TESIS DOCTORAL

OBTENCIÓN DE COMPONENTES BIOACTIVOS DE RESIDUOS DE LA INDUSTRIA CAFETERA MEDIANTE DISOLVENTES SUPRAMOLECULARES

EXTRACTION OF BIOACTIVE COMPOUNDS FROM COFFEE WASTE BY SUPRAMOLECULAR SOLVENTS

Laura Sofía Torres Valenzuela

TITULO: *OBTENCIÓN DE COMPONENTES BIOACTIVOS DE RESIDUOS DE LA INDUSTRIA CAFETERA MEDIANTE DISOLVENTES SUPRAMOLECULARES*

AUTOR: *Laura Sofía Torres Valenzuela*

© Edita: UCOPress. 2020
Campus de Rabanales
Ctra. Nacional IV, Km. 396 A
14071 Córdoba

https://www.uco.es/ucopress/index.php/es/
ucopress@uco.es

Tesis doctoral:

Obtención de componentes bioactivos de residuos de la industria cafetera mediante disolventes supramoleculares

Trabajo presentado, para optar al grado de Doctor, por

Laura Sofía Torres Valenzuela

que lo firma en Córdoba, a 20 de Enero de 2020

Firmado:
Laura Sofía Torres Valenzuela
Ingeniera Agrícola, M.Sc. en Ingeniería de Alimentos

con el VºBº de los directores,

Soledad Rubio Bravo y Ana María Ballesteros Gómez

RUBIO BRAVO MARIA SOLEDAD - 30428928C

Firmado digitalmente por RUBIO BRAVO MARIA SOLEDAD - 30428928C
Nombre de reconocimiento (DN): c=ES, serialNumber=IDCES-30428928C, givenName=MARIA SOLEDAD, sn=RUBIO BRAVO, cn=RUBIO BRAVO MARIA SOLEDAD - 30428928C
Fecha: 2020.01.22 15:33:06 +01'00'

Firmado:

Dra. Soledad Rubio Bravo

Catedrática del Departamento de Química Analítica de la Universidad de Córdoba

BALLESTEROS GOMEZ ANA MARIA - 30968640Z

Digitally signed by BALLESTEROS GOMEZ ANA MARIA - 30968640Z
Date: 2020.01.22 16:06:35 +01'00'

Firmado:

Dra. Ana María Ballesteros Gómez

Contratada Ramón y Cajal del Departamento de Química Analítica de la Universidad de Córdoba

TÍTULO DE LA TESIS:

Obtención de componentes bioactivos de residuos de la industria cafetera mediante disolventes supramoleculares

DOCTORANDO: Laura Sofía Torres Valenzuela

INFORME RAZONADO DE LOS DIRECTORES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

Las investigaciones desarrolladas en esta Tesis Doctoral han tenido como objetivo general la valorización de residuos de procesamiento del café (borras, pulpa y aguas residuales) para la obtención de compuestos bioactivos (alcaloides y polifenoles) mediante el uso de disolventes supramoleculares (SUPRAS). Para ello se han aplicado SUPRAS basados en componentes autorizados como aditivos alimentarios (ácidos carboxílicos, 1-hexanol, agua y etanol) y sintetizados por procesos espontáneos y simples de auto-ensamblaje y coacervación con el fin de facilitar su posterior implementación en las industrias agroalimentarias y farmacéuticas. Se han optimizado las principales variables implicadas en el proceso de extracción de los compuestos bioactivos mayoritarios (cafeína en las tres matrices ensayadas, ácido clorogénico en borras y ácido protocatéquico en pulpa). Los extractos enriquecidos bajo condiciones óptimas se han caracterizado mediante cromatografía de líquidos y espectrometría de masas en tándem para elucidar la presencia de los principales alcaloides y polifenoles presentes en los mismos. Finalmente, se ha determinado el poder antioxidante de los extractos utilizando diferentes métodos químicos. En el caso de las borras se ha determinado también su poder antimicrobiano.

La alta capacidad de extracción de compuestos bioactivos y el elevado poder antioxidante de los extractos de SUPRAS obtenidos de residuos comunes de la industria del café, así como su biocompatibilidad, demuestran la idoneidad de estos disolventes alternativos para la valorización de residuos agrícolas. Los resultados de las investigaciones realizadas se han materializado en 4 artículos científicos publicados en revistas indexadas y situadas en el primer cuartil (JCR) y se han presentado en 3 contribuciones a congresos (2 nacionales y 1 internacional).

En base a la originalidad de las investigaciones desarrolladas y expuestas en esta Memoria así como la formación científica adquirida por Dª. Laura Sofía Torres Valenzuela, autorizamos la presentación de esta Tesis Doctoral.

Córdoba, 20 de Enero de 2020

Firma de los directores

RUBIO BRAVO MARIA SOLEDAD - 30428928C

Firmado digitalmente por RUBIO BRAVO MARIA SOLEDAD - 30428928C
Nombre de reconocimiento (DN): c=ES, serialNumber=IDCES-30428928C, givenName=MARIA SOLEDAD, sn=RUBIO BRAVO, cn=RUBIO BRAVO MARIA SOLEDAD - 30428928C
Fecha: 2020.01.22 15:33:57 +01'00'

Firmado:

Prof. Soledad Rubio Bravo

BALLESTEROS GOMEZ ANA MARIA - 30968640Z

Digitally signed by BALLESTEROS GOMEZ ANA MARIA - 30968640Z
Date: 2020.01.22 16:07:17 +01'00'

Firmado:

Dra. Ana María Ballesteros Gómez

Agradecimientos

Quiero expresar mis agradecimientos a:

Mi familia que ha sido y será mi motor y razón de ser. En cada iniciativa que he emprendido he contado con el apoyo de mis padres y hermano. Gracias por acompañarme, cuidarme y enseñarme valores familiares que han hecho de mí la persona que soy y que han aportado a alcanzar esta meta, que es de todos. Gracias por aceptar y apoyar mis ausencias, aún en los momentos en que más me necesitaban. A Simón y Luciana por ser mi polo a tierra.

A quien fue mi amigo, mi novio y ahora será mi esposo. Fher, gracias por todo el apoyo, las sonrisas y el amor, gracias por ese espíritu alegre, noble y bonito que me contagia y me motiva a ser mejor cada día, gracias por este nuevo proyecto llamado Familia.

La Asociación Universitaria Iberoamericana de Postgrados, entidad que financió mi formación de doctorado y la Universidad La Gran Colombia, en cabeza del rector Dr. Jaime Bejarano, Vicerrectora Dra. Bibiana Medina y Decana Ing. Ximena Cifuentes, quienes permitieron que este proceso se gestara.

A mis compañeros de la “Facu”, especialmente a Johanna, Francia y Aleja quienes me han apoyado decididamente, a LuisMi por los títeres, a John Edward y Johan por los informes del tiempo; gracias por acompañarme aún a miles de kilómetros y un océano de distancia.

A la Dra. Soledad Rubio, quien me recibió en su grupo de investigación, gracias porque a pesar de que no ser “doctorando ideal” me permitió aprender, tener una nueva visión y aplicar el conocimiento a lo que para mí es una pasión y un orgullo: el café de mi Colombia.
A Ana Ballesteros, porque me recibió en el camino y me apoyó incansablemente, sus enseñanzas las llevaré para siempre a nivel personal y profesional.

A mis compañeros del grupo SAC, a Francesca por enseñarme a sintetizar mis primeros SUPRAS, a Noelia y Diego por sus enseñanzas, a Sole, Maria Jesús, Encarni y José Ángel por hacer el proceso más alegre.
A mis compañeros “Latinos” con quienes se inició este camino. Al profesor Arturo Chica por ser el padre de todo este proceso, que está haciendo realidad nuestros proyectos.

A mi Familia Española, gracias por darme el calor de hogar, por sus enseñanzas y cariño. A los “amigos de los viernes”, por las sonrisas y las amenas charlas.

A Edwin Noreña, productor del mejor café que he tomado, gracias por sus enseñanzas y proveerme las materias primas necesarias para esta tesis. A Sergio de la tienda Expedición café por iniciarme como chapolera y como barista de café.

A los trabajadores del campo, que con el fruto de su esfuerzo y el trabajo de sus manos hacen que cada día me sienta más orgullosa de mi Tierra, por y para los cafeteros colombianos.

A Hortensia, la flor que llenó mi vida de

ESPERANZA

A mi Abuelito Juan,

quien retó mi "chirimoya" a continuar aprendiendo

ÍNDICE

OBJETIVOS

La industria agroalimentaria genera billones de toneladas de subproductos y residuos anualmente. Existe un interés creciente en la valorización de esta biomasa mediante la recuperación de compuestos bioactivos para el desarrollo de alimentos ¨funcionales¨y nutraceúticos. Solamente la industria del café genera alrededor de 2 billones de toneladas de residuos agrícolas anualmente, tales como cascarilla, pulpa, aguas residuales y borras, lo cual representa una amenaza para el medio ambiente. En este ámbito y con el fin de afrontar uno de los grandes retos de la química verde, existe una creciente demanda de desarrollo y aplicación de disolventes alternativos a los disolventes orgánicos convencionales. Se persigue así la finalidad de incrementar el rendimiento en los procesos de extracción y de reducir los costes, toxicidad y el impacto ambiental asociados.

Las investigaciones desarrolladas en esta Tesis Doctoral han tenido como objetivo general la síntesis y aplicación de disolventes supramoleculares (SUPRAS) para la recuperación de sustancias bioactivas a partir de biomasa procedente de residuos del café.

Los objetivos específicos de esta Tesis fueron:

- Identificar mediante un estudio bibliográfico los principales tipos de residuos valorizables del café, las técnicas de extracción verdes existentes y sus principales ventajas y desventajas con el fin de proponer metodologías de valorización alternativas basadas en SUPRAS y adecuadas a las necesidades de mercado identificadas en este estudio (biocompatibilidad, eficiencia, bajo coste, etc.).

- Desarrollo de SUPRAS biocompatibles basados en componentes autorizados en alimentos con el fin de facilitar su posterior implementación industrial (alimentos, cosméticos o nutraceúticos) para la valorización de residuos sólidos y líquidos del café.

- Comparación y optimización de los componentes del SUPRAS y de los parámetros de extracción en función del tipo de matriz (sólida: borras y cáscara) y líquida (aguas residuales de café) para maximizar la extracción de los biocomponentes mayoritarios en los residuos de café (polifenoles y alcaloides).

- Caracterización de los extractos de SUPRAS enriquecidos con compuestos bioactivos: identificación de los componentes bioactivos secundarios por cromatografía de líquidos y espectroscopia de masas en tándem, medida de la capacidad oxidante y antimicrobiana y/o estudio de la estabilidad de los compuestos bioactivos en el extracto de SUPRAS.

- Estudio de la mejora de las propiedades físico-químicas de las aguas residuales del café tratadas con SUPRAS.

Además, han constituido un objetivo transversal y fundamental en la realización de esta Tesis Doctoral la formación de la doctoranda a través de actividades complementarias a la labor investigadora, tales como la asistencia y presentación de comunicaciones en congresos, la discusión crítica de los resultados, redacción de artículos científicos, etc.

CONTENIDO

El contenido de la Memoria de esta Tesis Doctoral se ha estructurado en cuatro Capítulos, precedidos de una Introducción en la que se desarrollan aspectos teóricos y prácticos de interés para la síntesis y aplicación de los SUPRAS en procesos de extracción. Adicionalmente, se presentan los aspectos más relevantes del proceso de producción y transformación del café, los residuos generados y las metodologías actuales para su aprovechamiento.

Los contenidos de los cuatro capítulos en los que se ha divido esta Memoria son los siguientes:

Capítulo 1. Disolventes verdes para la extracción de compuestos con alto valor añadido a partir de residuos agroalimentarios

En la industria agroalimentaria se producen grandes cantidades de subproductos, fracciones no comestibles y residuos de la producción de alimentos, desde la etapa de recepción de las materias primas hasta el consumo final. La valorización de esta biomasa para obtener compuestos de alto valor añadido ha sido objeto de múltiples investigaciones en la última década. El uso de metodologías sostenibles en este ámbito es esencial para reducir los impactos de las mismas en la salud y el medio ambiente. En el capítulo 1 de esta Memoria se incluye un estudio de revisión crítica en el que se presentan los principales tipos de disolventes verdes empleados hasta la fecha para valorización de residuos y subproductos agroalimentarios y se discute su potencial para reemplazar a los disolventes orgánicos convencionales, con el fin de disponer de procesos más sostenibles y menos contaminantes. Se revisa así el uso de fluidos supercríticos, disolventes neotéricos (líquidos iónicos y disolventes eutécticos), bio-disolventes y disolventes supramoleculares. Se analizan los parámetros que afectan la eficiencia de extracción, así como las ventajas y limitaciones que presenta cada tipo de disolvente para su uso a escala industrial.

Capítulo 2. Valorización de borras de café mediante la extracción de compuestos bioactivos con disolventes supramoleculares

En este capítulo se presenta el estudio llevado a cabo para la evaluación del potencial de los disolventes supramoleculares (SUPRAS) para la extracción de los compuestos

bioactivos presentes en borras, principal residuo de la preparación de la bebida de café. Para ello se investigó el uso de SUPRAS constituidos por agregados hexagonales inversos sintetizados a partir de ácido decanoico o 1-hexanol en fases hidro-orgánicas (etanol-agua y tetrahidrofurano-agua). El proceso de extracción con los cuatro SUPRAS investigados se optimizó a partir del rendimiento obtenido en la extracción de cafeína y ácido clorogénico (5-CGA). Los rendimientos de extracción para cafeína (3.32 mg g^{-1}) y 5-CGA (4.3 mg g^{-1}) fueron máximos para SUPRAS sintetizados a partir de 1-hexanol en mezclas de etanol-agua. Para estos extractos, se determinó el perfil de compuestos bioactivos, mediante cromatografía líquida acoplada a espectrometría de masas en tándem. Adicionalmente se evaluó el contenido total en compuestos fenólicos y las propiedades antioxidantes y antimicrobianas de los extractos. Éstos presentaron elevada capacidad antioxidante, determinada mediante los métodos ABTS, DPPH y FRAP, que fue concordante con su alto contenido en polifenoles totales (60.1 mg 5-CGA / mg). Los extractos presentaron también efecto antimicrobiano frente a S*. aureus, B. cereus, S. enterica y P. putida*. La extracción de compuestos bioactivos con SUPRAS presentó ventajas en términos de rapidez (1 minuto de extracción) y simplicidad del proceso (sólo se requiere agitación y centrifugación a temperatura ambiente). Se evitan así los elevados costos asociados al uso de alta temperatura o presión que son generalmente requeridas en la valorización de residuos de café utilizando extracción con disolventes orgánicos convencionales o fluidos supercríticos.

Capítulo 3. Extracción de compuestos bioactivos de pulpa de café empleando disolventes supramoleculares

Se investigó el potencial de los SUPRAS para la extracción de compuestos bioactivos presentes en la pulpa de café, que es uno de los mayores subproductos generado en la transformación primaria del café por las vías semi-seca y húmeda. Se emplearon SUPRAS de agregados hexagonales inversos de ácido octanoico y decanoico sintetizados en mezclas de etanol-agua, producidos espontáneamente a través de auto-ensamblaje y coacervación. Los SUPRAS generados se aplicaron a la extracción de cafeína y ácido protocatéquico hallándose que el rendimiento de extracción fue mayor para los SUPRAS formados por ácido octanoico (3.6±0.3 mg g^{-1} para cafeína y 0.9±0.1 mg g^{-1} para ácido protocatéquico). El proceso implicó la extracción de la muestra con un volumen reducido de disolvente (relación muestra:disolvente 1:4) siguiendo un procedimiento simple (5 min de agitación y 10 min de centrifugación a temperatura

ambiente). Finalmente, se determinó el perfil de alcaloides y compuestos fenólicos en los extractos de SUPRAS (cromatografía liquida acoplada a espectrometría de masas en tándem) así como las propiedades antioxidantes (45% por DPPH y 91% por ABTS). Se constató también que la eficiencia de extracción empleando SUPRAS fue significativamente superior respecto a la obtenida empleando disolventes orgánicos convencionales, como son metanol, etanol, acetona y acetonitrilo.

Capítulo 4. Disolventes supramoleculares para la extracción y pre-tratamiento de aguas residuales de la transformación primaria del café

En este último Capítulo se presentan los resultados obtenidos en el estudio de la aplicación de los disolventes supramoleculares (SUPRAS) a la recuperación de compuestos bioactivos de aguas residuales del café, un residuo abundante en los métodos de procesamiento por vía húmeda y semi-húmeda. Para esta aplicación, se investigó el uso de SUPRAS constituidos por agregados hexagonales inversos de 1-hexanol o ácido decanoico, sintetizados *in situ* en el agua residual mediante la adición del compuesto anfifilico y etanol. El proceso de extracción, evaluado con o sin agitación, generó una recuperación de cafeína entre 53 y 64 mg por litro de agua residual. Los extractos de SUPRAS presentaron buena capacidad antioxidante (52% por el método ABTS) y el contenido en cafeína de los mismos fue estable durante su almacenamiento (4 – 24 ºC durante 2 meses). Adicionalmente, el proceso de extracción con SUPRAS mejoró algunos parámetros de calidad del agua, como la demanda bioquímica de oxígeno, conductividad y sólidos suspendidos totales, por lo que la valorización del agua residual produce simultáneamente un pretratamiento de la misma.

A continuación se presentan las conclusiones que pueden extraerse de los resultados obtenidos y finalmente, se incluyen los siguientes anexos: (A) artículos publicados en revistas internacionales derivados de esta Tesis con índices de calidad (B) contribuciones a congresos nacionales e internacionales derivados de esta Tesis y (C) otros artículos científicos, capítulos de libro y contribuciones a congresos derivados de investigaciones complementarias al desarrollo de la Tesis y relacionados con el aprovechamiento de los residuos del café.

1 INTRODUCCIÓN

1.1 La industria del café

El café es uno de los productos más consumidos, solamente precedido del agua (1). Se consume una media de 2.25 billones de tazas de café diariamente en el mundo (2), por lo que se considera una de las materias primas más comercializadas (3). Los principales consumidores son las economías industrializadas, principalmente Europa y Estados Unidos (4). El café se cultiva en alrededor de 80 países, de los que el 90% son países en vía de desarrollo, por lo que su producción tiene una importancia social relevante (2). Destacan como zonas productoras las áreas tropicales de África, Java, Sumatra, India, Islas del Pacífico, México, Centro y Sur América (1).

Los granos de *café verde* son producidos por la planta denominada cafeto, perteneciente al sub-reino *Angiospermae,* familia botánica *Rubiaceae,* la cual está formada por cerca de 6,000 especies (4). La *Figura 1* recopila las especies de mayor importancia económica a nivel mundial. Las variedades Arábica (*Coffea arabica*) y Robusta (*Coffea canephora*) son las predominantes en los mercados internacionales (5). La variedad Arábica constituye más del 70% del café comercializado en el mundo (6), debido a las excelentes características organolépticas de la bebida que se asocian a la composición química del grano. (7) Esta variedad se cultiva principalmente en Colombia, Brasil, India, Kenia y Etiopía (8). El resto de la producción de café corresponde principalmente a la variedad Robusta que es producida principalmente en África, Brasil e Indonesia (9).

Algunas de las diferencias más representativas entre estas dos variedades se presentan en la *Tabla 1*.

Tabla 1. Principales diferencias entre las variedades de café arábica y robusta

Característica	Arábica	Robusta
Altura en la producción agrícola	0 – 700 msnm	1000 – 2000 msnm
Temperatura óptima	15 – 24 ºC	24 – 30 ºC
Porcentaje de la producción a nivel mundial	70%	30%
Resistencia a plagas	Baja	Alta
Requerimientos agronómicos para la producción	Altos	Bajos
Contenido de cafeína	0.8 – 1.4%	1.7 – 4.0%
Calidad de la taza	Alta	Baja
Precio de comercialización	Mayor	Menor

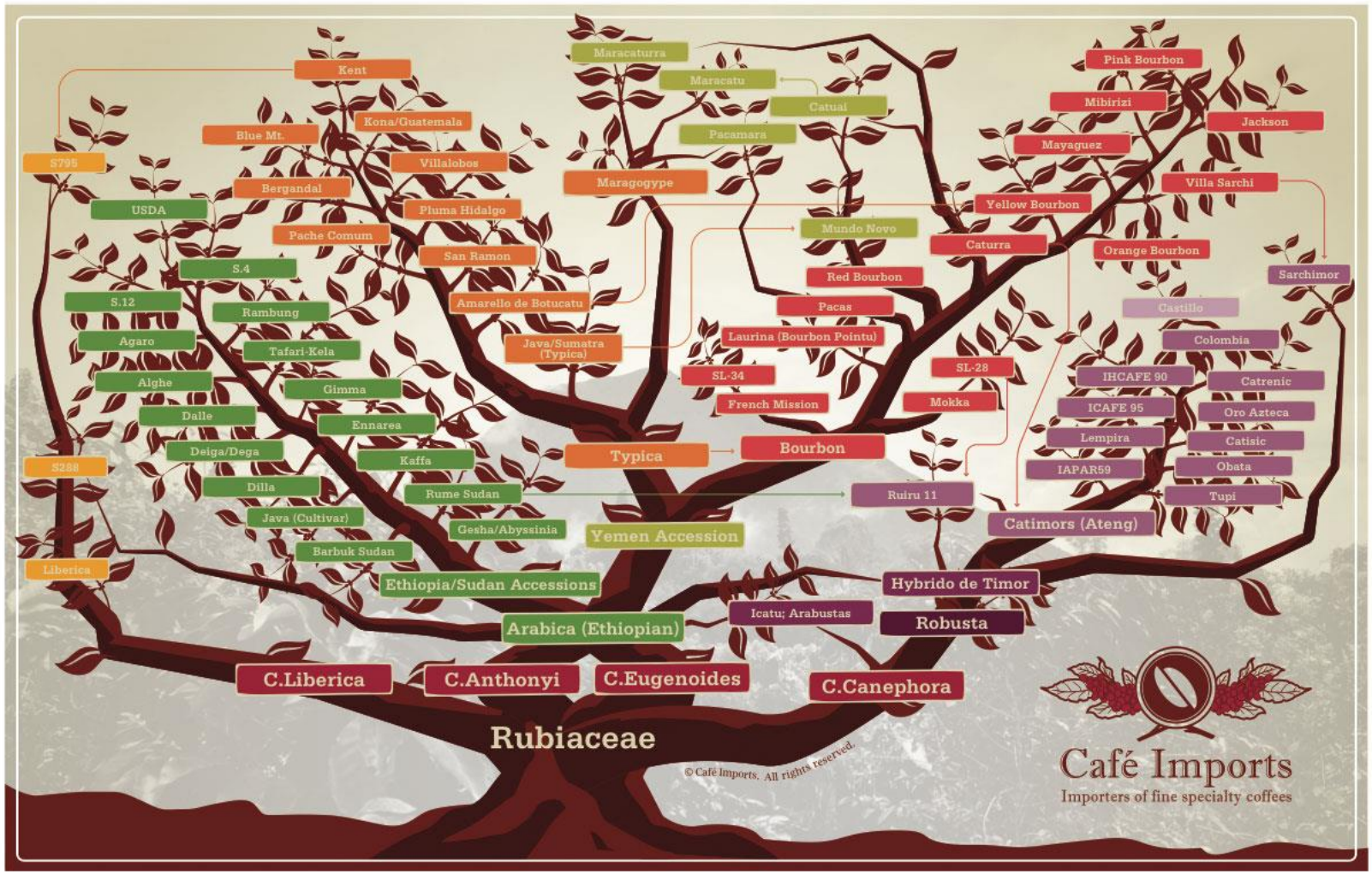


Figura 1. Árbol de variedades del café

Fuente: www.sprudge.com

Las especies de café Arábica presentan diferentes variedades, tales como Maragogipe, Bourbon, Tabi, Típica, Castillo, Caturra y Colombia. Las variedades Castillo, Caturra y Colombia son las que se cultivan en mayor proporción en Colombia.

Como se indicó anteriormente, el cultivo tiene importancia económica y social, principalmente en los países en vía de desarrollo. En el caso de Colombia, de acuerdo a lo reportado por el comité de cafeteros, entidad que asocia los productores a nivel nacional, hay 560,000 fincas dedicadas a la producción de café, con una ocupación de 948,000 hectáreas. El cultivo de café genera 785,000 empleos directos y 1.5 millones de empleos indirectos (www.federaciondecafeteros.org). Debido al impacto social y económico del café y a las características de la producción en el territorio colombiano, en el año 2011 el Comité de Patrimonio Mundial de Organización de las Naciones Unidas para la Educación, la Ciencia y la Cultura – Unesco, inscribió el *Paisaje Cultural Cafetero Colombiano* en la lista de Patrimonio Mundial. Para asegurar su conservación, el Consejo Nacional de Política Económica y Social (CONPES) de Colombia estableció la "Política para la preservación del *Paisaje Cultural Cafetero de Colombia".* Entre las estrategias propuestas destaca el fomento de la caficultura y la promoción e impulso del cultivo de cafés especiales en la región "con el objetivo de dinamizar la actividad cafetera y la generación de valor agregado y aumentar de esta manera la rentabilidad y sostenibilidad en el negocio del café" (10).

Los cafés especiales provienen de granos de excepcional calidad, cosechados de los mejores cultivos de *Coffea arabica* que se procesan para potenciar su sabor. Los atributos sensoriales de la bebida son evaluados por jueces certificados o catadores, de acuerdo a un protocolo definido por la Sociedad Americana de Cafés Especiales (SCA) (11). Las características sensoriales de este tipo de bebida se asocian a la percepción sensorial de una compleja variedad de compuestos químicos. Se han identificado más de 1,000 compuestos aromáticos en el café (12), más de los identificados en el vino, en el cual se han analizado 600 volátiles. Las características del terreno, el tipo de cultivo y la especie de café generan las características sensoriales primarias, mientras que el procesamiento post-cosecha genera el perfil sensorial final. Ambos factores confieren características distintivas a estos cafés en términos de cuerpo, aroma y sabor. La industria del café especial se ha introducido en el mercado de las *bebidas finas*, por lo cual los consumidores pagan precios elevados para apreciar el café por su origen y características individuales (11), generando de esta manera valor agregado a la agrocadena del café. Este tipo de café

representa un mercado creciente a nivel mundial. En Estados Unidos el 59% del café consumido corresponde a esta categoría (13).

En esta Tesis se han propuesto técnicas de valorización de residuos del procesamiento de café especial cultivado en Colombia (variedad Castillo). Se pretende así, proponer estrategias simples y poco costosas que añadan un valor agregado a la agrocadena del café y que ayuden a promover el mercado y aprovechamiento del café especial. Se cumple así con el marco de la "Política para la preservación del *Paisaje Cultural Cafetero de Colombia*", antes mencionado.

1.1.1 Producción de café verde

La primera etapa del proceso para la obtención del café es la cosecha, que se considera un paso importante para garantizar la calidad en la taza, debido a que esta se asocia con el estado de madurez del fruto. Los frutos maduros generan tazas de alta calidad, mientras los frutos verdes y/o sobremaduros disminuyen los atributos sensoriales en la bebida (1).

El proceso de maduración de los frutos no es homogéneo, es decir que en una misma planta se pueden encontrar frutos en diferentes estados de madurez. Esto es relevante para el proceso de cosecha, que puede realizarse de manera manual o mecánica. La cosecha manual se realiza recolectando de manera individual cada fruto, mientras la cosecha mecánica se realiza agitando la planta con un equipo que se acopla a las ramas o al tallo. El primer método es más costoso y requiere más tiempo, sin embargo se generan productos de mayor calidad, dado que se puede seleccionar la calidad y estado de madurez, mientras en el sistema mecanizado no se puede realizar el proceso de selección (1).

Los frutos del café están compuestos por la cáscara (o pericarpio), pulpa (o mesocarpio), mucílago (o capa de pectina), pergamino (o endocarpio), cascarilla plateada y en el centro del fruto se encuentra el grano de café, que es el endospermo del fruto (14). La Figura 2 presenta la composición del grano de café.

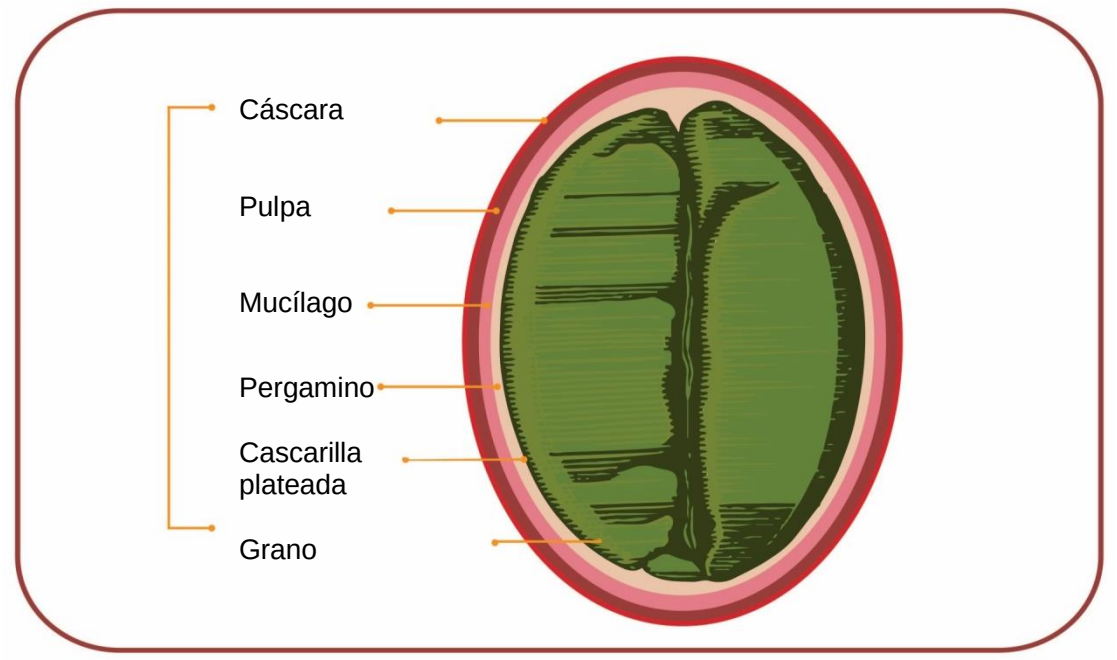


Figura 2. Composición del grano de café

El grano es la parte del fruto que se emplea para la posterior preparación de la bebida. La transformación primaria se realiza para separar los granos del resto del fruto, eliminando las capas externas. Los métodos más comunes para este propósito se denominan método húmedo y seco. La

Figura 3 presenta el diagrama de flujo de la transformación primaria del café.

En el procesado por vía seca, los frutos de café son secados al sol. Ocasionalmente se puede realizar una segunda etapa de secado (artificial), seguido de una etapa de descascarado, en la cual se elimina la pulpa, el mucílago, el pergamino y parte de la cascarilla plateada. El total de estos residuos se denomina en inglés *husk* (14). En el procesamiento húmedo se hace una primera etapa de separación por calidad y estado de madurez, empleando agua en la cual por diferencia de densidad los frutos maduros se van al fondo del tanque, mientras los verdes y con defectos quedan en la superficie (5). Posteriormente, la cáscara y la pulpa se eliminan empleando una máquina despulpadora (a esta fracción se le denomina pulpa). El despulpado es seguido de una etapa de fermentación, en la cual el mucílago y los restos de pulpa son eliminados. Por último los granos se secan y finalmente se realiza el proceso de trilla en el cual se elimina el

pergamino (5,14). En este proceso se genera un gran volumen de agua residual, proveniente de los procesos de lavado y fermentación.

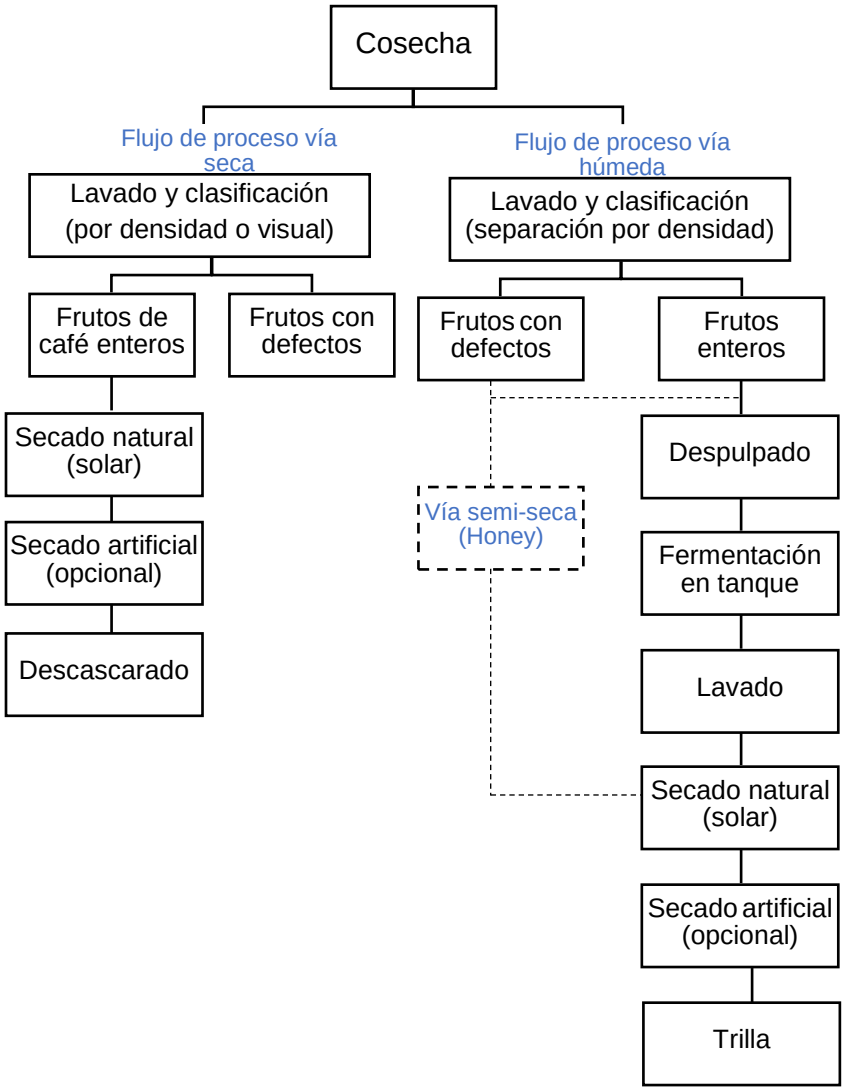


Figura 3. Diagrama de proceso de la transformación primaria de café

Recientemente se ha implementado un tercer proceso denominado semi-seco, también conocido como "*honey",* en cual los frutos se despulpan, pero se elimina la etapa de fermentación (5).

La etapa final del proceso de transformación (independiente del método de procesamiento) es el proceso de tostado, en el cual se eliminan los restos de la cascarilla plateada y se desarrollan los atributos de sabor, aroma y color característicos del café (15).

1.1.2 Residuos generados en la producción de café

La industria del café genera alrededor de 2 billones de toneladas de residuos, los cuales representan un alto riesgo de contaminación (16). Por cada 100 Kg de grano de café verde se producen 39 Kg de pulpa, 39 Kg de pergamino y cascarilla plateada y 22 Kg de mucílago. (17). Dependiendo del sistema de procesado del café también se generan altos volúmenes de aguas residuales, que se han estimado en 40–45 L/ kg de café en la vía húmeda (18). Aproximadamente el 40% del café mundial es producido por vía húmeda.

Finalmente, el residuo asociado a la producción de café instantáneo y preparación de bebidas de café, denominado borras (¨*spent coffee grounds*¨ en inglés) genera una cantidad relevante de residuos. En el caso de la industria de cafés solubles se producen 4.5 toneladas de borras por año (4) y la producción total de borras ha sido reportada en 6 millones de toneladas por año (19).

En la Tabla 2 se presentan los principales residuos de café en las distintas etapas de transformación y consumo final.

Tabla 2. Residuos generados en la industria del café (post-cosecha)

	Transformación primaria		**Transformación secundaria**	**Consumo final**
Procesamiento seco	**Procesamiento húmedo**	**Procesamiento semi-seco**	**Tostado**	**Preparación de la bebida**
¨Husks¨	Pulpa	Pulpa	Cascarilla plateada	Borras
Pergamino	Agua	Agua		
	Pergamino	Pergamino		

En los residuos sólidos del café se encuentran diferentes compuestos químicos de interés dentro de los cuales encontramos los alcaloides y los compuestos fenólicos (14,20–23). Las Tabla 3 y Tabla 4 muestran la composición química de los residuos sólidos del café.

Tabla 3. Composición química de la pulpa de café

	Armas, 2008 (24)	Murthy and Naidu (2012) (15)	Janissen and Huynh (2018) (22)	Preedy (2015) (1)
Humedad	N.D.	N.D.	81.4	N.D.
Proteína	13.30	11.5 ± 2.0	10 -12	4 – 12
Lípidos	1.73	2.0 ± 2.6	2.5	1 – 2
Fibra	N.D.	60.5 ± 2.9	18 - 21	N.D.
Carbohidratos	N.D.	N.D.	44-50	45 - 89
Azúcares totales	4.10	N.D.	N.D.	N.D.
Azúcares reductores	12.40	N.D.	N.D.	N.D.
Azúcares no reductores	2.00	N.D.	N.D.	N.D.
Ceniza	9.70	N.D.	8.9	6 – 10
Lignina	19.30	17.5 ± 2.2	N.D.	N.D.
Celulosa	18.30	63 ± 2.5	63	N.D.
Hemicelulosa	N.D.	2.3 ± 1.0	2.3	N.D.
Polifenoles	2.90	1.5 ± 1.5	N.D.	N.D.
Taninos	1.8-8.56	3.0± 5.0	1.8 – 8.6	1 – 9
Cafeína	1.30	1.5 ± 1.0	1.2 – 1.3	1
Ácido clorogénico	2.60	2.4 ± 1.0	10.7	N.D.
Ácido cafeico	1.60	N.D.	N.D.	N.D.

Valores expresados en porcentaje, base seca; N.D.: no determinado

Tabla 4. Composición química de las borras de café

	Jiménez-Zamora (2015) (3)	Ballesteros (2014) (25)	Janissen and Huynh (2018) (22)
Humedad	N.D.	N.D.	11.69
Proteína	13.6 ± 1.3	17.4 ± 0.1	13.6
Lípidos	1.6 ± 0.3	2.3 ± 0.3	6
Fibra	54.6 ± 6.3	60.4 ± 2.1	60.5
Carbohidratos	71.4 ± 6.3	N.D.	82
Azúcares totales	N.D.	N.D.	N.D.
Azúcares reductores	N.D.	N.D.	N.D.
Azúcares no reductores	N.D.	N.D.	N.D.
Ceniza	1.5 ± 0.2	1.3 ± 0.1	1.6
Lignina	N.D.	23.9 ± 1.7	N.D.
Celulosa	N.D.	12.4 ± 0.8	8.6
Hemicelulosa	N.D.	39.1 ± 1.9	36.7
Polifenoles	19.2 ± 2.3	N.D.	N.D.
Taninos	N.D.	N.D.	0.02
Cafeína	N.D.	N.D.	0.4
Ácido clorogénico	1.18 ± 0.07	N.D.	11.45
Ácido cafeico	N.D.	N.D.	N.D.

Valores expresados en porcentaje, base seca; N.D.: no determinado

En general, la disposición inadecuada de los residuos de café genera impactos ambientales negativos, asociados a su composición química y su alta acidez (15). Respecto a las aguas residuales generadas en la vía de procesamiento húmedo, tienen un alto contenido en sólidos que generan colores oscuros, adicionalmente contienen cafeína, azúcares, proteínas y compuestos fenólicos (2,24–26), por lo cual son susceptibles a la fermentación y a la generación de olores desagradables (24).

Cuando los residuos de café se vierten a corrientes hídricas se agota el oxígeno del agua, lo cual genera la asfixia de los organismos acuáticos. Por otro lado, la descarga de nutrientes puede generar eutrofización (27). Además, el vertido de estos residuos sobre los suelos (o el uso de las aguas residuales para riego) puede afectar negativamente al crecimiento de las especies vegetales. Estudios previos han demostrado la fitotoxicidad y citotoxicidad de las aguas residuales de la industria del café y la inhibición total de la germinación en lechugas (28).

Con el fin de evitar impactos ambientales negativos y de generar un valor añadido en la agrocadena del café se han propuesto diversas estrategias de valorización de los residuos del café. La pulpa, la cáscara y las borras de café han sido objeto de valorización con distintos fines tales como su uso como sustrato de cultivo para cultivo de hongos (29), sistemas para inmovilización de enzimas (30), producción de etanol (31) o compostaje (32). Otra importante vía de valorización es la recuperación de compuestos bioactivos tales como alcaloides y polifenoles para su uso en la industria alimentaria, farmacéutica y cosmética (33–36). La cafeína, como principal alcaloide en estas matrices, tiene efecto anti-inflamatorio e inmunosupresor (36). Por su parte los compuestos fenólicos tienen efecto antioxidante, antibacteriano, antiinflamatorio y actividad anticancerígena (33–35).

La Tabla 5 presenta una recopilación bibliográfica de los distintos usos que se han dado a los residuos del café para su valorización.

Tabla 5. Alternativas de aprovechamiento de residuos del café

Subproducto	Proceso	Uso final	Ref.
Pulpa fresca	Troceado	Alimentación animal	(37,38)
			(39)
Pulpa fresca y seca	Extracción con agua caliente	Extracción de compuestos antioxidantes y antimicrobianos	(40)
Pulpa fresca	Fermentación	Producción de etanol	(41,42)
Pulpa fresca y seca	Extracción con etanol asistida por ultrasonidos	Extracción de compuestos con actividad antioxidante	(43)
Pulpa fresca	Pre-tratamiento con *Mycotypha sp*. y biometanización	Producción de Biogás	(44)
	Pre-tratamiento con *Streptomyces sp.* y biometanización		(45)
Pulpa fresca y seca	Co-digestión y biometanización		(46–50)
Pulpa fresca	Compostaje con *Trichoderma* sp., *Streptomyces* sp. *Azotobacter* sp. y *Bacillus* sp.	Producción de compost	(51)
Pulpa fresca	Fermentación	Extracción de compuestos bioactivos (ácidos clorogénicos)	(52)
Pulpa fresca y mucílago	Fermentación	Producción de etanol	(53)
Pulpa de café (husk) y pulpa seca	Hidrólisis y esterilización; fermentación con *Rhodotorula mucilaginosa*	Extracción de compuestos bioactivos (carotenoides)	(54)
Pulpa de café seca	Extracción con fluidos supercríticos (CO_2)	Extracción de compuestos bioactivos (cafeína)	(55)
Pulpa de café	Combustión en horno y en sistema de lecho fluidizado	Producción de energía a través de pirólisis	(56)
Pulpa de café	Compostaje con *Eudrilus eugeniae* y *Trichoderma viride*	Producción de compost	(57)
Cascarilla plateada	Extracción con agua sub-crítica	Extracción de compuestos fenólicos y antioxidantes	(58)
	Hidrólisis enzimática	Producción de prebióticos y fibra alimentaria	(59)
Pergamino	Extracción con agua	Uso medicinal para inhibición de hialuronidasa (supresión	(60)

		de procesos inflamatorios o alergias)	
	Extracción con etanol	Extracción de compuestos bioactivos (ácido gálico, clorogénico, cumárico y sinápico y otros antioxidantes). Producción de aditivo antifúngico.	(61)
Pergamino	Hidrólisis y fermentación	Producción de etanol	(62)
Pergamino	Hidrólisis y biometanización	Producción de biogás	(63)
Pergamino	Pirólisis a través de calentamiento convectivo y con microondas	Producción de sintegas (H_2 + CO)	(64)
Hojas, cáscara, cascarilla plateada, pergamino y borras	Fermentación	Producción de hongos *Pleurotus florida*	(65)

En esta Tesis se proponen alternativas sostenibles para la extracción de compuestos bioactivos para el aprovechamiento de residuos de la producción de café especial obtenido a partir de café Arábica, variedad Castillo, cultivada en el Municipio de Circasia, Colombia (4°37′09″N 75°38′05″). Este municipio está incluido en la declaración de *Paisaje Cultural Cafetero Colombiano*. Los granos de café se cultivaron de manera manual y se procesaron inmediatamente por vía húmeda y secado al sol. Como residuos del procesamiento primario se investigaron las cáscaras (o pulpa) obtenida en la etapa de despulpado y las aguas residuales del proceso de lavado. Adicionalmente, se utilizaron las borras generadas después de la preparación de la bebida, empleando el método de preparación denominado filtrado por goteo en un sistema v60. Se utilizó café de tostado medio y molienda media (65% del café pasa a través del tamiz # 20) y agua a 96 ºC. La bebida se preparó en una relación 1:18 café:agua.

1.2 Técnicas empleadas para la extracción de compuestos bioactivos de café

La extracción de compuestos bioactivos es una de las estrategias de valorización más empleadas en el aprovechamiento de residuos de la industria cafetera. La eficiencia de extracción depende fundamentalmente del tipo de materia prima (o subproducto), el tipo de disolvente, las condiciones de extracción como temperatura, presión y tiempo y el uso de energías auxiliares tales como ultrasonidos, microondas, presión, etc. La Tabla 6 presenta una recopilación de los procesos de extracción empleados para la valorización de borra, pulpa, cascarilla plateada y pergamino de café.

