This research aims to investigate the feasibility and efficiency of using supercritical fluids and pulsed electric fields in combination, for the extraction of essential oil from allspice. The study seeks to provide a comprehensive comparative analysis of these green technologies against traditional extraction techniques, focusing on extraction performance, energy consumption and quality of the extracted oil.

Materials and methods

For the extraction of the essential oil, pepper berries harvested at the "El Pimiento" Ranch, located in La Mesa, Puebla, Mexico, were used. The pepper fruits were previously dried. To later be crushed and sieved with a 20 mesh. Carbon dioxide will be used (99.99% purity) acquired from the company INFRA (México) SA de CV, Toluca, State of Mexico.

Pretreatment with pulsed electric fields (PEF)

The dried and ground allspice was processed in batches of 100g in a PEF equipment, comprising a pulse generator (Makita EG4550A) connected to an oscilloscope (Owon Sds1022). The processing chamber is linked to the generator via 2 stainless steel electrodes. The operating settings were controlled through computer equipment, contacting the oscilloscope and pulse generator. Pulses with electrical potentials of 0.1, 1.0 and 3.0 kV/cm were applied to each sample for 1 second. Figure 3 illustrates the representation of the pretreatment experimental process utilized.

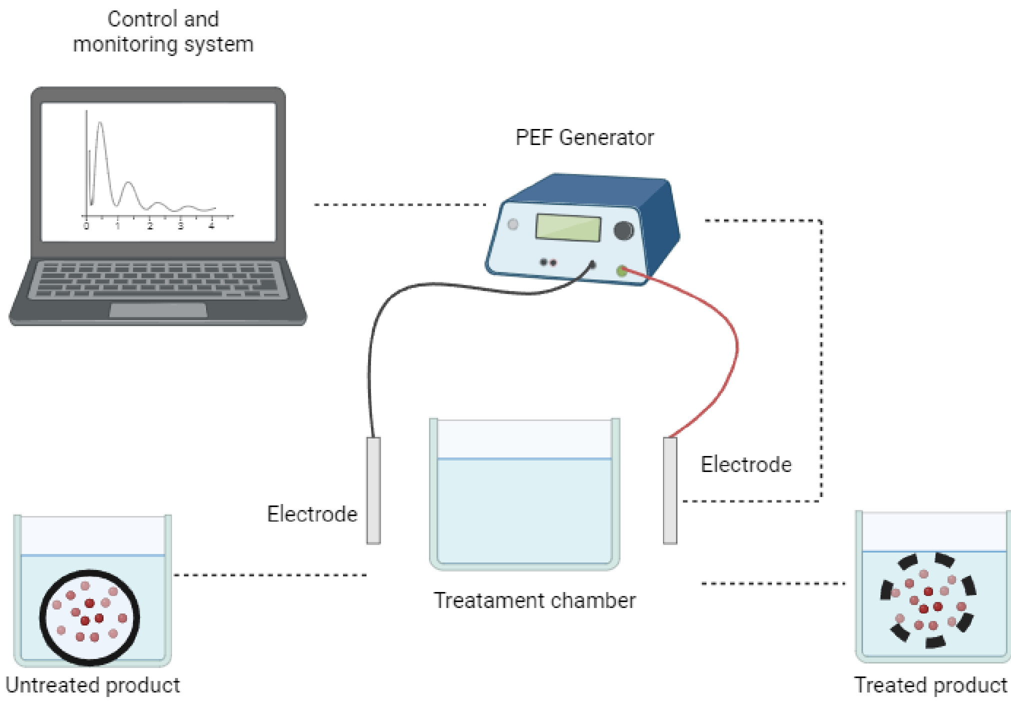

Figure 3. Pretreatment with pulsed electric fields (PEF)

Extraction with supercritical carbon dioxide

The high-pressure extraction cell was loaded with 100 g of dried, ground allspice previously treated with pulsed electric fields (PEF). The cell was connected to a tube line setup equipped with a temperature controller that monitors the operating temperature. CO2 flow was passed through the lines to displace the air in the tubes and extraction cell. The carbon dioxide is compressed employing a manual pressurization pump. Upon reaching the experimental temperature, the cell was pressurized with CO2 until the desired experimental pressure was attained. Pressure was gauged with a high-pressure transducer (Sensotec LA/7093-03). After stable pressure and temperature, the heterogeneous mixture of ground allspice with CO_2–SC was left in contact for 6 hours. After the specified contact time, the supercritical CO_2 with dissolved essential oil went through a micrometer heating valve and was subsequently released into a recovery cell, where the extracted oil was amassed. The outlet flow was carefully regulated by a micrometric valve since sudden expansion of the mixture can cause cooling and frost formation, hence obstructing the outlet. An analytical balance determined the quantity of allspice oil gathered in the recovery cell. The extracted oil accumulated in the outlet tubes was recovered with the solvent ethanol.