Tabla 6. Técnicas para la extracción de compuestos bioactivos de residuos de café

Residuo	Condiciones óptimas de extracción	Compuesto(s) bioactivo(s), actividad biológica	Rendimiento	Ref.
Borras	Extracción con metanol (60% v/v), 90 minutos, relación muestra (g):solvente (mL) 1:20	Polifenoles totales, actividad antioxidante	16 mg GAE g^{-1} 0.10 mM Fe(II) g^{-1}	(19)
Borras	Extracción Soxhlet con etanol, 2 horas, relación muestra (g):solvente (mL) 1:30	Cafeína, ácido clorogénico, polifenoles totales, actividad antioxidante	Rendimiento global de extracción 15 ± 2%. Actividad antioxidante 90.3%, 151 ± 12 mg GAE g^{-1}	(4)
Borras	Extracción sólido – líquido con etanol :agua (70% v/v), 50 ºC, 120 minutos, relación muestra (g):solvente (mL) 1:40	Polifenoles totales	19.98 mg GAE g^{-1}.	(66)
Borras	Extracción con agua a 90 ºC, 6 minutos, relación muestra (g):solvente (mL) 1:17	Cafeína	13.24 mg cafeína g^{-1}	(67)
Borras	Extracción asistida por microondas con etanol:agua (20% v/v), 40 segundos, relación muestra (g):solvente (mL) 1:9	Polifenoles totales, actividad antioxidante	Actividad antioxidante 90.69%, 398.95 mg GAE g^{-1}	(68)
Borras	Extracción asistida por ultrasonido con solventes eutécticos (cloruro de colina:1,6-hexanediol), 45 minutos, relación muestra (g): solvente (mL) 1:17.	Polifenoles totales, flavonoides totales, ácidos clorogénicos y actividad antioxidante	5 mg CQA g^{-1}, 15 mg GAE g^{-1}, 18 mg CE g^{-1} y 21 mg TE g^{-1}	(69)
Borras	Extracción con agua subcrítica a 50 bar y170 ºC, 30 minutos, relación muestra (g): solvente (mL) 1:50	Polifenoles totales, actividad antioxidante y 5-CQA	56.6 mg GAE g^{-1} 32.3 mmol TE/100g 1.4 mg 5-CQA g^{-1}	(70)
Borras	Extracción con agua a 92 ºC, 5 minutos, relación muestra (g): solvente (mL) 1:20	Melanoidinas y antioxidantes	25 g melanoidinas 100 g^{-1}, 500 mmol trolox g^{-1}	(3)
Borras	Extracción con líquidos presurizados, agua:etanol 70% (v/v), 160 ºC a 103 bar, 10 minutos, relación muestra (g): solvente (mL) 1:30	Polifenoles totales, cafeína y antioxidantes	22.7 mg GAE g^{-1} 9.2 mg cafeína g^{-1} 10 – 28 mg ácido ascórbico g^{-1}	(71)
Pulpa	Extracción Soxhlet con etanol y ultrasonidos, 2 horas, relación muestra (g): solvente (mL) 1:30	Capacidad antioxidante	Rendimiento global de extracción 3.1 ± 04%	(4)

			Actividad antioxidante: 32%, 587.7 ± 46.6 mg GAE g^{-1}	
Pulpa	Extracción con CO_2 supercrítico a 100 ºC y 300 bar, relación muestra (g):solvente (mL)1:197	Cafeína	84%	(72)
Pulpa	Extracción con agua a 100 ºC, 10 minutos, relación muestra (g): solvente (mL) 1:200	5-CQA, cafeína, polifenoles totales y flavonoides totales	1.7 mg CQA g^{-1} 13.9 mg cafeína g^{-1} 15.6 mg GAE g^{-1} 0.9 mg rutina g^{-1}	(73)
Pulpa	Extracción con agua a 85 ºC, 10 minutos, relación muestra (g): solvente (mL) 1:10	Polifenoles totales	9.17 mg GAE g-^{1}	(5)
Pulpa	Extracción con agua, 72 horas, relación muestra: solvente 1:5, 72 horas	Polifenoles totales y antioxidantes	2 mg GAE g-^{1} 3.8 mmol trolox g^{-1}	(74)
Cascarilla plateada	Extracción agua a 100 ºC, 10 minutos, relación muestra (g): solvente (mL) 1:200	5-CQA, cafeína, polifenoles totales y flavonoides	19.4 mg CQA g^{-1} 24 mg cafeína g^{-1} 44.8 mg GAE g^{-1} 3.4 mg rutina g^{-1}	(73)
Cascarilla plateada	Extracción con etanol 60% (v/v), 30 minutos a 80 ºC, relación muestra (g): solvente (mL)1:35	Polifenoles totales, antioxidantes, ácidos clorogénicos y cafeína	10.6 mg GAE g^{-1}, 39.4 μmol TE g^{-1} 2.7 mg CQA g^{-1} 12.1 mg cafeína g^{-1}	(75)
Cascarilla plateada	Extracción con agua subcrítica a 80 ºC (para cafeína y 5-CQA) y a 210 ºC (para 5-HMF), 10 minutos, relación muestra (g): solvente (mL) 1:50	Cafeína, 5-CQA y 5-HMF	4.4 mg cafeína g^{-1} 1.7 mg 5-CQA g^{-1} 6.9 mg 5-HMF g^{-1}	(58)
Pergamino	Extracción agua a 100 ºC, con agitación a 250 rpm, 10 minutos, relación muestra (g): solvente (mL) 1:200	5-CQA, cafeína, compuestos fenólicos y flavonoides	6.1 mg CQA g^{-1} 58.2 mg cafeína g^{-1} 68.2 mg GAE g^{-1} 6 mg rutina g^{-1}	(73)

Abreviaciones: GAE: Equivalentes de ácido gálico; CQA: ácido clorogénico; TE: Equivalentes de trolox, 5-HMF: 5-Hidroximetil furfural

1.3 Disolventes verdes en procesos de extracción

Los disolventes verdes se caracterizan por ser no tóxicos, no volátiles, reciclables, biodegradables y con bajos requerimientos energéticos para su síntesis (76). La Figura 4 presenta los principales tipos de disolventes alternativos que cumplen en diferentes niveles con la definición de disolventes verdes y que han sido empleados para la recuperación de compuestos bioactivos de residuos agrícolas.

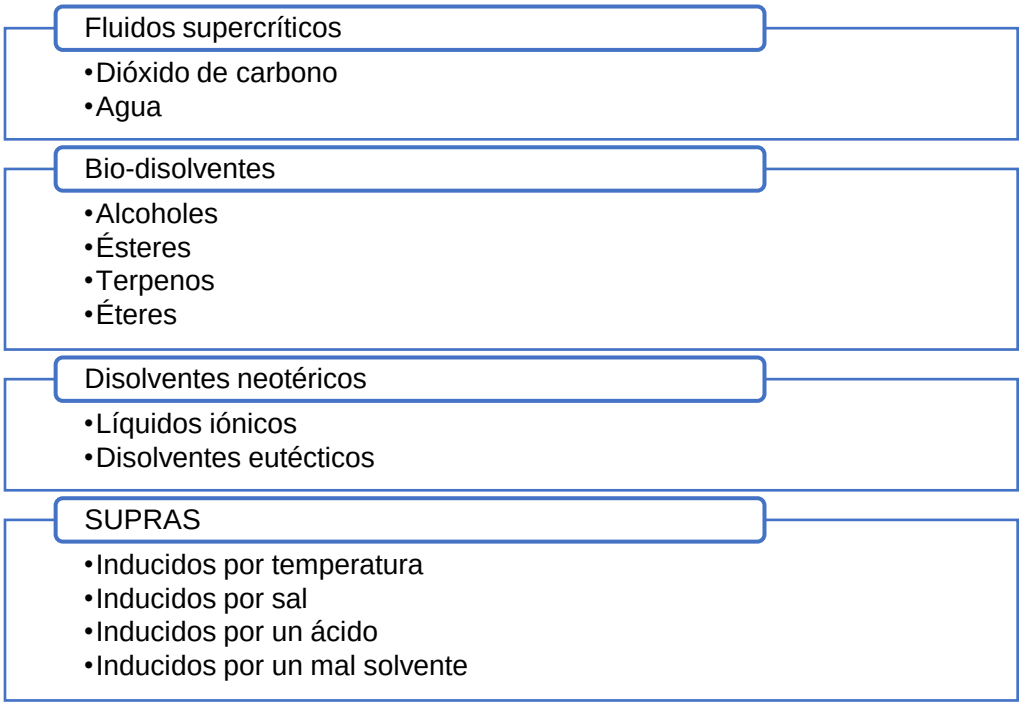


Figura 4. Disolventes verdes empleados en procesos de extracción de compuestos bioactivos de residuos agrícolas

1.3.1 Fluidos supercríticos

Los fluidos supercríticos son sustancias que tienen una presión y temperatura superior a la de su punto crítico (77,78), condición en la que se genera un cambio en sus propiedades (77). En cuanto a su viscosidad y difusividad tienen un comportamiento similar a los gases, pero respecto a su densidad y solvatación presentan las propiedades de un líquido. Este comportamiento favorece los procesos de extracción (78,79) dado que la baja viscosidad y

alta difusividad incrementan los procesos de transferencia de masa, facilitando la penetración del fluido a través de la fase sólida (80). Adicionalmente, la densidad y solubilidad pueden modificarse cambiando la presión y/o temperatura o por la adición de modificadores como disolvente polares, por ejemplo etanol (81). Los fluidos supercríticos se han utilizado extensamente en procesos de extracción en diversas industrias, como la alimentaria, cosmética, farmacéutica y química o de energía, entre otras (85, 87). A pesar de sus excelentes propiedades, los altos costos de procesamiento limitan su aplicación en industrias de pequeña y mediana escala (82).

1.3.2 Bio-disolventes

Los bio-disolventes se producen en bio-refinerías a partir de fuentes biológicas, como cultivos, productos forestales, biomasa acuática o residuos de las anteriores (83). Dentro de esta categoría se incluyen los alcoholes (etanol, glicerol), ésteres (acetato de etilo, lactato de etilo), terpenos (*α*-pineno, *p*-cimeno), furfurales y furano (83). Los bio-disolventes son una alternativa sostenible para reemplazar los disolventes derivados del petróleo (84). Sin embargo, a pesar de su potencial y de que existen ya productos en el mercado, la mayoría de las bio-refinerías se encuentran todavía a escala de laboratorio o planta piloto (84).

1.3.3 Disolventes neotéricos

En esta categoría se incluyen los líquidos iónicos y los eutécticos. Los líquidos iónicos están formados por sales compuestas por cationes y aniones y tienen un punto de fusión inferior a 100 ºC (85). Tiene propiedades ventajosas para los procesos de extracción, entre las cuales destacan una presión de vapor despreciable, alta estabilidad térmica y química, y alta solubilidad de sustancias orgánicas, inorgánicas y organometálicas (86). Sin embargo su clasificación como “disolvente verde” es controvertida debido a su elevado coste de síntesis y su potencial toxicidad (87,88).

Para superar esta problemática, se desarrollaron los líquidos eutécticos que constituyen una alternativa más sostenible (86). Los líquidos eutécticos están formados por una mezcla de ácidos y bases de Lewis o Brønsted y pueden sintetizarse a partir de una amplia variedad

de especies aniónicas y catiónicas. Los más comunes están constituidos por una sal de amonio cuaternaria y un metal o un donador de protones. Así entre sus constituyentes destacan el cloruro de colina, los ácidos carboxílicos y otros donadores de protones como urea, ácido cítrico y glicerol. Adicionalmente se han desarrollado líquidos eutécticos naturales constituidos por ácidos orgánicos, aminoácidos y azúcares. Los líquidos eutécticos tienen propiedades fisicoquímicas comparables a las de los líquidos iónicos, pero son más fáciles de sintetizar, más estables y más económicos. Adicionalmente, no son inflamables, tienen naturaleza dipolar, alta solubilidad, versatilidad y biodegradabilidad (89–91).

1.3.4 Disolventes Supramoleculares (SUPRAS)

Los disolventes supramoleculares son líquidos nanoestructurados producidos en disoluciones coloidales de compuestos anfifílicos a través de un fenómeno espontáneo y secuencial de auto-ensamblaje y coacervación (92). La coacervación es definida como “la separación de dos fases líquidas en sistemas coloidales: una fase concentrada denominada coacervado (SUPRAS) y otra fase denominada disolución en equilibrio“ (93).

Estos líquidos nanoestructurados fueron empleados por primera vez en procesos de extracción en el año 1978, cuando Watanabe y Tanaka desarrollaron un método para extraer zinc empleando “una dispersión micelar de tensioactivos no iónicos separados en dos fases” (94). El proceso se denominó “técnica de punto de nube” (¨*cloud point technique*¨). El término disolvente supramolecular se introdujo posteriormente para resaltar que está formado por agregados supramoleculares en los que las moléculas anfifílicas que los constituyen están unidos por enlaces no covalentes y se forman a través de fenómenos de autoensamblaje (95).

1.3.4.1. Síntesis de los SUPRAS

El autoensamblaje se define como la asociación espontánea y reversible de dos o más componentes que forman estructuras ordenadas a través de interacciones no covalentes. El proceso implica tres fenómenos: (i) reconocimiento molecular, (ii) crecimiento a través de un proceso secuencial y co-operativo de múltiples componentes y (iii) finalización del proceso mediante una determinada *señal de stop* que significa que el sistema ha alcanzado

su completitud (96). El auto-ensamblaje es intrínsecamente dinámico y adaptativo, así dependiendo de las condiciones del ambiente, se pueden obtener diferentes estructuras. Un cambio en las condiciones ambientales hace que los agregados se reorganicen y re-estructuren, permitiendo de esta manera el diseño de materiales a medida (96).

El auto-ensamblaje de compuestos anfifílicos en una disolución tiene lugar cuando se alcanza una determinada concentración, denominada *concentración de agregación crítica* (*cac*), a partir de la cual comienzan a formarse estructuras ordenadas (micelas, vesículas, etc.) con el fin de minimizar los efectos solvofóbicos. En la *cac* la interacción entre tensioactivos es más favorable energéticamente que la interacción tensioactivo-disolvente. Así, estas estructuras surgen de un balance entre interacciones soluto-soluto y soluto-disolvente (97). La agregación se considera un proceso *"start-stop*". Cuando se adicionan más moléculas, se forman nuevos agregados del mismo tamaño debido a la solvofobicidad, mientras que el proceso de parada es inducido por la repulsión entre los grupos cabeza (98).

La morfología del agregado supramolecular depende de la relación entre el tamaño del grupo polar y la cadena hidrófoba del tensioactivo. Esta morfología puede predecirse empleando la Ecuación 1 propuesta por Israelachvili (99).

$$g = \frac{V}{a_0 l_c} \qquad \textit{Ecuación 1}$$

Donde g es el denominado factor de empaquetamiento, V es el volumen de la cadena hidrófoba, a_0 es el área media de sección que ocupa la cabeza polar en el agregado y l_c es la longitud de la cadena del tensioactivo.

El parámetro g depende por tanto de la geometría molecular del tensioactivo y hace referencia al número de cadenas hidrocarbonadas y de átomos de carbono, el grado de saturación de la cadena y el tamaño y carga de la cabeza polar (ver

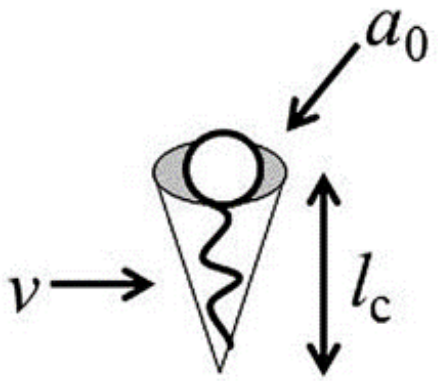


Figura 5). Adicionalmente, propiedades intrínsecas de la disolución, como pH, fuerza iónica, temperatura o la presencia de co-tensioactivos generan también efectos sobre el parámetro g, los cuales están implícitamente incluidos en los parámetros de cálculo.

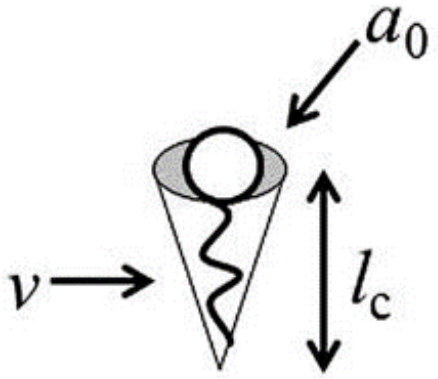
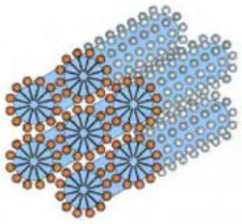
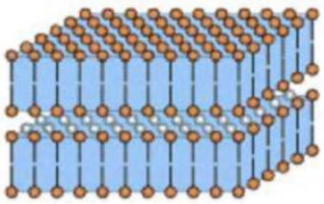
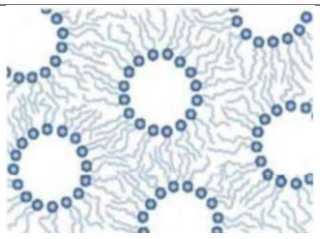


Figura 5. Parámetros que definen el factor de empaquetamiento del tensioactivo y por tanto la morfología del agregado supramolecular (V: volumen de la cadena hidrófoba, a_0: área media de sección que ocupa la cabeza polar, l_c : longitud de la cadena del tensioactivo)

En la Tabla 7 se presenta la morfología de las estructuras formadas por tenisoactivos en disolución en función del parámetro de empaquetamiento.

Tabla 7. Morfología de los agregados supramoleculares en función del parámetro de empaquetamiento

Tipo de agregado	Parámetro de empaquetamiento	Geometría del anfifilo	Estructura del agregado
Micelas esféricas	$\frac{V}{a_0 l_c} < \frac{1}{3}$		
Micelas cilíndricas	$\frac{1}{3} < \frac{V}{a_0 l_c} < \frac{1}{2}$		
Bicapas o vesículas flexibles	$\frac{1}{2} < \frac{V}{a_0 l_c} < 1$		
Bicapas planas	$\frac{V}{a_0 l_c} \sim 1$		
Micelas inversas	$\frac{V}{a_0 l_c} > 1$		

Después del primer proceso de auto-ensamblaje, que da lugar a una suspensión coloidal de agregados tridimensionales (micelas, vesículas, bicapas, etc.), para la formación del SUPRAS se debe producir un fenómeno de coacervación. El proceso de coacervación se induce por un agente externo que provoca que los agregados se ensamblen y se separen

como una nueva fase líquida que será inmiscible con su disolución de equilibrio. La coacervación se puede producir por dos mecanismos (100,101):

- Coacervación simple: el componente coloidal es una macromolécula neutra o cargada disuelta en agua, o una macromolécula neutra disuelta en un disolvente orgánico. El agente externo que provoca la coacervación puede ser un cambio en el pH o la temperatura, la adición de un electrolito, o bien la adición de un disolvente miscible con el medio de dispersión, pero en el que la macromolécula es poco soluble. La separación de fases se produce debido fundamentalmente a la desolvatación de las macromoléculas. En una suspensión coloidal, el disolvente interacciona con las macromoléculas a través de enlaces dipolo-dipolo, puentes de hidrógeno y/o fuerzas de Van der Waals; formando una capa alrededor de las macromoléculas que impide o limita la interacción entre las mismas. El agente inductor de la coacervación simple debe destruir la interacción disolvente-macromolécula favoreciendo de este modo la interacción entre macromoléculas. Los agregados formados son insolubles en la disolución a partir de la que se han generado y se separan de ella produciendo el coacervado.

ii. Coacervación compleja: se produce al añadir a una disolución acuosa de macromoléculas cargadas, una macromolécula de signo opuesto. En este tipo de coacervación el factor electrostático (densidad de carga de macromoléculas, fuerza iónica, etc.) es esencial para la formación del coacervado. Para favorecer la coacervación frente a la precipitación, la densidad de carga superficial no debe ser muy elevada y la distribución de la carga sobre los dos poli-iones no debe ser complementaria, es decir, el espaciado entre cargas debe ser asimétrico. Al producirse la interacción, el complejo resultante retiene contra-iones y una cantidad considerable de moléculas de disolvente.

En la *Tabla 8* se presentan las principales estrategias de coacervación dependiendo de la naturaleza del grupo polar de la molécula anfifílica (95).

Tabla 8. Estímulos externos para producir la coacervación en diferentes tipos de anfifilos

Tipo de anfifilo	Estímulo externo	Efecto
Compuesto no iónico	Cambio en la temperatura o adición de un mal disolvente para el anfifilo	Disminución de la cantidad de disolvente disponible para la solvatación
Compuesto iónico	Adición de sales orgánicas o inorgánicas	Neutralización de la carga
Compuesto ionizable	Adición de ácido	Neutralización de la carga

En resumen, la formación del SUPRAS conlleva las siguientes etapas (ver Figura 6):

a. Formación de agregados tridimensionales (micelas acuosas, inversas, vesículas, etc.) a partir de una disolución de monómeros de tensioactivo (Ver Figura 6a).

b. Agregación de los mismos y formación de una nueva fase líquida mediante coacervación dando lugar a "gotas de coacervado" (Ver Figura 6b).

c. Floculación de las gotas dando lugar a clústeres de mayor tamaño (Ver Figura 6c).

d. Separación de estas gotas de diferente densidad en una nueva fase dando lugar al disolvente supramolecular.

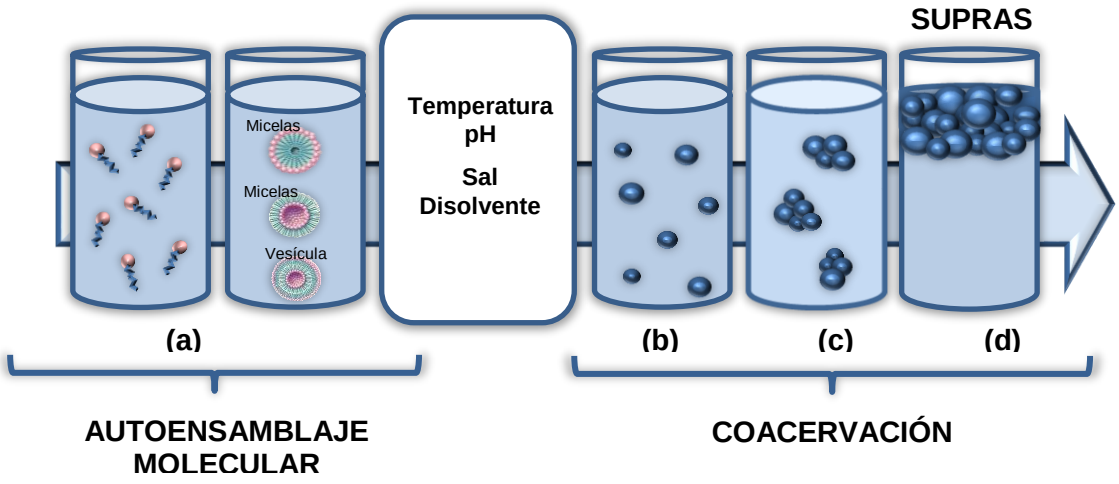


Figura 6. Proceso de formación del SUPRAS

Los SUPRAS están constituidos por elevadas concentraciones de tensioactivo y son inmiscibles en el disolvente (normalmente agua) a partir del cual se generan, a pesar de que este disolvente sea un componente mayoritario del mismo y constituya la fase continua en la que los agregados se dispersan (102). La disolución que queda en equilibrio con el disolvente supramolecular contiene los monómeros de tensioactivo a la concentración de agregación crítica.

1.3.4.2. Propiedades y formatos en procesos de extracción

Los SUPRAS presentan propiedades intrínsecas y operacionales ventajosas para sustituir a los disolventes orgánicos en procesos de extracción. Entre estas propiedades destacan:

1. Capacidad de solubilización simultánea de compuestos polares, apolares y anfifílicos;
2. Versatilidad en las estructuras y en los tipos de interacciones que proporcionan;
3. Elevado número de sustancias anfifílicas naturales y sintéticas comercialmente disponibles a bajo costo;
4. Procesos de síntesis de fácil implementación en cualquier laboratorio o industria;
5. Compatibilidad con las técnicas de detección más comunes.
6. Baja volatilidad e inflamabilidad, lo que permite desarrollar procesos de extracción menos contaminantes y más seguros.

Los SUPRAS contienen una elevada concentración de tensioactivo (~0.1-1 µg/mL) organizados en nanoestructuras con microambientes de distinta polaridad, lo que les confieren una alta capacidad de solubilización de compuestos. Todos los SUPRAS proporcionan así un ambiente apolar en la región hidrocarbonada de los agregados, idónea para la extracción de compuestos no polares a través de fuerzas de dispersión. Por otro lado, los grupos polares del anfifilo (óxidos de polietileno, ácidos carboxílicos, sulfatos, sulfonatos, carboxilatos e iones de amonio y piridinio, etc.) proporcionan interacciones polares, de tipo iónico, puentes de hidrógeno y, en el caso de contener anillos bencénicos, *π*-catión y *π*-*π*, que permiten la extracción de compuestos polares e iónicos. También poseen una elevada capacidad de extracción de compuestos anfifílicos mediante la formación de agregados mixtos a través de interacciones tanto apolares como polares, alcanzándose la máxima eficiencia de extracción para mezclas de tensioactivos de carga opuesta.

Los formatos para la extracción con SUPRAS se dividen en dos tipos: *in situ* y *ex situ*. La extracción de muestras líquidas con SUPRAS implica la formación *in situ* del mismo. Por lo tanto, la generación del disolvente y la extracción se produce en una etapa única (Ver Figura 7).

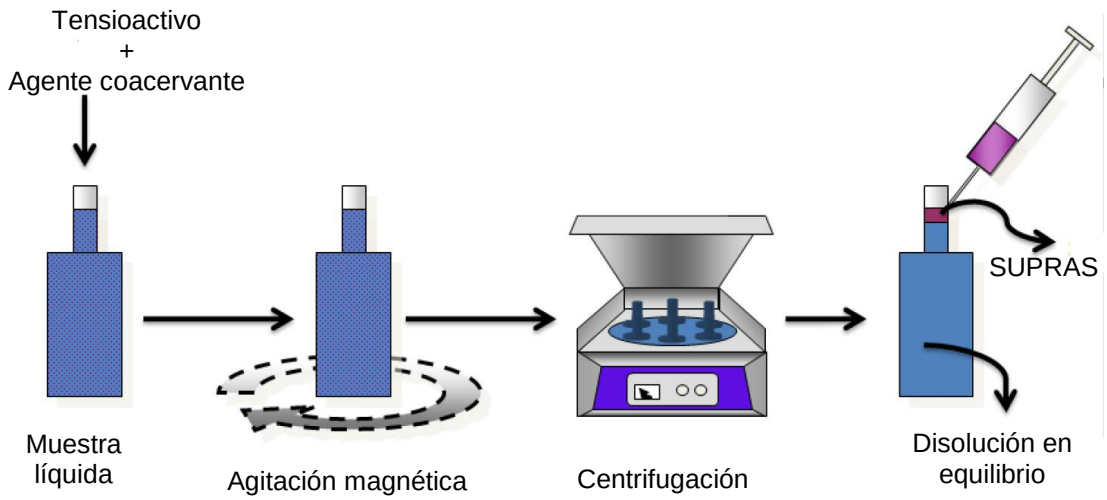


Figura 7. Extracción con disolventes supramoleculares en muestras líquidas (formato de extracción in situ).

La extracción de los compuestos de interés se realiza a través de agitación mecánica o magnética, seguida de una etapa de centrifugación para acelerar la separación de fases. Dependiendo de la densidad del SUPRAS, éste queda en la parte superior o inferior del tubo. Cuando la densidad del SUPRAS es inferior a la disolución en equilibrio, se separa manualmente empleando una pipeta, mientras la separación de SUPRAS con mayor densidad que el agua se suele hacer enfriando el tubo después de la centrifugación. De esta forma se incrementa la viscosidad del disolvente, que se adhiere a las paredes del tubo, facilitando así el desecho de la disolución de equilibrio por decantación.

En el caso de muestras sólidas, la síntesis del SUPRAS se puede realizar síntesis *in situ* o *ex situ* (Ver Figura 8). En la extracción *in situ,* la síntesis del SUPRAS se realiza en presencia de la muestra, para lo cual se añade la muestra sólida (0.1 – 1 g) a una disolución acuosa con los componentes necesarios para formar el SUPRAS (anfifilo, disolvente y estímulo externo). Posteriormente, se repiten las etapas empleadas para muestras líquidas (agitación, centrifugación y separación). El soluto se distribuye en tres fases al alcanzar el equilibrio: la muestra no solubilizada, la disolución acuosa de equilibrio y el SUPRAS.

La extracción *ex situ* se realiza con la síntesis inicial del SUPRAS y la posterior adición de este a la muestra (con o sin disolución de equilibrio) (95).

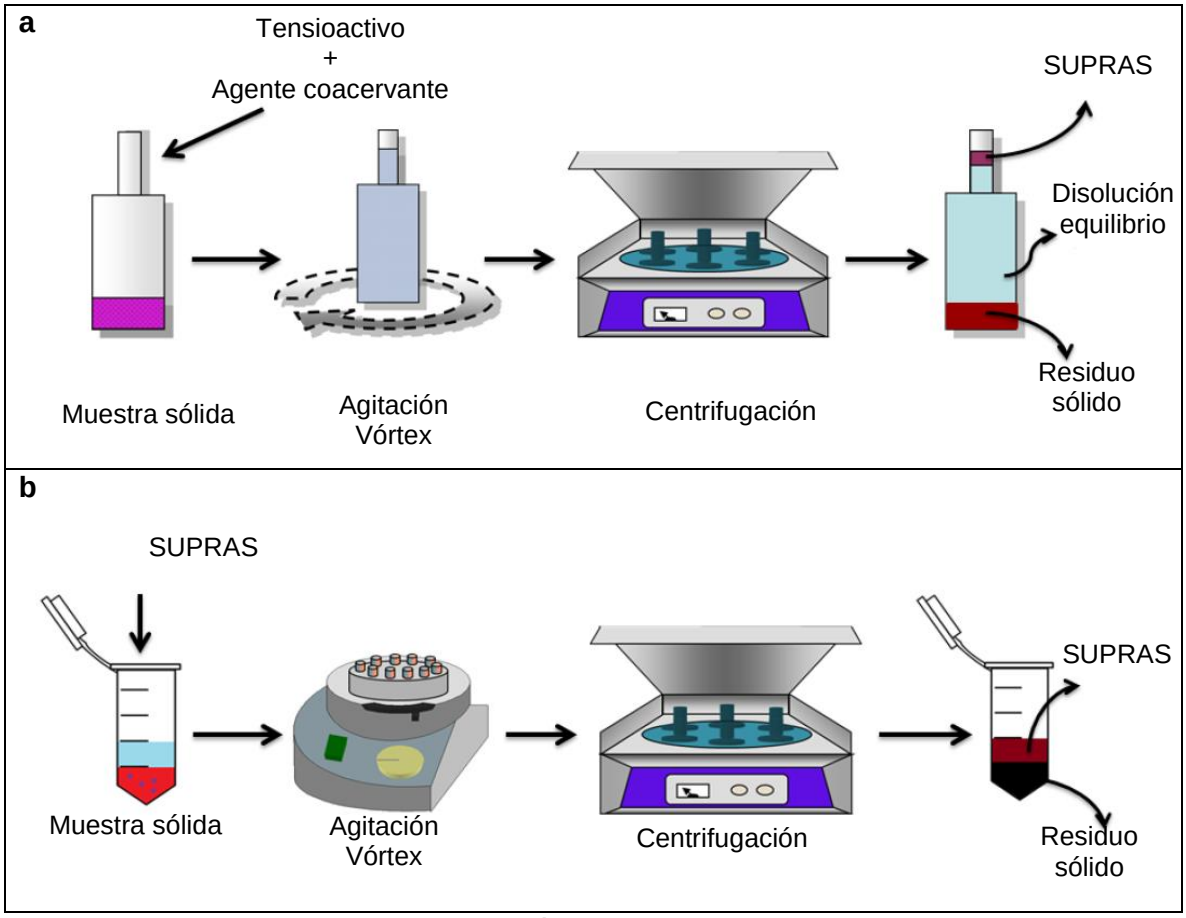


Figura 8. Formatos de extracción (a) in-situ, (b) ex-situ con disolventes supramoleculares en muestras sólidas.

La extracción *in situ* es favorable en el caso de compuestos apolares, debido a que el equilibrio de distribución está favorecido hacia el SUPRAS. Al mismo tiempo, la disolución de equilibrio puede retener compuestos polares de la matriz. Por el contrario, la extracción *ex situ* es favorable para compuestos muy solubles en agua, donde la disolución de equilibrio puede generar una competencia por los mismos. Sin embargo la adición de disolución de equilibrio en pequeñas dosis puede permitir la humectación de muestras altamente higroscópicas e incrementar de esta manera la eficiencia en la extracción.

Los SUPRAS se han aplicado con éxito a la separación, preconcentración y/o purificación de compuestos orgánicos como hidrocarburos aromáticos policíclicos, pesticidas, y pigmentos, procesos en los cuales se han obtenido elevadas eficiencias (92,95). Sin embargo, a pesar de su elevado potencial, su uso en la extracción de compuestos bioactivos a partir de biomasa residual ha sido escasa. Algunos estudios han reportado las

condiciones para la extracción de compuestos bioactivos a partir de residuos agroindustriales, como es el caso de polifenoles de lodo de vino (103), betaínas de melaza de remolacha (104), saponinas de residuos de *Agave sisalana* (105) y antraquinonas de cáscaras de aloe (106). Recientemente nuestro grupo de investigación ha empleado SUPRAS de ácidos carboxílicos para la extracción de astaxantina de microalgas (107). Sin embargo, no existen estudios del uso de SUPRAS para la valorización de residuos de café.

En esta Tesis se utilizan por primera vez los disolventes supramoleculares para la valorización de residuos de café especial cultivado en Colombia. Los resultados presentados a continuación evidencian el elevado potencial de este tipo disolventes verdes en este tipo de aplicación, debido a la simplicidad de su síntesis, su bajo costo y la alta eficiencia en el proceso de extracción. Se pretende así proponer estrategias simples y económicas para incrementar el valor de la agrocadena del café a pequeños y medianos productores de Colombia.

Referencias

1. Preedy VR. Coffee in Health and Disease Prevention [Internet]. Elsevier; 2015 [citado 9 de julio de 2019]. 1080 p. Disponible en: https://linkinghub.elsevier.com/retrieve/pii/C20120069591

2. Dadi D, Mengistie E, Terefe G, Getahun T, Haddis A, Birke W, et al. Assessment of the effluent quality of wet coffee processing wastewater and its influence on downstream water quality. Ecohydrol Hydrobiol. abril de 2018;18(2):201-11.

3. Jiménez-Zamora A, Pastoriza S, Rufián-Henares JA. Revalorization of coffee by-products. Prebiotic, antimicrobial and antioxidant properties. LWT - Food Sci Technol. 1 de abril de 2015;61(1):12-8.

4. Andrade KS, Gonçalvez RT, Maraschin M, Ribeiro-do-Valle RM, Martínez J, Ferreira SRS. Supercritical fluid extraction from spent coffee grounds and coffee husks: Antioxidant activity and effect of operational variables on extract composition. Talanta. 15 de enero de 2012;88:544-52.

5. Heeger A, Kosińska-Cagnazzo A, Cantergiani E, Andlauer W. Bioactives of coffee cherry pulp and its utilisation for production of Cascara beverage. Food Chem. 15 de abril de 2017;221:969-75.

6. Aerts R, Geeraert L, Berecha G, Hundera K, Muys B, De Kort H, et al. Conserving wild Arabica coffee: Emerging threats and opportunities. Agric Ecosyst Environ. 16 de enero de 2017;237:75-9.

7. Barbosa M de SG, Scholz MB dos S, Kitzberger CSG, Benassi M de T. Correlation between the composition of green Arabica coffee beans and the sensory quality of coffee brews. Food Chem. 15 de septiembre de 2019;292:275-80.

8. Puertas Quintero GI. Calidad en taza de las variedades de Coffea arabica L. cultivadas en Colombia. Cenicafé. 1998;49(4):265-78.

9. Sarasty Zambrano DJ. Alternativas de tratamiento del mucilago residual producto del beneficiadero de café [Internet] [Postgrade thesis]. [Colombia]: Universidad Industrial de Santander; 2012 [citado 9 de agosto de 2019]. Disponible en: https://studylib.es/doc/6437128/alternativas-de-tratamiento-del-mucilago-residual-product...

10. Consejo Nacionaal de Política Económica y Social. Documento CONPES 3803. Departamento Nacional de Planeación, República de COlombia; 2014.

11. Carvalho FM, Spence C. The shape of the cup influences aroma, taste, and hedonic

judgements of specialty coffee. Food Qual Prefer. 1 de septiembre de 2018;68:315-21.

12. Yeretzian C. Coffee. En: Springer handbook of odor. Cham, Switzerland: Springer International Publishing; 2017. p. 107-28.

13. Specialty Coffee Asociation. 2017 U.S. Specialty Coffee Consumption Trends | Specialty Coffee Association News [Internet]. 2017 [citado 14 de septiembre de 2019]. Disponible en: https://scanews.coffee/2017/11/29/2017-u-s-specialty-coffee-consumption-trends/

14. Esquivel P, Jiménez VM. Functional properties of coffee and coffee by-products. Food Res Int. mayo de 2012;46(2):488-95.

15. Murthy PS, Madhava Naidu M. Sustainable management of coffee industry by-products and value addition—A review. Resour Conserv Recycl. 1 de septiembre de 2012;66:45-58.

16. Dadi D, Mengistie E, Terefe G, Getahun T, Haddis A, Birke W, et al. Assessment of the effluent quality of wet coffee processing wastewater and its influence on downstream water quality. Ecohydrol Hydrobiol. abril de 2018;18(2):201-11.

17. Alves RC, Rodrigues F, Antónia Nunes M, Vinha AF, Oliveira MBPP. State of the art in coffee processing by-products. En: Galanakis CM, editor. Handbook of Coffee Processing By-Products [Internet]. Academic Press; 2017 [citado 28 de agosto de 2018]. p. 1-26. Disponible en: http://www.sciencedirect.com/science/article/pii/B9780128112908000013

18. Zayas Péerez T, Geissler G, Hernandez F. Chemical oxygen demand reduction in coffee wastewater through chemical flocculation and advanced oxidation processes. J Environ Sci. 1 de marzo de 2007;19(3):300-5.

19. Mussatto SI, Machado EMS, Martins S, Teixeira JA. Production, Composition, and Application of Coffee and Its Industrial Residues. Food Bioprocess Technol. 1 de julio de 2011;4(5):661.

20. Belščak-Cvitanović A, Komes D. Extraction and formulation of bioactive compounds. En: Handbook of Coffee Processing By-Products [Internet]. Elsevier; 2017 [citado 19 de septiembre de 2018]. p. 93-140. Disponible en: https://linkinghub.elsevier.com/retrieve/pii/B9780128112908000049

21. Galanakis CM. Food Waste Recovery. Processing Technologies and Industrial Techniques [Internet]. Elsevier; 2015 [citado 30 de noviembre de 2018]. Disponible en: https://linkinghub.elsevier.com/retrieve/pii/C20130160461

22. Janissen B, Huynh T. Chemical composition and value-adding applications of coffee industry by-products: A review. Resour Conserv Recycl. 1 de enero de 2018;128:110-7.

23. Rodrigues F, Nunes MA, Alves RC, Oliveira MBPP. Chapter 7 - Applications of recovered bioactive compounds in cosmetics and other products. En: Galanakis CM, editor. Handbook of Coffee Processing By-Products [Internet]. Academic Press; 2017. p. 195-220. Disponible en: http://www.sciencedirect.com/science/article/pii/B9780128112908000074

24. Armas Flores EA, Cornejo Mazariego NC, Murcia Zamora KM. Propuesta para el aprovechamiento de los subproductos del beneficiado del café como una alternativa para la diversificación de la actividad cafetera y aporte de valor a la cadena productiva. [Buenos Aires, Argentina]: Universidad del Salvador; 2008.

25. Ballesteros LF, Teixeira JA, Mussatto SI. Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. Food Bioprocess Technol. 1 de diciembre de 2014;7(12):3493-503.

26. Murthy PS, Naidu MM. Recovery of Phenolic Antioxidants and Functional Compounds from Coffee Industry By-Products. Food Bioprocess Technol. abril de 2012;5(3):897-903.

27. Rattan S, Parande AK, Nagaraju VD, Ghiwari GK. A comprehensive review on utilization of wastewater from coffee processing. Environ Sci Pollut Res. 1 de mayo de 2015;22(9):6461-72.

28. Aguiar LL, Andrade-Vieira LF, de Oliveira David JA. Evaluation of the toxic potential of coffee wastewater on seeds, roots and meristematic cells of Lactuca sativa L. Ecotoxicol Environ Saf. noviembre de 2016;133:366-72.

29. Murthy PS, Manonmani HK. Bioconversion of Coffee Industry Wastes with White Rot Fungus Pleurotus florida. Res J Environ Sci. 2008;2(2):145-50.

30. Buntić AV, Pavlović MD, Antonović DG, Šiler-Marinković SS, Dimitrijević-Branković SI. Utilization of spent coffee grounds for isolation and stabilization of Paenibacillus chitinolyticus CKS1 cellulase by immobilization. Heliyon. 1 de agosto de 2016;2(8):e00146.

31. Woldesenbet AG, Woldeyes B, Chandravanshi BS. Bio-ethanol production from wet coffee processing waste in Ethiopia. SpringerPlus [Internet]. 2 de noviembre de 2016 [citado 25 de julio de 2018];5(1). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5093098/

32. Hachicha R, Rekik O, Hachicha S, Ferchichi M, Woodward S, Moncef N, et al. Co-composting of spent coffee ground with olive mill wastewater sludge and poultry manure and effect of Trametes versicolor inoculation on the compost maturity. Chemosphere. 1 de julio de 2012;88(6):677-82.

33. Campos-Vega R, Loarca-Piña G, Vergara-Castañeda HA, Oomah BD. Spent coffee

grounds: A review on current research and future prospects. Trends Food Sci Technol. 1 de septiembre de 2015;45(1):24-36.

34. Kovalcik A, Obruca S, Marova I. Valorization of spent coffee grounds: A review. Food Bioprod Process. 1 de julio de 2018;110:104-19.

35. McNutt J, He Q (Sophia). Spent coffee grounds: A review on current utilization. J Ind Eng Chem. 25 de marzo de 2019;71:78-88.

36. Hall S, Desbrow B, Anoopkumar-Dukie S, Davey AK, Arora D, McDermott C, et al. A review of the bioactivity of coffee, caffeine and key coffee constituents on inflammatory responses linked to depression. Food Res Int. octubre de 2015;76(3):626-36.

37. Pedraza-Beltrán P, Estrada-Flores JG, Martínez-Campos AR, Estrada-López I, Rayas-Amor AA, Yong-Angel G, et al. On-farm evaluation of the effect of coffee pulp supplementation on milk yield and dry matter intake of dairy cows grazing tropical grasses in central Mexico. Trop Anim Health Prod. 26 de febrero de 2012;44(2):329-36.

38. Didanna HL. A critical review on feed value of coffee waste for livestock feeding. World J Biol Biol Sci. 2014;2(5):72-086.

39. Rathinavelu R, Graziosi G. Potential alternative use of coffee wastes and by-products Rajkumar. 2005.

40. Duangjai A, Suphrom N, Wungrath J, Ontawong A, Nuengchamnong N, Yosboonruang A. Comparison of antioxidant, antimicrobial activities and chemical profiles of three coffee (Coffea arabica L.) pulp aqueous extracts. Integr Med Res. 1 de diciembre de 2016;5(4):324-31.

41. Gouvea BM, Torres C, Franca AS, Oliveira LS, Oliveira ES. Feasibility of ethanol production from coffee husks. Biotechnol Lett. 2009;31(9):1315-9.

42. Bonilla VA. Reaproveitamento de resíduos da indústria do café como matéria prima para a produção de etanol. Universidad Federal de Lavras; 2014.

43. Magoni C, Bruni I, Guzzetti L, Dell'Agli M, Sangiovanni E, Piazza S, et al. Valorizing coffee pulp by-products as anti-inflammatory ingredient of food supplements acting on IL-8 release. Food Res Int. 1 de octubre de 2018;112:129-35.

44. Jayachandra T, Venugopal C, Anu Appaiah KA. Utilization of phytotoxic agro waste— Coffee cherry husk through pretreatment by the ascomycetes fungi Mycotypha for biomethanation. Energy Sustain Dev. 1 de marzo de 2011;15(1):104-8.

45. Orozco AL, Pérez MI, Guevara O, Rodríguez J, Hernández M, González-Vila FJ, et al. Biotechnological enhancement of coffee pulp residues by solid-state fermentation with Streptomyces. Py–GC/MS analysis. J Anal Appl Pyrolysis. 1 de marzo de 2008;81(2):247-

46. Corro G, Paniagua L, Pal U, Bañuelos F, Rosas M. Generation of biogas from coffee-pulp and cow-dung co-digestion: Infrared studies of postcombustion emissions. Energy Convers Manag. octubre de 2013;74:471-81.

47. Houbron E, Larrinaga A, Rustrian E. Liquefaction and methanization of solid and liquid coffee wastes by two phase anaerobic digestion process. Water Sci Technol J Int Assoc Water Pollut Res. 2003;48(6):255-62.

48. Chala B, Oechsner H, Latif S, Müller J. Biogas potential of coffee processing waste in Ethiopia. Sustain Switz. 2018;10(8):1-14.

49. Neves L, Oliveira R, Alves MM. Anaerobic co-digestion of coffee waste and sewage sludge. Waste Manag. 2006;26(2):176-81.

50. Juliastuti SR, Widjaja T, Altway A, Iswanto T. Biogas production from pretreated coffee-pulp waste by mixture of cow dung and rumen fluid in co-digestion. AIP Conf Proc. 2017;1840(May).

51. Dzung NA, Dzung TT, Khanh VT. Evaluation of Coffee Husk Compost for Improving Soil Fertility and Sustainable Coffee Production in Rural Central Highland of Vietnam. Resour Enviroment. 2013;3(4):77-82.

52. Santos da Silveira J, Durand N, Lacour S, Belleville M-P, Perez A, Loiseau G, et al. Solid-state fermentation as a sustainable method for coffee pulp treatment and production of an extract rich in chlorogenic acids. Food Bioprod Process. 1 de mayo de 2019;115:175-84.

53. Harsono SS, Salahuddin, Fauzi M, Purwono GS, Soemarno D, Kissinger. Second Generation Bioethanol from Arabica Coffee Waste Processing at Smallholder Plantation in Ijen Plateau Region of East Java. Procedia Chem. 1 de enero de 2015;14:408-13.

54. Moreira MD, Melo MM, Coimbra JM, Reis KC dos, Schwan RF, Silva CF. Solid coffee waste as alternative to produce carotenoids with antioxidant and antimicrobial activities. Waste Manag. 1 de diciembre de 2018;82:93-9.

55. Tello J, Viguera M, Calvo L. Extraction of caffeine from Robusta coffee (Coffea canephora var. Robusta) husks using supercritical carbon dioxide. J Supercrit Fluids. 1 de noviembre de 2011;59:53-60.

56. Saenger M, Hartge E-U, Werther J, Ogada T, Siagi Z. Combustion of coffee husks. Renew Energy. 1 de mayo de 2001;23(1):103-21.

57. Sathianarayanan a, Khan AB. An Eco-Biological Approach for Resource Recycling and Pathogen (Rhizoctoniae Solani Kuhn .) Suppression. J Environ Prot Sci. 2008;2(October):36-9.

58. Narita Y, Inouye K. High antioxidant activity of coffee silverskin extracts obtained by the treatment of coffee silverskin with subcritical water. Food Chem. 1 de diciembre de 2012;135(3):943-9.

59. Borrelli RC, Esposito F, Napolitano A, Ritieni A, Vicenzo F. Characterization of a New Potential Functional Ingredient: Coffee Silverskin. 2004;

60. Furusawa M, Narita Y, Iwai K, Fukunaga T, Nakagiri O. Inhibitory Effect of a Hot Water Extract of Coffee “Silverskin” on Hyaluronidase. Biosci Biotechnol Biochem. 23 de junio de 2011;75(6):1205-7.

61. Mirón-Mérida VA, Yáñez-Fernández J, Montañez-Barragán B, Barragán Huerta BE. Valorization of coffee parchment waste (Coffea arabica) as a source of caffeine and phenolic compounds in antifungal gellan gum films. LWT. 1 de marzo de 2019;101:167-74.

62. Mussatto SI, Machado EMS, Carneiro LM, Teixeira JA. Sugars metabolism and ethanol production by different yeast strains from coffee industry wastes hydrolysates. 2012;

63. Kivaisi A. Pretreatment of robusta coffee hulls and co-digestion with cow-dung for enhanced anaerobic digestion. Tanzan J Sci. 12 de octubre de 2002;28(2):1-10.

64. Menéndez JA, Domínguez A, Fernández Y, Pis JJ. Evidence of self-gasification during the microwave-induced pyrolysis of coffee hulls. Energy Fuels. 2007;21(1):373-8.

65. Murthy P, Manonmani M. Bioconversion of Coffee Industry Wastes with White Rot Fungus Pleurotus florida. Res J Environ Sci. 1 de febrero de 2008;2(2):145-50.

66. Zuorro A, Lavecchia R. Spent coffee grounds as a valuable source of phenolic compounds and bioenergy. Recent Clean Prod Adv Process Monit Optim. 1 de octubre de 2012;34:49-56.

67. Bravo J, Juániz I, Monente C, Caemmerer B, Kroh LW, De Peña MP, et al. Evaluation of spent coffee obtained from the most common coffeemakers as a source of hydrophilic bioactive compounds. J Agric Food Chem. 26 de diciembre de 2012;60(51):12565-73.

68. Pavlović MD, Buntić AV, Šiler-Marinković SS, Dimitrijević-Branković SI. Ethanol influenced fast microwave-assisted extraction for natural antioxidants obtaining from spent filter coffee. Sep Purif Technol. 30 de octubre de 2013;118:503-10.

69. Yoo DE, Jeong KM, Han SY, Kim EM, Jin Y, Lee J. Deep eutectic solvent-based valorization of spent coffee grounds. Food Chem. 30 de julio de 2018;255:357-64.

70. Xu H, Wang W, Liu X, Yuan F, Gao Y. Antioxidative phenolics obtained from spent coffee grounds (Coffea arabica L.) by subcritical water extraction. Ind Crops Prod. 15 de diciembre de 2015;76:946-54.

71. Shang YF, Xu JL, Lee WJ, Um BH. Antioxidative polyphenolics obtained from spent coffee grounds by pressurized liquid extraction. South Afr J Bot. 1 de marzo de 2017;109:75-80.

72. Tello J, Viguera M, Calvo L. Extraction of caffeine from Robusta coffee (Coffea canephora var. Robusta) husks using supercritical carbon dioxide. J Supercrit Fluids. 1 de noviembre de 2011;59:53-60.

73. Iriondo-DeHond A, Aparicio García N, Fernandez-Gomez B, Guisantes-Batan E, Velázquez Escobar F, Blanch GP, et al. Validation of coffee by-products as novel food ingredients. Innov Food Sci Emerg Technol. 22 de junio de 2018;In press.

74. Jaisan C, Chase S, Punbusayakul N. Antioxidant and antimicrobial activities of various solvents extracts of arabica coffee pulp. J Process Energy Agric. 2015;15(5):224-7.

75. Guglielmetti A, D'Ignoti V, Ghirardello D, Belviso S, Zeppa G. Optimisation of ultrasound and microwave-assisted extraction of caffeoylquinic acids and caffeine from coffee silverskin using response surface methodology. Ital J Food Sci [Internet]. 15 de marzo de 2017 [citado 14 de agosto de 2019];29(3). Disponible en: https://www.chiriottieditori.it/ojs/index.php/ijfs/article/view/727

76. Das S, Mondal A, Balasubramanian S. Recent advances in modeling green solvents. Curr Opin Green Sustain Chem. junio de 2017;5:37-43.

77. Cabeza LF, de Gracia A, Fernández AI, Farid MM. Supercritical CO2 as heat transfer fluid: A review. Appl Therm Eng. 1 de octubre de 2017;125:799-810.

78. Knez Ž, Markočič E, Leitgeb M, Primožič M, Knez Hrnčič M, Škerget M. Industrial applications of supercritical fluids: A review. Energy. 1 de diciembre de 2014;77:235-43.

79. Pitchaiah KC, Sujatha K, Deepitha J, Ghosh S, Sivaraman N. Recovery of uranium and plutonium from pyrochemical salt matrix using supercritical fluid extraction. J Supercrit Fluids [Internet]. 2 de noviembre de 2018; Disponible en: http://www.sciencedirect.com/science/article/pii/S0896844618304273

80. da Silva RPFF, Rocha-Santos TAP, Duarte AC. Supercritical fluid extraction of bioactive compounds. TrAC Trends Anal Chem. 1 de febrero de 2016;76:40-51.

81. Herrero M, Cifuentes A, Ibañez E. Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. Food Chem. 1 de enero de 2006;98(1):136-48.

82. Albarelli JQ, Santos DT, Ensinas AV, Maréchal F, Cocero MJ, Meireles MAA. Comparison of extraction techniques for product diversification in a supercritical water gasification-based sugarcane-wet microalgae biorefinery: Thermoeconomic and

environmental analysis. J Clean Prod. 10 de noviembre de 2018;201:697-705.

83. Li Z, Smith KH, Stevens GW. The use of environmentally sustainable bio-derived solvents in solvent extraction applications—A review. Chin J Chem Eng. 1 de febrero de 2016;24(2):215-20.

84. Vovers J, Smith KH, Stevens GW. Chapter 4 - Bio-Based Molecular Solvents. En: Pena-Pereira F, Tobiszewski M, editores. The Application of Green Solvents in Separation Processes [Internet]. Elsevier; 2017. p. 91-110. Disponible en: http://www.sciencedirect.com/science/article/pii/B9780128052976000048

85. Henderson RK, Jiménez-González C, Constable DJC, Alston SR, Inglis GGA, Fisher G, et al. Expanding GSK's solvent selection guide – embedding sustainability into solvent selection starting at medicinal chemistry. Green Chem. 5 de abril de 2011;13(4):854-62.

86. Dominguez de Maria P. Ionic liquids, switchable solvents and eutectic mixtures. En: Green solvents. Amsterdam, Netherlands: Elsevier; 2017. p. 533.

87. Passos H, Freire MG, Coutinho JAP. Ionic liquid solutions as extractive solvents for value-added compounds from biomass. Green Chem. 2014;16(12):4786-815.

88. Ventura SPM, e Silva FA, Quental MV, Mondal D, Freire MG, Coutinho JAP. Ionic-Liquid-Mediated Extraction and Separation Processes for Bioactive Compounds: Past, Present, and Future Trends. Chem Rev. 24 de mayo de 2017;117(10):6984-7052.

89. Satlewal A, Agrawal R, Bhagia S, Sangoro J, Ragauskas AJ. Natural deep eutectic solvents for lignocellulosic biomass pretreatment: Recent developments, challenges and novel opportunities. Biotechnol Adv [Internet]. septiembre de 2018 [citado 27 de septiembre de 2018]; Disponible en: https://linkinghub.elsevier.com/retrieve/pii/S0734975018301472

90. Zhang Q, Vigier KDO, Royer S, Jérôme F. Deep eutectic solvents: syntheses, properties and applications. Chem Soc Rev. 8 de octubre de 2012;41(21):7108-46.

91. Tomé LIN, Baião V, da Silva W, Brett CMA. Deep eutectic solvents for the production and application of new materials. Appl Mater Today. marzo de 2018;10:30-50.

92. Caballo C, Sicilia MD, Rubio S. Chapter 5 - Supramolecular Solvents for Green Chemistry. En: Pena-Pereira F, Tobiszewski M, editores. The Application of Green Solvents in Separation Processes [Internet]. Elsevier; 2017 [citado 25 de junio de 2018]. p. 111-37. Disponible en: https://www.sciencedirect.com/science/article/pii/B978012805297600005X

93. IUPAC. Compendium of Chemical Terminology. [Internet]. 1997 [citado 8 de noviembre de 2018]. Disponible en: https://goldbook.iupac.org/html/C/C01116.html

94. Watanabe H, Tanaka H. A non-ionic surfactant as a new solvent for liquid—liquid extraction of zinc(II) with 1-(2-pyridylazo)-2-naphthol. Talanta. 1 de octubre de

1978;25(10):585-9.

95. Ballesteros-Gómez A, Lunar L, Sicilia MD, Rubio S. Hyphenating Supramolecular Solvents and Liquid Chromatography: Tips for Efficient Extraction and Reliable Determination of Organics. Chromatographia. 24 de septiembre de 2018;1-14.

96. Lehn J-M. Toward complex matter: supramolecular chemistry and self-organization. Proc Natl Acad Sci U S A. 16 de abril de 2002;99(8):4763-8.

97. Steed JW, Turner DR, Wallace K. Core Concepts in Supramolecular Chemistry and Nanochemistry. Chippenham, Witshire: John Wiley & Sons, Ltd; 2007. 297 p.

98. Pramauro E, Pelizzetti E. Behaviour and properties of amphiphilic molecules and their assemblies. En: Surfactants in Analytical Chemistry [Internet]. Amsterdam, Netherlands: Elsevier; 1996 [citado 31 de julio de 2019]. Disponible en: https://www.elsevier.com/books/surfactants-in-analytical-chemistry/pramauro/978-0-444-89033-7

99. Israelachvili JN, Mitchell DJ, Ninham BW. Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers. J Chem Soc Faraday Trans 2. 1 de enero de 1976;72:1525-68.

100. Wang Y, Kimura K, Huang Q, Dubin PL, Jaeger W. Effects of Salt on Polyelectrolyte−Micelle Coacervation. Macromolecules. 1 de octubre de 1999;32(21):7128-34.

101. Mohanty B, Bohidar HB. Systematic of alcohol-induced simple coacervation in aqueous gelatin solutions. Biomacromolecules. agosto de 2003;4(4):1080-6.

102. Menger FM, Sykes BM. Anatomy of a Coacervate. Langmuir. 1 de julio de 1998;14(15):4131-7.

103. Chatzilazarou A, Katsoyannos E, Gortzi O, Lalas S, Paraskevopoulos Y, Dourtoglou E, et al. Removal of polyphenols from wine sludge using cloud point extraction. J Air Waste Manag Assoc 1995. abril de 2010;60(4):454-9.

104. Mohammadzadeh M, Honarvar M, Zarei AR, Mashhadi Akbar Boojar M, Bakhoda H. A new approach for separation and recovery of betaine from beet molasses based on cloud point extraction technique. J Food Sci Technol. abril de 2018;55(4):1215-23.

105. Dias Ribeiro B, Weingart Barreto D, Zarur Coelho MA. Use of micellar extraction and cloud point preconcentration for valorization of saponins from sisal (Agave sisalana) waste. Food Bioprod Process. 1 de abril de 2015;94:601-9.

106. Tan Z-J, Li F-F, Xing J-M. Cloud point extraction of aloe anthraquinones based on non-ionic surfactant aqueous two-phase system. Nat Prod Res. 2012;26(15):1423-32.

107. Salatti-Dorado JA, García-Gómez D, Rodriguez-Ruiz V, Gueguen V, Pavon-Djavid G, Rubio S. Multifunctional green supramolecular solvents for cost-effective production of highly stable astaxanthin-rich formulations from Haematococcus pluvialis. Food Chem. 1 de mayo de 2019;279:294-302.

CAPÍTULO I

DISOLVENTES VERDES PARA LA EXTRACCIÓN DE COMPUESTOS DE ALTO VALOR AÑADIDO A PARTIR DE RESIDUOS AGROALIMENTARIOS

Green solvents for the extraction of high added-value compouns from agri-food waste

Laura Sofía Torres-Valenzuela [a,b], Ana Ballesteros [b], Soledad Rubio[b]

Food Engineering Reviews (2019): https://doi.org/10.1007/s12393-019-09206-y

Abstract

Large amounts of agri-food by-products, non-edible food and waste are produced throughout the supply chain from the initial production to the final consumption stages. The valorization of this biomass to obtain high-value added compounds has been the focus of extensive research in the last decade. For this purpose, the use of green techniques is essential to reduce the negative impact on the health and the environment. In this review, we discuss the use of green solvents for the valorization of agri-food waste and by-products and we consider their potential to replace conventional organic solvents in order to provide more environmentally friendly and sustainable processes. The use of supercritical fluids, neoteric (ionic liquids and deep eutectic solvents), bio-based and supramolecular solvents is critically dicussed. Parameters affecting extraction efficiency are detailed for each type of solvent along with advantages and limitations for application at the industrial scale.

Keywords

Agri-food waste; green solvents, valorization; bio-based solvents; ionic liquids; deep eutectic solvents

1. Introduction

Agri-food waste is estimated at 5 billion tons of biomass residues per year globally (Naidu et al. 2018). Only in EU, the total annual biowaste is estimated at 76.5–102 million tonnes (Jablonský et al. 2018). Nowadays, the final disposal of agri-food waste has become a major challenge for food processing industries due its potential negative impact on the environment (Galanakis 2015). Thus, agri-food by-products account for 3.3 billion tonnes of carbon dioxide emissions each year, globally.

The Food and Agricultural Organization (FAO) estimates that one third of the edible food is annually wasted (Gustavsson et al. 2011). The valorization of nonedible crop residues is also relevant (peels, seed, leaves, pits, pulp, press cakes, etc.). Over the last years, the evaluation of these by-products as sources of biologically active compounds has attracted great interest (Carciochi et al. 2017) both to decrease the volume of residues and to obtain high added-value compounds (Strati and Oreopoulou 2014). Natural bioactive compounds from agri-food waste constitute a wide variety of molecules with different structures and functionalities for the production of nutraceuticals, functional foods, and cosmetics, such as polyphenols, lycopene, anthocyanins, lipids, sugars, alkaloids, proteins, dietary fibers and flavors (Kumar at al., 2017; see Table 1). Articles reviewing the valorization of certain industrial food waste, such as tomato (Strati and Oreopoulou 2014), wine (Teixeira et al. 2014; Kammerer et al. 2014), fruit juice (Kammerer et al. 2014) and olive oil (Roselló-Soto et al. 2015; Araújo et al. 2015) have been reported in the last years. Other valorization activities include the production of animal feed, compost, fuel, wood-based panels, bio-fertilizers and biofibers.

Many efforts have been devoted to find simple and inexpensive strategies for the exploitation of agrifood by-products. A variety of solvents and extraction methods, such as high pressure and temperature extraction, supercritical fluids, ultrasound- and microwave-assisted extractions, and enzymatic treatment have been proposed in an attempt to enhance process efficiency for recovery of high added-value compounds. Organic solvents, such as diethyl ether, *N,N*-dimethylformamide, ethanol, hexane, toluene and their aqueous solutions have been the main extractant phases (Byrne et al. 2016). However, many of the solvent-based extraction processes are nowadays considered inefficient because of the extended times needed to extract/purify the target compounds, the requirement of large solvent volumes per sample so that a high amount of toxic waste is generated. This waste possesses a negative impact on health, safety and the environment (Vian et al. 2017) and consequently, the search for solvent reduction consumption and greener solvents has been strongly fostered (Pena-Pereira and Tobiszewski 2017; Tomé et al. 2018; Cvjetko Bubalo et al. 2018).

Table 1. High-added value compounds in agri-food waste (examples)

By-product	Compounds	Use and/orbenefitial effects
Yellow pitahaya	Vitamin C, Polyphenols	Vitamin C: Dietary supplement (essential nutrient for repair of tissues, enzymatic production of certain neurotransmitters, immune system functions), antioxidant; polyphenols: antioxidants
Mangostino peel	Anthocyanins	Food coloring, antioxidants
Orange peel	Flavonoids, phenolics compounds	Antioxidants
Avocado peel and avocado seed	Essential oils, fat acids	Fragances and flavourings, food additives and preservatives
Grape seed	Resveratrol, polyphenols, anthocyanins	Antioxidants (resveratrol is used as dietary supplement too)
Passion fruit	Polyphenols	Antioxidants
Pineaple peel	Enzymes	
Soursop peel	Flavonoids	Antioxidants
Guava peel	Vitamin C	Dietary supplement, essensial nutrient, immune system functions, antioxidant
Papaya peel	Phenolic compounds	Antioxidants
Pupunha peel	Polyphenols	Antioxidants
Cocoa peel	Polyphenols	Antioxidants
Tamarind peel	Aromatic compounds	Fragances
Coffee peel and spent coffee grounds	Polyphenols	Antioxidants
Tomato peel and seed	Lycopene	Food coloring, antioxidant
Corncob	Lignin Glucose and xylose	Paper industry, textiles and fibers, food and pharmaceuticals additive, building materials, biofuel; sugars:
Coconut husk	Celullose Lignin	Paper industry, textiles and fibers, food and pharmaceuticals additive, building materials, biofuel

2. Green solvents: potential and limitations in the extraction and valorization of agri-food waste

Green solvents are non-toxic, non-volatile, recyclable, biodegradable and may not involve a high energy cost of synthesis (Das et al. 2017). A number of alternative solvents that fulfill, to a greater or lesser extent, this definition is included in Figure 1. They are grouped in four categories, namely supercritical fluids, neoteric, bio-based and supramolecular solvents. Replacement of a harmful solvent by a greener alternative in a separation process is not trivial and, in some cases, novel challenges and limitations can arise due to the different physicochemical properties of the solvents considered. In this review, we discuss briefly the extraction potential and limitations of green solvents for the valorization of agri-food waste.

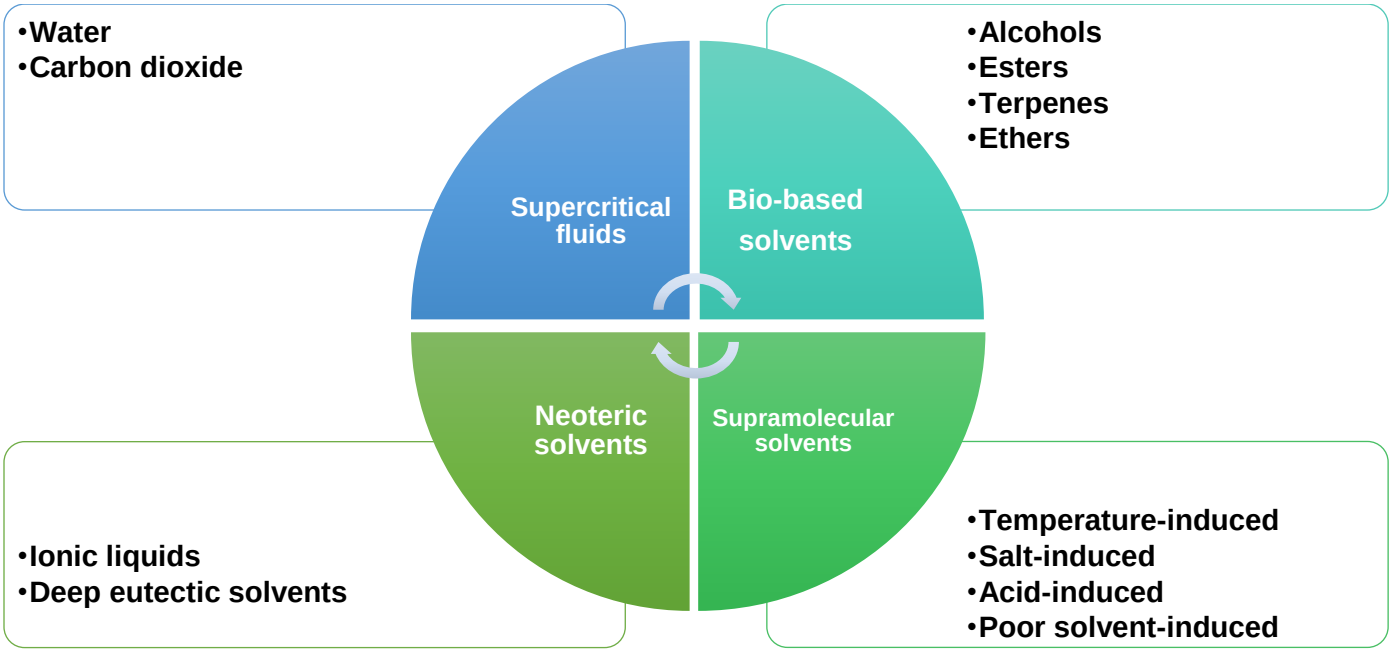


Figure 1. Green solvents covered in this review

2.1 Supercritical fluids

Supercritical fluids (SCFs) are substances for which both pressure and temperature are above their critical values (Knez et al. 2014; Cabeza et al. 2017). The SCFs are characterized by gas-liquid properties, i.e. gas-like viscosity and diffusivity and liquid-like density and solvating

properties. This makes them excellent solvents for extraction processes in the so-called supercritical fluid extraction, SFC (Knez et al. 2014; Pitchaiah et al. 2018). Thus, the fluid diffuses easily through solids and provides faster extraction yields (da Silva et al. 2016). Additionally, the SCFs density can be modified by changing its pressure and/or temperature and since density is related to solubility, the solvent strength of the fluid can be modified (Herrero et al. 2006). Furthermore, the fluid solubility strength can be tuned by the addition of modifiers. This versatility makes SFCs very interesting for different applications (Yoon and Lee 2018).

SCFs have been extensively used in the industry and scientific literature for fractionation of products, dyeing of fibers, treatment of contaminated soils, production of powders in micro/nanometer sizes and novel chemical reactions to replace organic solvents (e.g. catalytic hydrogenation reactions typical for petrochemical industry), energy industry applications and biofuel production (Knez et al. 2014). The most used SCFs are water, carbon dioxide, helium, refrigerants and hydrocarbon fuels, but health and safety benefits are especially evident in the use of supercritical CO_2 and supercritical water.

2.1.1 Water

Water is considered as the cleanest solvent. Supercritical water exists at temperatures above 374°C and pressures above 22.1 MPa. Supercritical water behaves as a nonpolar solvent because hydrogen bonding is lost under these extreme conditions (DeSimone 2002). Its use has increased during the last two decades and industrial applications have been developed looking for environment-friendly and energy-saving technologies (Gorbaty and Bondarenko 2017; Yang et al. 2019). However, despite extensive research efforts, corrosion problems have not been satisfactorily solved for application at industrial scale up to now. (Plaza and Turner 2015). An alternative is the use of pressurized hot water extraction (PHWE) or subcrital water extraction that uses water at temperatures above its boiling point (100 °C) but below the critical point of water (374°C, 22.1 MPa) (so, below the critical point of water) (Pagano et al.a, 2018; Plaza and Turner 2015). A variety of applications to the extraction of bioactives have been made, such as flavonoids from onion waste (Munir et al. 2018) or reducing sugars from wheat straw (Abdelmoez et al. 2014).However, the risk of hydrolysis and other degradation reactions during extraction are major drawbacks of this technique. (Plaza and Turner 2015)

2.1.2 Carbon dioxide

Supercritical fluid (SCF) extraction with carbon dioxide has widely contributed to the development of green extraction processes for bioactive compounds (da Silva et al. 2016). CO_2 is the most used because of its moderate critical temperature (31.3 °C) and pressure (7.38 MPa). CO_2 is non-carcinogenic, non-toxic, non-mutagenic, non-flammable and thermodynamically stable (Knez et al. 2014) and generally recognized as safe and green solvent (Herrero et al. 2006).

The applicability of SFE to high-added value compounds from vegetable matrices (agri-waste, algae, etc.) has been reviewed by several authors (Oliveira et al. 2011; Sharif et al. 2014; Knez et al. 2014; da Silva et al. 2016; Cabeza et al. 2017; Djas and Henczka 2018). The bioactive compounds extracted by SFE include a wide variety, such as phenolic compounds from passion fruit seeds (Oliveira et al. 2017) and grape seeds (Pérez et al. 2015), phytochemical compounds from soy bean expeller (Alvarez et al. 2019), essential oil from orange peel (Xhaxhiu and Wenclawiak 2015), phenols from olive oil mill waste (Lafka et al. 2011), phytosterol from roselle seeds (Nyam et al. 2010b), limonoid glucosides from grapefruit molasses (Yu et al. 2006), solanesol from tobacco waste (Wang and Gu 2018) and saponins from Agave salmiana bagasse (Santos-Zea et al. 2019) (see Table 2). Most of these studies investigate the influence of pressure and temperature in the extraction yield. Extractions are usually carried out at temperatures and pressures in the ranges 35 – 80 ºC and 10 – 70 MPa, respectively. The flux ranges from 1.5 to 5,000 mL CO_2/min and the extraction times from 25 to 150 min. The use of experimental design is common for understanding linear and complex interactions among variables. However, as Sharif et al., 2014 pointed out, the successful application of an experimental design in SFE relies on the in-depth understanding of both SFE and experimental design techniques (Sharif et al. 2014).

When compared to other extraction techniques, CO_2-SFE was superior to ultrasound-assisted extraction for isolation of essential oils from orange peel extracts (Xhaxhiu and Wenclawiak 2015), while for more polar compounds, such as phenolics from olive oil mill waste, CO_2-SFE was acceptable but less efficient than extraction with polar solvents (e.g. ethanol). In this sense, many authors propose the use of co-solvents, such as ethanol, for improving recoveries of polar and medium polar compounds (Braga et al. 2008; Xhaxhiu and Wenclawiak 2015; Campone et al. 2018). Since CO_2 is a gas with low polarity, the addition of a polar solvent (4.7 – 10%) improves its solubility for compounds with polar functional groups (such as vitamin E, γ-oryzanols and

xanthophylls). Another advantage of SFE processes is the fact that this technology can be easily transferred at industrial scale to extract large quantities of matrix and obtaining great amount of extract in a single step (Campone et al. 2018).

However, despite the excellent extraction properties and great versatility, the high processing costs and the complex industrial equipment are limiting factors. For example, the economical assessment of SFE into a sugarcane-microalgae biorefinery by Albarelli et al (2018) led to the conclusion that the process was not economically attractive, as it increased the total investment by 71% (respect to traditional biorefinery) and presented a very high energy demand that would lead to high operational costs (Albarelli et al. 2018).

2.2 Neoteric solvents

Neoteric solvents is a term that refers to solvents structurally novel or unconventional and usually characterized by physical and chemical properties that can be finely tuned for a range of applications by varying the chemical constituents (Gutiérrez-Arnillas et al. 2016). Among neoteric solvents, fluorous solvents, ionic liquids and eutectic solvents have received the highest attention.

Fluorous solvents are made from highly fluorinated compounds, such as perfluorooctane, perfluorohexane, perfluoro (methyl cyclohexane), perfluorodecaline, perfluorotributylamnine and perfluoropolyether (Matsuda et al. 2013). They are so-called the “third liquid phase,” because of their immiscibility with both water and organic phases, which make easier their reuse and application in separation processes. Furthermore, perfluorocarbons have advantages as solvents, because they are chemically unreactive, non-flammable and have low toxicity (Kerton 2009). Main drawbacks are their high cost, limited applicability to very non polar solutes and the concern about their sustainability due to their high environmental persistence and global warming potential (greenhouse gases) (Clark and Tavener 2007). Fluorous solvents have been employed for extraction of metals and organic compounds. However, to the best of our knowledge, their applicability to the extraction of bioactive compounds for agri-waste has not been explored yet. So, in this review, we focus our discussion on ionic liquids and eutectic solvents.

Table 2. Extraction of bioactive compounds from agroindustrial by-products using SCFs and subcritical water

Agri-food waste	SCFs	Sample size	Extraction rate/time	Bioactive compound	Extraction efficiency/performance	Reference
Soy bean expeller	CO_2 at 35 ºC and 40 MPa, 3% w/w ethanol	50 g	0.5 kg/h	Phytochemical compounds	Up to 16.0 mg GAE/ 100 g and up to 65 mg QE/ 100 g	(Alvarez et al. 2019)
Grape seeds	CO_2 at 40 ºC and 30 MPa	6 g	1.5 mL/min	Phenolic compounds	25 mg GAE/g	(Pérez et al. 2015)
Maritime pine bark	CO_2 at 30 ºC and 25 MPa, 10% v/v ethanol	-	95 – 167 g / min, 90 min.	Catechin+epicatechin	0.35 mg/g	(Braga et al. 2008)
Onion skin	CO_2 at 40 ºC and 10 MPa, 4.7% v/v ethanol	1 g	10.5 mL/min, 120 min	Phenolic compounds	3.7 mg/g quercetin 1.4 mg/g protocathechiuc acid (among others)	(Campone et al. 2018)
Orange peel	CO_2 at 50 ºC and 40 MPa	0.5 g	1.6 mL/min, 15min	Limonene, β-myrcene, decanal, α-pinene, linalool, valencene	~0.25 % limonene; ~0.004-0.005 % linalool, β-myrcene and decanal,; ~0.003-0.004 % α-pinene, linalool, valencene	(Xhaxhiu and Wenclawiak 2015)

Table 2. Extraction of bioactive compounds from agroindustrial by-products using SCFs and subcritical water

Agri-food waste	SCFs	Sample size	Extraction rate/time	Bioactive compound	Extraction efficiency/performance	Reference
Rice bran	CO_2 at 43ºC and 34.5 MPa, 10% ethanol	4 g	60 min	Rice brain essences (with vitamin E, total γ-oryzanols and total xanthophylls)	0.68-16.65, 1410-2480 and non deected-0.1 μg/g of vitamin E, γ-oryzanols and xanthophylls	(Sookwong et al. 2016)
Brazilian cherry seeds	CO_2 at 45 ºC, 17 MPa, 10% ethanol	72 g	2 g CO_2/min, 22 h	Sesquiterpenes (Germacrone and γ−Elemene)	380 mg/g germacrone and 460 mg/g γ−Elemene	(Santos et al. 2015)
Kalahari melon and Roselle seeds	CO_2 at 60 ºC and 30 MPa (melon) and at 80, ºC and 20MPa (roselle seeds)	1 g	20 mL/min, 3 h	Tocopherol	266.87 and 94.88 mg/100 g from Kalahari melon and roselle seed	(Nyam et al. 2010a)
Citrus junos seed	CO_2 at 70 ºC and 50 MPa	5 g	3 mL/min, 120 min	N-methylanthranyl acid methyl, β-sitosterol, squalene	1.1, 1.85 and 0.11 x 10^4 mg/g of N-methylanthranyl acid methyl, β-sitosterol and squalene, respectively	(Ueno et al. 2008)
Olive oil mill waste	CO_2 at 25 ºC and 35 MPa	2 g	2 g/min, 60 min	Phenolic compounds	0.76% (w/w)	(Lafka et al. 2011)

Table 2. Extraction of bioactive compounds from agroindustrial by-products using SCFs and subcritical water

Agri-food waste	**SCFs**	**Sample size**	**Extraction rate/time**	**Bioactive compound**	**Extraction efficiency/performance**	**Reference**
Roselle seeds	CO_2 at 40, ºC and 40 MPa, 10% v/v ethanol	-	20 mL/min	Oil with phytosterol	108.7 % recovery of oil containing 7,263 mg/Kg of phytosterol	(Nyam et al. 2010b)
Grapefruit molasses	CO_2 at 50 ºC and 48.3 MPa, 10% v/v ethanol	60 g	5 L/min, 40 min	Limonoid glucosides	0.61 mg/g molasses	(Yu et al. 2006)
Industrial tobacco waste	CO_2 at 40 ºC and 30 MPa	7 g	1L/min, 120 min, pretreatment with organic solvent extraction	Solanesol	0.9 % (with pretreatment), 0.1 % (without pretreatment)	(Wang and Gu 2018)
Agave salmiana bagasse	CO_2 at 60 ºC and 30 MPa, 10% v/v ethanol	10 g	1.7 g/min, 60 min	Antioxidants	17.6 µmol Trolox equivalents/g	(Santos-Zea et al.2019)
Apple by-products	Water, 125 ºC (flavonoids) and 175 °C (polyphenols) and 10.3 MPa	5 g (11mL cells were filled with water)	3 min	Phenolic compounds	1.8 µmol GAE/g 1.3 µmol QE/g	(Plaza et al 2013)

Table 2. Extraction of bioactive compounds from agroindustrial by-products using SCFs and subcritical water

Agri-food waste	**SCFs**	**Sample size**	**Extraction rate/time**	**Bioactive compound**	**Extraction efficiency/performance**	**Reference**
Onion waste (skin)	Water, 230 ºC (flavonoids) and 175 °C (polyphenols) and 3 MPa	0.6 L water suspension of onion peel (2% wt solids)	30 min	Phenolic compounds	63-75 mg GAE/g 23-26 QE/g	(Munir et al 2018)
Potato peel	Water, 90 ºC and 4 MPa	0.5 g	3 mL/min, 9 min	Carbohydrates and phenolic compounds	610 mg glucose equivalent/ g 20 mg GAE/g	(Alvarez et al.2014)

GAE: gallic acid equivalents (total polyphenolic content); QE: quercetin equivalent (total flavonoids contents)

2.2.1 Ionic liquids

Ionic liquids (ILs) have been widely applied to the extraction of bioactive compounds (Passos et al. 2014; Ventura et al. 2017). They are a class of salts composed of discrete cations and anions with melting points below 100 °C (Henderson et al. 2011), unique physicochemical properties and pre-organized and tunable solvent structures (Strati and Oreopoulou 2016). Some of their special properties are negligible vapor pressure, excellent thermal and chemical stability, wide electrochemical potential window and outstanding solubility for organic, inorganic and organometallic substances. These properties, along with the extraordinary degree of tunability for both cations and anions, make ionic liquids interesting materials for extraction processes (Henderson et al. 2011).

Although the use of ILs in food processes is not regulated by the Federal Drug Administration, FDA (Martins et al. 2017), the extraction of alkaloids, terpenoids, flavonoids, phenolic compounds, saponins, etc. from natural sources (mainly plants) has been widely investigated (Ventura et al. 2017). However, their applicability to agri-food waste is somehow more limited (see Table 3). Among ILs, 1-alkyl-3-methylimidazolium-based ILs are by far the most studied and are usually combined with $[BF_4]$, Cl^-, and Br^- counterions. The application of greener ILs, e.g. ammonium-based cations, such as cholinium, is still scarce (Ventura et al. 2017).

Regarding agri-food waste, ILs have been applied to the extraction of reducing sugars from corn stalk (Li et al. 2008) and soybean hulls (Hu et al. 2014), levulinic acid from rice husk (Khan et al. 2018), lactic acid from deoiled cottonseed cake, wheat straw and sugarcane bagasse (Grewal and Khare 2018), oleanolic acid from olive tree leaves (Cláudio et al. 2018), cellulose from coconut husk (Zahari et al. 2018), tyrosol from olive mill wastewater (Larriba et al. 2016) and lignin from sugarcane bagasse (Saha et al. 2017). The use of high temperature for extraction is usual (up to 140 ºC) as well as long extraction times (2 h); additionally ultrasonic extraction has been frequently reported. The viscosity of ILs is high and can be lowered by temperature, which is an important factor in the mass tranfer process and fluid flow (Khan et al. 2018). Additionally, the high temperature promotes the biomass dissolution (Hou et al. 2015) and ILs are mostly thermally stable above 200 °C (Khan et al. 2018). ILs concentration and composition are the other most investigated parameters for extraction processes based on these solvents.

Table 3. Extraction of bioactive compounds from agroindustrial by-products using ILs

Agri-food waste	**Type of ILs**	**Ratio sample size (g):ILs volume (mL)[a]**	**Extraction conditions**	**Bioactive compound**	**Extraction efficiency/performance**	**Reference**
Corn stalk	C_4mimBr, C_4mimCl, C_4mimHSO_4, C_6mimCl, 1-Allyl-3-methylimidazolium chloride, **C_4mimCl**	0.2:4	100 ºC , 60 min, HCl/sample ratio 7%	Total reducing sugars	71%	(Li et al. 2008)
Rice husk	$[C_4(Mim)_2][(2HSO_4)(H_2SO_4)_0]$, **$[C_4(Mim)_2][(2HSO_4)(H_2SO_4)_2]$** $C_4(Mim)_2][(2HSO_4)(H_2SO_4)_4]$	0.025:0.75	110 ºC , 60 min IL:water 10:1	Levulinic acid	47.52%	(Khan et al. 2018)
Olive tree leaves	$[C_6$mim]Cl, $[C_8$mim]Cl, $[C_{10}$mim]Cl, **$[C_{12}$mim]Cl**, $[C_{12}$mim]Br, $[C_{12}$mim]I, **$[C_{14}$mim]Cl**, $[C_{16}$mim]Cl and $[C_{18}$mim]Cl	1:10	80 °C for 2h or microwave assisted extraction for 30 min; IL in water (500mM)	Oleanolic acid	2.5 % (wt%)	(Cláudio et al. 2018)
Coconut husk	$[N_{2220}][HSO_4]$	9:100 w/w IL:water 80:20 v/v	120 ºC, 2 h	Cellulose, lignin	56.5% (cellulose) 12.8% (lignin)	(Zahari et al. 2018)
Olive mill wastewater	**$[P_{4441}][Tf_2N]$**, $[N_{4441}][Tf_2N]$, and $[N_{8881}][Tf_2N]$	1:5	30 ºC , 2 h	Tyrosol	78%	(Larriba et al. 2016)
Sugarcane bagasse	C_3mim acetate	1:20 w/w	140 °C, 120 min	Lignin	90.1%	(Saha et al. 2017)

Table 3. Extraction of bioactive compounds from agroindustrial by-products using ILs

Agri-food waste	**Type of ILs**	**Ratio sample size (g):ILs volume (mL)[a]**	**Extraction conditions**	**Bioactive compound**	**Extraction efficiency/performance**	**Reference**
Soybean hulls	$[C_4(Mim)_2]$ hydrogen sulfate + pretreatment with 1-allyl-3-imidazolium chloride [AMIM]Cl	1:4.8 w/w	95 °C, 1 h; ultrasonic-assisted extraction; water/sample 20:1	Reducing sugars	275.4 mg/g	(Hu et al. 2014)

[a] or per gram when indicated (% w/w); Optimal ILs shown in bold; $C_n$mim: 1-alkyl-3-methylimidazolium cation; [Tf2N]: bis(tri-fuoromethylsulfonyl)imide anion; $[N_{2220}]$: Triethylammonium catión; , $[N_{4441}]$: tributyl(methyl)phosphonium catión; $[N_{8881}]$: tricaprylmethylammonium; [P441]: tributylmethylphosphonium cation

A special advantage of ILs for the extraction of bioactives is their ability to permeate and modify biomass cell walls and tissues and facilitate the release of compounds. Protic ILs may facilitate the hydrolysis of polysaccharides and other components for cell lysis via strong hydrogen bonding. This has been exploited for the extraction of asthaxanthin for algae and levulinic acid from lignocellulosic biomass (Shankar et al. 2017; Khan et al. 2018). The extraction of levunilic acid also involved a catalytic process favoured by acidic ILs (Khan et al. 2018). Acidic ionic liquids have been proposed for furher favouring the hydrolysis of lignocellulosic materials (Li et al. 2008).

The versatility of ILs and the wide range of experimental conditions for its use make them very attractive for extraction processes. However, their further practical use has been limited so far, mainly due to their inherent high costs and potential toxicity. The development of more environmentally- benign ILs for extraction purposes is still in its infancy (Passos et al. 2014; Dominguez de Maria 2017). To reduce costs, the utilization of co-solvents, such as methanol, and solvent reuse based on the different solubility of ILs and bioactives in organic solvents and water, are available options (Cooney and Benjamin 2016). Thus, Khan et al. (2018) proposed the recycling of the IL by re-extraction of the levulinic acid with ethyl acetate (in which the IL was not soluble) and solubilization of the IL in water (in which levunilic acid was not soluble). The IL was then recovered by evaporation using vacuum rotary and could be reused four times with reasonable yield. The yield of levulinic acid was between 47 and 48 %. Saha et al. (2017) proposed to recycle the IL and to recover lignin from soybean hulls by adding a mixture of acetone: water (1:1 v/v) to the bagasse:ionic liquid solution 10:1(v/v). This caused the precipitation of the cellulosic material and left a filtrate solution containing lignin and the IL. Ligning was recovered after evaporation of acetone and the IL was obtained after the further evaporation of water under vacuum. The yield of lignin for the whole process was 90.1% and the efficient recovery of the IL was proved by thermogravimetric analysis.

2.2.2 Deep eutectic solvents

Deep eutectic solvents (DESs) were developed to overcome the environmental issues of ILs (Dominguez de Maria 2017). They have physical and chemical properties comparable to ionic liquids, but they are easier to synthesize and more stable and cost-competitive and, typically, most of them are environmental-friendly (Zhang et al. 2012; Satlewal et al. 2018). DESs have shown a great potential in emerging green extraction technologies and they are expected to be widely transferred to industry in coming years (Alonso et al. 2016).

DESs are eutectic mixtures of Lewis or Brønsted acids and bases which can contain a variety of anionic and/or cationic species (Smith et al. 2014a). They are usually produced by the complexation of a quaternary ammonium salt with a metal salt or hydrogen bond donor. The charge delocalization trough the hydrogen bonding results in a decrease of the melting point of the mixture. This is due to the fact that DESs consist of large, non-symmetric ions with low lattice energy and hence, low melting points (Smith et al. 2014a).

DESs are prepared by simply mixing the components and are classified depending on the nature of the complexing agent into four categories (See figure 2). They can be composed of a quaternary ammonium salt and a metal chloride (type I), a metal chloride hydrate (type II) or a hydrogen bond donor (type III) and of a hydrogen bond donor and a metal chloride (type IV). A range of hydrogen bond donors have been studied such as amides, carboxylic acids, and alcohols (Smith et al. 2014a).

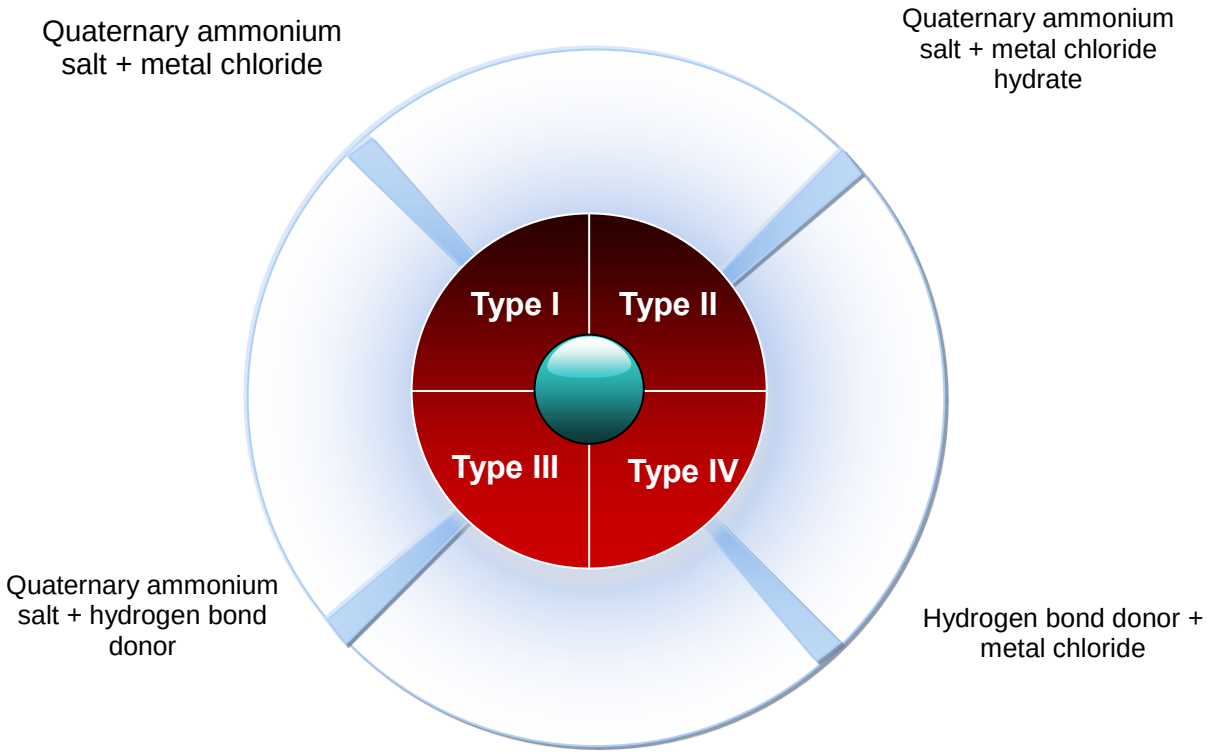


Figure 2. DES classification

Adapted from: (Smith et al. 2014a)

One of the attractive features of DES is their tunability. Thus, a huge number of eutectic mixtures with varying viscosity, density, miscibility and polarity can be obtained by simply changing one or both components in the mixture. In this way, DESs can be easily tailored for specific applications including extraction processes (Huddleston et al. 1998; Tomé et al. 2018; Cunha and Fernandes 2018).

Regarding the applicability of DESs in the valorization of agri-waste, type III DESs have been the most studied and have the greater potential in biomass processing due to their quick and easy preparation, non-reactivity with water, biodegradable nature and cost effectiveness (Smith et al. 2014a; Loow et al. 2017). The most used DES has been made up of choline chloride (ChCl) mixed with different chemical functional groups such as amine, alcohol, acid, and sugar, which act as hydrogen bond donors. Choline is nontoxic, have low cost and is classified as a provitamin in Europe (Smith et al. 2014a).