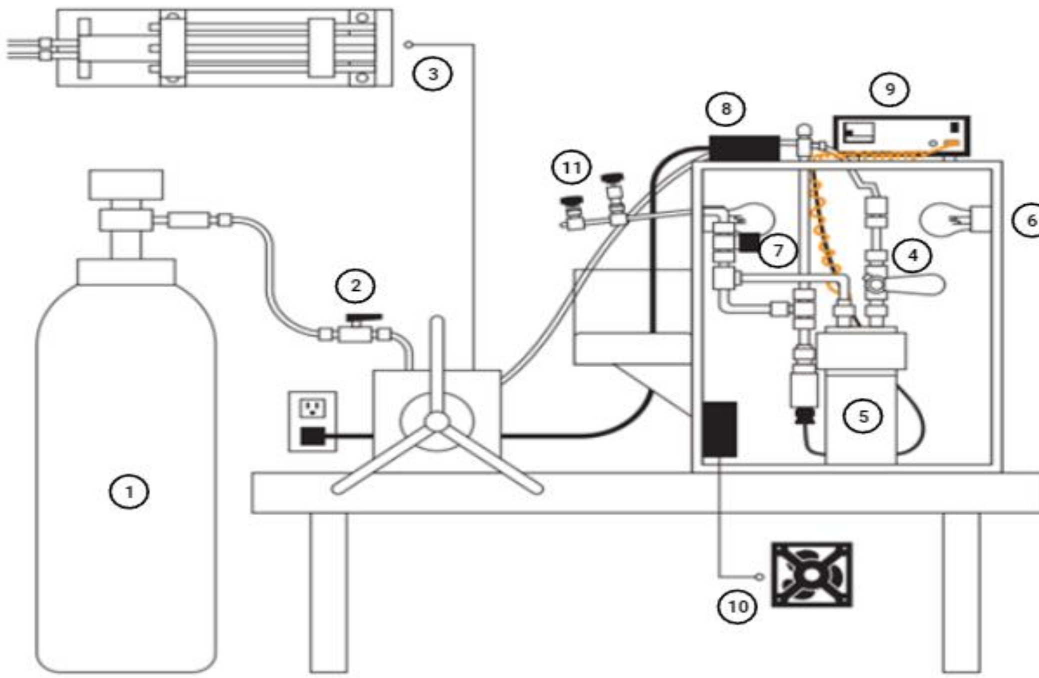

Figure 3. Diagram of supercritical extraction experimental equipment. CO_2 tank (1), Shut-off valves (2,4,7), Manual pressurization pump (3), Extraction cell (5), Heat sources (6), Pressure indicator (8), Temperature indicator (9), Fan (10) and CO2 outlet micrometric valve (11).

Product performance and quality results.

Extractions were performed using the supercritical carbon dioxide method of allspice essential oil with and without PEF pretreatment. Notable differences were obtained between the amount of extract obtained with both methods, as can be seen in the results of Table 1.

Table 1. Amount of extract obtained with SC-CO_2 and PEF/SC-CO_2

Without PEF	0.1kV/cm	1.0 kV/cm	3.0 kV/cm

T(K)	318.15	318.15	318.15	318.15
P (bar)	100	100	100	100
Amount of extract (g)	0.894	0.972	1.262	1.396

According to the results obtained, it can be observed that the amount of extract increases with pretreatment. This behavior has already been observed in similar methodologies that have been carried out by several researchers, for example, in the extraction process of anthocyanins obtained from strawberries[27]. Pretreatment with PEF can alter the cellular structure of ground pepper, facilitating the release of the essential oil. This alteration increases the accessibility of the target molecules to the supercritical fluid during the extraction process, leading to more efficient extraction. based on the electroporation phenomenon generated by PEF. By increasing the permeability of cell membranes by creating temporary pores in the cell walls, this improved permeability allows the supercritical fluid to penetrate the plant material more effectively, promoting essential oil extraction.

The results showed that PEF treatment significantly increased the yield of phenolic compounds from allspice fruits. Pulsed electric field (PEF) pretreatment can influence the chemical composition of essential oils through several mechanisms. Applying short bursts of high-voltage electrical pulses to plant tissues before extraction can cause changes in cellular structure, permeability, and biochemical processes within the plant material.