DESs have been reported for the extraction of tocols from crude palm oil (Hadi et al. 2015), anthocyanins from wine (Radošević et al. 2016; Bosiljkov et al. 2017), genistin, genistein and apigenin from Pigeon pea roots (Cui et al. 2015) and lignin from rice straw (Kumar et al. 2016; Hou et al. 2018). Polyphenols have been extracted from lemon peels, olive leaves, onion solid wastes, red grape pomace and wheat bran (Mouratoglou et al. 2016), grape skins (Radošević et al. 2016), Cajanus cajan leaves (Wei et al. 2015), Morus alba L. leaves (Zhou et al. 2018), olive pomace (Chanioti and Tzia 2018) and spent coffee grounds (Yoo et al. 2018). The extraction time and yield for the bioactives varied according to the type of DES, the structure of the bio-compound, the extraction temperature applied and the use of auxiliary energy (such as microwave or ultrasound). Extraction times varied from 11 min to 24 h with temperatures in the range 40-90 ºC and frequent dilution with water (5-30 % w/w). Table 4 lists valorization processes of agri-waste with DESs.

The physicochemical properties of DESs greatly influence extraction rates (Zainal-Abidin et al. 2017). Polarity and viscosity are two very influencial factors when optimizing the extraction of bioactive compounds with DESs. The high viscosity of DES is a major disadvantage since it reduces the mass transfer of bioactive compounds. Viscosity can be lowered by increasing the temperature at which extraction occurs and by mixing DES with water. For instance, in the case of DESs made up of ChCl:glycerol (1:1), the viscosity decreased by 1/5 at 5% of water and to 1/80 at 20% of water (Zainal-Abidin et al. 2017).

Table 4. Extraction of bioactive compounds from agroindustrial by-products using DES

Agri-food waste	**Type of DES**	**Ratio sample size (g):DES volume (mL)[a]**	**Extraction conditions**	**Bioactive compound**	**Extraction rate efficiency/performance**	**Reference**
Crude palm oil	choline chloride (ChCl):acetic acid, **ChCl: malonic acid**, ChCl: citric acid	1:3 w/w	3 h IL diluted in methanol, sample diluted in hexane	Tocols	14,689-18,525 mg/Kg	(Hadi et al. 2015)
Wine lees (*Merlot grapes*)	ChCl:citric acid, **ChCl: malic acid,** ChCl: oxalic acid, ChCl: glucose, ChCl: fructose, ChCl: xylose, ChCl: glycerol	1:60	30 min and ultrasound assisted extraction Water in NADES 35.4 w/w	Anthocyanins and related compounds	5.2-6.5 mg/g (total anthocyanins)	(Bosiljkov et al. 2017)
Pigeon pea roots	ChCl:sucrose ChCl:1,2-propanediol, ChCl: glucose, ChCl: sorbitol, ChCl: glycol, ChCl: glycerol, ChCl:1,3-Butanediol, ChCl: 1,4-Butanediol, **ChCl: 1,6-Hexanediol**, glucose: L-proline, glucose: lactid acid	2.5:100	11 min, 80 ºC, microwave assisted extraction	Genistin, genistein apigenin	0.449 mg genistin /g, 0.617 mg genistein/g and 0.221 mg apigenin/g	(Cui et al. 2015)
Rice straw	Lactic acid: betaine; **lactic acid: ChCl**	0.5-10, 1:10 w/w	12 h ,60 ºC	Lignin	68 mg/g	(Kumar et al. 2016)

Table 4. Extraction of bioactive compounds from agroindustrial by-products using DES

Agri-food waste	Type of DES	Ratio sample size (g):DES volume (mL)[a]	Extraction conditions	Bioactive compound	Extraction rate efficiency/performance	Reference
			DES with 5% water v/v			
Lemon peels, olive leaves, onion solid wastes, red grape pomace, spent filter coffee and wheat bran	**Glycerol: choline chloride** / sodium acetate, glycerol: sodium–potassium, tartrate:water	0.1:10	90 min, 80 ºC, – ultrasound-assisted extraction DES and 10 % water	Phenolic compounds	88.03 mg GAE/g in onion solid wastes (with glycerol: sodium–potassium, tartrate:water), 53.76 mg GAE/g in lemon waste peels, 36.75 mg GAE/g in olive leaves, 53.63 mg GAE/g in red grape pomace, 22.59 mg GAE/g in spent coffee grounds and 17.78 mg GAE/g in wheat bran	(Mouratoglou et al. 2016)
Corncob	**ChCl: glycerol**, **ChCl: imidazole**, ChCl: urea	1:16	15 h, 80 ºC (ChCl: imidazole) 15 h, 180 ºC (ChCl: glicerol) Washing and evaporation to	Fermentable sugar	Glucose 91.5-92.3% Xylose 59.5-95.5 %	(Procentese et al. 2015)

Table 4. Extraction of bioactive compounds from agroindustrial by-products using DES

Agri-food waste	Type of DES	Ratio sample size (g):DES volume (mL)[a]	Extraction conditions	Bioactive compound	Extraction rate efficiency/performance	Reference
			remove DES+enzymatic treatment			
Grape skins	ChCl: glucose, ChCl: fructose, ChCl: xylose, ChCl: glycerol, **ChCl: malic acid**	1:10	50 min, 65 ºC, ultrasound-assisted extraction	Phenolic compounds, anthocyanins	91 mg/g polyphenols and 24 mg /g anthocyanins	(Radošević et al. 2016)
Cajanus cajan leaves	ChCl: glycerol, ChCl: 1,4-butanediol, ChCl: ethylene glycol, ChCl: glucose ChCl: sucrose, **ChCl: maltose**, ChCl: sorbitol, ChCl: citric acid, ChCl: malic acid, ChCl: lactic acid, citric acid: glucose, citric acid: sucrose, lactic acid: glucose, lactic acid: sucrose	1:30	12 min , 60 ºC, microwave-assisted extraction DES and 20% water	Phenolic compounds (*n*=14)	Stilbenes cajaninstilbene acid 6.9 mg/g; longistyline C 4.4 mg/g	(Wei et al. 2015)
Corncob	ChCl: lactic acid, ChCl: glycolic acid, ChCl: levulinic acid, ChCl: malonic acid, ChCl: glutaric acid, ChCl:	1:20 w/w	24 h, 90 ºC Enzymatic treatment	Lignin removal and glucose yield	71.3 % (lignin); 96.4% (glucose)	(Zhang et al. 2016)

Table 4. Extraction of bioactive compounds from agroindustrial by-products using DES

Agri-food waste	Type of DES	Ratio sample size (g):DES volume (mL)[a]	Extraction conditions	Bioactive compound	Extraction rate efficiency/performance	Reference
	oxalic acid, ChCl: malic acid, ChCl: ethylene glycol, **ChCl: glycerol**					
Grape skins	ChCl: glycerol ChCl: oxalic acid ChCl: malic acid ChCl: sorbose **ChCl: proline: malic acid**	1:10	50 min, 65ºC, ultrasound-assisted extraction DES and 25% water	Flavonoids	~25 mg/g (sum of anthocyanins and cynidine-3-O-glucosides)	(Cvjetko Bubalo et al. 2016)
Morus alba L. leaves	ChCl: Urea, ChCl: Ethylene glycol, ChCl: Glycerol, **ChCl: Citric acid**, ChCl: malic acid, Betaine: levulinic acid, betaine: lactic acid, betaine: glycerol, proline: malic acid, proline: Glycerol, L-proline: levulinic acid, L-proline: lactic acid	1:20	30 min, 40 ºC, ultrasonic-assisted extraction DES:water 3:1 v/v	Phenolic compounds	22.66 mg/g	(Zhou et al. 2018)
Olive pomace	**ChCl: citric acid**, ChCl: lactic acid, ChCl: maltose, ChCl: glycerol	1:12.5	30 min, 60 ° , homogenate-assisted extraction	Phenolic compounds	35 mg GAE/g and homogenization	(Chanioti and Tzia 2018)

Table 4. Extraction of bioactive compounds from agroindustrial by-products using DES

Agri-food waste	Type of DES	Ratio sample size (g):DES volume (mL)[a]	Extraction conditions	Bioactive compound	Extraction rate efficiency/performance	Reference
			20% v/v water			
Spent coffee grounds	ChCl: urea, ChCl: acetamide, ChCl: glycerol, ChCl: sorbitol, ChCl: ethylene glycol, ChCl: 1,4-Butanediol, **ChCl: 1,6-hexanediol**, ChCl: malonic acid, ChCl: citric acid, ChCl: fructose, ChCl: xylose, ChCl: sucrose, ChCl: glucose	1:17	45 min, ultrasonic-assisted extraction DES and 30% water	Phenolic compounds	15 mg GAE/g	(Yoo et al. 2018)

[a] or per gram when indicated (% w/w); optimal DES shown in bold; GAE: gallic acid equivalents

Additionally, the polarity of DES increased along with the water content (Huang et al. 2017). Different hydrogen bond donors (i.e. sugars, polyhydric alcohols and organic acids) were tested by Cui et al. (2015) to lower viscosity and increase polarity of choline-based DESs in the extraction of genistin, genistein and apigenin from pigeon pea root (Cui et al. 2015). The viscosity of DESs with sugars was the greatest while the polarity was higher for sugars and polyhydric alcohols compared to organic acids. Finally, DESs made up of 30% water in 1,6-hexanediol/ChCl (7:1, mol/mol) were selected as optimal. Microwave assisted extraction and 80 °C were applied to enhance the extraction yield. Procedures for the recovery of DES and bioactives with solvent back-extraction are proposed, such as a washing step with water:ethanol for ChCl:glycerol enriched with glucose and xylose and further drying at 38 ºC (Procentese et al. 2015). In this way the yield of glucose and xylose were in the ranges 91.5-92.3% and 59.5-95.5 %, respectively. Hadi et al. (2015) investigated the reuse of other chloine-based DES after extraction of tocols from crude palm oil. A mixture of water–hexane (4:1 v/v) was employed for liquid-liquid separation. The hexane layer contained the tocols that were later recovered by evaporation at 60 °C. The DES-rich layer, which contained a mixture of methanol, water and traces of hexane, was dried to remove methanol and water (15 h). The yield of the recycled DES slightly decreased from 18,525 ± 882 to 11,741 ± 566 mg/kg (total tocols concentration).

2.3 Bio-based solvents

Bio-based solvents are defined as solvents produced from renewable biomass sources such as energy crops, forest products, aquatic biomass and waste materials (Naidu et al. 2018). They are produced in a biorefinery (Vovers et al. 2017) which aims for the maximum recovery and production of high added-value products (Carmona-Cabello et al. 2018). Some bio-based solvents are alcohols (ethanol), esters (ethyl lactate), glycerols, terpenes, furfurals (furfural, furfural alcohol, levulinic acid), and furan (Li et al. 2016). Viscosities are low, which make them easy to handle in extraction processes. Despite their great potential, the scale of biorefineries is still mainly limited to lab-scale or pilot plants (Vovers et al. 2017). However, some of them are already commercially available.

2.3.1 Alcohols

The first generation of bio-based ethanol was derived from sources like starch, sugar, animal fats and vegetable oil. The main problem was the food-versus-fuel debate (Pandiyan et al. 2019). The second generation was produced from a non-food biomass, such as lignocellulosic materials. The third generation was derived from microalgae (Pandiyan et al. 2019). Methanol can also be produced from biomass, but it has toxicity issues (Vovers et al. 2017). Other bio-alcohols with low toxicity are bio-butanol, bio-2-octanol, bio-1,3-propanediol and bio-1,3-butanediol (Calvo-Flores et al. 2018). On the other hand, glycerol has been widely obtained as by-product in biodiesel production (Vovers et al. 2017).

2.3.2 Esters

Ethyl acetate is an industrially relevant ester, non-toxic and fully biodegradable (Chan and Su 2008). This bio-solvent is mainly produced by esterification of acetic acid and ethanol in liquid or vapor phase, acetylation of ethylene, and ethanol dehydrogenation (Santaella et al. 2015). Yeasts, such as *Saccharomyces cerevisiae, Wickerhamomyces anomalus* and *Kluyveromyces marxianus* can also convert sugar into ethyl acetate (Kruis et al. 2017). Ethyl lactate is widely used as a green solvent to replace chlorinated hydrocarbons (Pighin et al. 2017). It is very suitable and environmental benign for food applications. It is also allowed as pharmaceutical and food additive by the FDA (Bermejo et al. 2013).

2.3.3 Terpenes

α-Pinene is a bicyclic monoterpene hydrocarbon and is one of the most abundant components in the essential oils of various plant species (Kim et al. 2018). It has potential for the pharmaceutical, bioenergy, fine chemistry and flavor industries (Ndongou Moutombi et al. 2018). D-Limonene is a colorless liquid cyclic terpene extracted from orange peels in orange juice industry. It is widely accepted for cosmetics and food (Chemat et al. 2012). Finally, p-Cymene is another bio-based molecule. It is used for the synthesis of p-cresol and fine chemicals for perfumes, fungicides and pesticides and as a solvent of dyes and varnishes (Lycourghiotis et al. 2018). It can be obtained for conversion of limonene into p-cymene, also is present in pine trees (Yao et al. 2019).

2.3.4 Extraction of compounds from agroindustrial by-products using bio-based solvents

The extraction of bioactive compounds from agri-food waste with bio-based solvents have been applied in a lesser extent than with SCFs. Studies are mainly focused on extraction from algae or natural resources (not residues) (Bermejo et al. 2013; Villanueva-Bermejo et al. 2017; Ben-Youssef et al. 2017; de Jesus et al. 2018). Table 5 shows research studies concerning the use bio-based solvents to extract bioactive compounds from agri-waste.

Bio-based solvents have been used to extract rosmarinic and caffeic acids from basil wastewater (Pagano et al. 2018), carotenoids and phenols from tomato waste (Strati and Oreopoulou 2011, 2016; El-Malah et al. 2015; Silva et al. 2018), polyphenols, flavonoids, anthocyanins and ellagic acid from pomegranate peel (Masci et al. 2016), phenolic compounds, flavonoids and sinapine from seeds of rapeseed, mustard crambe and sunflower (Matthäus 2002), oil from rice bran (Liu and Mamidipally 2005) and volatile compounds from Cooperage woods in winemaking (Alañón et al. 2017). Ethyl lactate and ethyl acetate, sometimes in mixtures with water, have been by far the most used bio-based solvents. It is usual to employ high temperatures (usually 30-80 ºC and up to 170 ºC) and repetitive extractions to reach adequate recovery of bioactives, which is highly dependent on extraction time and the presence (or not) of auxiliary energy such as microwave or ultrasound.

Bio-based solvents have been reported to extract bioactive compounds as efficiently (or with higher efficiency) than conventional organic solvents. In the extraction of rice bran oil, the use of D-limonene showed superior extraction yield (24.6%) than hexane (18.6%). Similarly, in olive oil extraction, the use of D-Limonene increase the lipid yield in 8.3% more than hexane (Virot et al. 2008). Yara-Varón et al (2016) also reported that α-pinene and d-limonene extracted more carotenoids from carrot than n-hexane (95.4, 94.8 and 78.1% respectively). Commonly, energy assisted-extraction techniques are used for enhancing recoveries. Thus, ultrasound extraction increased in 9.4% the lycopene yield in tomato pomace with ethyl lactate – ethyl acetate mixtures (Silva et al. 2018). Also, pressurized liquid extraction was suitable for the extraction of phenolic compounds from basil waste using mixtures of water (75% v/v) and ethanol or ethyl lactate at 150 ºC, with extraction rates up to 93.9 an 99.2% respectively (Pagano et al. 2018).

Table 5. Extraction of bioactive compounds from agroindustrial by-products using bio-based solvents

Agri-food waste	Solvent	Relationship Sample(g):solvent (mL)	Extraction conditions	Bioactive compound	Extraction rate or extraction efficiency	References
Distillation wastewater of basil	Ethyl lactate : water 25:75 % v/v	0.250 g:- not specified	Pressurized liquid extraction, 50 ºC, 10 MPa, static mode, 20 min	Rosmarinic acid (RA), caffeic acid (CA)	104.1 and 94.2% for rosmarinic acid and caffeic acid, respectively	(Pagano et al. 2018)
Tomato waste (skin and seeds)	Ethyl lactate and ethyl acetate	1:10	30 min, 70 ºC	Carotenoids	243 mg/kg (ethyl lactate) and 46.21 mg/kg (ethyl acetate)	(Strati and Oreopoulou 2011)
Tomato waste (skin + seeds)	Ethyl lactate	1:10	40 min at 20 ºC and 40 min at 60 ºC with ultrasound-assisted extraction	Phenolic compounds, flavonoids and lycopene	1.4 mg GAE/g (20 ºC), 0.5 mg catechin / g (20 º C) and 0.05 mg lycopene /g (60 ºC)	(El-Malah et al. 2015)
Pomegranate peel	Ethyl acetate	1:4	Soxhlet extraction, 6 h	Phenolic compounds, flavonoids, anthocyanins, punicalagings, ellagic acid	2.4-3.2 mmol GAE/g; 0.7 mmol rutin/g;0.05-0.4 µmol cyaniding-3-O-glucoside/g; punicalagings 6.8-7.3 mg/g; ellagic acid 37.7-63.6 mg/g	(Masci et al. 2016)
Seeds of rapeseed, mustard,	Ethyl acetate/water (70:30)	1:5	Overnight with shaking+ 45 min with ultrasound-assisted extraction;	Phenolic compounds, flavonoids and sinapine	2.6 – 9.2 mg TPC/g, 2.1 – 59.8 mg flavonoids/g and 0 – 60.9 mg sinapine /g	(Matthäus 2002)

Table 5. Extraction of bioactive compounds from agroindustrial by-products using bio-based solvents

Agri-food waste	Solvent	Relationship Sample(g):solvent (mL)	Extraction conditions	Bioactive compound	Extraction rate or extraction efficiency	References
crambe and sunflower			pretreatment for defafting with petroleum benzene			
Olive leaves	Glycerol (60% in water) + 7% w/v 2-hydroxypropyl - β-cyclodextrin	1:50	180 min at 60 ºC	Phenolic compounds	54.3 mg GAE/g	(Mourtzinos et al. 2016)
Apple pomace	Ethyl acetate	1:20	3 times extraction for 3 s each and microwave-assisted extraction	Phenolic compounds, flavonoids	~200 mg GAE/L and ~150 mg rutin/L	(Grigoras et al. 2013)
Potato peel	Ethyl acetate	1:10	30 ºC (several extraction steps)	Phenolic compounds	44-83 mg GAE/g	(Arun et al. 2015)
Olive leaves	Aqueous glicerol (9.3 w/v)	1:60	80 ºC, 165 min	Phenolic compounds	51.9 mg GAE/g	(Apostolakis et al. 2014)
Tomato pomace	Ethyl lactate + 35% v/v ethyl acetate	1:100	20 min at 63.4 ºC, ultrasound-assisted extraction	Lycopene	1.3 mg /g	(Silva et al. 2018)
Cooperage woods in winemaking	Ethyl lactate	1:3	10 min 80 ºC in 2-extraction cycles and pressurized liquid extraction	Phenolic compounds, volatile	15 mg GAE/g ~30 µg/g (volatile compunds as sum of total furanic compounds,	(Alañón et al. 2017)

Table 5. Extraction of bioactive compounds from agroindustrial by-products using bio-based solvents

Agri-food waste	**Solvent**	**Relationship Sample(g):solvent (mL)**	**Extraction conditions**	**Bioactive compound**	**Extraction rate or extraction efficiency**	**References**
				compounds (natural flavoring)	β-methyl-γ-octalactones and terpenes and norisoprenoids)	
Tomeate peel and seeds	Ethyl lactate	1:10	30 min at 70 ºC	Lycopene, carotene, lutein	166.4 mg lycopene/kg, 26.4 mg carotene/kg and 10.8 mg lutein/kg	(Strati and Oreopoulou 2016)

GAE: gallic acid equivalents

2.4 Supramolecular solvents (SUPRASs)

Supramolecular solvents (SUPRASs) are nanostructured liquids produced in colloidal suspensions of amphiphiles by spontaneous, sequential phenomena of self-assembly and coacervation (Caballo et al. 2017). Coacervation is defined as “the separation into two liquid phases in colloidal systems. The phase more concentrated in colloid component is the coacervate, and the other phase is the equilibrium solution” (IUPAC 1997).

These nanostructured liquids have been used for extraction since Watanabe and Tanaka in 1978 developed a method to extract zinc using “a micellar solution of a non-ionic surfactant that separates in two phases” also known as the cloud point technique (Watanabe and Tanaka 1978). The name SUPRAS was introduced later, to highlight the differences between these liquid phases and molecular and ionic solvents, to underline the nanostructures formed by non-covalent interactions and to emphasize the synthesis process, which is based on amphiphile self-assembly (Ballesteros-Gómez et al. 2018).

The SUPRAS synthesis is made in two steps. First, “an aqueous or organic colloidal suspension of the amphiphile is prepared above its critical aggregation concentration”. This suspension contains supramolecular aggregates, typically aqueous or reverse micelles or vesicles (Ballesteros-Gómez et al. 2018). The formation of these architectures primarily depends on the packing parameter, which in turn depends of the volume and the length of the hydrophobic segment and the cross-sectional area of the head group (Liu et al. 2015).

In the second step, the generated nanostructures self-assembly in larger aggregates by the action of an external stimulus (coacervating agent) that diminishes the repulsion among the aggregates (Sarkar et al. 2018) and separate from the bulk solution as an immiscible liquid via coacervation (Ballesteros-Gómez et al. 2010, p.; Rezaei et al. 2016). The most used stimulus for the coacervation are pH, temperature, inorganic and organic salts and poor solvents for the amphiphile (Ballesteros-Gómez et al. 2018) (See Figure 3).

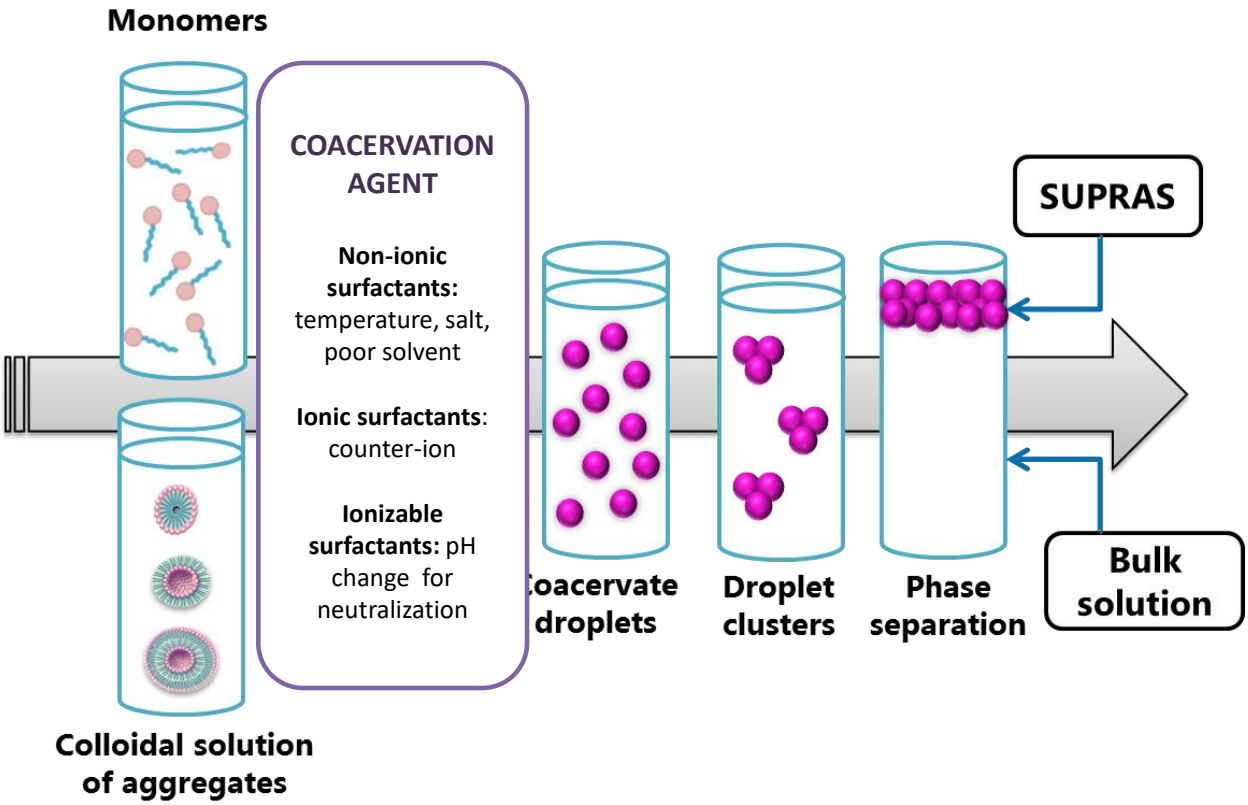


Figure 3. SUPRAS sequential formation process by self-assembly and coacervation

Supramolecular solvents have a unique array of physicochemical properties that render them very attractive to replace conventional organic solvents in extractions (Ballesteros-Gómez et al. 2010). Thus, SUPRAS offer mixed-mechanisms for solute solubilization and produce high extractions rates for solutes covering a wide polarity range. Multiple binding interactions are available which depends on the nature of the amphiphile (Ballesteros-Gómez et al. 2010), and due to its internal structure, different polarity regions are generated (Ballesteros-Gómez et al. 2018). Another important characteristic is that they can be tailored to offer programmed characteristics such as molecular restricted access behavior (Ballesteros-Gómez and Rubio 2012).

SUPRASs have proved high efficiency for the separation, preconcentration, or purification of organic compounds such as such as polycyclic aromatic hydrocarbons, pesticides, surfactants, bioactive compounds and dyes (Caballo et al. 2017; Ballesteros-Gómez et al. 2018). In terms of green chemistry, they are good alternatives to the conventional extraction systems because of their high performance, low toxicity and low cost (Liu et al. 2007; Santalad et al. 2009; Caballo et

al. 2017; Ballesteros-Gómez et al. 2018). Furthermore, they are non-volatile and non-flammable and many amphiphiles are bio-compatible and renewable, such as carboxylic acids and rhamnolipids. In summary, sustainable and economical SUPRAS-based extraction processes can be implemented taking into account that the synthesis can be developed with green natural amphiphiles at low cost and thought energyless processes (Ballesteros-Gómez et al. 2018).

Despite their great potential, only a few studies have been related to the extraction of bioactives from agroindustrial by-products (Table 6). These studies have focused on the extraction of polyphenols from wine sludge (Chatzilazarou et al. 2010), betaine from beet molasses (Mohammadzadeh et al. 2018), saponins from sisal (*Agave sisalana)* waste (Dias Ribeiro et al. 2015) and anthraquinones from aloe peel (Tan et al. 2012).

The most used amphiphiles were non-ionic surfactants from the Triton X series and the most employed coacervating agent was the temperature. High recoveries have been reported with these solvents. Good recoveries have been also obtained with other non-ionic surfactants, such as those reported by Chatzilazarou et al (2010) (Chatzilazarou et al. 2010).

Thus, recoveries found for phenol from wine sludge were 98.5% using PEG 8000 as amphiphile (at pH 2.5, 55 °C) in a fast process that took 30 min. On the other hand, Ribeiro et al. (2015) found that SUPRASs were superior for extraction of saponins from sisal waste (98.4%) compared to an ethanolic solution 30% v/v (38.6%) under the same conditions of time (4h), temperature (50 ºC) and sample mass/volume ratio (0.17 g/mL) (Dias Ribeiro et al. 2015). Some authors investigated the recovery of bioactives from the surfactant rich-phase. Mohammadzadeh et al. (2018) proposed the recovery of betaine (nearly 100%) extracted from beet molasses from the surfactant-rich phase with an aqueous phase at pH at 2.5.

The recovery of bioactives from the surfactant-rich phase by a change of pH in aqueous solution was also proposed by Tan et al. (2012) for the recovery of anthraquinones from aloe peel with an efficiency of 70%.

Table 6. Extraction of bioactive compounds from agroindustrial by-products, using supramolecular solvents

Raw material	Amphiphile	External stimulus for phase separation and conditions	Bioactive compound	Extraction efficiency	References
Wine sludge	Genapol X-080, **PEG 8000**	**Temperature** Conditions: 10mL sample, NaCl 5%, 10% v/v of PEG 8000 (pH 3.5, 55 °C, 30 min)	Phenolic compounds	98.5%	(Chatzilazarou et al. 2010)
Beet molasses	**Triton X-114,** Triton X-100, Sodium dodecyl sulfate, Cetyltrimethyl ammonium bromide	**Temperature** Conditions:surfactant concentration 0.5% (w/v), molasses concentration 27.5% (w/v), incubation time 20 min, pH 6.1, extraction time 30 min	Betaine	80%	(Mohammadzadeh et al. 2018)
Sisal waste	Triton X-100	**Temperature and salts** Conditions: ratio sisal/solvent 0.17 g/mL, surfactant concentration 7.5% (v/v), sodium carbonate 20% (m/v), 50 °C, extraction time 4 h	Saponins	89.1%	(Dias Ribeiro et al. 2015)
Aloe peel	Triton X-114	**Temperature, acids, salts**	Anthraquinones	96.9%	(Tan et al. 2012)

Table 6. Extraction of bioactive compounds from agroindustrial by-products, using supramolecular solvents

Raw material	Amphiphile	External stimulus for phase separation and conditions	Bioactive compound	Extraction efficiency	References
		Conditions: surfactant concentration 10% (w/v), NaCl 2.0% (w/v) l, 40° C, pH 3.0, extraction time 20 min			

Optimal amphiphile in bold

3. Future perspectives

This review aimed to provide an overview of the application of green solvents for the extraction of different classes of bioactive compounds from agri-food waste, mainly small organic extractable compounds (phenolic compounds, carotenoids, tocols, among others) and other high-added value compounds (fermentable sugars, lignin, oils, etc.). Research in this area is increasing in the last years and constitutes an urgent demand since disposal of agri-waste represents both cost and potential negative impact on the environment. In general, it can be concluded that if properly selected, green solvents are able to afford high extraction yields in different agri-food wastes. The sustainable character and costs associated with the extraction depend on the selected solvent, the source of bioactive compound, the temperature and processing time and the presence – or not - of assisted-extraction modes, such as the use of microwave, ultrasound or the use of re-flux.

Despite the efforts made by different authors to develop alternative green solvents and to evaluate different extraction approaches and conditions, many studies are still based on ionic liquids and SFCs. However, the use of SCFs is too expensive and the toxicity of ILs is controversial. Bio-based solvents, natural deep eutectic solvents (NADES) and supramolecular solvents appear to be a more promising and greener option due to their bio-compatibility and low toxicity. The term NADES refers to deep eutectic solvents synthetized from natural compounds, i.e. choline chloride, mixed with natural acids, amines and alcohols (Kumar et al. 2018). For these non-volatile (or hardly volatile) green solvents, strategies for the recovery or back-extraction and concentration of bioactives are key for their implementation at industrial scale. However, only few studies investigate possible procedures.

The evaluation of the economic viability and implementation at industrial scale are necessary to broaden the applicability for green solvents. The development of cost-effective and more sustainable extraction and separation processes is the critical step toward the recovery and commercialization of new and low-cost bioactive products for the nutraceutical, cosmetic, and pharmaceutical sectors. Research in extraction processes with green solvents needs to take into account in the near future: (i) the life cycle analysis of their processes and products, (ii) processes able to be scaled-up and (iii) economic analyses of the extraction process, solvent, and material costs.

Acknowledgments

Authors gratefully acknowledge financial support from Spanish MINECO (Project CTQ2017-83823-R). A. Ballesteros-Gómez acknowledges the funding from Spanish Ministry of Science, Innovation and Universities for a Ramón y Cajal contract (RYC-2015-18482). L.S. Torres-Valenzuela thanks AUIP for her doctoral fellowship.

References

Abdelmoez W, Nage SA, Bastawess A et al (2014). Subcritical water technology for wheat straw hydrolysis to produce value added products. J Cleaner Prod 70:68-77. doi:10.1016/j.jclepro.2014.02.011

Alañón ME, Alarcón M, Marchante L, et al (2017) Extraction of natural flavorings with antioxidant capacity from cooperage by-products by green extraction procedure with subcritical fluids. Industrial Crops and Products 103:222–232. doi: 10.1016/j.indcrop.2017.03.050

Albarelli JQ, Santos DT, Ensinas AV, et al (2018) Comparison of extraction techniques for product diversification in a supercritical water gasification-based sugarcane-wet microalgae biorefinery: Thermoeconomic and environmental analysis. Journal of Cleaner Production 201:697–705. doi: 10.1016/j.jclepro.2018.08.137

Alonso DA, Baeza A, Chinchilla R, et al (2016) Deep Eutectic Solvents: The Organic Reaction Medium of the Century. European Journal of Organic Chemistry 2016:612–632. doi: 10.1002/ejoc.201501197

Alvarez MV, Cabred S, Ramirez CL, Fanovich MA (2019) Valorization of an agroindustrial soybean residue by supercritical fluid extraction of phytochemical compounds. The Journal of Supercritical Fluids 143:90–96. doi: 10.1016/j.supflu.2018.07.012

Alvarez VH, Cahyadi J, Xu D et al. (2014) Optimization of phytochemicals production from potato peel using subcritical water: experimental and dynamic modeling. J Supercrit Fluids, 90: 8-17. doi: 10.1016/j.supflu.2014.02.013

Apostolakis A, Grigorakis S, Makris DP (2014) Optimisation and comparative kinetics study of polyphenol extraction from olive leaves (Olea europaea) using heated water/glycerol mixtures. Separation and Purification Technology 128:89–95. doi: 10.1016/j.seppur.2014.03.010

Araújo M, Pimentel FB, Alves RC, Oliveira MBPP (2015) Phenolic compounds from olive mill wastes: Health effects, analytical approach and application as food antioxidants. Trends in Food Science & Technology 45:200–211. doi: 10.1016/j.tifs.2015.06.010

Arun KB, Chandran J, Dhanya R, et al (2015) A comparative evaluation of antioxidant and antidiabetic potential of peel from young and matured potato. Food Bioscience 9:36–46. doi: 10.1016/j.fbio.2014.10.003

Ballesteros-Gómez A, Lunar L, Sicilia MD, Rubio S (2018) Hyphenating Supramolecular Solvents and Liquid Chromatography: Tips for Efficient Extraction and Reliable Determination of Organics. Chromatographia 1–14. doi: 10.1007/s10337-018-3614-1

Ballesteros-Gómez A, Rubio S (2012) Environment-responsive alkanol-based supramolecular solvents: characterization and potential as restricted access property and mixed-mode extractants. Anal Chem 84:342–349. doi: 10.1021/ac2026207

Ballesteros-Gómez A, Sicilia MD, Rubio S (2010) Supramolecular solvents in the extraction of organic compounds. A review. Analytica Chimica Acta 677:108–130. doi: 10.1016/j.aca.2010.07.027

Ben-Youssef S, Fakhfakh J, Breil C, et al (2017) Green extraction procedures of lipids from Tunisian date palm seeds. Industrial Crops and Products 108:520–525. doi: 10.1016/j.indcrop.2017.07.010

Bermejo DV, Luna P, Manic MS, et al (2013) Extraction of caffeine from natural matter using a bio-renewable agrochemical solvent. Food and Bioproducts Processing 91:303–309. doi: 10.1016/j.fbp.2012.11.007

Bosiljkov T, Dujmić F, Cvjetko Bubalo M, et al (2017) Natural deep eutectic solvents and ultrasound-assisted extraction: Green approaches for extraction of wine lees anthocyanins. Food and Bioproducts Processing 102:195–203. doi: 10.1016/j.fbp.2016.12.005

Braga MEM, Santos RMS, Seabra IJ, et al (2008) Fractioned SFE of antioxidants from maritime pine bark. The Journal of Supercritical Fluids 47:37–48. doi: 10.1016/j.supflu.2008.05.005

Byrne FP, Jin S, Paggiola G, et al (2016) Tools and techniques for solvent selection: green solvent selection guides. Sustainable Chemical Processes 4:7. doi: 10.1186/s40508-016-0051-z

Caballo C, Sicilia MD, Rubio S (2017) Chapter 5 - Supramolecular Solvents for Green Chemistry. In: Pena-Pereira F, Tobiszewski M (eds) The Application of Green Solvents in Separation Processes. Elsevier, pp 111–137

Cabeza LF, de Gracia A, Fernández AI, Farid MM (2017) Supercritical CO2 as heat transfer fluid: A review. Applied Thermal Engineering 125:799–810. doi: 10.1016/j.applthermaleng.2017.07.049

Calvo-Flores FG, Monteagudo-Arrebola MJ, Dobado JA, Isac-García J (2018) Green and Bio-Based Solvents. Topics in Current Chemistry 376:18. doi: 10.1007/s41061-018-0191-6

Campone L, Celano R, Lisa Piccinelli A, et al (2018) Response surface methodology to optimize supercritical carbon dioxide/co-solvent extraction of brown onion skin by-product as source of nutraceutical compounds. Food Chemistry 269:495–502. doi: 10.1016/j.foodchem.2018.07.042

Carciochi RA, D'Alessandro LG, Vauchel P, et al (2017) Chapter 4 - Valorization of Agrifood By-Products by Extracting Valuable Bioactive Compounds Using Green Processes. In:

Grumezescu AM, Holban AM (eds) Ingredients Extraction by Physicochemical Methods in Food. Academic Press, pp 191–228

Carmona-Cabello M, Garcia IL, Leiva-Candia D, Dorado MP (2018) Valorization of food waste based on its composition through the concept of biorefinery. Current Opinion in Green and Sustainable Chemistry 14:67–79. doi: 10.1016/j.cogsc.2018.06.011

Chan W-C, Su M-Q (2008) Biofiltration of ethyl acetate and amyl acetate using a composite bead biofilter. Bioresource Technology 99:8016–8021. doi: 10.1016/j.biortech.2008.03.045

Chanioti S, Tzia C (2018) Extraction of phenolic compounds from olive pomace by using natural deep eutectic solvents and innovative extraction techniques. Innovative Food Science & Emerging Technologies 48:228–239. doi: 10.1016/j.ifset.2018.07.001

Chatzilazarou A, Katsoyannos E, Gortzi O, et al (2010) Removal of polyphenols from wine sludge using cloud point extraction. J Air Waste Manag Assoc 60:454–459. doi:10.3155/1047-3289.60.4.454

Chemat S, Tomao V, Chemat F (2012) Limonene as Green Solvent for Extraction of Natural Products. In: Mohammad A (ed) Green Solvents I: Properties and Applications in Chemistry. Springer Netherlands, Dordrecht, pp 175–186

Clark JH, Tavener SJ (2007) Alternative Solvents: Shades of Green. Org Process Res Dev 11:149–155. doi: 10.1021/op060160g

Cláudio AFM, Cognigni A, de Faria ELP, et al (2018) Valorization of olive tree leaves: Extraction of oleanolic acid using aqueous solutions of surface-active ionic liquids. Separation and Purification Technology 204:30–37. doi: 10.1016/j.seppur.2018.04.042

Cooney MJ, Benjamin K (2016) Ionic Liquids in Lipid Extraction and Recovery. In: Xu X, Guo Z, Cheong L-Z (eds) Ionic Liquids in Lipid Processing and Analysis. AOCS Press, pp 279–316

Cui Q, Peng X, Yao X-H, et al (2015) Deep eutectic solvent-based microwave-assisted extraction of genistin, genistein and apigenin from pigeon pea roots. Separation and Purification Technology 150:63–72. doi: 10.1016/j.seppur.2015.06.026

Cunha SC, Fernandes JO (2018) Extraction techniques with deep eutectic solvents. TrAC Trends in Analytical Chemistry 105:225–239. doi: 10.1016/j.trac.2018.05.001

Cvjetko Bubalo M, Ćurko N, Tomašević M, et al (2016) Green extraction of grape skin phenolics by using deep eutectic solvents. Food Chemistry 200:159–166. doi: 10.1016/j.foodchem.2016.01.040

Cvjetko Bubalo M, Vidović S, Radojčić Redovniković I, Jokić S (2018) New perspective in extraction of plant biologically active compounds by green solvents. Food and Bioproducts Processing 109:52–73. doi: 10.1016/j.fbp.2018.03.001

da Silva RPFF, Rocha-Santos TAP, Duarte AC (2016) Supercritical fluid extraction of bioactive compounds. TrAC Trends in Analytical Chemistry 76:40–51. doi: 10.1016/j.trac.2015.11.013

Das S, Mondal A, Balasubramanian S (2017) Recent advances in modeling green solvents. Current Opinion in Green and Sustainable Chemistry 5:37–43. doi: 10.1016/j.cogsc.2017.03.006

de Jesus SS, Ferreira GF, Fregolente LV, Maciel Filho R (2018) Laboratory extraction of microalgal lipids using sugarcane bagasse derived green solvents. Algal Research 35:292–300. doi: 10.1016/j.algal.2018.09.001

DeSimone JM (2002) Practical Approaches to Green Solvents. Science 297:799. doi: 10.1126/science.1069622

Dias Ribeiro B, Weingart Barreto D, Zarur Coelho MA (2015) Use of micellar extraction and cloud point preconcentration for valorization of saponins from sisal (Agave sisalana) waste. Food and Bioproducts Processing 94:601–609. doi: 10.1016/j.fbp.2014.07.004

Djas M, Henczka M (2018) Reactive extraction of carboxylic acids using organic solvents and supercritical fluids: A review. Separation and Purification Technology 201:106–119. doi: 10.1016/j.seppur.2018.02.010

Dominguez de Maria P (2017) Ionic liquids, switchable solvents and eutectic mixtures. In: Green solvents. Elsevier, Amsterdam, Netherlands, p 533

El-Malah MH, Hassanein MM, Helmy Areif M, Al-Amrousi EF (2015) Utilization of Egyptian Tomato Waste as a Potential Source of Natural Antioxidants Using Solvents, Microwave and Ultrasound Extraction Methods. American Journal of Food Technology 10:14–25. doi: 10.3923/ajft.2015.14.25

Galanakis CM (2015) Food Waste Recovery. Processing Technologies and Industrial Techniques. Elsevier

Gorbaty Y, Bondarenko GV (2017) Transition of liquid water to the supercritical state. Journal of Molecular Liquids 239:5–9. doi: 10.1016/j.molliq.2016.06.040

Grewal J, Khare SK (2018) One-pot bioprocess for lactic acid production from lignocellulosic agro-wastes by using ionic liquid stable Lactobacillus brevis. Bioresource Technology 251:268–273. doi: 10.1016/j.biortech.2017.12.056

Grigoras CG, Destandau E, Fougère L, Elfakir C (2013) Evaluation of apple pomace extracts as a source of bioactive compounds. Industrial Crops and Products 49:794–804. doi: 10.1016/j.indcrop.2013.06.026

Gustavsson J, Cederberg C, Sonesson U, et al (2011) Global food losses and food waste. Food and Agriculture Organization of the United Nations, Rome

Gutiérrez-Arnillas E, Álvarez MS, Deive FJ, et al (2016) New horizons in the enzymatic production of biodiesel using neoteric solvents. Renewable Energy 98:92–100. doi: 10.1016/j.renene.2016.02.058

Hadi NA, Ng MH, Choo YM, et al (2015) Performance of Choline-Based Deep Eutectic Solvents in the Extraction of Tocols from Crude Palm Oil. Journal of the American Oil Chemists' Society 92:1709–1716. doi: 10.1007/s11746-015-2720-6

Henderson RK, Jiménez-González C, Constable DJC, et al (2011) Expanding GSK's solvent selection guide – embedding sustainability into solvent selection starting at medicinal chemistry. Green Chem 13:854–862. doi: 10.1039/C0GC00918K

Herrero M, Cifuentes A, Ibañez E (2006) Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. Food Chemistry 98:136–148. doi: 10.1016/j.foodchem.2005.05.058

Hou Q, Li W, Ju M, et al (2015) Separation of polysaccharides from rice husk and wheat bran using solvent system consisting of BMIMOAc and DMI. Carbohydrate Polymers 133:517–523. doi: 10.1016/j.carbpol.2015.07.059

Hou X-D, Lin K-P, Li A-L, et al (2018) Effect of constituents molar ratios of deep eutectic solvents on rice straw fractionation efficiency and the micro-mechanism investigation. Industrial Crops and Products 120:322–329. doi: 10.1016/j.indcrop.2018.04.076

Hu XM, Zhang BX, Dong SJ, et al (2014) Hydrolisis of soybean by-products to prepare reducing sugar in ionic liquids. Asian Journal of Chemistry 26:8475–8478

Huang Y, Feng F, Jiang J, et al (2017) Green and efficient extraction of rutin from tartary buckwheat hull by using natural deep eutectic solvents. Food Chemistry 221:1400–1405. doi: 10.1016/j.foodchem.2016.11.013

Huddleston JG, Willauer HD, Swatloski RP, et al (1998) Room temperature ionic liquids as novel media for 'clean' liquid–liquid extraction. Chem Commun 1765–1766. doi: 10.1039/A803999B

IUPAC (1997) Compendium of Chemical Terminology.

Jablonský M, Škulcová A, Malvis A, Šima J (2018) Extraction of value-added components from food industry based and agro-forest biowastes by deep eutectic solvents. Journal of Biotechnology 282:46–66. doi: 10.1016/j.jbiotec.2018.06.349

Kammerer DR, Kammerer J, Valet R, Carle R (2014) Recovery of polyphenols from the by-products of plant food processing and application as valuable food ingredients. Food Research International 65:2–12. doi: 10.1016/j.foodres.2014.06.012

Kerton F (2009) Alternative Solvents for Green Chemistry. Royal Society of Chemistry, Cambridge, U.K.

Khan AS, Man Z, Bustam MA, et al (2018) Efficient conversion of lignocellulosic biomass to levulinic acid using acidic ionic liquids. Carbohydrate Polymers 181:208–214. doi: 10.1016/j.carbpol.2017.10.064

Kim M, Sowndhararajan K, Park SJ, Kim S (2018) Effect of inhalation of isomers, (+)-α-pinene and (+)-β-pinene on human electroencephalographic activity according to gender difference. European Journal of Integrative Medicine 17:33–39. doi: 10.1016/j.eujim.2017.11.005

Knez Ž, Markočič E, Leitgeb M, et al (2014) Industrial applications of supercritical fluids: A review. Energy 77:235–243. doi: 10.1016/j.energy.2014.07.044

Kruis AJ, Levisson M, Mars AE, et al (2017) Ethyl acetate production by the elusive alcohol acetyltransferase from yeast. Metabolic Engineering 41:92–101. doi: 10.1016/j.ymben.2017.03.004

Kumar AK, Parikh BS, Pravakar M (2016) Natural deep eutectic solvent mediated pretreatment of rice straw: bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue. Environmental Science and Pollution Research 23:9265–9275. doi: 10.1007/s11356-015-4780-4

Kumar K, Yadav AN, Kumar V at al (2017) Food waste: a potential bioresource for extraction of nutraceuticals and bioactive compounds. Bioresources and Bioprocessing 4:18. doi: 10.1186/s40643-017-0148-6

Kumar AK, Sharma S, Shah E, Patel A (2018) Technical assessment of natural deep eutectic solvent (NADES) mediated biorefinery process: A case study. Journal of Molecular Liquids 260:313–322. doi: 10.1016/j.molliq.2018.03.107

Lafka T-I, Lazou AE, Sinanoglou VJ, Lazos ES (2011) Phenolic and antioxidant potential of olive oil mill wastes. Food Chemistry 125:92–98. doi: 10.1016/j.foodchem.2010.08.041

Larriba M, Omar S, Navarro P, et al (2016) Recovery of tyrosol from aqueous streams using hydrophobic ionic liquids: a first step towards developing sustainable processes for olive mill wastewater (OMW) management. RSC Adv 6:18751–18762. doi: 10.1039/C5RA26510J

Li C, Wang Q, Zhao ZK (2008) Acid in ionic liquid: An efficient system for hydrolysis of lignocellulose. Green Chem 10:177–182. doi: 10.1039/B711512A

Li Z, Smith KH, Stevens GW (2016) The use of environmentally sustainable bio-derived solvents in solvent extraction applications—A review. Chinese Journal of Chemical Engineering 24:215–220. doi: 10.1016/j.cjche.2015.07.021

Liu SX, Mamidipally PK (2005) Quality Comparison of Rice Bran Oil Extracted with d-Limonene and Hexane. Cereal Chemistry 82:209–215. doi: 10.1094/CC-82-0209

Liu W, Zhao W, Chen J, Yang M (2007) A cloud point extraction approach using Triton X-100 for the separation and preconcentration of Sudan dyes in chilli powder. Analytica Chimica Acta 605:41–45. doi: 10.1016/j.aca.2007.10.034

Liu Y, Liu B, Nie Z (2015) Concurrent self-assembly of amphiphiles into nanoarchitectures with increasing complexity. Nano Today 10:278–300. doi: 10.1016/j.nantod.2015.04.001

Loow Y-L, New EK, Yang GH, et al (2017) Potential use of deep eutectic solvents to facilitate lignocellulosic biomass utilization and conversion. Cellulose 24:3591–3618. doi: 10.1007/s10570-017-1358-y

Lycourghiotis S, Makarouni D, Kordouli E, et al (2018) Activation of natural mordenite by various acids: Characterization and evaluation in the transformation of limonene into p-cymene. Molecular Catalysis 450:95–103. doi: 10.1016/j.mcat.2018.03.013

Martins PLG, Braga AR, de Rosso VV (2017) Can ionic liquid solvents be applied in the food industry? Trends in Food Science & Technology 66:117–124. doi: 10.1016/j.tifs.2017.06.002

Masci A, Coccia A, Lendaro E, et al (2016) Evaluation of different extraction methods from pomegranate whole fruit or peels and the antioxidant and antiproliferative activity of the polyphenolic fraction. Food Chemistry 202:59–69. doi: 10.1016/j.foodchem.2016.01.106

Matsuda H, Hirota Y, Kurihara K, et al (2013) Liquid–liquid equilibria containing fluorous solvents as environmentally benign solvent. Fluid Phase Equilibria 357:71–75. doi: 10.1016/j.fluid.2012.12.037

Matthäus B (2002) Antioxidant Activity of Extracts Obtained from Residues of Different Oilseeds. J Agric Food Chem 50:3444–3452. doi: 10.1021/jf011440s

Mohammadzadeh M, Honarvar M, Zarei AR, et al (2018) A new approach for separation and recovery of betaine from beet molasses based on cloud point extraction technique. J Food Sci Technol 55:1215–1223. doi: 10.1007/s13197-017-2999-4

Mouratoglou E, Malliou V, Makris DP (2016) Novel Glycerol-Based Natural Eutectic Mixtures and Their Efficiency in the Ultrasound-Assisted Extraction of Antioxidant Polyphenols from Agri-Food Waste Biomass. Waste Biomass Valor 7:1377–1387. doi: 10.1007/s12649-016-9539-8

Mourtzinos I, Anastasopoulou E, Petrou A, et al (2016) Optimization of a green extraction method for the recovery of polyphenols from olive leaf using cyclodextrins and glycerin as co-solvents. J Food Sci Technol 53:3939–3947. doi: 10.1007/s13197-016-2381-y

Munir MT, Kheirkhah H, Baroutian S at al (2018). Subcritical water extraction of bioactive compounds from waste onion skin. J Cleaner Prod 183: 487-494. doi: 10.1016/j.jclepro.2018.02.166

Naidu DS, Hlangothi SP, John MJ (2018) Bio-based products from xylan: A review. Carbohydrate Polymers 179:28–41. doi: 10.1016/j.carbpol.2017.09.064

Ndongou Moutombi FJ, Selka A, Fabiano-Tixier A-S, et al (2018) Highly selective solvent-free hydrogenation of pinenes to added-value cis-pinane. Comptes Rendus Chimie. doi: 10.1016/j.crci.2018.09.002

Nyam KL, Tan CP, Karim R, et al (2010a) Extraction of tocopherol-enriched oils from Kalahari melon and roselle seeds by supercritical fluid extraction (SFE-CO2). Food Chemistry 119:1278–1283. doi: 10.1016/j.foodchem.2009.08.007

Nyam KL, Tan CP, Lai OM, et al (2010b) Optimization of supercritical fluid extraction of phytosterol from roselle seeds with a central composite design model. Food and Bioproducts Processing 88:239–246. doi: 10.1016/j.fbp.2009.11.002

Oliveira DA, Mezzomo N, Gomes C, Ferreira SRS (2017) Encapsulation of passion fruit seed oil by means of supercritical antisolvent process. The Journal of Supercritical Fluids 129:96–105. doi: 10.1016/j.supflu.2017.02.011

Oliveira ELG, Silvestre AJD, Silva CM (2011) Review of kinetic models for supercritical fluid extraction. Chemical Engineering Research and Design 89:1104–1117. doi: 10.1016/j.cherd.2010.10.025

Pagano I a, Piccinelli AL, Celano, R, at al (2018) Pressurized hot water extraction of bioactive compounds from artichoke by- products. Electrophoresis 39:1899–1907 doi: 10.1002/elps.201800063

Pagano I b, Sánchez-Camargo ADP, Mendiola JA, et al (2018) Selective extraction of high-value phenolic compounds from distillation wastewater of basil (Ocimum basilicum L.) by pressurized liquid extraction. Electrophoresis. 39:1884–1891 doi: 10.1002/elps.201700442

Pandiyan K, Singh A, Singh S, et al (2019) Technological interventions for utilization of crop residues and weedy biomass for second generation bio-ethanol production. Renewable Energy 132:723–741. doi: 10.1016/j.renene.2018.08.049

Passos H, Freire MG, Coutinho JAP (2014) Ionic liquid solutions as extractive solvents for value-added compounds from biomass. Green Chem 16:4786–4815. doi: 10.1039/C4GC00236A

Pena-Pereira F, Tobiszewski M (2017) The Application of Green Solvents in Separation Processes | ScienceDirect. Elsevier, Amsterdam, Netherlands

Pérez C, Ruiz del Castillo ML, Gil C, et al (2015) Supercritical fluid extraction of grape seeds: extract chemical composition, antioxidant activity and inhibition of nitrite production in LPS-stimulated Raw 264.7 cells. Food Funct 6:2607–2613. doi: 10.1039/c5fo00325c

Pighin E, Díez VK, Di Cosimo JI (2017) Kinetic study of the ethyl lactate synthesis from triose sugars on Sn/Al2O3 catalysts. Catalysis Today 289:29–37. doi: 10.1016/j.cattod.2016.10.002

Pitchaiah KC, Sujatha K, Deepitha J, et al (2018) Recovery of uranium and plutonium from pyrochemical salt matrix using supercritical fluid extraction. J Supercritical Fluids. doi: 10.1016/j.supflu.2018.10.015

Plaza M, Abrahamsson V, Turner C (2013) Extraction and neoformation of antioxidant compounds by pressurized hot water extraction from apple byproducts. J Agric Food Chem 61: 5500-5510

Plaza M, Turner C (2015) Pressurized hot water extraction of bioactives. TrAC Trends in Analytical Chemistry 71:39–54. doi: 10.1016/j.trac.2015.02.022

Procentese A, Johnson E, Orr V, et al (2015) Deep eutectic solvent pretreatment and subsequent saccharification of corncob. Bioresource Technology 192:31–36. doi: 10.1016/j.biortech.2015.05.053

Radošević K, Ćurko N, Gaurina Srček V, et al (2016) Natural deep eutectic solvents as beneficial extractants for enhancement of plant extracts bioactivity. LWT-Food Science and Technology 73:45–51. doi: 10.1016/j.lwt.2016.05.037

Rezaei F, Yamini Y, Asiabi H, et al (2016) Supercritical fluid extraction followed by nanostructured supramolecular solvent extraction for extraction of levonorgestrel and megestrol from whole blood samples. The Journal of Supercritical Fluids 107:392–399. doi: 10.1016/j.supflu.2015.10.005

Roselló-Soto E, Barba FJ, Parniakov O, et al (2015) High Voltage Electrical Discharges, Pulsed Electric Field, and Ultrasound Assisted Extraction of Protein and Phenolic Compounds from Olive Kernel. Food Bioprocess Technol 8:885–894. doi: 10.1007/s11947-014-1456-x

Saha K, Dasgupta J, Chakraborty S, et al (2017) Optimization of lignin recovery from sugarcane bagasse using ionic liquid aided pretreatment. Cellulose 24:3191–3207. doi: 10.1007/s10570-017-1330-x

Santaella MA, Orjuela A, Narváez PC (2015) Comparison of different reactive distillation schemes for ethyl acetate production using sustainability indicators. Chemical Engineering and Processing: Process Intensification 96:1–13. doi: 10.1016/j.cep.2015.07.027

Santalad A, Srijaranai S, Burakham R, et al (2009) Cloud-point extraction and reversed-phase high-performance liquid chromatography for the determination of carbamate insecticide residues in fruits. Anal Bioanal Chem 394:1307–1317. doi: 10.1007/s00216-009-2663-6

Santos DN e, Souza LL de, Ferreira NJ, Oliveira AL de (2015) Study of supercritical extraction from Brazilian cherry seeds (Eugenia uniflora L.) with bioactive compounds. Food and Bioproducts Processing 94:365–374. doi: 10.1016/j.fbp.2014.04.005

Santos-Zea L, Gutiérrez-Uribe JA, Benedito J (2019) Effect of ultrasound intensification on the supercritical fluid extraction of phytochemicals from Agave salmiana bagasse. The Journal of Supercritical Fluids 144:98–107. doi: 10.1016/j.supflu.2018.10.013

Sarkar D, Choudhury P, Dinda S, Das PK (2018) Vesicle formation by cholesterol based hydrazone tethered amphiphiles: Stimuli responsive dissipation of self-assembly. Journal of Colloid and Interface Science 530:67–77. doi: 10.1016/j.jcis.2018.06.064

Satlewal A, Agrawal R, Bhagia S, et al (2018) Natural deep eutectic solvents for lignocellulosic biomass pretreatment: Recent developments, challenges and novel opportunities. Biotechnology Advances. doi: 10.1016/j.biotechadv.2018.08.009

Shankar M, Chhotaray PK, Agrawal A, et al (2017) Protic ionic liquid-assisted cell disruption and lipid extraction from fresh water Chlorella and Chlorococcum microalgae. Algal Research 25:228–236. doi: 10.1016/j.algal.2017.05.009

Sharif KM, Rahman MM, Azmir J, et al (2014) Experimental design of supercritical fluid extraction – A review. Journal of Food Engineering 124:105–116. doi: 10.1016/j.jfoodeng.2013.10.003

Silva YPA, Ferreira TAPC, Celli GB, Brooks MS (2018) Optimization of Lycopene Extraction from Tomato Processing Waste Using an Eco-Friendly Ethyl Lactate–Ethyl Acetate Solvent: A Green Valorization Approach. Waste and Biomass Valorization 1–11. doi: https://doi.org/10.1007/s12649-018-0317-7

Smith EL, Abbott AP, Ryder KS (2014a) Deep Eutectic Solvents (DESs) and Their Applications. Chem Rev 114:11060–11082. doi: 10.1021/cr300162p

Smith EL, Abbott AP, Ryder KS (2014b) Deep Eutectic Solvents (DESs) and Their Applications. Chemical Reviews 114:11060–11082. doi: 10.1021/cr300162p

Sookwong P, Suttiarporn P, Boontakham P, et al (2016) Simultaneous quantification of vitamin E, γ-oryzanols and xanthophylls from rice bran essences extracted by supercritical CO2. Food Chemistry 211:140–147. doi: 10.1016/j.foodchem.2016.05.001

Strati IF, Oreopoulou V (2016) Recovery and Isomerization of Carotenoids from Tomato Processing By-products. Waste and Biomass Valorization 7:843–850. doi: 10.1007/s12649-016-9535-z

Strati IF, Oreopoulou V (2014) Recovery of carotenoids from tomato processing by-products – a review. Food Research International 65:311–321. doi: 10.1016/j.foodres.2014.09.032

Strati IF, Oreopoulou V (2011) Effect of extraction parameters on the carotenoid recovery from tomato waste. International Journal of Food Science & Technology 46:23–29. doi: 10.1111/j.1365-2621.2010.02496.x

Tan Z-J, Li F-F, Xing J-M (2012) Cloud point extraction of aloe anthraquinones based on non-ionic surfactant aqueous two-phase system. Nat Prod Res 26:1423–1432. doi: 10.1080/14786419.2011.601415

Teixeira A, Baenas N, Dominguez-Perles R, et al (2014) Natural Bioactive Compounds from Winery By-Products as Health Promoters: A Review. International Journal of Molecular Sciences 15:. doi: 10.3390/ijms150915638

Tomé LIN, Baião V, da Silva W, Brett CMA (2018) Deep eutectic solvents for the production and application of new materials. Applied Materials Today 10:30–50. doi: 10.1016/j.apmt.2017.11.005

Ueno H, Tanaka M, Machmudah S, et al (2008) Supercritical Carbon Dioxide Extraction of Valuable Compounds from Citrus junos Seed. Food and Bioprocess Technology 1:357–363. doi: 10.1007/s11947-007-0015-0

Ventura SPM, e Silva FA, Quental MV, et al (2017) Ionic-Liquid-Mediated Extraction and Separation Processes for Bioactive Compounds: Past, Present, and Future Trends. Chem Rev 117:6984–7052. doi: 10.1021/acs.chemrev.6b00550

Vian M, Breil C, Vernes L, et al (2017) Green solvents for sample preparation in analytical chemistry. Current Opinion in Green and Sustainable Chemistry 5:44–48. doi: 10.1016/j.cogsc.2017.03.010

Villanueva-Bermejo D, Reglero G, Fornari T (2017) Recent advances in the processing of green tea biomolecules using ethyl lactate. A review. Trends in Food Science & Technology 62:1–12. doi: 10.1016/j.tifs.2016.12.009

Virot M, Tomao V, Ginies C, Chemat F (2008) Total Lipid Extraction of Food Using d-Limonene as an Alternative to n-Hexane. Chroma 68:311–313. doi: 10.1365/s10337-008-0696-1

Vovers J, Smith KH, Stevens GW (2017) Chapter 4 - Bio-Based Molecular Solvents. In: Pena-Pereira F, Tobiszewski M (eds) The Application of Green Solvents in Separation Processes. Elsevier, pp 91–110

Wang Y, Gu W (2018) Study on supercritical fluid extraction of solanesol from industrial tobacco waste. The Journal of Supercritical Fluids 138:228–237. doi: 10.1016/j.supflu.2018.05.001

Watanabe H, Tanaka H (1978) A non-ionic surfactant as a new solvent for liquid—liquid extraction of zinc(II) with 1-(2-pyridylazo)-2-naphthol. Talanta 25:585–589. doi: 10.1016/0039-9140(78)80151-9

Wei Z, Qi X, Li T, et al (2015) Application of natural deep eutectic solvents for extraction and determination of phenolics in Cajanus cajan leaves by ultra performance liquid chromatography. Separation and Purification Technology 149:237–244. doi: 10.1016/j.seppur.2015.05.015

Xhaxhiu K, Wenclawiak B (2015) Comparison of Supercritical CO2 and Ultrasonic Extraction of Orange Peel Essential Oil from Albanian Moro Cultivars. Journal of Essential Oil Bearing Plants 18:289–299. doi: 10.1080/0972060X.2015.1010603

Yang X, Cheng K, Jia G (2019) The molecular dynamics simulation of hydrogen bonding in supercritical water. Physica A: Statistical Mechanics and its Applications 516:365–375. doi: 10.1016/j.physa.2018.10.022

Yao G, Wang L, Chen X, et al (2019) Measurement and correlation of vapor–liquid equilibrium data for binary and ternary systems composed of (−)-β-caryophyllene, p-cymene and 3-carene at 101.33 kPa. The Journal of Chemical Thermodynamics 128:215–224. doi: 10.1016/j.jct.2018.08.015

Yoo DE, Jeong KM, Han SY, et al (2018) Deep eutectic solvent-based valorization of spent coffee grounds. Food Chemistry 255:357–364. doi: 10.1016/j.foodchem.2018.02.096

Yoon TJ, Lee Y-W (2018) Current theoretical opinions and perspectives on the fundamental description of supercritical fluids. The Journal of Supercritical Fluids 134:21–27. doi: 10.1016/j.supflu.2017.11.022

Yu J, Dandekar DV, Toledo RT, et al (2006) Supercritical fluid extraction of limonoid glucosides from grapefruit molasses. J Agric Food Chem 54:6041–6045. doi: 10.1021/jf060382d

Zahari SMSNS, Amin ATM, Halim NM, et al (2018) Deconstruction of Malaysian agro-wastes with inexpensive and bifunctional triethylammonium hydrogen sulfate ionic liquid. AIP Conference Proceedings 1972:030024. doi: 10.1063/1.5041245

Zainal-Abidin MH, Hayyan M, Hayyan A, Jayakumar NS (2017) New horizons in the extraction of bioactive compounds using deep eutectic solvents: A review. Anal Chim Acta 979:1–23. doi: 10.1016/j.aca.2017.05.012

Zhang C-W, Xia S-Q, Ma P-S (2016) Facile pretreatment of lignocellulosic biomass using deep eutectic solvents. Bioresource Technology 219:1–5. doi: 10.1016/j.biortech.2016.07.026

Zhang Q, Vigier KDO, Royer S, Jérôme F (2012) Deep eutectic solvents: syntheses, properties and applications. Chem Soc Rev 41:7108–7146. doi: 10.1039/C2CS35178A

Zhou P, Wang X, Liu P, et al (2018) Enhanced phenolic compounds extraction from Morus alba L. leaves by deep eutectic solvents combined with ultrasonic-assisted extraction. Industrial Crops and Products 120:147–154. doi: 10.1016/j.indcrop.2018.04.071

CAPÍTULO II

VALORIZACIÓN DE BORRAS DE CAFÉ MEDIANTE LA EXTRACCIÓN DE COMPUESTOS BIOACTIVOS CON DISOLVENTES SUPRAMOLECULARES

Valorization of spent coffee grounds by supramolecular solvent extraction

Laura Sofía Torres-Valenzuela [a,b], Ana Ballesteros [a*], Alejandra Sanin [b], Soledad Rubio[a]

Separation and Purification Technology, 228 (2019): 115759

Abstract

In this study, we assess the potential of supramolecular solvents (SUPRAS) for valorization of spent coffee grounds (SCG). SUPRAS, made up of self-assembled amphiphilic aggregates dispersed in an aqueous or hydro-organic medium, are excellent extractants that provide multiple binding interactions (hydrogen bonds, dispersion, dipole-dipole, etc.) and microenvironments of different polarity due to their special internal architecture. In this work, SUPRAS made up of different amphiphiles (decanoic acid and hexanol) and hydro-organic media (water-ethanol and water-tetrahydrofuran) were investigated for extraction of bioactives from SCG. Extraction was optimized from the yield obtained for caffeine and 5-chlorogenic acid, that were considered as model compounds. Under optimal extraction conditions, the profile of bioactive compounds in the extracts was screened by liquid chromatography tandem mass spectrometry and the total phenolic content was estimated. The antioxidants and antimicrobial properties of the extracts were also evaluated. Bioactive compounds were extracted from wet SCG up to 3.32 $mg.g^{-1}$and 4.3 $mg.g^{-1}$ SCG of caffeine and chlorogenic acid, respectively. Extracts showed antioxidant capacity by different assays (DPPH, TEAC, FRAP) in accordance with their high total phenolic content (60.1 mg CGA per mg of extracted dry SCG). SUPRAS offered advantages in terms of rapidity (extraction for 1 min) and simplicity (the process involved stirring and centrifugation at room temperature), thus avoiding costly processes based on high pressure and temperature. Furthermore, SUPRAS extracts exhibited certain degree of antimicrobial effects against, *S. aureus* and *B. cereu*s and a high effect against *S. enterica* and *P. putida.*

Keywords: supramolecular solvents; spent coffee grounds; bioactive compounds; valorization

1. Introduction

The agricultural world production is continuously increasing as a result of the rising global demand for food and generates billion tons of by-products each year [1]. There is a growing interest in the recovery of bioactive compounds from agro-waste for application in functional foods and nutraceutical formulations [2]. The coffee industry alone generates about 2 billion tons of agro-waste, which represent a great pollution hazard [3]. Coffee pulp, husks, silverskin, peel and spend coffee grounds are common coffee by-products [4] and have been reported of interest as substrates for mushroom cultivation [5], immobilization of enzymes [6], production of bioethanol [7] composting [8], and extraction of bioactive compounds [9–11].

Spent coffee grounds (SCG), a high humidity residue (up to ~80%) obtained in coffee beverage preparation and instant coffee manufacturing, is the most abundant coffee by-product (45-50%) [12,13]. SCG is produced at a rate of 6 million tons a year [12]. Valorization of coffee by-products through the recovery of bioactives, particularly alkaloids and polyphenols, has increasingly become of interest for food, pharmaceutical and cosmetic industries [13–16]. The major alkaloid in coffee by-products is caffeine, which shows anti-inflammatory and immunosuppressant effects [16]. Regarding polyphenols, they include a broad range of compounds including tannins, flavanols, flavones, anthocyanins, proanthocyanidins, and phenolic, hydroxybenzoic and hydroxycinnamic acids [17]. Polyphenols have demonstrated antioxidant, anti-bacterial, anti-inflammatory and anti-carcinogenic activities [13–15].

Extraction of bioactives from SCG has been investigated using different solvents and techniques, including conventional solid-liquid extraction (SLE) [18,19], supercritical fluid extraction (SFE), with and without co-solvent [20], Soxhlet extraction [20], and ultrasound (USAE) [20–22] or microwave (MAE) [21,23] assisted extraction. Extraction efficiencies, usually given as total phenolic compounds (TPC) and expressed as gallic acid (GAE) or chlorogenic acid (CAE) equivalents [24], are highly dependent on the type of solvent, the solvent/solid ratio, the number of extraction steps and the extraction time and temperature, among others factors [15]. Extractions have been carried out using polar (e.g. methanol and ethanol) and medium or non-polar (e.g. dichloromethane, ethyl acetate, hexane) solvents [18–20,23], supercritical fluids [20], subcritical water [21], and deep eutectic solvents [22].

Common conditions for conventional SLE include solvent/solid ratios of around 30-40 mL/g SCG, extraction temperatures in the range 50-65 ºC and extraction times for 1-2 h, which give extraction efficiencies for TPCs of about 16-18 mg GAE/g SCG [18–20]. Extraction efficiencies for TPCs in SFE increase in the presence of ethanol as co-solvent (e.g. around 42 mg CAE per gram of extract using 8% ethanol, which is equivalent to ~4 mg CAE/g SCG taking into account yields of about 10%) [20]. Extraction of phenolic compounds have been also reported for energy-assisted techniques, namely Soxhlet extraction [20], USAE [20] or MAE [23]. Thus, TPCs were in the range 119-167 mg CAE/g extract (18-22 mg CAE/g SCG; extraction yields 12-15%) with Soxhlet extraction using solvents of different polarity, solvent /solid ratios of 30, and 6 h of extraction at the boiling temperature of the solvent [20]. Likewise, the application of USAE for 2 h, at room temperature and solvent/solid ratios of 30, permitted to achieve extraction efficiencies for TPCs in the range of 221-588 mg CAE/g extract (21.9-71.7 mg CAE/g SCG; extraction yields 10-12%) [20]. Application of MAE was also assessed; it provided up to 399 mg GAE/g extract (21.5 mg/g SCG; extraction yield 5.4) with 40 s of irradiation and a solvent/solid ratio of 9 [23]. All these figures indicate that SCG is a valuable source for bioactives and that further research should be intended to reduce extraction efforts in order to make their valorization simpler and more cost-effective.

In this paper, we propose for the first time the use of supramolecular solvents (SUPRASs) for the extraction of bioactives from SCG. SUPRASs are nanostructured liquids spontaneously produced in colloidal suspensions of amphiphiles through a bottom-up approach based on sequential self-assembly phenomena [25,26]. The synthesis is made by a simple two-step process. First, amphiphiles spontaneously assemble into three-dimensional individual aggregates (mainly micelles and/or vesicles). The second stage generates a new highly packed phase by the assembly of the aggregates into a nano or microestructured liquid (SUPRAS phase). This second phase is triggered by an external stimuli such pH or temperature changes, addition of salt or addition of a poor solvent for the amphiphile, which diminishes the repulsion among the aggregates and promotes their assembly [25]. The SUPRAS phase remains in equilibrium with the bulk solution, which contains the amphiphile at the critical aggregation concentration. SUPRAS can be collected and stored if required (keeping its structure and properties) for application to solid samples or applied together with the equilibrium solution, which acts as a wetting and dispersion phase for the matrix [27].

The capability of SUPRASs for developing efficient processes for extraction of bioactives is based on the presence of different polarity microenvironments into their ordered structures, the high concentration of amphiphiles make up them (up to 1 mg/µL), and the possibility of producing tailored SUPRASs by selection of the amphiphile or the environment for self-assembly [28]. Thus, SUPRASs are able to efficiently extract compounds spanning a wide polarity range using low solvent/solid ratios [27]. On the other hand, SUPRASs with restricted access properties (SUPRAS-RAM) have been reported that permit the extraction of low molecular weight compounds while excluding macromolecules [29]. These properties have allowed the development of innovative strategies for sample preparation in the determination of organic contaminants and metals in food, the environment and biological fluids [25,26]. More recently, SUPRASs have also proved promising for the extraction of bioactives from microalgae [30] and the removal of contaminants in wastewater [31].

The suitability of SUPRASs for the extraction of bioactives from SCG obtained by the drip filter method was here explored. For this purpose, two types of SUPRASs, synthesized from decanoic acid [32] and hexanol [33] in hydro-organic media (water and ethanol or tetrahydrofuran) were investigated. Extraction efficiencies were evaluated by monitoring caffeine and chlorogenic acid, two major representatives of alkaloids and polyphenols, respectively. Under the optimized conditions, the SUPRAS extracts were further analysed to identify the main bioactives, to estimate their total phenolic content and evaluate their antioxidant and antimicrobial properties. Below, the more relevant results are presented and discussed.

2. Materials and methods

2.1 Chemicals

Caffeine (1,3,7-trimethylxantine, HPLC grade), 5-chlorogenic acid (5-O-Caffeoylquinic acid, 5-CGA, 98%), (±)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (98,1%, Trolox), 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2 diphenyl-1-picrylhydrazyl (DPPH), decanoic acid (98%), ethanol (HPLC grade), methanol (99,9%), 2,3,5-triphenyltetrazolium chloride (TTC), glacial acetic acid and tetrahydrofuran (HPLC grade) were purchased from Sigma–Aldrich Co. (St. Louis, USA). 1-hexanol (98%), hydrochloric acid (37%), and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) were supplied by Merck (Darmstadt,

Germany). Potassium persulfate was purchased from Panreac (Barcelona, Spain), ferric chloride from Carlo Erba (Val-de-Reuil, France) and potassium acetate (99,4%) from JT Baker (Madrid, Spain). All chemicals were analytical reagent-grade and were used as supplied. Pure water was prepared using a Milli-Q, Ultrapure water purification system equipped with a 0.22-µm filter (MA, USA).

Three reagents were prepared for evaluation of the antioxidant capacity of SUPRAS extracts containing coffee bioactives. The DPPH reagent was freshly prepared by dissolving 1 mg of DPPH in 50 mL of methanol and diluted with methanol to give an absorbance of 1.057±0.005 at 529 nm. It was kept in the dark at room temperature when not used. The $ABTS^{+}$ radical reagent was freshly prepared by dissolving 97 mg of ABTS and 16.5 mg of potassium persulfate in 25 mL of distilled water and keeping the solution for 16 hours under dark. Then, it was diluted with ethanol to yield an absorbance of 0.635±0.005 at 732 nm. The reagent FRAP (ferric reducing antioxidant power) was prepared by the mixing of three solutions in a thermostatic bath at 35 ºC; 250 mL of acetic acid/acetate buffer (40 mM, pH 3.6), 2.5 mL of an aqueous solution of ferric chloride (20 mM) and 2.5 mL of TPTZ (10 mM) in 40 mM HCl. The absorbance of the reagent solution was 0.107±0.005 at 595 nm.

2.2 Apparatus

A high-performance liquid chromatograph (HPLC) coupled to a UV Detector (Shimadzu, Japan) was employed for the quantification of caffeine and 5-CGA. The stationary phase was an Ultra C_8 column (5 µm particle size, 150 mm length, 4.6 mm i.d.) from Restek (France). All data were acquired and processed using the LabSolutions Software (Shimadzu, Japan). For the target screening of bioactive compounds in SUPRAS extracts under optimal conditions (section 2.5) we performed LC-MS/MS analysis. The equipment consisted in an Agilent Technologies 1200 LC system with a column ACE 3 C18-PFP column (3 mm i.d., 150 mm length, 3.0 µm particle size) preceded by a precolumn Phenomenex KJ 0-4282 Security Guard Cartridge Kit, Ea. The detector was an Agilent Technologies 6420 Triple Quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operating in negative and positive modes. Raw data were controlled and processed using Agilent MassHunter Software® (version B.07.00). Other instrumentation used for sample preparation were a vortex-shaker REAX Top (Heidolph, Schwabach, Germany) and a 12 x 1.5 – 2 mL angle rotor Minicen centrifuge from Ortoalresa

(Madrid, Spain). Optimization of the extraction of coffee byproducts was carried out in 2 mL-microtubes Safe-Lock from Eppendorf Iberica (Madrid, Spain). A vortex shaker from Vorterex (Heathrow Scientific, Vernon Hills, IL, USA) with an attachment for 4 tubes, and a high-speed brushless centrifuge BX 24 (Unico, USA) were used for sample preparation. Antimicrobial activity was evaluated in a laminar flow cabinet Physis (AirFlux, Malasya).

2.3 Spent coffee grounds

Spent coffee grounds (SCG) were obtained from a drip filter brewing method consisting in flowing water at 92–96 °C through a ground coffee bed so that the extract drips from the brewing chamber into the pot. The coffee used in all the experiments was the variety Castillo produced in Circasia (Colombia). The water content in the SCG was 74.0±0.8%. SCG samples were not dried and immediately processed or stored at -18 ºC.

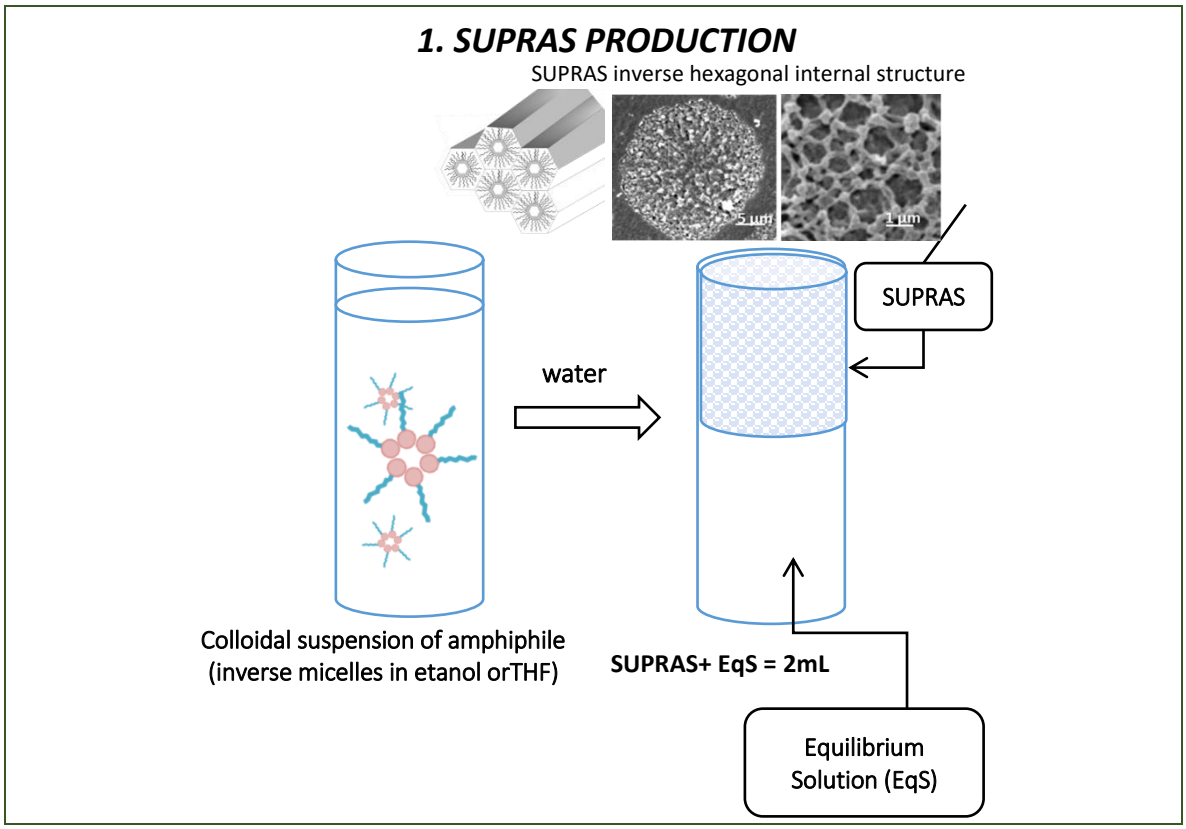
2.4. SUPRAS production

SUPRASs of different composition were produced by adding ultrapure water to a colloidal suspension of decanoic acid or hexanol in THF or ethanol (total volume of the mixture: 2 mL). Under addition of water, the decanoic acid or hexanol aggregates in the colloidal suspension gave spontaneously oily droplets that associated as clusters and finally separated from the bulk solution as a new liquid phase named SUPRAS. The whole solution, containing both the SUPRAS (at the top) and the hydro-organic equilibrium solution, was added to the SCG. Figure 1 shows a schematic of the general procedure followed for SUPRAS production.

2.5 SUPRAS-based extraction of bioactives from SCG

The following variables were considered for the optimization of SUPRAS-based extraction of bioactives from SCG: (a) type of organic solvent used to produce the colloidal suspension (ethanol or THF); (b) type of amphiphile (decanoic acid or hexanol) making up the SUPRAS; (c) amphiphile concentration in the SUPRAS synthetic solution (8, 16 and 24 % v/v), and (d) organic solvent concentration in the SUPRAS synthetic solution (20, 30 and 40 % v/v). The extraction of bioactives was performed by adding 0.35 g of wet SCG to the SUPRAS synthetic solution (see section 2.4) in polypropylene centrifuge microtubes. The

composition of the SUPRAS synthetic solution was varied as follows: hexanol or decanoic acid (66-574 µL), ethanol or THF (176-1024 µL) and distilled water (656-1560 µL). The sample size was kept constant at 0.35 g to ensure good sample dispersibility at the SUPRAS volume/sample size ratio that was set for the laboratory scale. The mixtures were vortex-shaken for 1 min at 3,000 rpm for the extraction of bioactives and then centrifuged for 20 minutes at 4,519 g to accelerate the separation of SUPRAS from the bulk equilibrium phase (in the middle) and precipitate (at the bottom). The volume of SUPRAS was measured using a digital caliper [33]. The volume of SUPRAS produced varies under different synthetic conditions (usually increasing with the concentration of both the amphiphile and the organic solvent) and consequently this affects concentration factors (ratio of SUPRAS volume/sample size). SUPRAS volumes varied in the range 61 – 1476 µL under the tested conditions. Experiments were done in triplicate. Figure 1 shows a schematic picture of the SUPRAS extraction procedure.



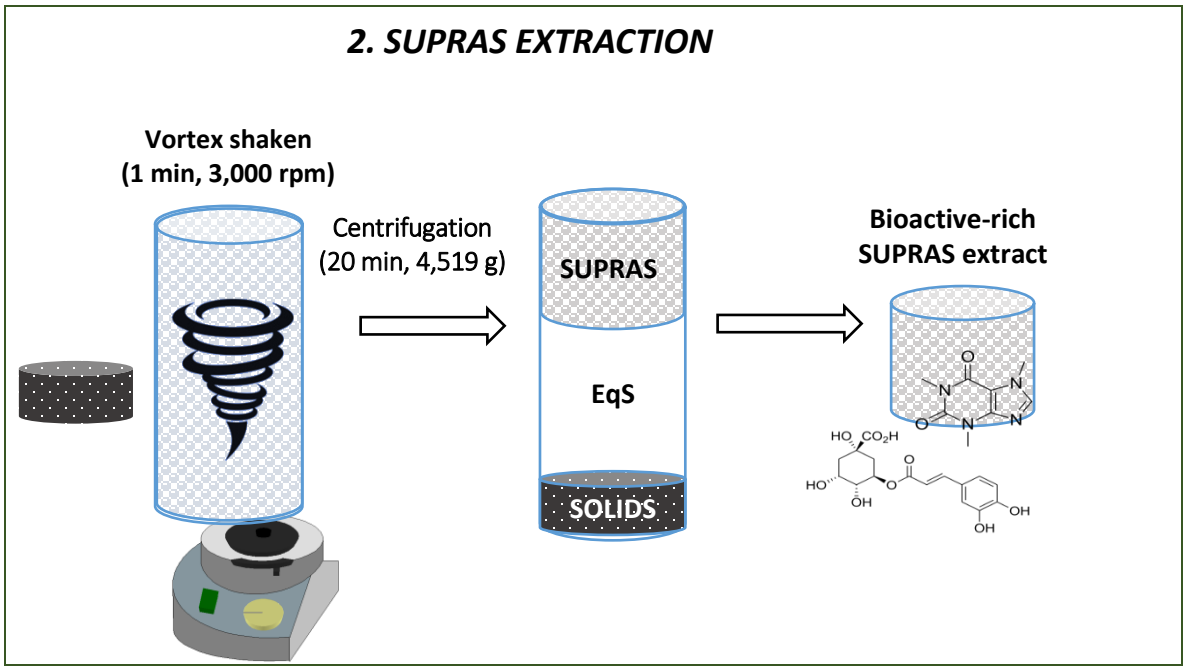


Figure 1. Schematic picture of SUPRAS production and SUPRAS-based extraction of SCGs

The final optimal SUPRAS synthesis conditions were 24% v/v hexanol and 30% v/v ethanol. The average SUPRAS volume was 980±10 µL (2.8 mL SUPRAS/g wet SCG). These conditions were finally tested for identification of bioactives, estimation of the total phenolic content and antioxidant and antimicrobial activity

2.6 Analysis of caffeine and chlorogenic acid by HPLC-UV

Caffeine and 5-CGA acid contents in the SUPRAS extracts were determined by HPLC-UV. The detector wavelength was set at 254 nm. The mobile phase consisted of 69.9% v/v of water, 30% v/v of methanol and 0.1% v/v of acetic in isocratic mode. The flow rate was set at 0.6 mL min^{-1} and the sample injection volume was 20 µL. Quantitative analysis was conducted by external calibration using standard solutions of caffeine and 5-CGA prepared in ultrapure water in the concentration range of 5 – 100 µg L^{-1}.

2.7 Profile of bioactive compounds in SUPRAS extracts by HPLC-MS/MS and estimation of total phenolic content

The presence of the main bioactives compounds present in SUPRAS extracts under optimal conditions (section 2.5.) was confirmed by target screening with LC-MS/MS experiments.

The mobile phase was made up of Milli-Q water with 0.1% acetic acid (A) and MeOH:Acetonitrile 50:50 v/v (B) at a flow rate of 0.3 mL·min^{-1}. The injection volume was 5 µL. The gradient was as follow: initial 5% B hold for 0.1 min, linear gradient to 30% B in 25 min and to 40% B in the next 10 min. Finally, B was increased to 100% at 35.1 min and maintained for 10 min to remove possible hydrophobic compounds form the column. The column was re-conditioned for 10 min before injection. The MRM transitions for target masses of the bioactives identified in SUPRAS extracts are given in Table 1. The MS parameters were: fragmentor 100 V, collision energy 15 eV, cell accelerator voltage 4 V, dwell 20 ms. Source parameters were: gas temperature, 350°C; gas flow, 12 L·min^{-1}; nebulizer gas pressure, 30 psi; capillary voltage, -4000 V. Total phenolic content was estimated from the sum of chromatographic peaks of the identified phenolic compounds with external calibration against 5-CGA, due to the lack of authentic standards for all of them.

2.8 Antioxidant activity assays

The antioxidant activity of the SUPRAS extracts obtained under the optimal conditions specified in section 2.5 was evaluated by the DPPH, TEAC [34] and FRAP [35] methods. Control assays with Trolox were run in parallel for TEAC. The decrease of the absorbance of the reagent solutions, measured as inhibition, was calculated from the following equation [34]:

$$\%inhibition = \frac{Abs_0 - Abs_{30}}{Abs_0} * 100$$

where Abs_0 is the absorbance of DPPH, $ABTS^+$ or FRAP reagent solution at time zero and Abs_{30} is the absorbance of the reagent in the presence of the bioactive coffee compounds at 30 minutes of reaction (as mentioned below).

2.8.1 DPPH radical scavenging assay

Aliquots of 100 µL of SUPRAS (previously diluted in 1:10 with methanol) or methanol as blank were mixed with 2 mL of DPPH solution. The mixture was vortexed for a minute and placed in the dark for 30 min. Finally, the absorbance of the mixture was measured at 529 nm. The final concentration of extract tested was ~4.1 mg SUPRAS extract /mL.

2.8.2 Trolox equivalent antioxidant capacity (TEAC) assay

The assays were made by mixing 50 µL of methanol as blank or SUPRAS extracts (previously diluted in 1:10 with methanol) and 1450 µL of the free radical $ABTS^{+}$ stock solution prepared as indicated in section 2.1. The mixture was vortexed for a minute and placed in the dark for 30 min. The absorbance was measured at 732 nm and the percentage of inhibition was referred to TEAC. The final concentration of extract tested was 2.7 mg SUPRAS extract/mL.

2.8.3 Ferric reducing antioxidant potential (FRAP) assay

Aliquots of 30 µL of SUPRAS extracts (previously diluted in 1:10 with methanol) or methanol as blank, 90 µL of water and 900 µL of the FRAP reagent were mixed and incubated during 30 minutes at 37 ºC. The absorbance was measured at 595 nm. The final concentration of extract tested was 2.5 mg SUPRAS extract/mL.

2.9 Antimicrobial susceptibility testing method

The colorimetric broth microdilution method with 2,3-diphenyl-5-thienyl-(2)-tetrazolium chloride (TTC) [36,37] was used to determine the lowest concentration of the assayed antimicrobial agent (minimal inhibitory concentration, MIC). Suspensions of *S. enterica* (ATCC 0363), *S. aureus* (ATCC 0496), *P. putida* (ATCC 49128) and *B. cereus* (ATCC 14579) were growth at 37°C in Tryptic Soy broth (TSB) until a concentration of 10^6 colonies forming units (cfu)/mL was reached. Initially, 100 µL of TSB with 1% of TTC (indicator of metabolic activity) were added in each well of a sterile 96-well microplate followed by 100 µL of SUPRAS extracts (undiluted and diluted at 1:10 and 1:100 with distilled water). Finally, 100 µL of the previously standardized microorganisms were inoculated. Final extract concentrations were 287, 28.7 and 2.87 mg SUPRAS extract/mL. After incubating for 24 hours, a color change in the wells was observed and those showing microbial growth were pink-colored.

3. Results and discussion

3.1 SUPRAS-based extraction of bioactives from SCG

The ability of SUPRASs to develop efficient and cost-effective processes for extraction of bioactives from SCG was evaluated by monitoring the extraction yield for caffeine and chlorogenic acid (5-CGA), which were selected as model compounds for alkaloids and polyphenols, respectively. These compounds can establish donor and/or acceptor hydrogen bonds, and polar and dispersion interactions, so the components making up the SUPRAS were selected to maximize these types of interactions.

Two amphiphiles (decanoic acid and hexanol) and two hydro-organic media (THF:water and ethanol:water) were chosen for SUPRAS production. Both, carboxylic acids [32] and alkanols [29] have been reported to give SUPRASs made up of inverted hexagonal aggregates where the polar groups surround aqueous cavities and the hydrocarbon chains disperse in the organic solvent (see schematic in Figure 1). The amphiphile functional groups (-OH, -COOH) provide hydrogen bonds and polar interactions, while the alkyl chains give dispersion interactions, so both alkaloids and polyphenols can be solubilized in the hexagonal nanostructures of the SUPRAS by mixed mode mechanisms, which should enhance extraction. On the other hand, ethanol and THF, used to produce the colloidal suspension of the amphiphile, were selected on the basis of their different polarity, which should also influence the extraction of the target compounds.

Optimization of the SUPRAS-based extraction was carried out according to the procedure specified in section 2.5. The SCG obtained by the drip filter method were subjected to extraction as collected (viz. without drying the by-product) in order to reduce costs and speed up the valorization process. Although bioactives in the SCG were solubilized in the SUPRAS, the equilibrium solution generated in SUPRAS formation (see Figure 1) was also used in the extraction process with the aim of facilitating both the dispersion of the SCG and the SUPRAS extract overflows.