Table 2. Composition of essential oils obtained from SC-CO_2 and PEF/SC-CO_2

Oil obtained by SC-CO_2			**Oil obtained by PEF SC-CO_2**		
	%	Compounds		%	Compounds
1	52.91	Eugenol	1	64.81	Eugenol
2	0.33	Terpinen-4-ol	2	0.37	Terpinen-4-ol

3	9.02	Caryophyllene	3	0.12	Caryophyllene
4	43.45	Methyl eugenol	4	32.25	Methyl eugenol

Table 2 shows the chemical composition for the sample without PEF pretreatment and the sample treated at 3.0 kV/cm with PEF pretreatment. These results showed that PEF (pulsed electric field) pretreatment led to further cell disruption and release of free eugenol compounds, enhancing their extractability by supercritical CO_2. Concurrently, some methyl eugenol may have become bound or broken down, decreasing its final concentration. Additionally, the PEF pretreatment could have induced some matrix effects that altered the solubility or mass transfer kinetics of eugenol versus methyl eugenol into the supercritical CO_2. This could enable preferential extraction of eugenol.

Conclusions

The growing shift towards sustainable extraction methods has led researchers to explore integrating supercritical fluid extraction (SFE) and pulsed electric field (PEF) technology to extract essential oils from allspice. This combined green approach offers potential advantages over traditional methods, including higher yields, superior oil quality, and a reduced environmental impact. SFE utilizes supercritical CO_2 is unique diffusive and solvating abilities to penetrate plant cells, while PEF permeabilizes cell membranes through electroporation to facilitate release of intracellular contents like essential oils. Early research on applying SFE-PEF to allspice oil extraction shows promising results - PEF pretreatment before SFE increased oil yields in a voltage-dependent manner, indicating cell disruption enhances subsequent SFE performance. Optimization of PEF parameters along with supercritical extraction conditions will be key to achieving maximum and consistent enhancement.

While more research is still needed on scaling up the SFE-PEF process for commercial implementation, the initial data highlights the immense potential of this green, synergistic technique to transform essential oil extraction to be more efficient and sustainable. Given the rising market demand for high-quality botanical oils and shift away from traditional solvent-based methods, integrated SFE-PEF techniques could establish new processing standards emphasizing eco-responsibility without sacrificing extraction efficacy. Advancements optimizing this approach could position SFE-PEF extraction as an ideal sustainable platform for next-generation essential oil production.

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CONCLUSIONES

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La aplicación de un pretratamiento con campo eléctrico pulsado (PEF) antes de la extracción con dióxido de carbono supercrítico (SC-CO_2) de bayas de pimienta de Jamaica mostró resultados interesantes. Por una parte, el uso de PEF mejoró significativamente el rendimiento de extracción, con un aumento de hasta un 56.2% en comparación con la muestra sin pretratamiento con PEF. Además, el pretratamiento con PEF influyó en la composición química del aceite esencial extraído, lo que llevó a una mayor proporción del compuesto bioactivo eugenol (hasta 64,81%) y un menor contenido de metil eugenol (32.25 %).

El aumento observado en el contenido de eugenol después del pretratamiento con PEF se atribuye al fenómeno de electroporación, que altera las estructuras celulares y facilita la liberación de eugenol de los compartimentos intracelulares. Así mismo, el PEF puede tener una actividad enzimática mejorada, contribuyendo a la hidrólisis de los glucósidos de eugenol y liberando más eugenol libre para la extracción.

En cuanto a la actividad antioxidante, el contenido fenólico total (TPC) del aceite esencial extraído mostró un aumento progresivo con intensidades de PEF más altas, lo que indica que la optimización de las condiciones de PEF podría mejorar la eficiencia de extracción de compuestos fenólicos utilizando SC-CO_2.

La investigación contribuye a la comprensión científica de los mecanismos subyacentes a los efectos sinérgicos del SC-CO_2 y el PEF en la extracción de compuestos naturales. También proporciona información valiosa sobre la optimización de los parámetros del proceso, como la intensidad del campo eléctrico y la duración del pulso, para maximizar la eficiencia de la extracción.

La combinación de pretratamiento con campo eléctrico pulsado (PEF) y extracción de dióxido de carbono supercrítico (SC-CO2) representa un método novedoso e innovador para mejorar la eficiencia y el rendimiento de la extracción de compuestos naturales de materiales vegetales. Este enfoque integrado tiene el potencial de abordar las limitaciones asociadas con las técnicas de extracción convencionales, como el uso de solventes orgánicos peligrosos y altas temperaturas que pueden degradar los compuestos extraídos. Al minimizar la dependencia de disolventes nocivos y reducir la necesidad de temperaturas elevadas, este método ofrece una alternativa respetuosa con el medio ambiente que reduce el impacto ambiental, previene la degradación térmica de los compuestos extraídos y reduce el consumo de energía durante el proceso de extracción. La técnica combinada de pretratamiento PEF y extracción SC-CO_2 presenta una solución prometedora, ecológica y eficiente para la producción de aceite esencial de pimienta de Jamaica, con potencial de ampliación e implementación a nivel industrial.

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