Figures 2 and 3 show the average extraction recoveries obtained for caffeine and 5-CGA, respectively, when the SCG were subjected to extraction with each of the SUPRAS investigated. Results are expressed as mg of bioactive per g of dry SCG in order to facilitate

comparison with previous reported procedures. Each SUPRAS was produced at different proportions (expressed as volume percentages) of the ternary mixture (viz. amphiphile:organic solvent:water), which permitted to vary both SUPRAS composition and volume [29,32]. Thus, increased volume of SUPRAS was obtained by increasing the concentration of the amphiphile at constant organic solvent/water volume ratios in the synthesis. On the other hand, increased volume of SUPRAS was obtained by increasing the organic solvent/water volume ratios in the synthesis at constant amphiphile concentration.

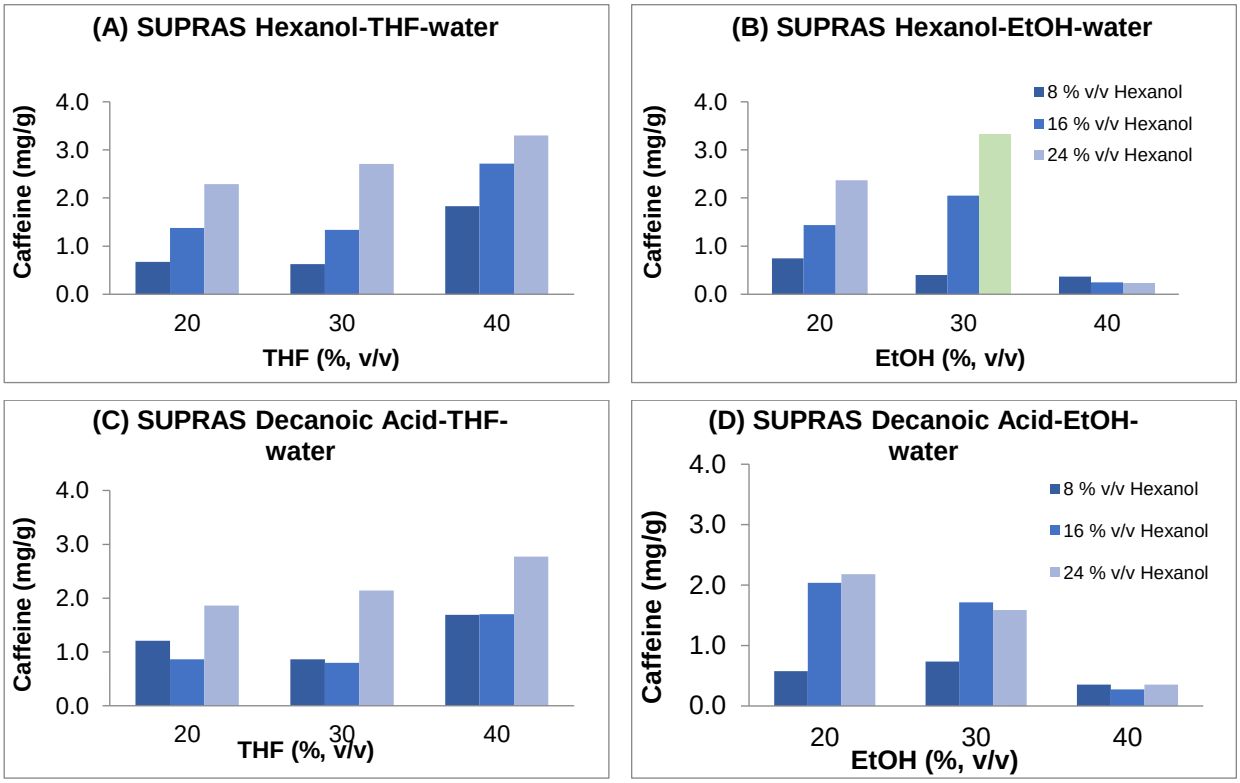


Figure 2. Extraction rate of caffeine (average of three replicates, relative standard deviation, RSD: 5-10%) from SCG with SUPRAS synthesized under different percentages of organic solvent (% v/v ethanol or THF) and amphiphiles (% v/v hexanol or decanoic acid). The optimal conditions are shown in a different color.

According to the results (Fig. 2 and 3), hexanol was better extractant for both caffeine and 5-CGA than decanoic acid. The stronger hydrogen bonding ability of hexanol over decanoic acid (which is related to its shorter alkyl chain length) could explain this behavior. In general, recoveries for both bioactives increased or kept constant as a function of amphiphile concentration, at least in the range 8-24%, due to the increase of available binding interactions. Regarding the organic solvent, maximal extraction yields were usually obtained for 40% of THF and 30% of ethanol, being the recovery slightly greater for ethanol. Since,

in addition, this solvent is more biocompatible and authorized for use in author industry, ethanol was selected for the production of the colloidal suspension of hexanol. Hexanol is also an authorized food additive by FDA and EU (flavouring substance).

The maximum extraction rates of caffeine and of CGA (expressed both in dry weight) were 3.32 ± 0.07 mg g^{-1} and 4.3 ± 0.1 mg g^{-1}, respectively, by extraction of the SCG with a SUPRAS obtained from 24% v/v hexanol, 30% v/v ethanol and 46% v/v water. These extracts were selected as optimal for further characterization of functional properties.

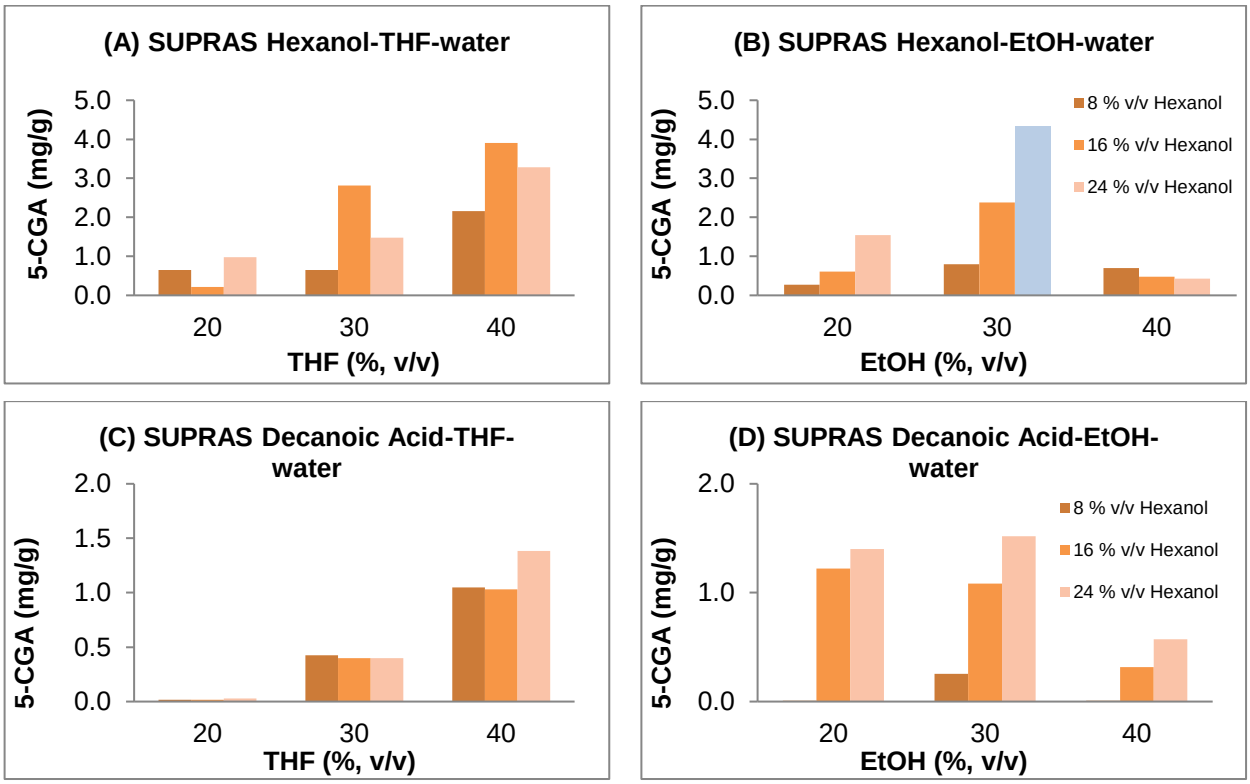


Figure 3. Extraction rate of 5-CGA (average of three replicates, RSD: 5-10%) from SCG with SUPRAS synthesized under different percentages of organic solvent (% v/v ethanol or THF) and amphiphiles (% v/v hexanol or decanoic acid). The optimal conditions are shown in a different color.

The contents of caffeine and 5-CGA in SCG have been reported to be highly dependent on the extraction process and the SCG source [13, 38,39]. Caffeine and 5-CGA contents were previously reported in the ranges 3.59-8.09 mg.g^{-1} and 1.18-3.59 mg.g^{-1}, respectively, in freeze-dried SCG from *Robusta* and *Arabica* varieties. The extraction procedure involved the drying of the SCG, the defatting with petroleum ether (1:11, w/v) for 3 h at 60 °C in a Soxhlet extraction system, the extraction of the SCG residue with water at 90 ºC for 6 min

(16 mL/g SCG) and the freeze-drying of the extract [38], which is not cost-effective for SCG valorization. The concentration for both caffeine and 5-CGA obtained by the SUPRAS-based extraction were similar to those previously reported for the drip filter method [38] taking into account that actual concentrations will be influenced by coffee variety and roasting degree [39].

Since the optimization was done on the basis of caffeine and 5-CGA only, optimal SUPRAS extracts were further analysed by LC-MS/MS to confirm the presence of common bioactives expected in SCG (alkaloids, phenolic compounds and niacin) [40]. Abundant MS peaks corresponding to *n*-O-dicaffeoyl quinic acids, *n*-O-feruloylquinic acids, *n*-O-caffeoylquinic acids, *n*-O-feruloylquinic lactones, *n*-O-coumaroylquinic acids, *n*-O-caffeoylshikimic acid, *n*-O-caffeoylquinic lactones, caffeine, niacin, trigonelline and N-methylpyridinium were obtained (Table 1).

Table 1. Polyphenolic compounds, alkaloids and niacin identified in SUPRAS extracts, two main fragments were monitored for each class according reference [40]

Compound class	**Abreviation**	**Parent ion**	**Fragment 1**	**Fragment 2**	**Retention times**	**[a]Area (sum of peaks)**	**Polarity**
n-O-Dicaffeoylquinic acids	n-DCQAs	515	179	135	31.1, 31.8, 32.4, 35.2	43804	-
n-O-Feruloylquinic acids	n-FQAs	367	193	191	17.3, 18.4, 22.8,23.7	118109	-
n-O-Caffeoylquinic acids	n-CQAs	353	191	173	13.0, 16.1, 17.7, 18.5	220745	-
n-O-Feruloylquinic lactones	n-FQLs	349	175	193	26.8,28.9, 30.3, 30.9, 32.8	829621	-
n-O-Coumaroylquinic acids	n-CouQAs	337	191	173	21.8, 22.2	5273	-
n-O-caffeoylshikimic acid	n-CSAs	335	179	173	20.6, 23.8	152796	-
n-O-caffeoylquinic lactones	n-CQLs	335	135	161	25.3, 26.4	1157812	-
Trigonelline	T	138	92	94	2.2	52738	+
Niacin	N	124	106	80	3.2	4776	+
Caffeine	C	195	138		17.8	14961897	+

Fig. 4 A and B shows the MRM chromatograms recorded in negative and positive acquisition modes (only the most abundant isomer of the main classes are labelled).

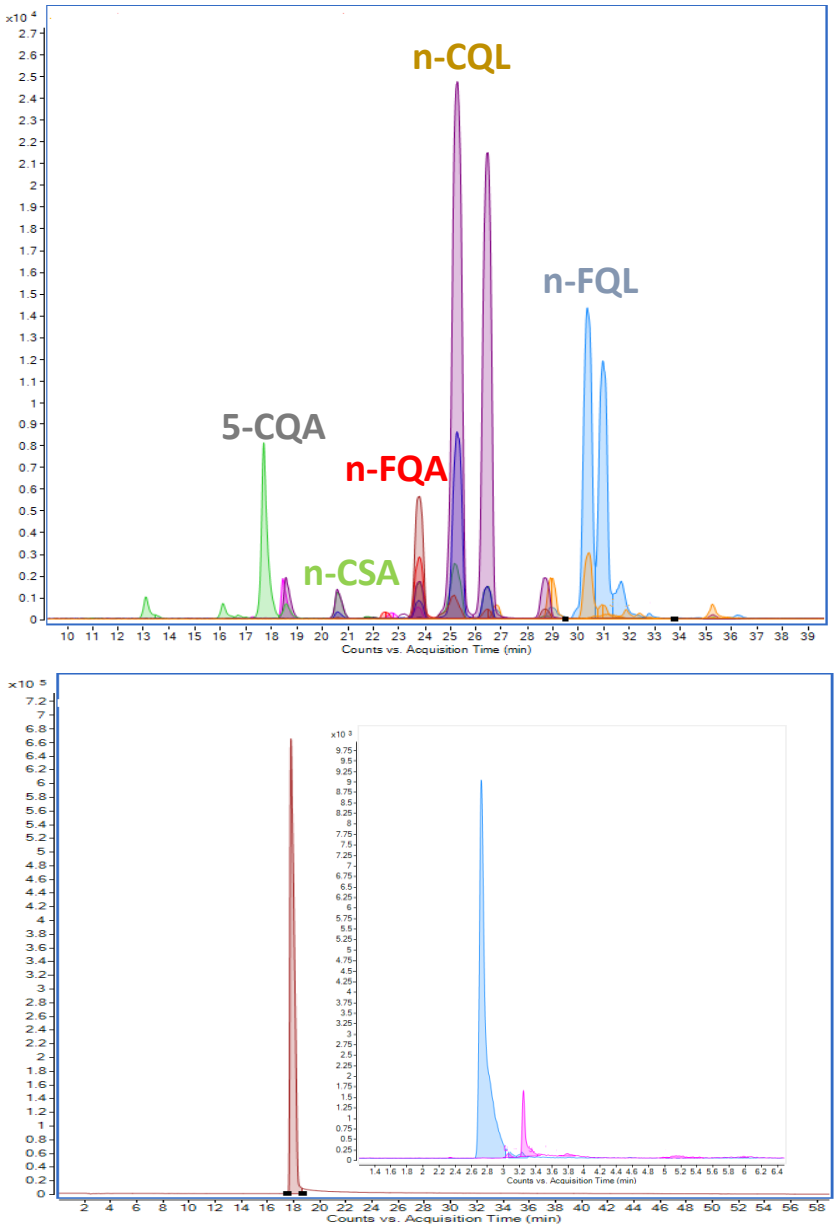


Figure 4. LC-(ESI) MS-MS peaks corresponding to the extracted ion chromatograms of n-O-dicaffeoyl quinic acids, n-O-feruloylquinic acids (n-FQA), n-O-caffeoylquinic acids (n-CQA), n-O-feruloylquinic lactones (n-FQL), n-O-coumaroylquinic acids (n-CSA), n-O-caffeoylshikimic acid, n-O-caffeoylquinic lactones (n-CQL), caffeine (C), niacin (N), trigonelline (T) and N-methylpyridinium (Table 1). Fig. A and B shows the MRM chromatograms recorded in negative and positive acquisition modes, respectively. Only the most intense isomers of the most abundant classes are labelled.

3.2 Functional and microbiological properties of SUPRAS extracts

3.2.1 Total phenolic content (TPC)

Phenolic compounds are the main contributors to the strong antioxidant activity of coffee brews and processing by-products [39]. The use of HPLC measurements instead of the standard Folin-Ciocalteu assay has been recommended by different authors to avoid overestimation due to the presence of reducing sugars, proteins and ascorbic acids, among others [41,42]. As mentioned before, different isomeric peaks of the major groups of polyphenolic compounds present in SCG were identified, namely *n*-O-dicaffeoyl quinic acids (n=4), *n*-O-feruloylquinic acids (n=4), *n*-O-caffeoylquinic acids (n=4, being 5-CGA the most abundant), *n*-O-feruloylquinic lactones (n=5), *n*-O-coumaroylquinic acids (n=2), *n*-O-caffeoylshikimic acid (n=2) and *n*-O-caffeoylquinic lactones (n=2). Their concentration were estimated by external calibration against 5-CGA due to the lack of authentic standards for all of them.

The TPC obtained with the SUPRAS extraction at optimal conditions (see section 3.1) was 14,4 ± 0.5 mg CGA/ g wet SCG (equivalent to 60.1 mg CGA per mg of extracted dry SCG). This value was near the TPC reported for USAE (71.7 mg CAE/g SCG, extraction for 2 h at room temperature and ethanol/solid ratio of 30) [20], that is, for the best of our knowledge, the highest reported for SCG. The high extraction efficiency of SUPRAS for phenolic compounds, the wide variety of phenolics extracted (see Table 1 and Figure 4), and the fact that samples were immediately processed, without further treatment, could account for the high TPC value found in our experiments. Values for SUPRAS were higher than those reported for conventional SLE (16-18 mg GAE/g) [18-20], SFE (~4 mg CAE/g) [20] or Soxhlet (18-22 mg CAE/g). However, it is known that TPC values depend on variables such as the roasting process [43], the preparation method (grinding degree or particle size, coffee:water ratio, water temperature, extraction time, etc.) and the technique followed for TPC estimation too, so that these factors can also influence results and differences between reported levels. Some advantages of SUPRAS were the low solvent/solid ratio (11.7 mL/g dry SCG) and the fact that the extraction was done at room temperature during 1 min.

3.2.2 Antioxidant activity

SUPRAS extracts, rich in TPC, were further tested for antioxidant activity using three assays (see section 2.8). The maximum value for the antioxidant capacity (100%) means that the respective reagent was reduced by the effect of the antioxidants present in the SUPRAS extract. The antioxidant capacity with DPPH (4.1 mg SUPRAS extract/mL) and FRAP (2.5 mg SUPRAS extract/mL) was 21 ± 3% and 68 ± 4%, respectively. Values of DPPH antioxidant capacity have been reported in the range 14.4-93.5% in extracts from dry SCG, with those techniques enhancing TPC extraction too, such as Soxhlet, USAE and MAE (EC_{50} concentration values in the range 0.2-1 mg extract/mL) [20,23]. The same Soxhlet and USAE extracts gave values in the range 160-381 µM TEAC/g extract. [20], while for SUPRAS a value of 405 ± 6 µM TEAC/g extract was measured. These results are in line with their high TPC content.

3.2.3 Antimicrobial activity

Previous studies have reported that phenolic substances, alkaloids and melanoidins present in the coffee have antibacterial activity [13]. However, even though the antimicrobial activity of coffee by-products can be attributed to any of their compounds, some studies suggest that bacteria are highly sensitive to phenolic acids [44], while other authors report that caffeine is the cause for the inhibition of growth in gram-negative bacteria, and that chlorogenic acid is less efficient against *S. enterica* [45].

The antimicrobial activity of SUPRAS was tested against *P. putida, S. enterica, S. areus and B. cereus.* Both *P. putida* and *S. enterica* have the ability to form biofilms [46], which is a strategy developed by bacteria to protect themselves from harmful substances such as antibiotics. For this reason, multiple studies are conducted to control these microorganisms in food. *S. aureus*, can produce different infections such pneumonia [47]. Respect to *B. cereus,* it has been reported to produce five enterotoxins and one emetic toxin and their spores are resistant to many processes as low and high temperatures, desiccation, disinfectant agents, ionization, radiation and ultraviolet light [48]. *A priori,* the complex mixture of compounds that could be present in SUPRAS extracts from SCG, could be used for enhancing functional properties such as antimicrobial activity to be used in the food

industry as a preservative, extending the shelf life of food, or even in the pharmaceutical and cosmetic sector.

The minimum inhibitory capacity (MIC), considered as the lowest concentration of SUPRAS extract that inhibited the growth of the microorganism tested (two gram-positive and two-gram negative bacteria), were calculated. SUPRAS extracts showed antimicrobial activities toward the growth of the target bacteria at varying degrees of concentrations. Thus, gram-positive bacteria (*B. cereus, S aureus)* were found more resistant, with a MIC value of 287 mg SUPRAS extract/mL. On the contrary, gram-negative bacteria (*S. enterica, P. putida)* were very sensitive with a MIC value of 2.87 mg SUPRAS extract/mL.

The literature reporting the antibacterial capacity of coffee waste is very scarce. Values ranged between 5 and 60 mg extract/mL for SCG extracted with subcritical water [21]. In this study, gram-positive (*B. cereus, S. aureus*) and gram-negative (*E. Coli, S. typhi*) bacteria were tested with methods involving different modifiers and pretreatments. MIC values were in the ranges 20-40 mg extract/mL for *B. cereus*, 5 mg extract/mL for *S. aureus,* 10-20 mg extract /mL for *E. Coli and* 20-60 mg extract/mL for *S. typhi.*

4. Conclusions

This study shows the first insights on the potential of SUPRAS, nanostructured solvents made up of assembled amphiphile aggregates, for valorization of coffee waste. Results proved that these solvents offer good extraction capacity of high-added value compounds from coffee by-products with interest for the food, pharmaceutical and cosmetic industry. Furthermore, extracts showed antioxidant capacity and antimicrobial effects to gram-negative bacteria. SUPRAS extraction offer rapid, simple and low cost methods and could be directly applied to the extraction of bioactives from wet by-products. Given the high number of biocompatible amphiphiles commercially available, the use of SUPRAS for agrifood by-product valorization is promising.

Acknowledgments

Authors gratefully acknowledge financial support from Spanish MINECO (Project CTQ2017-83823-R). A. Ballesteros-Gómez acknowledges the funding from Spanish Ministry of

Science, Innovation and Universities for a Ramón y Cajal contract (RYC-2015-18482). L.S. Torres-Valenzuela thanks AUIP for her doctoral fellowship.

References

[1] Lai WT, Khong NMH, Lim SS, Hee YY, Sim BI, Lau KL, Lai OM. A review: Modified agricultural by-products for the development and fortification of food products and nutraceuticals, Trends Food Sci. Technol. 2017; 59:148–160. doi:10.1016/j.tifs.2016.11.014.

[2] Bandara N, Chalamaiah M. Bioactives From Agricultural Processing By-products, in: Reference Module in Food Science, Elsevier, 2018. doi:10.1016/B978-0-08-100596-5.22408-6.

[3] Dadi D, Mengistie E, Terefe G, Getahun T, Haddis A, Birke W, Beyene A, Luis P, Van der Bruggen B. Assessment of the effluent quality of wet coffee processing wastewater and its influence on downstream water quality. Ecohydrol. Hydrobiol. 2018; 18:201–211. doi:10.1016/j.ecohyd.2017.10.007.

[4] Janissen B, Huynh T. Chemical composition and value-adding applications of coffee industry by-products: A review, Resources, Conservation and Recycling. 2018; 128:110–117. doi:10.1016/j.resconrec.2017.10.001.

[5] Murthy PS, Manonmani HK. Bioconversion of Coffee Industry Wastes with White Rot Fungus Pleurotus florida, Res. J. Environ. Sci. 2008; 2:145–150. doi:10.3923/rjes.2008.145.150.

[6] Buntić AV, Pavlović MD, Antonović DG, Šiler-Marinković SS, Dimitrijević-Branković SI. Utilization of spent coffee grounds for isolation and stabilization of Paenibacillus chitinolyticus CKS1 cellulase by immobilization. Heliyon. 2016; 2 e00146. doi:10.1016/j.heliyon.2016.e00146.

[7] Woldesenbet AG, Woldeyes B, Chandravanshi BS. Bio-ethanol production from wet coffee processing waste in Ethiopia, Springerplus. 2016; 5: 1903. doi:10.1186/s40064-016-3600-8.

[8] Hachicha R, Rekik O, Hachicha S, Ferchichi M, Woodward S, Moncef N, Cegarra J, Mechichi T. Co-composting of spent coffee ground with olive mill wastewater sludge and poultry manure and effect of Trametes versicolor inoculation on the compost maturity, Chemosphere. 2012; 88:677–682. doi:10.1016/j.chemosphere.2012.03.053.

[9] Jiménez-Zamora A, Pastoriza S, Rufián-Henares JA. Revalorization of coffee by-

products. Prebiotic, antimicrobial and antioxidant properties, LWT - Food Sci. Technol. 2015; 61:12–18. doi:10.1016/j.lwt.2014.11.031.

[10] Iriondo-DeHond A, Aparicio García N, Fernandez-Gomez B, Guisantes-Batan E, Velázquez Escobar F, Blanch GP, San Andres MI, Sanchez-Fortun S, del Castillo MD. Validation of coffee by-products as novel food ingredients. Innovative Food Sci. Emerging Technol. 2019; 51: 194-204. doi:10.1016/j.ifset.2018.06.010.

[11] Esquivel P, Jiménez VM, Functional properties of coffee and coffee by-products. Food Res. Int. 2012; 46:488–495. doi:10.1016/j.foodres.2011.05.028.

[12] Mussatto SI, Machado EMS, Martins S, Teixeira JA. Production, Composition, and Application of Coffee and Its Industrial Residues, Food Bioprocess. Technol. 2011; 4: 661. doi:10.1007/s11947-011-0565-z.

[13] Campos-Vega R, Loarca-Piña G, Vergara-Castañeda HA, Oomah BD. Spent coffee grounds: A review on current research and future prospects. Trends Food Sci. Technol.2015; 45:24–36. doi:10.1016/j.tifs.2015.04.012.

[14] Kovalcik A, Obruca S, Marova I. Valorization of spent coffee grounds: A review. Food Bioprod. Process.2018; 110:104–119. doi:10.1016/j.fbp.2018.05.002.

[15] McNutt J, He QS. Spent coffee grounds: A review on current utilization, J. Ind. Eng. Chem. 2019; 71:78–88. doi:10.1016/j.jiec.2018.11.054.

[16] Hall S, Desbrow B, Anoopkumar-Dukie S, Davey AK, Arora D, McDermott C, Schubert MM, Perkins AV, Kiefel MJ, Grant GD. A review of the bioactivity of coffee, caffeine and key coffee constituents on inflammatory responses linked to depression, Food Res. Int. 2015; 76:626–636. doi:10.1016/j.foodres.2015.07.027.

[17] Pathak L, Agrawal Y, Dhir A. Natural polyphenols in the management of major depression, Expert Opin. Investig. Drugs. 2013; 2:863–880. doi:10.1517/13543784.2013.794783.

[18] Mussatto SI,. Ballesteros LF, Martins S, Teixeira JA. Extraction of antioxidant phenolic compounds from spent coffee grounds. Sep. Purif. Technol. 2011; 83:173–179. doi:10.1016/j.seppur.2011.09.036.

[19] Zuorro A, Lavecchia R. Spent coffee grounds as a valuable source of phenolic compounds and bioenergy. J. Cleaner Prod 2012;. 34:49–56. doi:10.1016/j.jclepro.2011.12.003.

[20] Andrade KS, Gonçalvez RT, Maraschin M, Ribeiro-do-Valle RM, Martínez J, Ferreira SRS. Supercritical fluid extraction from spent coffee grounds and coffee husks: Antioxidant activity and effect of operational variables on extract composition. Talanta;

2012; 88:544–552. doi:10.1016/j.talanta.2011.11.031.

[21] Getachew AT, Chun BS. Influence of pretreatment and modifiers on subcritical water liquefaction of spent coffee grounds: A green waste valorization approach, J. Cleaner Prod. 2017; 142: 3719–3727. doi:10.1016/j.jclepro.2016.10.096.

[22] Yoo DE, Jeong KM, Han SY, Kim EM, Jin Y, Lee J. Deep eutectic solvent-based valorization of spent coffee grounds, Food Chem. 2018; 255:357–364. doi:10.1016/j.foodchem.2018.02.096.

[23] Pavlović MD, Buntić AV, Šiler-Marinković SS, Dimitrijević-Branković SI. Ethanol influenced fast microwave-assisted extraction for natural antioxidants obtaining from spent filter coffee, Sep. Purif. Technol. 2013; 118 :503–510. doi:10.1016/j.seppur.2013.07.035.

[24] Chun OK, Kim DO. Consideration on equivalent chemicals in total phenolic assay of chlorogenic acid-rich plums. Food Res. Int. 2004; 37:337–342. doi:10.1016/j.foodres.2004.02.001.

[25] Caballo C, Sicilia MD, Rubio S, Chapter 5 - Supramolecular Solvents for Green Chemistry, in: F. Pena-Pereira, M. Tobiszewski (Eds.), The Application of Green Solvents in Separation Processes, Elsevier, 2017: pp. 111–137. doi:10.1016/B978-0-12-805297-6.00005-X.

[26] Ballesteros-Gómez A, Sicilia MD, Rubio S. Supramolecular solvents in the extraction of organic compounds. A review, Anal. Chim. Acta. 2010; 677:108–130. doi:10.1016/j.aca.2010.07.027.

[27] Ballesteros-Gómez A, Lunar L, Sicilia MD, Rubio S. Hyphenating Supramolecular Solvents and Liquid Chromatography: Tips for Efficient Extraction and Reliable Determination of Organics, Chromatographia. 2019; 82:111–124. doi:10.1007/s10337-018-3614-1.

[28] Steed JW, Turner DR, Wallace K. Core Concepts in Supramolecular Chemistry and Nanochemistry, John Wiley & Sons, Ltd, Chippenham, Witshire, 2007.

[29] Ballesteros-Gómez A, Rubio S. Environment-responsive alkanol-based supramolecular solvents: characterization and potential as restricted access property and mixed-mode extractants. Anal. Chem. 2012; 84: 342–349. doi:10.1021/ac2026207.

[30] Salatti-Dorado JA, García-Gómez D, Rodriguez-Ruiz V, Gueguen V, Pavon-Djavid G, Rubio S. Multifunctional green supramolecular solvents for cost-effective production of highly stable astaxanthin-rich formulations from Haematococcus pluvialis. Food Chem. 2019; 279:294–302. doi:10.1016/j.foodchem.2018.11.132.

[31] Ballesteros-Gómez A, Caballero-Casero N, García-Fonseca S, Lunar L, Rubio S. Multifunctional vesicular coacervates as engineered supramolecular solvents for wastewater treatment. Chemosphere. 2019; 223:569–576. doi:10.1016/j.chemosphere.2019.02.089.

[32] Ruiz FJ, Rubio S, Pérez-Bendito D. Water-induced coacervation of alkyl carboxylic acid reverse micelles: phenomenon description and potential for the extraction of organic compounds, Anal. Chem. 2007; 79: 7473–7484. doi:10.1021/ac0708644.

[33] Salatti-Dorado JA, Caballero-Casero N, Sicilia MD, Lunar ML, Rubi S. The use of a restricted access volatile supramolecular solvent for the LC/MS-MS assay of bisphenol A in urine with a significant reduction of phospholipid-based matrix effects, Anal. Chim. Acta. 2017; 950:71–79. doi:10.1016/j.aca.2016.11.026.

[34] Régnier P, Bastias J, Rodriguez-Ruiz V, Caballero-Casero N, Caballo C, Sicilia D, Fuentes A, Maire M, Crepin M, Letourneur D, Gueguen V, Rubio S, Pavon-Djavid G. Astaxanthin from Haematococcus pluvialis Prevents Oxidative Stress on Human Endothelial Cells without Toxicity, Mar Drugs. 2015; 13:2857–2874. doi:10.3390/md13052857.

[35] Jones A, Pravadali-Cekic S, Dennis GR, Bashir R, Mahon PJ, Shalliker RA. Ferric reducing antioxidant potential (FRAP) of antioxidants using reaction flow chromatography. Anal. Chim. Acta. 2017; 967:93–101. doi:10.1016/j.aca.2017.02.032.

[36] D'Amato S, Mazzarrino G, Rossi C, Serio A, López CC, Celano GV, Paparella A. Thymus Vulgaris (Red Thyme) and Caryophyllus Aromaticus (Clove) Essential Oils to Control Spoilage Microorganisms in Pork Under Modified Atmosphere. Ital. J. Food Saf. 2016; 5:5785. doi:10.4081/ijfs.2016.5785.

[37] Pellegrini M, Ricci A, Serio A, Chaves-López C, Mazzarrino G, D'Amato S, Lo Sterzo C, Paparella A. Characterization of Essential Oils Obtained from Abruzzo Autochthonous Plants: Antioxidant and Antimicrobial Activities Assessment for Food Application, Foods. 2018; 7:19. doi:10.3390/foods7020019.

[38] Bravo J, Juániz I, Monente C, Caemmerer B, Kroh LW, De Peña MP, Cid C. Evaluation of spent coffee obtained from the most common coffeemakers as a source of hydrophilic bioactive compounds, J. Agric. Food Chem. 2012; 60:12565–12573. doi:10.1021/jf3040594.

[39] Belščak-Cvitanović, A, Komes D. Extraction and formulation of bioactive compounds, in: Handbook of Coffee Processing By-Products, Elsevier, 2017: pp. 93–140.

doi:10.1016/B978-0-12-811290-8.00004-9.

[40] Angelino D, Tassotti1 M, Brighenti F, Del Rio D, Mena P. Niacin, alkaloids and (poly)phenolic compounds in the most widespread Italian capsule-brewed coffees. Sci. Rep. 2018; 8:17874. doi:10.1038/s41598-018-36291-6

[41] Escarpa A, González MC. Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods Anal. Chim. Acta 2001; 427: 119–127. doi: 10.1016/S0003-2670(00)01188-0

[42] Tsao, R., Yang, R. Optimization of a new mobile phase to know the complex and real polyphenolic composition: towards a total phenolic index using high-performance liquid chromatography. J. Chromatogr. A, 2003; 1018:29–40. doi: 10.1016/j.chroma.2003.08.034

[43] Duarte SMS, de Abreu CMP, de Menezes HC, dos Santos MH, Gouvêa CMCP. Effect of processing and roasting on the antioxidant activity of coffee brews, Food Sci. Technol.. 2005; 25: 387–393. doi:10.1590/S0101-20612005000200035.

[44] Monente C, Bravo J, Vitas AI, Arbillaga L, De Peña MP, Cid C. Coffee and spent coffee extracts protect against cell mutagens and inhibit growth of food-borne pathogen microorganisms, J. Funct. Foods. 2015; 12: 365–374. doi:10.1016/j.jff.2014.12.006.

[45] Almeida AAP, Farah A, Silva DAM, Nunan EA, Glória MBA. Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria, J. Agric. Food Chem. 2006; 54:8738–8743. doi:10.1021/jf0617317.

[46] Liu H, Xiao Y, Nie H, Huang Q, Chen W. Influence of (p)ppGpp on biofilm regulation in Pseudomonas putida KT2440, Microbiol. Res. 2017; 204:1–8. doi:10.1016/j.micres.2017.07.003.

[47] Paling FP, Wolkewitz M, Bode LGM, Klein Klouwenberg PMC, Ong DSY, Depuydt P, de Bus L, Sifakis F, Bonten MJM, Kluytmans JAJW. Staphylococcus aureus colonization at ICU admission as a risk factor for developing S. aureus ICU pneumonia, Clin. Microbiol. Infect. 2017; 23: 49 doi:10.1016/j.cmi.2016.09.022.

[48] Sadek ZI, Abdel-Rahman MA, Azab MS, Darwesh OM, Hassan MS. Microbiological evaluation of infant foods quality and molecular detection of Bacillus cereus toxins relating genes, Toxicol. Rep. 2018; 5: 871-877. doi:10.1016/j.toxrep.2018.08.013.

CAPÍTULO III

EXTRACCIÓN DE COMPUESTOS BIOACTIVOS DE PULPA DE CAFÉ EMPLEANDO CON DISOLVENTES SUPRAMOLECULARES

Supramolecular solvent extraction of bioactives from coffee cherry pulp

Laura Sofía Torres-Valenzuela [a,b], Ana Ballesteros [a*], Alejandra Sanin [b], Soledad Rubio[a]

Journal of Food Engineering (2020): In press

Abstract

The potential of supramolecular solvents (SUPRAS) is investigated for the extraction of bioactive compounds from coffee cherry pulp, one of the major by-products generated in the coffee industry. SUPRAS made up of hexagonal inverted aggregates of octanoic acid in ethanol:water mixtures provided good extraction yields for bioactives (3.6±0.3 mg caffeine g^{-1} and 0.9±0.1 mg protocatechuic acid g^{-1}) at a low solvent:sample ratio of 4:1 v/w and under mild operations conditions (5 min extraction at room temperature). SUPRAS-based extraction was optimized and extracts were analyzed to identify the main phenolic and alkaloid compounds. A variety of bioactives were present and extracts showed high antioxidant capacity by different assays (45% for DPPH and 91% for ABTS). Extraction efficiencies with SUPRAS were clearly superior than those obtained with organic solvents commonly used for valorization of coffee residues.

Keywords: supramolecular solvents; coffee cherry pulp; bioactive compounds; valorization

1. Introduction

Coffee is the most popular beverage with a production of over 9 billion kg of beans per year and it is cultivated in around 70 countries (International Coffee Organization, 2017). Coffee berries contain the beans surrounded by different layers: first the silverskin, then the parchment, the mucilage, the pulp and finally the skin (Esquivel and Jiménez, 2012). During the dry process, coffee cherries are sun-dried and then they are dehusked to remove the skin, the pulp, the mucilage, the parchment and part of the silverskin (Esquivel and Jiménez, 2012). These by-products are known as coffee husks. In the wet and semi-wet processes, after separating ripened from unripe berries with water, fruits are de-pulped to remove the skin and the pulp. The by-product or waste that is generated at this step is known as coffee

cherry pulp or coffee pulp (Murthy and Madhava Naidu, 2012; Pandey et al., 2000). In the wet process, the beans are further fermented to remove the mucilage and the remaining pulp, then dehusked and finally dried. After any of these three processes, the beans are roasted and generate as by-product the silverskin. Considering that each 100 kg of mature fruits are composed by around 39 kg of pulp, 22 kg of mucilage and 39 kg of parchment coffee, it is easily concluded that the amount of residues generated is extremely high (Alves et al. 2017). Finally, spent-coffee grounds are generated during the production of instant coffee and coffee brewing (Kovalcik et al. 2018).

In coffee producing countries, the unsafe disposal of the corresponding wastes has a negative impact on the environment due to their high concentration in caffeine, polyphenols and tannins and high acidity (Murthy and Naidu, 2012). The large-scale management of coffee waste is still challenging. A very attractive strategy is its valorization to obtain benefits as compost, fuel, animal feed, bio-solvents or bioactive compounds, among others. Bioactive compounds obtained from coffee by-products are mainly alkaloids, melanoidins and polyphenolic compounds that exert beneficial antioxidant, anti-bacterial or anti-fungal effects of interest for the food, pharmaceutical and cosmetic industries (Belščak-Cvitanović and Komes, 2017; Esquivel and Jiménez, 2012; Galanakis, 2015; Janissen and Huynh, 2018; Rodrigues et al., 2017).

Extraction of bioactives from coffee by-products has been investigated using different solvents and techniques. Moderate polar solvents are usually employed, such as methanol, ethanol or isopropanol, sometimes mixed with water (up to 40% v/v) under typical sample to solvent ratios in the range 1:10-1:100 v/w. Supercritical fluids (Andrade et al., 2012), subcritical water (Getachew and Chun, 2017), and deep eutectic solvents (Yoo et al., 2018) have been also used. Techniques include conventional solid-liquid extraction (Mussatto et al., 2011; Zuorro and Lavecchia, 2012), Soxhlet extraction (Murthy et al., 2012), supercritical fluid extraction (SFE) with and without co-solvent (Andrade et al., 2012), ultrasound (USAE) (Andrade et al., 2012; Getachew and Chun, 2017; Yoo et al., 2018) and microwave assisted extraction (MAE) (Getachew and Chun, 2017; Pavlović et al., 2013). Spent coffee grounds have been the most investigated coffee waste for the extraction of bioactives (Kovalcik et al., 2018) and in a lesser extent coffee husks (Andrade et al., 2012) and coffee silverskin (Narita and Inouye, 2014). As mentioned above, coffee cherry pulp is one of the main by-products of the wet processing of coffee (~40% of the coffee is wet processed, Garde et al.

2017). However, it is still hardly investigated for the extraction of bioactives despite its good antioxidant properties (Heeger et al., 2017, Murthy et al., 2012).

In this study, we investigate the suitability of supramolecular solvents (SUPRAS) for the extraction of bioactives from coffee cherry pulp that was obtained by a wet process. SUPRAS are nanostructured liquids produced spontaneously from colloidal suspensions of amphiphiles by self-assembly processes (Ballesteros-Gómez et al., 2018; Caballo et al., 2017). SUPRAS production involves the application of an external stimuli (pH or temperature change, addition of salt or addition of a poor solvent for the amphiphile) to the colloidal suspension where the amphiphiles arrange as three-dimensional aggregates, which are usually normal or inverted micelles or vesicles (Ballesteros-Gómez et al., 2010). The application of an external stimulus diminishes the repulsion among the polar groups of the amphiphilic molecules, which causes the growth of the aggregates that finally separate as a new liquid phase named coacervate or SUPRAS (Ballesteros-Gómez et al., 2018). The organized structures in the supramolecular phase are held together by intermolecular interactions, such as ion–ion, ion–dipole, dipole–dipole, hydrogen bonding, π–π and cation–π. Although these interactions are weaker than covalent bonds they can produce very stable assemblies and provide multiple biding forces for extraction, which makes them very efficient extractants (Caballo et al., 2017; Steed et al., 2007).

SUPRAS are tunable solvents since by changing the environmental conditions and/or the amphiphile functional group/s is possible to tailor their composition and structure (Ballesteros-Gómez et al., 2018). Thus, SUPRAS have been designed to exclude proteins and carbohydrates from extraction by chemical and physical mechanisms, respectively (Ballesteros-Gómez and Rubio, 2012). These versatile and efficient extraction materials have proved successful for the recovery of a variety of compounds for analytical purposes (e.g. PAHs, mycotoxins, perfluorinated compounds, drugs, dyes, etc.) (Ballesteros-Gómez et al., 2010; Caballo et al., 2017). However, their application to the extraction of bioactives from biomass or waste is still limited (Salatti-Dorado et al., 2019; Torres-Valenzuela et al, 2019).

Here, we investigate the suitability of SUPRAS produced by the addition of water to colloidal suspensions of decanoic or octanoic acid in ethanol (Ruiz et al., 2007) for the extraction of bioactives from coffee cherry pulp. SUPRAS components were selected from *Generally*

Recognized As Safe (GRAS) chemicals in order to produce a green and biocompatible solvent for further application in the development of cosmetics, nutraceuticals or functional foods. SUPRAS extraction was optimized on the basis of the extraction yields for caffeine and protocatechuic acid, the two most abundant bioactives in this by-product (Heeger et al., 2017). SUPRAS extracts were further screened to elucidate the profile of phenolic and alkaloid compounds and to measure their antioxidant activity.

2. Materials and methods

2.1. Chemicals and solutions

The list of chemicals and solutions is provided in the Supplementary Material (SI).

2.2. Coffee cherry pulp

Coffee cherry pulp was obtained using the wet method by the mechanical peeling of ripe coffee fruits freshly harvested from an experimental lot located in Armenia City, Colombia (latitude 4°32'54'' north, longitude 75°39'54'' west and altitude 1500 MAS). Coffee cheery pulp was dried to reduce water activity and extend its shelf-life. The process was carried out at 60 ºC during 8 hours, up to reach around 9.5% of water content. Finally, sample size was reduced by using a coffee mill until obtaining a homogeneous powder (particle size <2 mm).

2.3. SUPRAS production and composition

A variety of SUPRAS were produced by dissolving decanoic or octanoic acid in ethanol and then adding water (pH ~3) to induce the growth of the aggregates of the amphiphiles. The volume of the ternary mixture, consisting of variable percentages of ethanol and water and 5% v/v of amphiphile, was 50 mL. The mixture was shaken on a vortex (Vorterex, Heathrow Scientific, Vernon Hills, IL, USA) for 1 min at 500 rpm and then centrifuged (Mixtasel BLT, Selecta, Cham, Suiza) for 5 min at 3,000 rpm. The SUPRAS separated as a new liquid top phase in equilibrium with the bulk solution. Then both phases (SUPRAS and equilibrium solution, EqS) were independently collected and stored in closed glass containers at room temperature until use (~20-25 °C, within one week). Figure 1.1 shows a schematic for SUPRAS production. The composition of each SUPRAS, which is dependent on the

ethanol:water ratio in the synthetic solution, was determined. The concentration of amphiphile, water and ethanol in the SUPRAS was calculated as weight percent (w/w, %). Water content was determined by coloumetric Karl Fischer titration (KF 831 model, Methrom, Herisau, Switzerland) after proper dilution with methanol. The amphiphile content was determined by weighting SUPRAS aliquots (~200 µL) before and after the evaporation of water and ethanol. Finally, the ethanol content in the SUPRAS was calculated by weight difference.

2.4. SUPRAS extraction

Figure 1.2 shows a schematic of the SUPRAS extraction procedure. Extractions were done in 2 mL-microtubes Safe-Lock (Eppendorf Iberica, Madrid, Spain) by mixing 200 mg of coffee cherry pulp and variable volumes of the different types of SUPRAS (and corresponding EqS) that were previously produced as explained in section 2.3. The mixtures were vortex-shaken at 2,990 rpm for 5 minutes and then centrifuged for 10 minutes at 10,000 rpm. A sequential design was used to determine significant variables affecting the extraction yields of caffeine and protocatechuic acid, the two bioactives chosen as models for valorization of coffee cherry pulp .Variables were optimized by varying each factor at a time because of the different composition of the SUPRAS that were investigated, which can be considered as different solvents. First, we investigated the effect of the amphiphile (decanoic acid or octanoic acid) and of the ethanol concentration employed for SUPRAS formation. Conditions giving the maximum yield for both target compounds were selected as optimal. Then we evaluted the effect of the ratios EqS:SUPRAS v/v and sample:extraction solvent w/v. All the experiments were carried out at room temperature. For each experiment, results were presented as mean±standard deviation for three individual extractions. Statistical comparisons were performed with Minitab software Ver. 18 (Minitab Inc, State College, Pennsylvania, USA) using one-way analysis of variance (ANOVA) and Tukey's tests (p-value < 0.05).

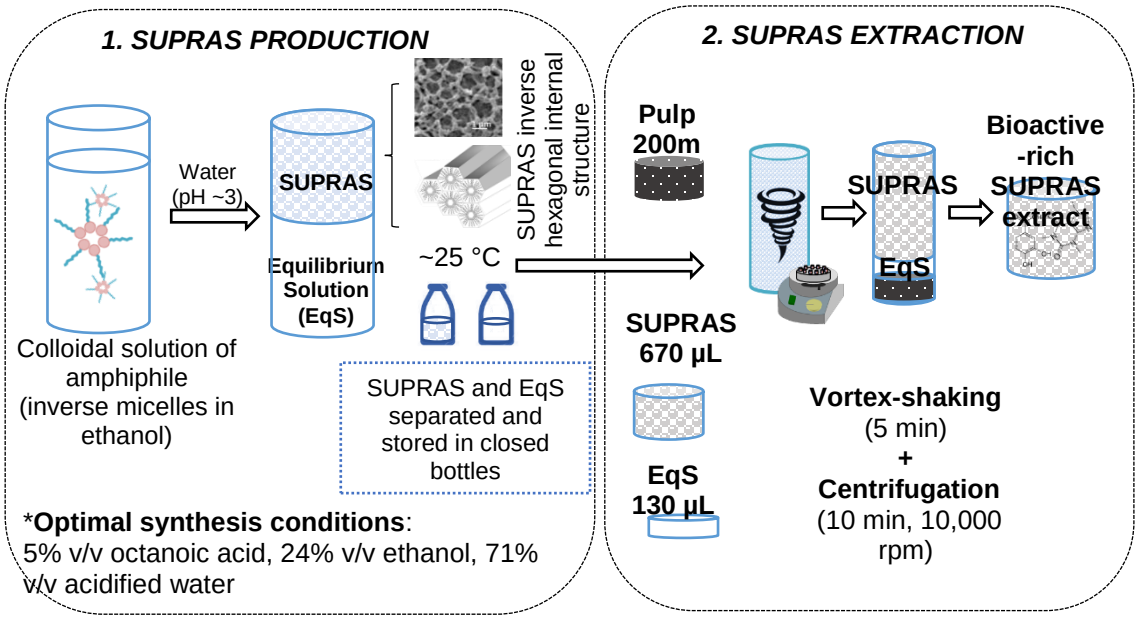


Figure 1. Schematic picture of the SUPRAS production and the extraction of coffee cherry pulp

2.5. Analysis of bioactive compounds by LC-MS/MS

Caffeine, protocatechuic acid, 5-chlorogenic acid, gallic acid and caffeic acid were quantified by LC-MS/MS in diluted SUPRAS extracts (dilution with methanol 1:5-1:100 v/v). Calibration curves were prepared in methanol (0.1-20 mg L^{-1}). Other common bioactives found in the extracts were tentatively identified based on characteristic transitions (Table S1) and semi-quantified against 5-chlorogenic acid (due to the lack of authentic standards): rutin, 5-O-feruloylquinic acid, 3-O-coumaroylquinic acid, p-coumaric acid, caffeic acid,n-O-dicaffeoyl quinic acids (three isomers), 4-chlorogenic acid , 3-chlorogenic acid and 4-O-feruloylquinic acid. Quantification and target screening of bioactives was carried out by LC-MS/MS (conditions specified in SI).

2.6. Antioxidant activity assays

SUPRAS extracts obtained under optimal conditions (200 mg of coffee peel, 670 µL of SUPRAS and 130 µL of EqS) were tested for the evaluation of the antioxidant activity by the DPPH and ABTS assays. The inhibition percentage (or antioxidant capacity) was calculated (Régnier et al., 2015). For the DPPH assay, SUPRAS (blanks or extracts diluted 1:0 to

1:1000 v/v in methanol) were mixed with 250 µL of DPPH solution. The absorbance of the mixture was measured at 517 nm at 20 second-intervals during 20 min in a multilabel plate reader Victor 3 1420 (Perkin Elmer, Waltham, Massachusetts, USA). For the Trolox equivalent (TE) antioxidant capacity assay, the $ABTS^{\bullet+}$ radical cation was produced as described in SI. The assays were run by mixing 66 µL of Trolox (0, 10, 30, 50 and 70 µM diluted in methanol) or of SUPRAS (blanks or extracts diluted 1:0 at 1:1000 v/v in methanol) with 154 µL of $ABTS^{\bullet+}$ solution. The absorbance of the mixture was measured at 732 nm at 40 second-intervals for 60 min and the percentage of inhibition expressed as TEAC (Apak et al., 2013).

3. Results and discussion

3.1. SUPRAS production and composition

SUPRAS of different composition were prepared from ternary mixtures of octanoic or decanoic acid, ethanol and water. Alkyl carboxylic acids have been previously reported to produce SUPRAS in hydro-organic media (Ruiz et al., 2007), being tetrahydrofuran:water the mixture more used for analytical purposes (Ballesteros-Gómez et al., 2018). These amphiphiles give inverted micelles in water-miscible organic solvents (e.g. THF, acetone, dioxane, propanol, butanol, acetonitrile, etc.) and the addition of water (a “poor solvent” for the amphiphile) triggers the assembly of the aggregates into the SUPRAS, a new highly packed phase with an inverted hexagonal arrangement (Figure 1.1). In this structure, the carboxylic groups surround aqueous cavities while the hydrocarbon chains are dispersed in the organic solvent. The SUPRAS is in equilibrium with a hydro-organic solution (EqS) containing the amphiphile at the critical aggregation concentration. Both the SUPRAS and the EqS are immiscible. In this paper, a mixture of ethanol:water was selected for the production of SUPRAS. Ethanol was selected against THF because of its lower toxicity. Indeed, ethanol, decanoic and octanoic acid are authorized food ingredients. These carboxylic acids were selected because they usually provide better extraction yields and they also form SUPRAS in a wider range of conditions than acids with higher and lower hydrocarbon chain length (Ballesteros-Gómez et al., 2018).

Table 1 shows the composition of the SUPRAS that were produced by mixing octanoic or decanoic acid (5% v/v) with variable volume ratios of ethanol and water. These ratios were

determined by the minimal and the maximal percentage of organic solvent required to form decanoic and octanic acid-based SUPRAS (Ruiz et al., 2007). The volume percentages of ethanol varied in the intervals 9.5-33 % and 19-38% for octanoic and decanoic acid, respectively. As shown in Table 1, the concentration of amphiphile in the SUPRAS was very high. So, SUPRAS contained a huge number of binding sites for bioactives. This should enable the efficient extraction of the target compounds even at low SUPRAS/coffee pulp ratios.

Table 1. Chemical composition of SUPRAS obtained from different ternary mixtures of amphiphile:ethanol:water (%, v/v). The optimal SUPRAS is shown in bold.

	Chemical composition of the synthetic solution (%, v/v)			SUPRAS Chemical composition (%, v/v)		
	Amphiphile	**Ethanol**	**Water**	**Amphiphile**	**Ethanol**	**Water**
Octanoic acid	5	9.5	85.5	89 ± 1	5 ± 1	5.5 ± 0.3
	5	19	76	76 ± 1	14.7 ± 0.2	9.7 ± 0.4
	5	**24**	**71**	**67 ± 1**	**21 ± 1**	**12.3 ± 0.3**
	5	33	62	45 ± 2	32 ± 2	23.2 ± 0.4
Decanoic acid	5	19	76	83 ± 1	11 ± 1	6.05 ± 0.07
	5	24	71	75.7± 0.2	16.1 ± 0.1	8.1 ± 0.1
	5	33	62	59.9 ± 0.5	25.6 ± 0.5	14.4 ± 0.2
	5	38	57	48.2 ± 0.6	32.7 ± 0.7	19 ±1

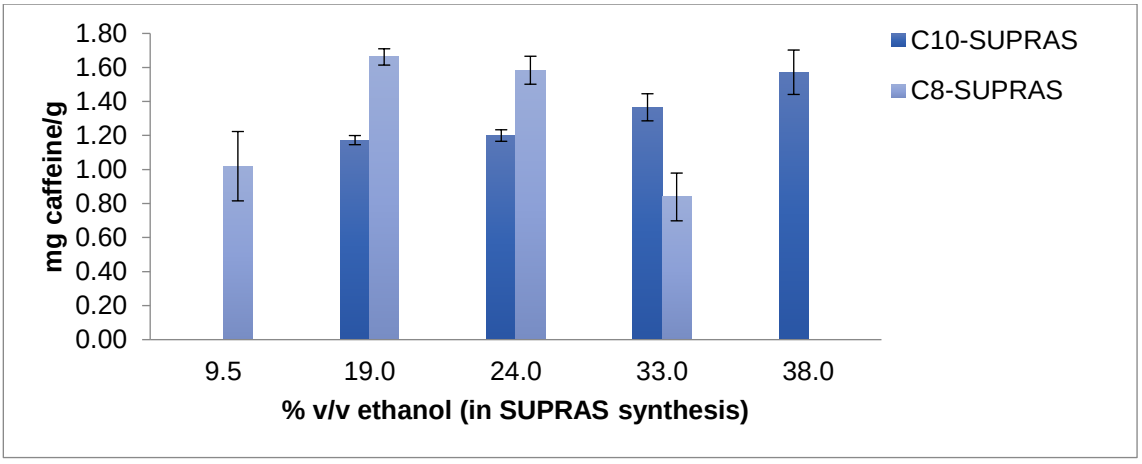
The concentration of water and ethanol in the SUPRAS increased as the percentage of ethanol did in the synthetic solution (r-Pearson = 0.95-0.98 and 0.990-0.995, for water and ethanol with octanoic and decanoic acid, respectively), while the concentration of amphiphile decreased accordingly (r-Pearson = -0.95-(-0.98) and -0.986-(-0.995) with octanoic and decanoic acid, respectively). So, these SUPRAS can be considered as environment-responsive (Ballesteros-Gómez and Rubio, 2012). Accordingly, SUPRAS composition can be tailored by adequate selection of the composition of the synthetic solution (Table 1). Likewise, as water is progressively incorporated into the SUPRAS, the size of the aqueous cavities of the hexagonal aggregates increases and this behavior opens the door to the use of these SUPRAS as restricted access liquids (Ballesteros-Gómez and Rubio, 2012). Thus, exclusion of polar macromolecules (e.g. polysaccharides), takes place by size-exclusion mechanisms due to the small pores of the SUPRAS network. On the other hand, proteins precipitate owing to the decrease of the dielectric constant and the formation of

macromolecular complexes with carboxylic acids. In this way, SUPRAS will extract bioactives and simultaneously exclude major matrix components in coffee cherry pulp.

3.2. SUPRAS-based extraction of bioactives from coffee pulp

Optimization was carried out following the procedure specified in section 2.4 (see also Figure 1.2) and considering the extraction efficiency obtained for caffeine and protocatechuic acid. These bioactives were expected to be solubilized in the SUPRAS by mixed-mode mechanisms, namely dispersion interactions with the hydrocarbon chains and hydrogen bonds with the carboxylic groups.

First, we investigated the influence of the composition of the eight synthesized SUPRAS (Table 1) on the extraction yields of the target bioactives. For this purpose, both the SUPRAS and EqS, which are immiscible, were added to the sample at a ratio of 1:1:2 sample:SUPRAS:EqS, g:mL:mL. The role of the EqS was to favor the wetting and dispersibility of the sample. Figure 2 shows the results expressed as a function of the percentage of ethanol used in the synthesis of the SUPRAS. Statistical operations including ANOVA table and results of Tukey's tests are shown in Tables S2 and S3 of Supplementary Information.The maximal extraction yields (i.e. around 1.6 mg/g caffeine and 0.39 mg/g protocatechuic acid) were not significantly different for the octanoic and decanoic acid. However, the optimal conditions were achieved at different synthetic conditions (at 33-38% and at 24% of ethanol for decanoic and octanoic acid, respectively).



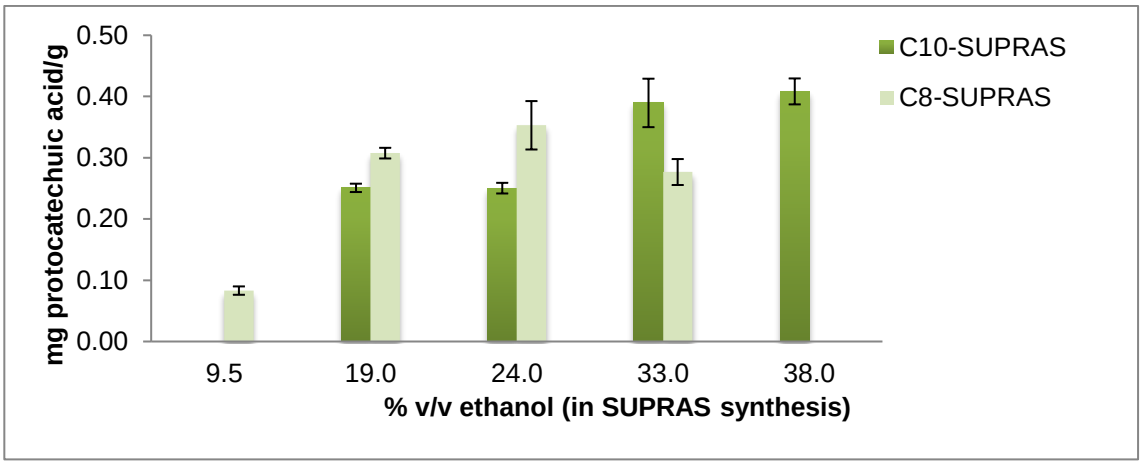


Figure 2. Extraction yields of caffeine and protocatechuic acid as mean ± standard deviation (n=3). Significant differences are indicated by different letters on the top of the bars (Tukey tests). Extraction of coffee cherry pulp (200 mg) with SUPRAS (400 µL) and corresponding EqS (800 µL) produced by mixing decanoic or octanoic acid in ethanol:water mixtures according to the conditions specified in Table 1. Percentages of ethanol on the X-axis represent the concentrations of this solvent in the synthetic solution.

Regarding the composition of these optimal SUPRAS, the decanoic acid-based SUPRAS had a higher ethanol (25.6-32.7%) and water (14.4-19%) contents compared to the octanoic acid-based SUPRAS (i.e. 21% of ethanol and 12.3% of water) (see Table 1). This different behavior can be qualitatively interpreted as follows: the two binding forces driving the extraction of the target bioactives (hydrogen bonding and dispersion) decrease and increase, respectively, with increasing length of the hydrocarbon chain (Burke, 1984). Short-chain carboxylic acids are better proton donors than longer carboxylic acids. So, considering the high polarity of the target bioactives (log K_{ow} -0.07 for caffeine and 0.86 for protocatechuic acid, source: DrugBank) and the number of hydrogen bonds acceptors (3 for caffeine and 4 for protocatechuic acid), it is reasonable to assume that extraction will be favored with SUPRAS made up of octanoic acid, because stronger hydrogen bonds can be established. In the case of SUPRAS synthesized from decanoic acid, more water and ethanol were necessary, particularly for caffeine, to increase hydrogen bonding. In fact, recoveries for the target bioactives were highly correlated with SUPRAS water content (r-Pearson 0.96 and 0.97 for caffeine and protocatechuic acid, respectively for decanoic acid-based SUPRAS). Overall, maximum extraction of bioactives at the lowest consumption of ethanol (cost-benefit ratio taking into account that similar prices are expected for food grade natural octanoic and decanoic acids), was found with SUPRAS made up from 5% v/v of

octanoic acid, 24% v/v of ethanol and 71 % v/v of water. These conditions were selected as optimal for further studies.

Secondly, we optimized the influence of the ratio SUPRAS:EqS. For this aim, extractions were carried out with a total volume of 1.2 mL of SUPRAS+EqS and the content of the SUPRAS phase varied from 33 to 100%. Results are shown in Figure 3 and the corresponding statistical operations including ANOVA table and results of Tukey's tests are shown in Tables S4 and S5. The results clearly show that extraction yields increased as the SUPRAS phase did, especially for caffeine, a highly polar compound that partitioned between the SUPRAS and the EqS. Lower losses of protocatechuic acid were observed in the EqS due to its lower polarity. Maximum extraction efficiencies for both bioactives were reached for extractant phases containing 83% and 100% of SUPRAS, so these phases were selected for further studies.

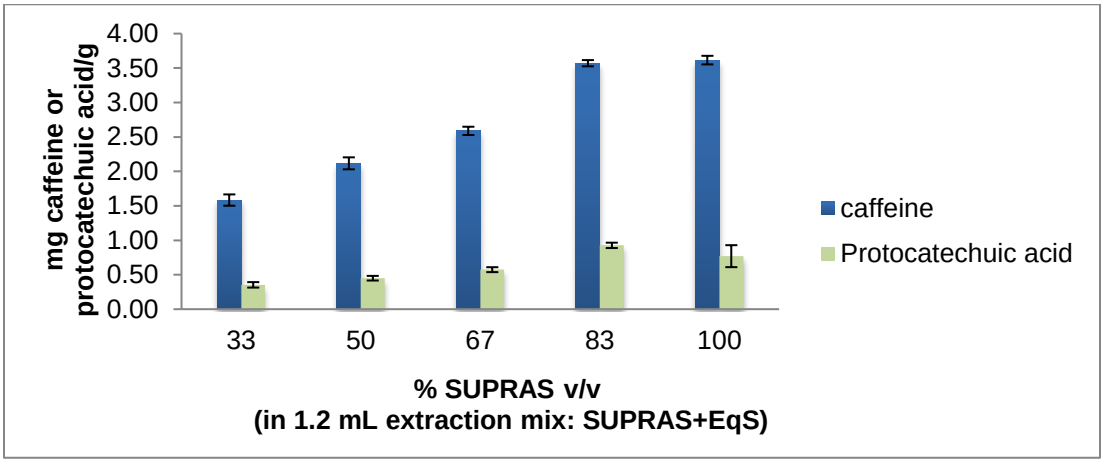


Figure 3. Extraction yields of caffeine and protocatechuic acid as mean ± standard deviation (n=3). Significant differences are indicated by different letters on the top of the bars (Tukey tests). Extraction of coffee cherry pulp (200 mg) using an extractant phase consisting in SUPRAS + EqS. The content of the SUPRAS phase varied from 33 to 100% and the volume of the extractant phase was 1.2 mL. The ratio solvent to sample was 6:1 v/w. SUPRAS synthesis conditions: octanoic acid 5% v/v, ethanol 24 % v/v and water 71% v/v.

Next, we studied the sample:extractant phase ratios (mg:mL) in the range 1:3 to 1:6. For this purpose, the amount of sample was kept constant (i.e. 200 mg) and the volume of the extractant phase varied from 0.6 to 1.2 mL. As can be seen in Figure 4 (see also Tables S6 and S7 for Tukey tests), not significant differences were found in the extraction efficiencies for both bioactives in the whole interval for SUPRAS:EqS and in the interval 1:4.5-1:6 for SUPRAS. Since absolute values were slightly higher for SUPRAS:EqS in the interval 1:4-1:6, a ratio 1:4 was selected as optimal. Significantly higher solvent to sample ratios have been reported for the extraction of bioactives from solid coffee waste, many times using pretreatment, high temperature or energy-assisted techniques. For example standard solvent extraction procedures based on aqueous methanol or ethanol have been reported at ratios of 30-40 mL/g and carried out at room temperature or 50 °C (Mussato et al., 2011; Zuorro and Lavechia, 2011). Lower ratios (9-10 mL/g) were reported by Murthy et al. (2012) with Soxhlet extraction using aqueous isopropanol at 50 °C and pretreatment with viscozyme and by Pavlović et al. (2013) by microwave-assisted extraction. The use of deep eutectic solvents also required high ratios of 17-100 mL/g under ultrasonic assisted extraction at room temperature or at 80 °C (Mouratoglou et al, 2016; Yoo and Lee, 2018).

Extraction yields of 3.6±0.3 mg caffeine g^{-1} and 0.9±0.1 mg protocatechuic acid g^{-1} were obtained under optimal conditions, which are specified in Figure 1. These values were in agreement with those previously reported by Heeger et al. (2017). The content of caffeine and protocatechuic acid in coffee cherry pulp obtained by wet or semi-dry processes from six varieties varied from 3.4 to 6.8 mg g^{-1} and from ~0.2 to 3.1 mg g^{-1}, respectively.

3.3. *Comparison of the extraction efficiency of SUPRAS and conventional organic solvents for bioactives in coffee pulp*

The extraction efficiency of SUPRAS for caffeine and protocatechuic acid was compared with that obtained by organic solvents commonly used for extraction of bioactives from coffee residues (e.g. methanol, ethanol, acetone, and acetonitrile). Aqueous mixtures with polar solvents are also commonly employed for this aim. However, since these mixtures limit the extraction of less polar bioactives (e.g. flavonoids), we preferred to do a comparison with pure polar solvents for wide scope extraction purposes. We employed the same extraction procedure for polar solvents than that optimized for SUPRAS (i.e. solvent:sample ratio of 4:1 v/w and experimental conditions as reported in Figure 1.2). SUPRAS extracted ~7-fold

more caffeine (3.6±0.3 mg g^{-1}) than methanol (0.31 ± 0.05 mg g^{-1}). Values for ethanol (0.187 ± 0.004 mg g^{-1}), acetone (0.205 ± 0.009 mg g^{-1}) and acetonitrile (0.22 ± 0.02 mg g^{-1}) were even lower. Results for protocatechuic acid were also better for SUPRAS that extracted this bioactive ~11-fold more efficiently (0.9±0.1 mg g^{-1}) than methanol (0.12± 0.07 mg g^{-1}). Ethanol (0.055 ± 0.009 mg g^{-1}), acetone (0.041± 0.006 mg g^{-1}) and acetonitrile (0.0272± 0.0007 mg g^{-1}) were even less efficient. Results for other phenolic compounds identified in the following section are shown in Figure S1. The multiple binding sites and adequate balance of hydrogen bonds and dispersion interactions provided by SUPRAS could be the reason for this higher extraction efficiency.

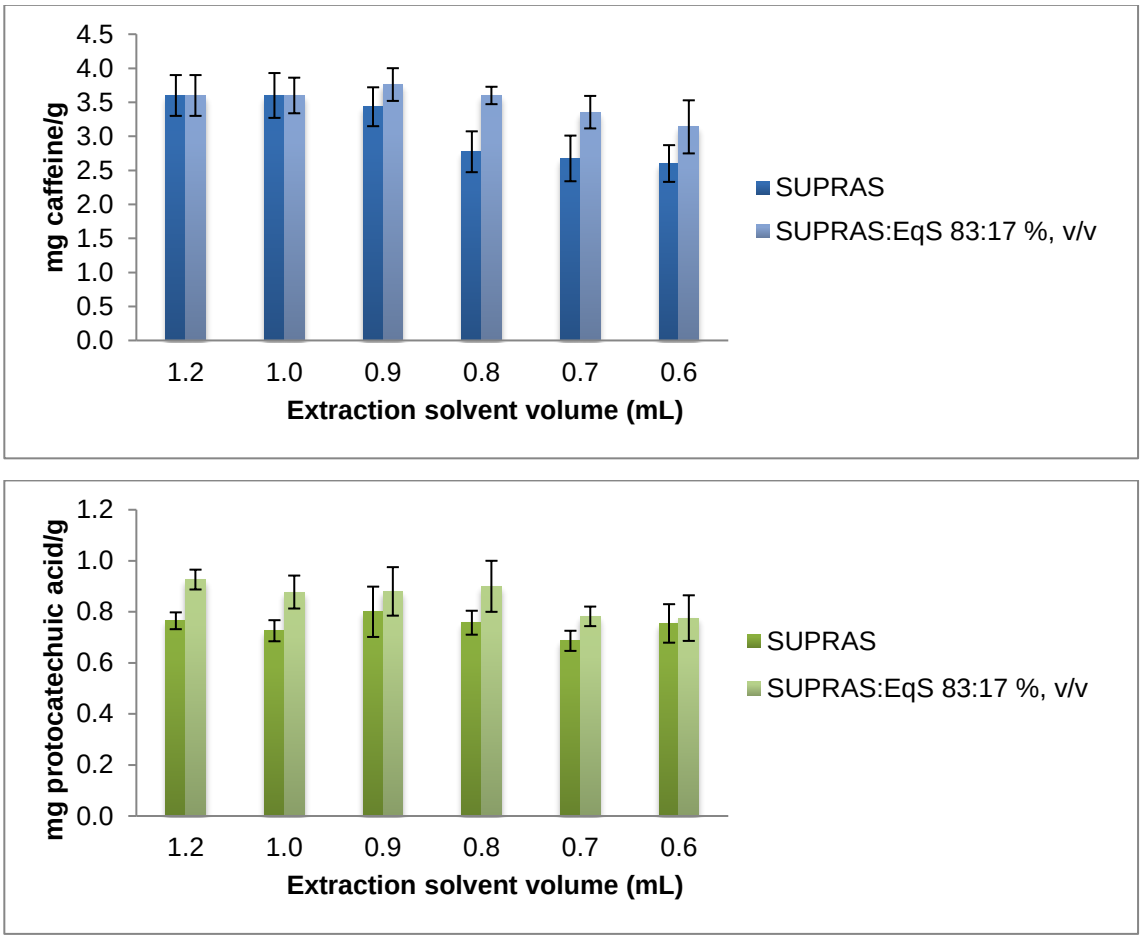


Figure 4. Extraction yields of caffeine and protocatechuic acid as mean ± standard deviation (n=3). Significant differences are indicated by different letters on the top of the bars (Tukey tests). Extraction of coffee cherry pulp (200 mg) with SUPRAS or SUPRAS:EqS 88:17 v/v and different solvent to sample ratios 3:1-6:1 v/w. SUPRAS synthesis conditions: octanoic acid 5% v/v, ethanol 24 % v/v and water 71% v/v.

3.4. *Phenolic compounds and alkaloids profile in SUPRAS extracts*

SUPRAS extracts obtained under optimal conditions were analyzed by LC-MS/MS to investigate their content in phenolic compounds and alkaloids. The most abundant phenolic compounds were protocatechuic acid (0.9±0.1 mg g^{-1}), gallic acid (0.25±0.02 mg g^{-1}) and 5-chlorogenic acid (0.13±0.01 mg g^{-1}). Other phenolic compounds were present at significantly lower levels: rutin (30.5±2 µg g^{-1}), 5-O-feruloylquinic acid (17.3±0.5 µg g^{-1}), 3-O-coumaroylquinic acid (13.4±0.4 µg g^{-1}), p-coumaric acid (4.5±0.1 µg g^{-1}), caffeic acid (2±0.2 µg g^{-1}), n-O-dicaffeoyl quinic acids (1.6±0.1 µg g^{-1}, sum of three isomers), 4-chlorogenic acid (1.1±0.05 µg g^{-1}), 3-chlorogenic acid (0.34±0.04 µg g-1) and 3-O-feruloylquinic acid (0.4±0.03 µg g^{-1}). The isomeric form of each class of compound was assigned on the basis of relative retention times and main fragments according to (Angelino et al., 2018). Figure 5 (LC-MS/MS chromatogram) shows the most abundant phenolic compounds in SUPRAS extracts. Regarding alkaloids, caffeine (3.6±0.3 mg g^{-1}) was the major compound followed by trigonelline which was detected with about 15-fold lower abundance.

Samples were also extracted with conventional solvents (i.e. methanol, ethanol, acetone and acetonitrile) under the same experimental conditions that those used for SUPRAS and the extracts analyzed by LC-MS/MS to quantify the most abundant phenolic compounds (protocatechuic acid, gallic acid, 5-CGA and rutin). Figure S1 shows the results obtained, which prove the higher efficiency of SUPRAS compared to the most used organic solvents. Levels of 5-chlorogenic acid in *Castillo* variety were lower than those reported from other varieties cultivated in South America (*Caturra, Catuai, Maragogype* from Honduras and *Bourbon* from El Salvador, 0.7-0.9 mg g^{-1}, Heeger et al., 2017). The same study reported gallic acid and rutin at levels below 100 µg g^{-1} in these varieties. A higher value of 0.73 mg g^{-1} of gallic acid was reported for a *Bourbon* variety cultivated in Congo.

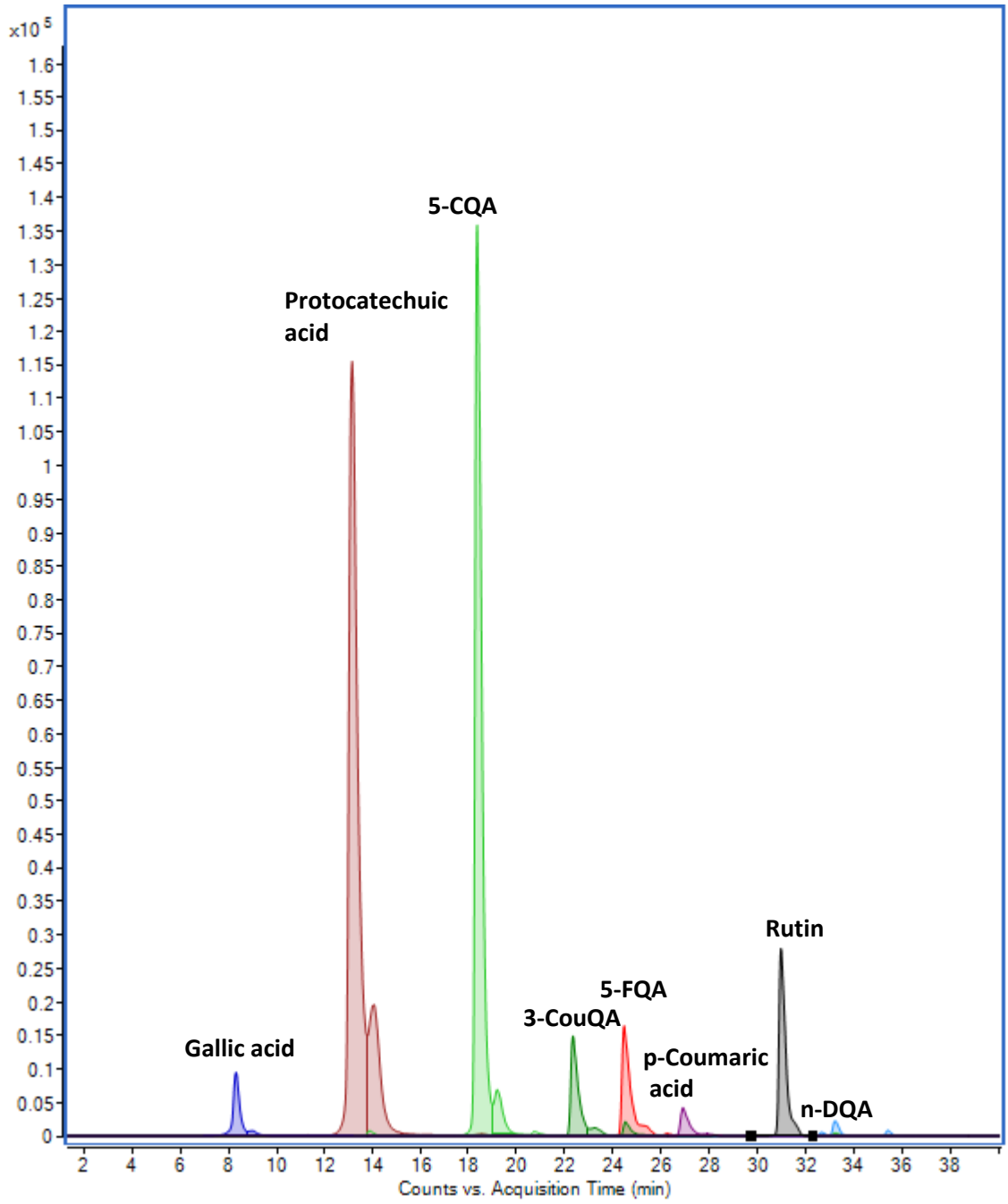


Figure 5. LC-(ESI-)MS/MS extracted ion chromatograms for the most abundant phenolic compounds in optimal SUPRAS extracts. Abbreviations: 5-CQA (5-O-Caffeoylquinic acid); 3-CouQA (3-O-Coumaroylquinic acid); 5-FQA (5-O-Feruloylquinic acid); n-DQA (n-O-Dicaffeoylquinic acids).

3.5 Antioxidant activity of SUPRAS extracts

Phenolic compounds are the main contributors to the antioxidant activity of coffee and by-products (Belščak-Cvitanović et al., 2017). The antioxidant activity of SUPRAS blanks was negligible under any dilution or experimental condition. The antioxidant activity of SUPRAS extracts was measured by the DPPH and ABTS assays. For this purpose diluted SUPRAS with concentrations in the ranges 0.14-36 g L^{-1} and 0.3-287 g L^{-1} for DPPH and ABTS assays, respectively, were tested. Linearity for antioxidant activity versus SUPRAS concentration was observed up to 15 g SUPRAS L^{-1} and 25 g SUPRAS L^{-1} in the DPPH and ABTS assays, respectively. At these concentrations, the DPPH and ABTS antioxidant capacity were 45±1% and 91±4%. The Trolox equivalent (TE) antioxidant capacity at 25 g SUPRAS L^{-1} was 334 µM.

The DPPH antioxidant capacity of coffee pulp extracts obtained under different extraction conditions, (which are hardly comparable) has been previously reported (Murthy and Naidu, 2012; Silva et al., 2013). Thus, DPPH activity of 65% has been found for extracts (0.5 g L^{-1}) obtained by Soxhlet extraction of 100 g sample with isopropanol:water 60:40 v/v at a solvent to sample ratio 10:1 v/w followed by evaporation to dryness (total yield 18.1%) (Murthy and Naidu, 2012). A similar DPPH antioxidant capacity (65.3%) was measured in coffee pulp extracts (20 g L^{-1}) obtained by extracting the samples by three consecutive times with ethanol (sample:solvent ratio 1:10 w/v) and evaporating the extracts to dryness (Silva et al., 2013). On the other hand, the values here obtained by the ABTS assay (corresponding to 40 µmol TE g^{-1} coffee pulp) were in accordance with those reported reported by Heeger et al. (2017) in the range 51-64.9 µmol TE g^{-1} coffee pulp (in weight, ~10% humidity) with different varieties of coffee cultivated in South America.

4. Conclusions

SUPRAS provides an efficient alternative extraction approach for the isolation of bioactive compounds from coffee cherry pulp, a less investigated coffee by-product than coffee husks, coffee silverskin or spent coffee grounds, but of major importance in the wet processing of coffee. SUPRAS extracts, rich in caffeine and polyphenols, can be of interest for the development of nutraceuticals, functional food or cosmetics. The extraction approach is simple (it is carried out at room temperature and atmospheric pressure in a single step and without external energy, such as ultrasound- or microwave-assisted extraction). While drying

and grinding are common industrial steps, vortexing and centrifugation can be replaced by a more gentle mixing approach during longer extraction times (to keep the extraction efficiency rate) followed by decantation and filtration. Further purification steps for concentration/separation of bioactives and for recovery and reuse of SUPRAS components are also probably required for industrial applicability. For this aim, different strategies could be employed, such as evaporation and/or freeze-drying for water and ethanol removal, back-extraction of bioactives with a poor solvent for the amphiphile (aqueous polar solvent mixture), dry fractionation for removal of octanoic acid and lipids or anionic exchange resins to retain octanoate after changing the pH to ~7. Nevertheless, the presence of octanoic acid in the final commercialized products should not be problematic since it is a food authorized ingredient and also can benefit the stability of bioactives For further implementation at industrial scale, the benefits of the amphiphile-rich extracts for enhancing the stability and bio-availability of bioactives will be addressed in the future.

Acknowledgment

Authors gratefully acknowledge financial support from Spanish MINECO (Project CTQ2017-83823-R). A. Ballesteros-Gómez acknowledges the funding from Spanish Ministry of Science, Innovation and Universities for a Ramón y Cajal contract (RYC-2015-18482). L.S. Torres-Valenzuela thanks AUIP for her doctoral fellowship.

References

Alves, R.C., Rodrigues, F., Antónia Nunes, M., Vinha, A.F., Oliveira, M.B.P.P., 2017. State of the art in coffee processing by-products, in: Galanakis, C.M. (Ed.), Handbook of Coffee Processing By-Products. Academic Press, pp. 1–26. https://doi.org/10.1016/B978-0-12-811290-8.00001-3

Andrade, K.S., Gonçalvez, R.T., Maraschin, M., Ribeiro-do-Valle, R.M., Martínez, J., Ferreira, S.R.S., 2012. Supercritical fluid extraction from spent coffee grounds and coffee husks: Antioxidant activity and effect of operational variables on extract composition. Talanta 88, 544–552. https://doi.org/10.1016/j.talanta.2011.11.031

Angelino, D., Tassotti, M., Brighenti, F., Del Rio, D., Mena, P., 2018. Niacin, alkaloids and (poly)phenolic compounds in the most widespread Italian capsule-brewed coffees. Scientific Reports 8, 17874. https://doi.org/10.1038/s41598-018-36291-6

Apak, R., Gorinstein, S., Böhm, V., Schaich, K.M., Özyürek, M., Güçlü, K., 2013. Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). Pure Appl. Chem. 85, 957–998. https://doi.org/10.1351/PAC-REP-12-07-15

Ballesteros-Gómez, A., Lunar, L., Sicilia, M.D., Rubio, S., 2018. Hyphenating Supramolecular Solvents and Liquid Chromatography: Tips for Efficient Extraction

and Reliable Determination of Organics. Chromatographia 1–14. https://doi.org/10.1007/s10337-018-3614-1

Ballesteros-Gómez, A., Rubio, S., 2012. Environment-responsive alkanol-based supramolecular solvents: characterization and potential as restricted access property and mixed-mode extractants. Anal. Chem. 84, 342–349. https://doi.org/10.1021/ac2026207

Ballesteros-Gómez, A., Sicilia, M.D., Rubio, S., 2010. Supramolecular solvents in the extraction of organic compounds. A review. Analytica Chimica Acta 677, 108–130. https://doi.org/10.1016/j.aca.2010.07.027

Belščak-Cvitanović, A., Komes, D., 2017. Extraction and formulation of bioactive compounds, in: Handbook of Coffee Processing By-Products. Elsevier, pp. 93–140. https://doi.org/10.1016/B978-0-12-811290-8.00004-9

Caballo, C., Sicilia, M.D., Rubio, S., 2017. Chapter 5 - Supramolecular Solvents for Green Chemistry, in: Pena-Pereira, F., Tobiszewski, M. (Eds.), The Application of Green Solvents in Separation Processes. Elsevier, pp. 111–137. https://doi.org/10.1016/B978-0-12-805297-6.00005-X

Esquivel, P., Jiménez, V.M., 2012. Functional properties of coffee and coffee by-products. Food Research International 46, 488–495. https://doi.org/10.1016/j.foodres.2011.05.028

Galanakis, C.M., 2015. Food Waste Recovery. Processing Technologies and Industrial Techniques. Elsevier. https://doi.org/10.1016/C2013-0-16046-1

Garde W. K., Buchberger S. G., Wendell D., Kupferle M.J. 2017. Application of Moringa Oleifera seed extract to treat coffee fermentation wastewater. Journal of Hazardous Materials 329, 102–109. https://doi.org/10.1016/j.jhazmat.2017.01.006

Getachew, A.T., Chun, B.S., 2017. Influence of pretreatment and modifiers on subcritical water liquefaction of spent coffee grounds: A green waste valorization approach. Journal of Cleaner Production 142, 3719–3727. https://doi.org/10.1016/j.jclepro.2016.10.096

Heeger, A., Kosińska-Cagnazzo, A., Cantergiani, E., Andlauer, W., 2017. Bioactives of coffee cherry pulp and its utilisation for production of Cascara beverage. Food Chemistry 221, 969–975. https://doi.org/10.1016/j.foodchem.2016.11.067

International Coffee Organization, 2017. Total production by all exporting countries. URL http://www.ico.org/prices/po-production.pdf (accessed 5.22.19).

Janissen, B., Huynh, T., 2018. Chemical composition and value-adding applications of coffee industry by-products: A review. Resources, Conservation and Recycling 128, 110–117. https://doi.org/10.1016/j.resconrec.2017.10.001

Kovalcik, A., Obruca, S., Marova, I., 2018. Valorization of spent coffee grounds: A review. Food and Bioproducts Processing 110, 104–119. https://doi.org/10.1016/j.fbp.2018.05.002

Mouratoglou E., Malliou V., Makris D.P. 2016. Novel Glycerol-Based Natural Eutectic Mixtures and Their Efficiency in the Ultrasound-Assisted Extraction of Antioxidant Polyphenols from Agri-Food Waste Biomass. Waste Biomass Valor. 7, 1377–1387. https://doi: 10.1007/s12649-016-9539-8

Murthy, P.S., Madhava Naidu, M., 2012. Sustainable management of coffee industry by-products and value addition—A review. Resources, Conservation and Recycling 66, 45–58. https://doi.org/10.1016/j.resconrec.2012.06.005

Murthy, P.S., Naidu, M.M., 2012. Recovery of Phenolic Antioxidants and Functional Compounds from Coffee Industry By-Products. Food and Bioprocess Technology 5, 897–903. https://doi.org/10.1007/s11947-010-0363-z

Mussatto, S.I., Ballesteros, L.F., Martins, S., Teixeira, J.A., 2011. Extraction of antioxidant phenolic compounds from spent coffee grounds. Separation and Purification Technology 83, 173–179. https://doi.org/10.1016/j.seppur.2011.09.036

Narita, Y., Inouye, K., 2014. Review on utilization and composition of coffee silverskin. Food Research International 61, 16–22. https://doi.org/10.1016/j.foodres.2014.01.023

Pandey, A., Soccol, C.R., Nigam, P., Brand, D., Mohan, R., Roussos, S., 2000. Biotechnological potential of coffee pulp and coffee husk for bioprocesses. Biochemical Engineering Journal 6, 153–162. https://doi.org/10.1016/S1369-703X(00)00084-X

Pavlović, M.D., Buntić, A.V., Šiler-Marinković, S.S., Dimitrijević-Branković, S.I., 2013. Ethanol influenced fast microwave-assisted extraction for natural antioxidants obtaining from spent filter coffee. Separation and Purification Technology 118, 503–510. https://doi.org/10.1016/j.seppur.2013.07.035

Régnier, P., Bastias, J., Rodriguez-Ruiz, V., Caballero-Casero, N., Caballo, C., Sicilia, D., Fuentes, A., Maire, M., Crepin, M., Letourneur, D., Gueguen, V., Rubio, S., Pavon-Djavid, G., 2015. Astaxanthin from Haematococcus pluvialis Prevents Oxidative Stress on Human Endothelial Cells without Toxicity. Mar Drugs 13, 2857–2874. https://doi.org/10.3390/md13052857

Rodrigues, F., Nunes, M.A., Alves, R.C., Oliveira, M.B.P.P., 2017. Chapter 7 - Applications of recovered bioactive compounds in cosmetics and other products, in: Galanakis, C.M. (Ed.), Handbook of Coffee Processing By-Products. Academic Press, pp. 195–220. https://doi.org/10.1016/B978-0-12-811290-8.00007-4

Ruiz, F.-J., Rubio, S., Pérez-Bendito, D., 2007. Water-induced coacervation of alkyl carboxylic acid reverse micelles: phenomenon description and potential for the extraction of organic compounds. Anal. Chem. 79, 7473–7484. https://doi.org/10.1021/ac0708644

Salatti-Dorado, J.A., García-Gómez, D., Rodriguez-Ruiz, V., Gueguen, V., Pavon-Djavid, G., Rubio, S., 2019. Multifunctional green supramolecular solvents for cost-effective production of highly stable astaxanthin-rich formulations from Haematococcus pluvialis. Food Chemistry 279, 294–302. https://doi.org/10.1016/j.foodchem.2018.11.132

Silva, R.M.G., Brigatti, J.G.F., Santos, V.H.M., Mecina, G.F., Silva, L.P., 2013. Allelopathic effect of the peel of coffee fruit. Scientia Horticulturae 158, 39–44. https://doi.org/10.1016/j.scienta.2013.04.028

Steed, J.W., Turner, D.R., Wallace, K., 2007. Core Concepts in Supramolecular Chemistry and Nanochemistry. John Wiley & Sons, Ltd, Chippenham, Witshire.

Yoo, D.E., Jeong, K.M., Han, S.Y., Kim, E.M., Jin, Y., Lee, J., 2018. Deep eutectic solvent-based valorization of spent coffee grounds. Food Chemistry 255, 357–364. https://doi.org/10.1016/j.foodchem.2018.02.096

Zuorro, A., Lavecchia, R., 2012. Spent coffee grounds as a valuable source of phenolic compounds and bioenergy. Journal of Cleaner Production 34, 49–56. https://doi.org/10.1016/j.jclepro.2011.12.003

SUPPLEMENTARY INFORMATION

Supramolecular solvent extraction of bioactives from coffee cherry pulp

Laura Sofía Torres-Valenzuela [a,b], Ana Ballesteros-Gómez [a*], Soledad Rubio[a]

Chemicals and solutions

All reagents were analytical reagent-grade and were used as supplied. Acetone, acetonitrile, ethanol, ethyl acetate, methanol, hydrochloric acid (37%) and acetic acid glacial (99.5%) were acquired from Panreac (Barcelona, Spain). The following reagents were purchased from Sigma–Aldrich Co. (St. Louis, USA): octanoic acid (99%), decanoic acid (98%), potassium persulfate (99%), protocatechuic acid, chlorogenic acid, gallic acid, caffeic acid, caffeine, 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2 diphenyl-1-picrylhydrazyl (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid (97%), catechin hydrate (96%) and (±)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (98,1%, Trolox). Stock solutions of bioactive compounds (1000 mg L^{-1}) were prepared in methanol and the respective calibration standards were prepared by dilution in methanol. The DPPH reagent was prepared by dissolving 1 mg of DPPH in 50 mL of methanol and diluting this solution with methanol to give an absorbance of 1.00 at 590 nm. The $ABTS^{\bullet+}$ radical was obtained by mixing 1 mL of ABTS 14 mM with 1 mL of potassium persulfate 4.9 mM. The solution was stored in darkness during 16 hours and its absorbance was adjusted at 1.0 (measured at 620 nm) with ultrapure water (Milli-Q system equipped with a 0.22-µm filter, MA, USA).

Analysis of bioactive compounds by LC-MS/MS

For this aim we employed an HPLC system (Agilent Technologies 1200) with a ACE 3 C18-PFP column (3 mm i.d., 150 mm length, 3.0 µm particle size) and precolumn (Phenomenex KJ 0-4282) coupled to a Triple Quadrupole mass spectrometer (Agilent 6420) equipped with an electrospray ionization (ESI) source operating in negative and positive modes. The mobile phase was made up of (A) Milli-Q water with 0.1% acetic acid and (B) MeOH:Acetonitrile 50:50 v/v at a flow rate of 0.3 $mL \cdot min^{-1}$. The injection volume was 5 µL. The gradient was as follow: initial 5% B hold for 0.1 min, linear gradient to 30% B in 25 min and to 40% B in the next 10 min. Finally, B was increased

to 100% at 35.1 min and maintained for 10 min to remove possible hydrophobic compounds from the column. The column was re-conditioned for 10 min before injection. The MRM transitions for target masses of the bioactives identified in SUPRAS extracts as well as MS parameters are given in Table S1.

Table S1. Polyphenolic compounds (and alkaloids) identified in SUPRAS extracts

Compound class	Abreviation	Parent ion	Fragment 1	Fragment 2	Retention time/s	Polarity
Trigonelline	-	138	92	94	2.7	+
Gallic acid	-	169	125	-	8.3	-
Protocatechuic acid	-	153	109	-	13.1	-
3-O-Caffeoylquinic acid	3-CQA	353	191	179	13.8	-
Caffeine	-	195	138	-	18.9	+
5-O-Caffeoylquinic acid	5-CQA	353	191	179	18.3	-
3-O-Feruloylquinic acid	3-FQA	367	173	193	18.4	-
4-O-Caffeoylquinic acid	4-CQA	353	173	179	19.2	-
Caffeic acid	-	179	135	-	21.6	-
3-O-Coumaroylquinic acid	3-CouQA	337	191	179	22.2	-
5-O-Feruloylquinic acid	5-FQA	367	191	173	24.4	-
p-coumaric acid	-	163	119	-	26.9	-
n-O-Dicaffeoylquinic acids	n-DQAs	515	179	135	31.8, 32.4, 35.2	-
Rutin	-	609	300	-	30.9	-

MS parameters were: fragmentor 100 V, collision energy 15 eV, cell accelerator voltage 4 V, dwell 20 ms.
Source parameters were: gas temperature, 350°C; gas flow, 12 $L \cdot min^{-1}$; nebulizer gas pressure, 30 psi; capillary voltage, -4000 V.
MS data were processed with Agilent MassHunter Software® (version B.07.00).

Table S2. One-way ANOVA and Tukey pairwise comparisons corresponding to Figure 2A

ANOVA

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	7	1.8182	0.259745	26.46	0.000
Error	16	0.1571	0.009817		
Total	23	1.9753			

Factors (n=8): C1-C8, null hypothesis: all means are equal (significance level α = 0.05)

Tukey tests (*means that do not share a letter are significantly different, A for optimal values*)

Factor	% v/v etanol /amphiphile	N	Mean	SD	Grouping			
C6	**19/C8**	3	1.66	0.05	**A**			
C7	**24/C8**	3	1.58	0.08	**A**			
C4	**38/C10**	3	1.57	0.08	**A**			
C3	**33/C10**	3	1.40	0.04	**A**	B		
C2	24/C10	3	1.23	0.03		B	C	
C1	19/C10	3	1.17	0.03		B	C	
C5	9.5/C8	3	1.0	0.2			C	D
C8	33/C8	3	0.8	0.1				D

Table S3. One-way ANOVA and Tukey pairwise comparisons corresponding to Figure 2B

ANOVA

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	7	0.227400	0.032486	59.06	0.000
Error	16	0.008800	0.000550		
Total	23	0.236200			

Factors (n=8): C1-C8, null hypothesis: all means are equal (significance level α = 0.05)

Tukey tests (*means that do not share a letter are significantly different, A for optimal values*)

Factor	% v/v etanol /amphiphile	N	mean	SD	Grouping			
C4	**38/C10**	**3**	**0.41**	0.02	**A**			
C3	**33/C10**	**3**	**0.39**	0.04	**A**			
C7	**24/C8**	**3**	**0.35**	0.04	**A**	B		
C6	19/C8	3	0.31	0.01		B	C	
C8	33/C8	3	0.28	0.02			C	
C2	19/C10	3	0.25	0.01			C	
C1	24/C10	3	0.25	0.01			C	
C5	9.5/C8	3	0.08	0.01				D

Table S4. One-way ANOVA and Tukey pairwise comparisons corresponding to Figure 3 (caffeine)

ANOVA

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	9.58123	2.39531	503.03	0.000
Error	10	0.04762	0.00476		
Total	14	9.62884			

Factors (n=8): C1-C5, null hypothesis: all means are equal (significance level α = 0.05)

Tukey tests (*means that do not share a letter are significantly different, A for optimal values*)

Factor	% v/v SUPRAS	N	mean	SD	Grouping			
C5	**100**	**3**	3.61	0.06	**A**			
C4	**83**	**3**	3.57	0.04	**A**			
C3	67	3	2.58	0.06		B		
C2	50	3	2.11	0.08			C	
C1	33	3	1.58	0.08				D

Table S5. One-way ANOVA and Tukey pairwise comparisons corresponding to Figure 3 (protocatechuic acid)

ANOVA

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	0.65557	0.163893	26.49	0.000
Error	10	0.06187	0.006187		
Total	14	0.71745			

Factors (n=8): C1-C5, null hypothesis: all means are equal (significance level α = 0.05)

Tukey tests (*means that do not share a letter are significantly different, A for optimal values*)

Factor	% v/v SUPRAS	N	mean	SD	Grouping			
C4	**83**	**3**	0.92	0.04	**A**			
C5	**100**	**3**	0.76	0.16	**A**	B		
C3	67	3	0.57	0.03		B	C	
C2	50	3	0.45	0.03			C	D
C1	33	3	0.35	0.03				D

Table S6. One-way ANOVA and Tukey pairwise comparisons corresponding to Figure 4A

ANOVA

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	11	5.598	0.50889	6.12	0.000
Error	24	1.995	0.08312		
Total	35	7.593			

Factors (n=8): C1-C12, null hypothesis: all means are equal (significance level α = 0.05)

Tukey tests (*means that do not share a letter are significantly different, A for optimal values*)

Factor	mL/% v/v SUPRAS	N	mean	SD	Grouping		
C9	**0.9/83**	**3**	3.7	0.3	**A**		
C10	**0.8/83**	**3**	3.6	0.3	**A**	B	
C8	**1.0/83**	3	3.6	0.3	**A**	B	
C7	**1.2/83**	3	3.6	0.3	**A**	B	
C2	**1.0/100**	3	3.6	0.3	**A**	B	
C1	**1.2/100**	3	3.6	0.3	**A**	B	
C3	**0.9/100**	3	3.4	0.2	**A**	B	C
C11	**0.7/83**	3	3.3	0.3	**A**	B	C
C12	**0.6/83**	3	3.1	0.3	**A**	B	C
C4	0.8/100	3	2.8	0.1		B	C
C5	0.7/100	3	2.7	0.2			C
C6	0.6/100	3	2.6	0.4			C

Table S7. One-way ANOVA and Tukey pairwise comparisons corresponding to Figure 4B

ANOVA

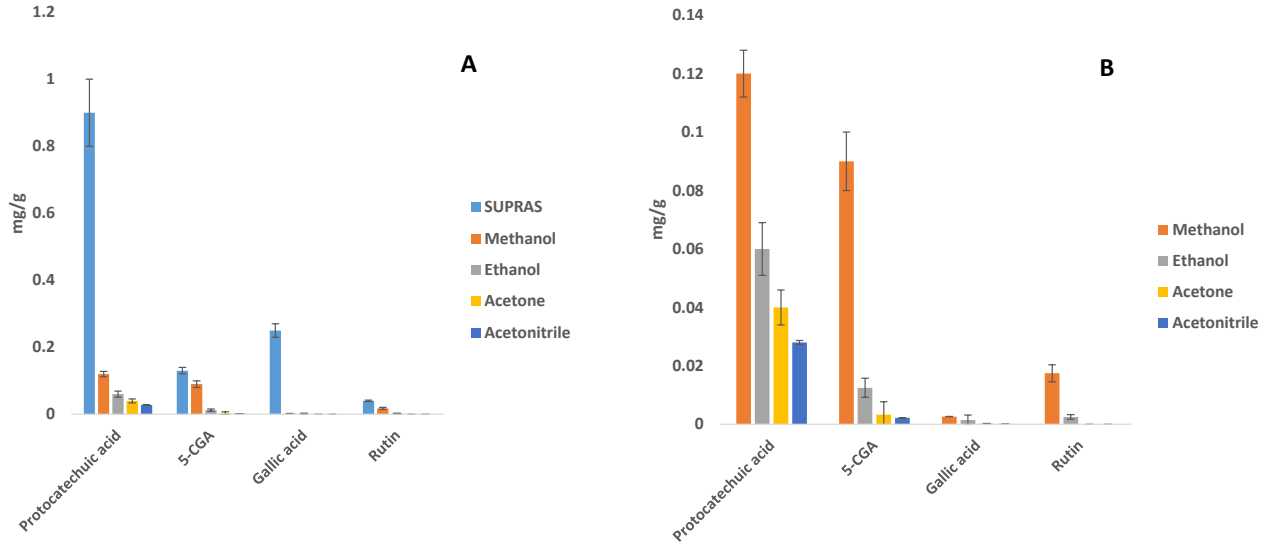
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	11	0.1883	0.017117	3.66	0.004
Error	24	0.1122	0.004676		
Total	35	0.3005			

Factors (n=8): C1-C12, null hypothesis: all means are equal (significance level α = 0.05)

Tukey tests (*means that do not share a letter are significantly different, A for optimal values*)

Factor	mL/% v/v SUPRAS	N	mean	SD	Grouping	
C7	**1.2/83**	**3**	0.93	0.04	**A**	
C10	**0.8/83**	**3**	0.9	0.1	**A**	
C9	**0.9/83**	3	0.88	0.09	**A**	B
C8	**1.0/83**	3	0.88	0.06	**A**	B
C3	**0.9/100**	3	0.8	0.1	**A**	B
C11	**0.7/83**	3	0.78	0.04	**A**	B
C12	**0.6/83**	3	0.78	0.09	**A**	B
C1	**1.2/100**	3	0.76	0.03	**A**	B
C4	0.8/100	3	0.76	0.05	**A**	B
C6	0.6/100	3	0.75	0.07	**A**	B
C2	1.0/100	3	0.72	0.04	**A**	B
C5	0.7/100	3	0.69	0.04		B

Figure S1. Extraction rate (mg/g) of the four most abundant compounds phenolic compounds with SUPRAS, methanol, ethanol, acetone and acetonitrile (solvent:sample ratio 4:1 v/w). Figure S1-A compares the results for SUPRAS and conventional organic solvents and Figure S1-B magnifies the results for conventional organic solvents.



CAPÍTULO IV

DISOLVENTES SUPRAMOLECULARES PARA LA EXTRACCIÓN Y PRE-TRATAMIENTO DE AGUAS RESIDUALES DE LA TRANSFORMACIÓN PRIMARIA DEL CAFÉ

Supramolecular solvents for the valorization of coffee wastewater

Laura Sofía Torres-Valenzuela [a,b], Ana Ballesteros [a*], Johanna Serna [b], Andrea Arango [b], Soledad Rubio[a]

Environmental Science Water Research & Technology (2020): DOI: 10.1039/c9ew01095e

Abstract

Supramolecular solvents (SUPRAS) were investigated for the recovery of bioactives from coffee wastewater (CWW), an abundant residue in the wet and semi-wet processing methods of coffee beans. SUPRAS were made up of hexagonal inverted aggregates of hexanol or decanoic acid and were spontaneously produced in the wastewater through self-assembly processes. SUPRAS components were food authorized ingredients this facilitating future industrial applicability. Under passive extraction (energy-less procedure), caffeine was recovered with a yield of 54 to 65 mg per liter of wastewater. SUPRAS extracts showed good antioxidant capacity (up to 53% $ABTS^{\bullet+}$) and were stable for at least 2 months in the range of temperatures investigated (4-24 ºC) for the preservation of bioactives. Additionally, the SUPRAS-based extraction of bioactives improved substantially some wastewater quality parameters (e.g. BOD, total suspended solids and conductivity), so this process also helped to purify wastewater before dumping it to surface waters.

Keywords

Supramolecular solvents; coffee wastewater; wastewater treatment; bioactive compounds; valorization

1. Introduction

Coffee is one of the most popular products in the world. It is cultivated in about 80 countries across the globe (90% takes place in developing countries) with an estimated production above 8.2 million tons. Over 2.25 billion cups of coffee are consumed every day globally mainly in the industrialized economies.[1]

There are two primary processing methods to obtain green coffee (traded coffee beans). In the dry process, coffee cherries are laid out in the sun to dry. In the wet process the fruit covering the

beans (exocarp, coffee pulp and parchment) is removed (mechanically and by fermentation) before they are dried. Approximately 40% of all coffee around the world is wet processed [2], because it is considered to produce better taste.[1,3] However, this process produces a considerable amount of wastewater and solid waste that can pollute the environment. It has been estimated that 40–45 L of wastewater are produced per kilogram of coffee.[4]

The wastewater produced from wet coffee processing is rich in organic compounds such as caffeine, sugars, proteins and phenolic compounds.[1,3,5,6] However, it presents high acidity (pH below 4), dark color and high concentration of organic matter that can deplete the oxygen content of water and elevate the risk of eutrophication (up to 20,000 mg/L and 50,000 mg/L for BOD and COD, respectively).[7] For these reasons, it is unsuitable to directly dispose it into the soil or waterway.

Discharges of wastewater from coffee processing has become a global issue of concern since the high volume of wastewater generated during the harvest period causes management problems,[8] and usually it is simply dumped into the river. Different alternatives to mitigate the negative effects of these discharges have been evaluated. These include, among others, coagulation with *Moringa oleifera* seed extract,[2] chemical coagulation-flocculation and advanced oxidation processes,[4] enzymatic oxidation,[5] biomethanation,[9,10] aeration and biofiltration with vegetal species[11,12] and electrocoagulation-electrooxidation.[13] The aim of these technologies is that physical-chemical parameters of the treated water (e.g. COD, BOD, total nitrogen and phosphorus content, etc.) are within the accepted ranges for discharge to surface water. However, to the best of our knowledge, the recovery of bioactive compounds (caffeine, polyphenolic compounds, etc.), which are of interest for the pharma, cosmetic and food industry, have been not reported yet, despite it has been strongly encouraged.[1]

In this study, we developed an approach for the valorization of coffee wastewater (CWW) based on the use of green supramolecular solvents (SUPRAS). This technology could be applied before conventional treatments for purification in order to valorize the residue before the discharge.

SUPRASs are green liquids spontaneously produced by a sequential self-assembly of amphiphiles.[14] First, amphiphiles assemble into tridimensional aggregates (such as micelles or vesicles) above a critical aggregation concentration (cac). Then, under the action of a coacervation inducing agent, the size of these aggregates increases or the amphiphiles rearrange in new nanostructured packed phases that separate from the bulk solution.[15] Common inducing agents of coacervation include modifications in the temperature or the pH of the solution or the

addition of salts or a poor solvent for the amphiphile.[14] SUPRAS are excellent extraction materials since they offer different polarity microenvironments, a high number of binding sites (the concentration of amphiphile can reach up to 1 mg/µL) and a variety of binding interactions (dispersive, polar, ionic, hydrogen bonds, etc.). They can efficiently extract compounds spanning a wide polarity range and are easily tailored for each application by the selection of the proper functional groups of amphiphiles and/or the nature of the environment for their self-assembly. SUPRAS have been successfully used in analytical extractions and more recently, also proved promising in the extraction of bioactives from microalgae [15] and solid coffee waste.[16,17]

This study assess the suitability of SUPRAS, synthesized from decanoic acid or 1-hexanol (amphiphiles), ethanol and water, for the recovery of high-added value compounds from CWW. For this purpose, inverted aggregates of the amphiphile were produced in ethanol above its cac and then wastewater (a poor solvent for the amphiphile) was used as the coacervation inducing agent. In this way, SUPRAS formation and extraction of bioactives took place simultaneously.

The extraction of bioactives was investigated through the recovery of caffeine, one of the most valuable compounds in the wastewater, and optimal conditions were further assessed for the extraction of total phenolic compounds. General quality parameters (pH, BOD, dissolved oxygen, COD, conductivity, total suspended solids, etc.) of the wastewater after SUPRAS extraction were also determined. The approach was simple and rapid and it could constitute a sustainable and valuable strategy for the valorization of CWW prior to conventional treatment.

2. Materials and methods

2.1 Chemicals and apparatus

Caffeine (HPLC grade), decanoic acid (98%), 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and HPLC grade ethanol, methanol and acetic acid glacial were purchased from Sigma–Aldrich Co. (St. Louis, USA). 1-hexanol (98%) and hydrochloric acid (37%) were obtained from Merck (Darmstadt, Germany). Potassium persulfate was purchased from Panreac (Barcelona, Spain) and potassium acetate (99,4%) from JT Baker (Madrid, España). All chemicals were analytical reagent-grade and were used as supplied. Pure water was prepared using a Milli-Q ultrapure water purification system equipped with a 0.22-µm filter (MA, USA).

The reagent $ABTS^{+}$ radicals was prepared by dissolving 97 mg of ABTS and 16.5 mg of potassium persulfate in 25 mL of distilled water and keeping the mixture for 16 hours under dark. Then, it was diluted with ethanol to give an absorbance of 0.709±0.005 at 732 nm.

A high-performance liquid chromatography (HPLC) coupled to a UV Detector (Shimadzu, Japan) was employed for caffeine quantification. The stationary phase was an Ultra C_8 column (5 µm particle size, 150 mm length, 4.6 mm i.d.) from Restek (France). All data were acquired and processed using the LabSolutions Software (Shimadzu, Japan). The extractions were made in 2 mL-microtubes Safe-Lock from Eppendorf Iberica (Madrid, Spain). A vortex shaker from Vorterex (Heathrow Scientific, Vernon Hills, IL, USA) with an attachment for 4 tubes, and a high-speed brushless centrifuge BX 24 (Unico, USA) were used for sample preparation. The antioxidant activity was measured in a spectrophotometer Genesys 10UV-VIS, Thermospectronic (ThermoScientific, USA). Stability experiments were done in an environmental chamber (1000L. Dies. Colombia). For physical-chemical characterization of wastewater a forced circulation stove (Binder, Germany), muffle (Terrigeno, Colombia), potentiometer (Jenway, England) and dissolved oxygen meter (Milwaukee, Hungría) were employed.

2.2 Coffee wastewater

Wastewater was obtained from a wet processed coffee from *Castillo* variety that was produced in Circasia (Colombia) at 1650 MASL. In this process, skin, pulp and mucilage are removed using water and aerobic fermentation. The coffee wastewater was not subjected to further treatment before SUPRAS extraction.

2.3 Experimental design and statistical analysis

Recovery of caffeine from wastewater was optimized by extraction with SUPRAS of different composition that were produced from two types of amphiphiles (decanoic acid and 1-hexanol) at several proportions of ingredients (amphiphile, ethanol and water).

The optimization was done with a central composite design (CCD) and response surface methodology (RSM). The independent variables were the amphiphile (1-hexanol or decanoic acid X1, 2.9 – 17.1% v/v) and solvent (ethanol; X2, 3.8 – 46.2%v/v) concentration in the synthetic solution, while the dependent variable was caffeine extraction (recovery). Both the composition and volume of SUPRAS formed in ternary mixtures of amphiphile-organic solvent-water have been widely proved to be dependent on the proportion of amphiphile and organic solvent in the

mixture.[18,19] Considering two independent variables and two levels (low (−) and high (+)); the total number of experiments was 13, as determined by the expression: 2^n (2^2 = 4: factor points) + 2n (2 × 2 = 4: axial points) + 5 (center point: five replicates). The 5 replicates of the central point level (10%v/v amphiphile and 25%v/v organic solvent) give an estimate of the experimental error (See Table 1).

The response surface regression (RSREG) and significance tests were conducted with Minitab 18.1 software. The parameters obtained from the RSM analysis were adjusted by a second-order polynomial model equation (See equation 1). Statistical analysis of variance (ANOVA) was used to evaluate the fit quality of the experimental results to the developed polynomial model. The optimum point was further estimated by a ridge analysis.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_{ij} + \varepsilon \qquad \text{Equation 1}$$

where Y is the extraction of caffeine (recovery), β_0, β_i, β_{ii} and β_{ij} are the regression coefficients of the model. k is number of studied factors and ε is the error. X_i, X_i^2 and X_{ij} are linear, quadratic, and interaction terms of model, respectively.

Table 1. Experiments (CCD design) for the optimization of the influence of SUPRAS produced from different proportions of ingredients on the recovery of caffeine.

[a]Amphiphile	[a]Ethanol	[a]Wastewater	[a]Distilled water
5.0	10.0	31.25	53.75
15.0	10.0	31.25	43.75
5.0	40.0	31.25	23.75
15.0	40.0	31.25	13.75
2.9	25.0	31.25	40.82
17.1	25.0	31.25	26.68
10.0	3.8	31.25	54.96
10.0	46.2	31.25	12.54
[b]10.0	25.0	31.25	33.75

[a]Concentrations expressed as volume percentages (v/v, %). Total volume of the solution: 1.6 mL. [b]The central point condition was measured 5-times in accordance with the experimental design

2.4 *Optimization of SUPRAS extraction*

Experiments were done at a laboratory scale. The supramolecular solvent was synthesized *in situ* in the wastewater sample by addition of 1-hexanol or decanoic acid and ethanol. In order to maintain constant the variety and concentration of ingredients of the wastewater during the optimization process, the volume of wastewater remained unchanged. The extraction was performed in 2 mL polypropylene centrifuge microtubes. The SUPRAS synthesis solution (1.6 mL) consisted in wastewater (500 µL), amphiphile (decanoic acid or hexanol, 45 – 273 µL), ethanol (61 – 739 µL) and distilled water (220 – 879 µL).

The mixtures were vortex-shaken for 1 min at 3,000 rpm (for both SUPRAS formation and caffeine extraction), then centrifuged for 20 minutes at 4.519 g for phase separation of the SUPRAS (upper phase) from the bulk solution (bottom phase). The volume of the top enriched SUPRAS phase was measured using a digital caliper and calculated as cylinder volume. For caffeine quantification SUPRAS extracts were diluted with methanol and then vortex-shaken for 1 min at 300 rpm. The standard deviation was calculated based on 5 replicates of the central point level.

Additionally, the caffeine content in CWW was quantified. For this aim, the wastewater was with ultrapure water and then centrifuged (30 minutes at 4.519 g), filtered using a syringe filter (0.20 µm) and subjected to HPLC-UV analysis as described in the following section.

Optimal SUPRAS conditions were tested to simulate nearly passive extraction conditions, which could be advantageous for application at the industrial scale. For this purpose, solutions containing wastewater and optimal SUPRAS components were gently mixed and they were allowed to stand for spontaneous phase separation of the SUPRAS from the bulk solution. The recovery of caffeine as a function of time was measured at 3.5, 6, 8, 10, 13, 16, 20 and 30 minutes.

2.5 Analysis of bioactive compounds by HPLC-UV

Caffeine content in SUPRAS made up from different synthetic conditions and wastewater were analyzed by high performance liquid chromatography (HPLC) and ultraviolet detection (UV). The detector wavelength was set at 254 nm. The mobile phase consisted of 69.9% v/v of water, 30% v/v of methanol, and 0.1% v/v of acetic acid and was operated in isocratic mode. The flow rate was set at 0.6 mL/min and the sample injection volume was 20 µL. Quantitative analysis was

conducted using authentic standards of caffeine prepared in water:methanol 70:30 v/v in the concentration range of 5 – 100 mg L^{-1}. The equation of the calibration curve for caffeine was $y = 27{,}219\,x - 1{,}100$ ($R^2 = 0.9998$) (see Figure S1 for statistics and graph).

2.6 Antioxidant activity determination

The antioxidant activity of SUPRAS extracts, obtained under the optimal conditions for hexanol and decanoic acid, was evaluated by the $ABTS^{\bullet+}$ method. The inhibition was calculated by the change in the absorbance of the reagent solution ($ABTS^{\bullet+}$) from the following equation:[20]

$$\%inhibition = \frac{Abs_0 - Abs_{30}}{Abs_0} * 100 \qquad \text{Equation 2}$$

where Abs_0 is the absorbance of $ABTS^{+}$ reagent solution at time zero and Abs_{10} is the absorbance of the reagent in the presence of SUPRAS extracts (previously diluted in 1:10 with methanol) at 30 minutes of reaction.

For the $ABTS^{\bullet+}$ assay, aliquots of 330 µL of SUPRAS extracts or methanol as blank and 670 µL of the $ABTS^{\bullet+}$ reagent were mixed and incubated during 30 minutes at 25 ºC. The absorbance was measured at 732 nm. The final concentration of SUPRAS extract tested was 91.7 mg SUPRAS/mL.

2.7 Caffeine stability in SUPRAS extracts.

The caffeine extracts obtained with SUPRAS under optimal conditions were stored in an environmental chamber (1000L. Dies. Colombia) at 4, 14 and 24 ± 1 °C and 85% relative humidity. The caffeine content was measured at 0, 1, 2, 6, 9, 16, 23, 30, 50 and 60 days (storage time). Caffeine concentration in SUPRAS (CA), ln(CA) or 1/CA was plotted against time (days) to determine the reaction order of the degradation of caffeine according to equation 3:

$$-r_{CA} = kC_{CA}^{\propto} \qquad \text{Equation 3}$$

where $-r_{CA}$: caffeine degradation rate; k : caffeine degradation constant; CA: caffeine concentration in SUPRAS, mg/L (as a function of time) and α: reaction order

2.8 Physico-chemical parameters

The physico-chemical parameters of the wastewater were characterized before and after SUPRAS treatment using the optimal value with both amphiphiles. The measured parameters were total suspended solids, pH, conductivity, dissolved oxygen, phosphates, nitrates, biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD). Total suspended solids were measured by gravimetry according to the AOAC 940.26 official method. Values of pH and conductivity were directly measured by using a potentiometer and dissolved oxygen using an apparatus DO Meter (Milwaukee, Hungría).

The other parameters were measured with different analytical kits from Hanna Instruments, USA (phosphates, kit HI 3833; nitrates, kit HI 3874) and NANOCOLOR, Macherey-Nagel, Germany (BOD_5, kit 8-22 and COD, Test 0-29).

3. Results and discussion

3.1 SUPRAS synthesis

Because of the aim of this research was to recover bioactive compounds from CWW, two conditions were imposed for SUPRAS selection. First, components making up the SUPRAS should be authorized as food ingredients [21,22] or extractants,[23] and second, the SUPRAS should be immiscible with wastewater. To meet the first requirement, SUPRAS produced from fatty acids [19] and fatty alcohols [18] in ethanol-water mixtures were evaluated for the extraction of the targeted bioactives. The amphiphiles selected were decanoic acid and 1-hexanol, which are both authorized as food ingredients.[20,21] Alcohols are also authorized as extraction solvents in the production of foodstuffs, with no residues limits established for ethanol and 10 mg kg^{-1} for longer alkyl chains.[23]

The formation of SUPRAS was carried out by dissolving decanoic acid or 1-hexanol in ethanol, where they form inverse aggregates above the cac (i.e. reverse micelles), and then, adding wastewater as the coacervation-inducing agent. The addition of wastewater to the colloidal suspension of fatty acids and alcohols in organic solvents caused the formation of oily droplets that associated in clusters of individual droplets, and finally separated as a SUPRAS top layer in equilibrium with the wastewater solution. Liquid-liquid phase separation was the result of the increase in the size of the reverse micelles, as previously reported by optical and scanning electron microscopy,[18,19] due to the partial removal of ethanol molecules available for their

solvation when adding wastewater. Aggregate growth in the presence of a mixture of miscible solvents, one of which is a poor solvent for the micelles, is a known mechanism for coacervation of non-ionic surfactants.[24,25] The volume of SUPRAS formed depended linearly on the amount of amphiphile and exponentially on the percentage of ethanol in the ternary mixture, as previously reported for SUPRAS formed in distilled water.

Bioactive compounds contained in wastewater, such as caffeine and polyphenols, were expected to enrich the SUPRAS due to its multi-binding capacity via hydrogen bonding, polar and dispersion interactions. Since the wastewater was employed for SUPRAS synthesis, both the formation of the solvent and the extraction of bioactives took place in a single step after stirring and centrifugation. Figure 1 shows a schematic picture of the SUPRAS formation/extraction of bioactives from wastewater.

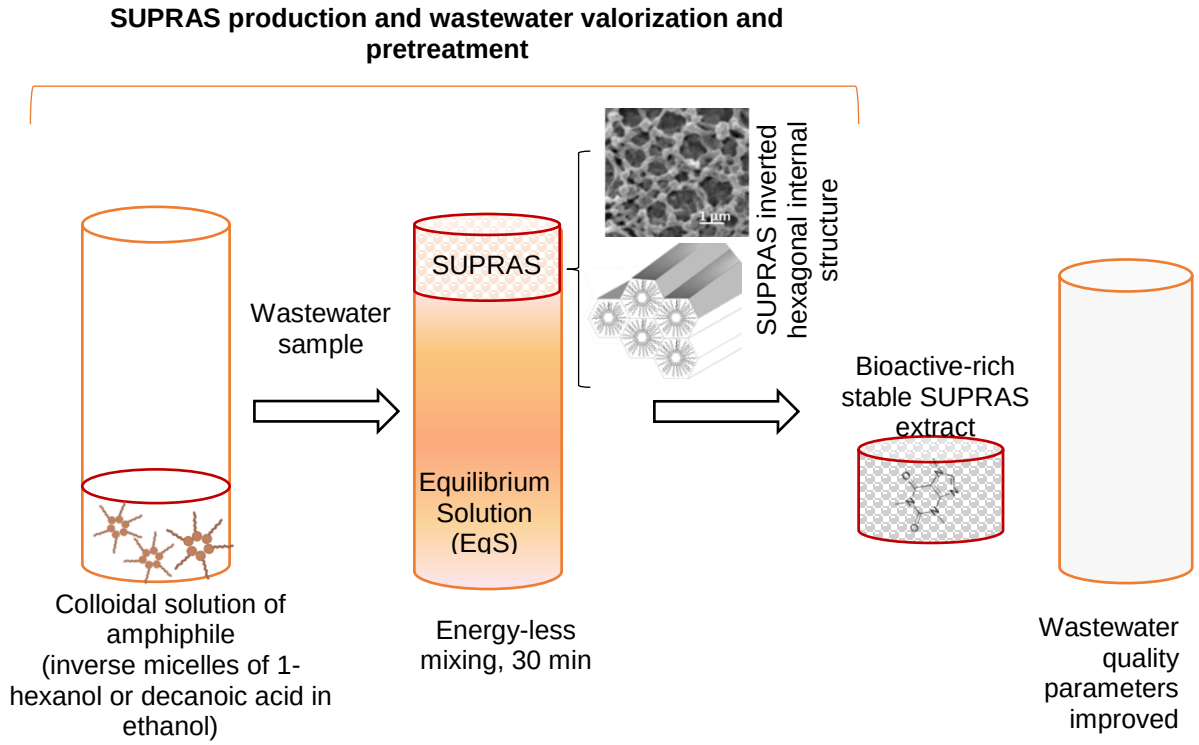


Figure 1. Schematic picture of the in situ production of SUPRAS and extraction of bioactives from coffee wastewater.

3.2 Optimization of caffeine extraction

The concentration of caffeine in the wastewater sample was 90 ± 6 $mg.L^{-1}$. Studies on CWW composition in the literature are very scarce and mainly focused on general quality parameters (DBO, total nitrogen content, pH, etc.). A value of 23 $mg.L^{-1}$ of caffeine has been reported in de-pulping water.[26]

The predictive caffeine extraction yield in SUPRAS reached a maximum of ~60% for hexanol and ~70% for decanoic acid, which are acceptable taking into account the high polarity of caffeine (logP -0.1) and consequently high water solubility (21.6 mg L^{-1}). The extraction was influenced by both the amphiphile and ethanol concentration.

The model equations for predicting the extraction of caffeine from both SUPRAS are shown below.

For hexanol-based SUPRAS:

*Caffeine (R, %) = 22.6 – 2.71 H - 0.425 E + 0.2592 H*H - 0.00218 E*E +0.0292 H*E*

where R= recovery, H= hexanol and E=ethanol. The determination coefficient (R^2) was 94.4%, and the estimated standard deviation of the model (S) was 5.1%

For decanoic-based SUPRAS:

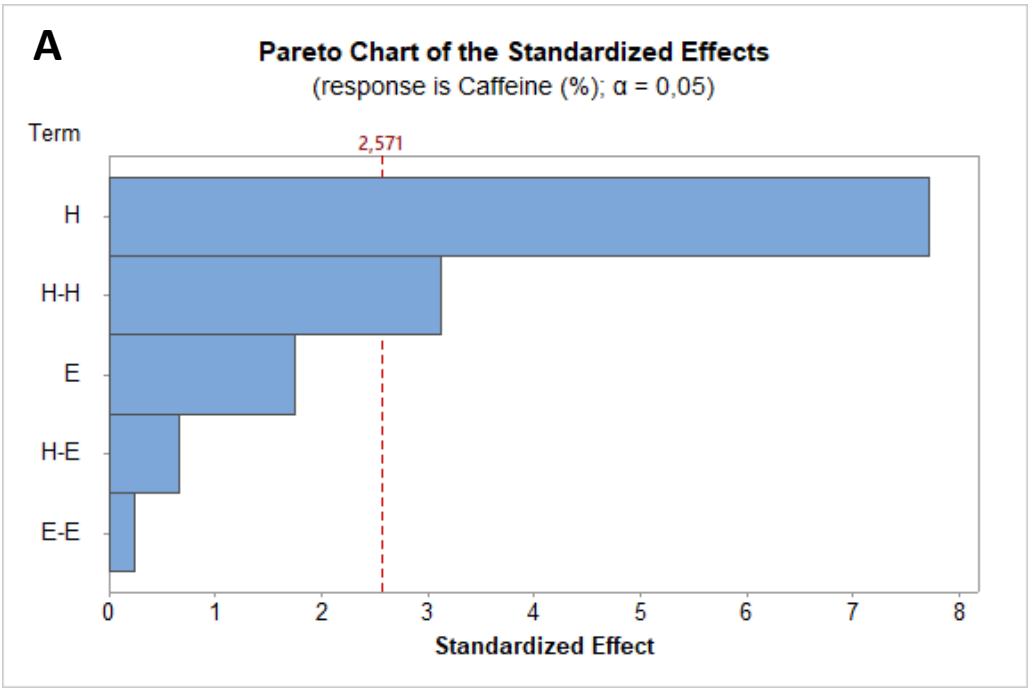
*Caffeine (R, %) = -10.8 - 0.26 DA + 0.858 E+ 0.053 DA*DA - 0.0239 E*E+ 0.1054 DA*E*

where DA= decanoic acid; R^2= 90.7%, S= 7.4 %

The Pareto graph for the equation of the hexanol-based SUPRAS (Figure 2A) showed that the most relevant factor was the percentage of hexanol (linear; ANOVA p=0.001), followed by its quadratic interaction H*H (ANOVA p=0.026). The percentage of ethanol (ANOVA p=0.141), the interaction between the two factors (H*E, ANOVA p=0.533) and the quadratic interaction E*E (ANOVA p=0.823) were less significant. At α=0.05, only hexanol (linear and quadratic interaction) was a statistically significant variable.

Regarding the Pareto graph for the equation of decanoic acid-based SUPRAS (Figure 2B), the most relevant factor was the percentage of decanoic acid (linear; ANOVA p<0.001), followed by

ethanol (linear; ANOVA *p*= 0.005), the interaction between the two factors (DA*E, ANOVA *p*=0.07) and the quadratic interaction E*E (ANOVA *p*=0.09); while the quadratic interaction DA*DA was the less significant (ANOVA p=0.6). At α=0.05, both decanoic acid and ethanol were statistically significant variables.



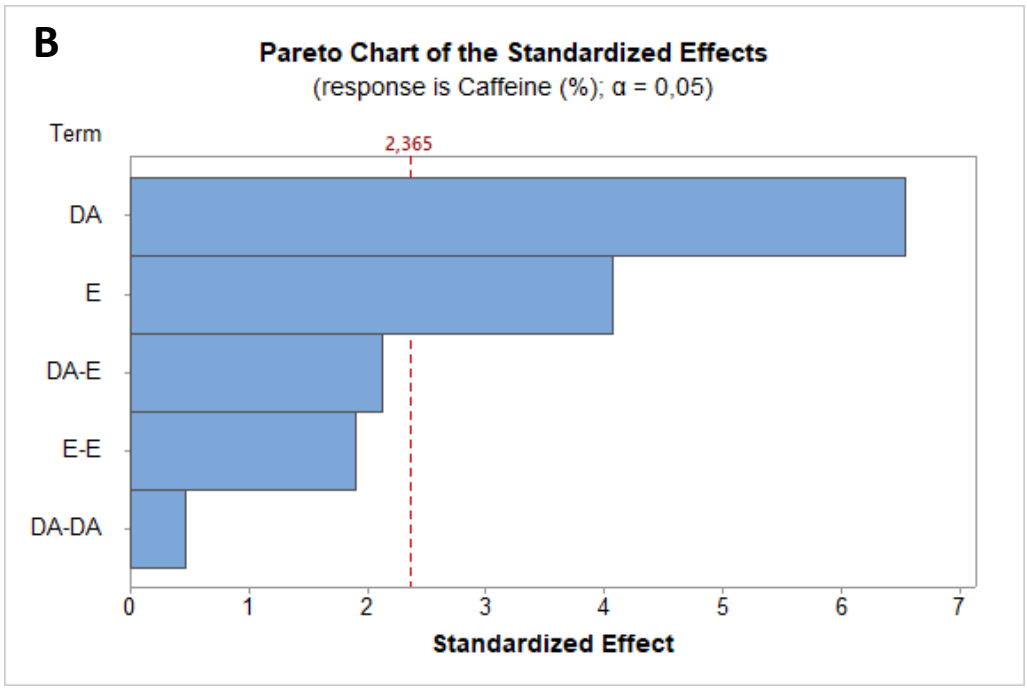


Figure 2. Pareto charts of the standardized effects in caffeine extraction with SUPRAS synthetized with A) hexanol-ethanol and B) decanoic acid – ethanol

Figure 3 (A and B) shows the modelled response surface of SUPRAS based on hexanol and decanoic acid. The extraction increased when the amount of hexanol in the synthetic solution did (Pearson correlation coefficient, PCC 0.52). In the case of decanoic acid-based SUPRAS, both the amphiphile and the solvent had a directly proportional relationship respect to the extraction rate (PCC 0.70 and 0.51 for decanoic acid and ethanol, respectively).

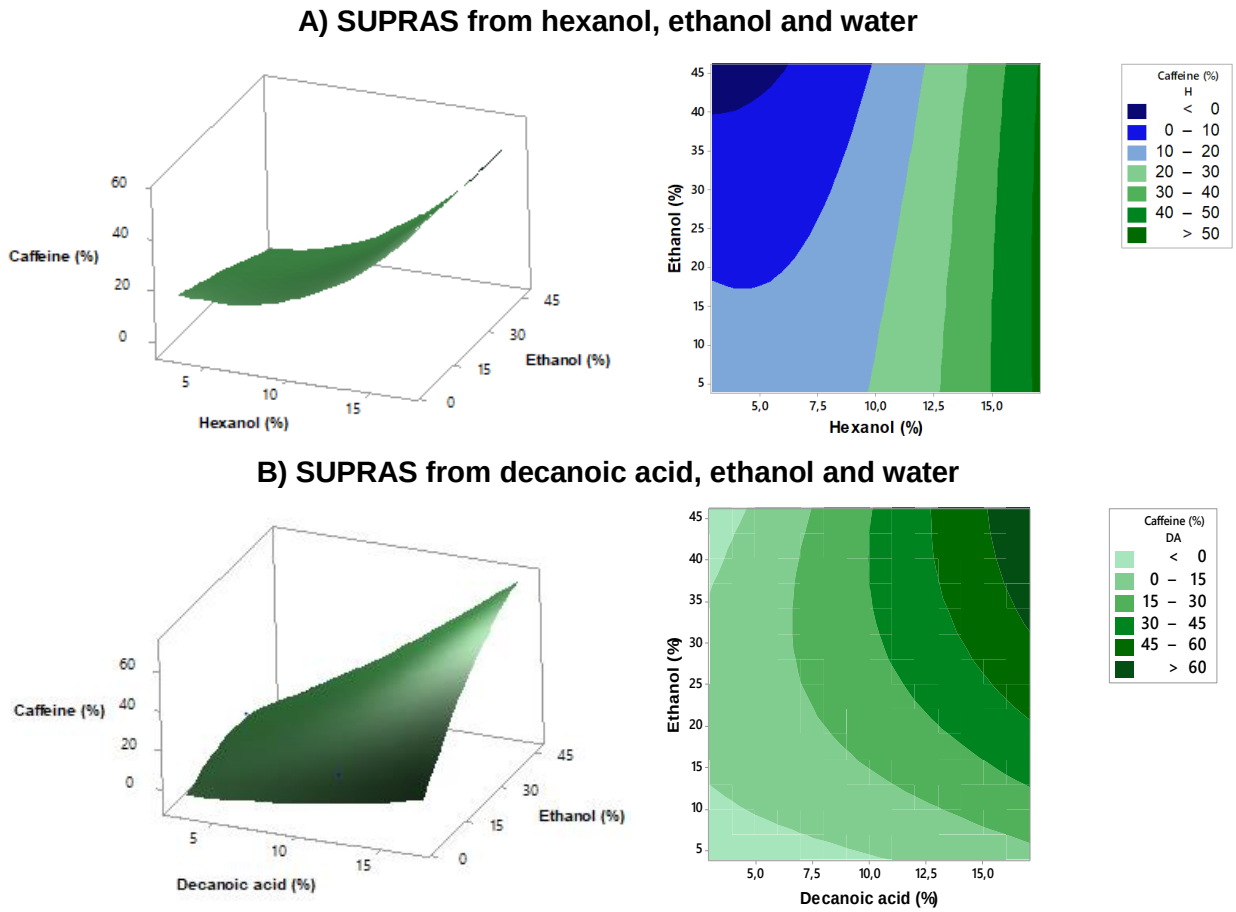


Figure 3. Surface response and contour plot for extraction yields obtained for caffeine from coffee wastewater using SUPRAS from A) hexanol – ethanol and B) decanoic acid - ethanol

The maximal modelled optimal responses were of 72% recovery at 17% v/v decanoic acid and 46% v/v ethanol and of 60% recovery at 17% v/v hexanol and 25% v/v ethanol. For decanoic-acid based SUPRAS modelled recoveries decreased rapidly at lower ethanol percentages, e.g. 51% at 25% v/v ethanol. So, recoveries were more favorable for hexanol-based SUPRAS compared to those obtained for decanoic acid-based SUPRAS as the same concentrations of ingredients were used.

This behavior can be explained considering the composition and properties of both types of SUPRAS. Thus, SUPRAS consist of the amphiphile (decanoic acid or hexanol), ethanol and water and, consequently, they offer different types of interactions, among which hydrogen bonding and polar interactions are expected to primarily govern the extraction of polar bioactives such as caffeine. As previously reported,[19,27] the proportion of both ethanol and water in the SUPRAS increases exponentially as the content of ethanol in the synthetic solution does. So, for SUPRAS made up of nonpolar amphiphiles such as decanoic acid (log K_{ow} 4.09), higher extraction yields are expected as the percentage of ethanol in the synthetic solution increases (see figure 3B) owing to the higher content of ethanol and water in the SUPRAS. On the other hand, SUPRAS made up from polar amphiphiles such as hexanol (log K_{ow} 2.03) are expected to be less dependent on their content in water and ethanol, as clearly inferred from the results in figure 3A. So, it seems that less organic solvent (e.g. ethanol) will be required for recovery of polar bioactives from CWW using SUPRAS made up of polar amphiphiles.

Actual recoveries for caffeine agreed with those of modelled responses (e.g. 71 ± 3% and 59 ± 2% for SUPRAS based on decanoic acid and hexanol, respectively). So, an amount between around 54 and 65 mg of caffeine, the modeled compound, can be recovered per liter of wastewater. Sequential extractions steps could be carried out to further recover caffeine from wastewater, although values in the first extraction seem to be a good compromise solution between the consumption of reagents and the extraction efficiency for valorization purposes. Under optimal conditions, the SUPRAS/sample volume ratio was 0.32 and 0.44 for SUPRAS formed from decanoic acid and hexanol.

Figure S2 shows representative LC-UV chromatograms obtained from caffeine standard, untreated CWW and SUPRAS extracts obtained using optimal experimental conditions.

Extraction of caffeine from CWW by SUPRAS was extremely fast under stirring. Preliminary experiments showed that recoveries for caffeine did not change after 1 min extraction as subjected the mixture CWW-SUPRAS to vortex-shaken at 3,000 rpm (see section 2.4). This behavior is very common for SUPRAS since they consist of oily droplets that keep as individual entities, so these solvents have intrinsically a high superficial area thus facilitating solute mass transfer in extraction processes.[14,25]

In order to prove if an energy-less process was an option for CWW valorization, we try the extraction of caffeine under resting conditions, just mixing ethanol, the amphiphile and wastewater at optimal percentages. The solution was allowed to stand for 3.5, 6, 8, 10, 13, 16, 20 and 30 minutes before measuring the caffeine content in the SUPRAS. Figure S3 shows the results obtained. As it is shown, an induction period is observed in the kinetic curve for both SUPRAS under resting conditions, which could be attributable to the time required for diffusion of caffeine to the SUPRAS. Within 20 min values, recoveries for caffeine into SUPRAS reached the same values that those obtained using stirring and centrifugation. So, SUPRAS have the potential for an easy and cheap implementation in CWW valorization.

3.3 Antioxidant activity determination

The antioxidant activity obtained by the $ABTS^{\bullet+}$ assay from SUPRAS blanks was negligible for both hexanol (1.7%) and decanoic acid (6.9%). The SUPRAS extracts obtained from CWW under optimal conditions exhibited good antioxidant activity (53.2% for hexanol and 41.8% for decanoic acid, see table S1), thus confirming the possibility of CWW valorization. Bioactive compounds expected to be responsible of the antioxidant activity of SUPRAS extracts were mainly polyphenols.[16] To the best of our knowledge, the extraction of antioxidants from CWW has not been reported previously.

3.4 Stability of caffeine in SUPRAS extracts

SUPRAS extracts were evaluated for stability of the extracted bioactives by monitoring caffeine. For this purpose, SUPRAS extracts stored at 4, 14 and 24 °C only showed a maximum ~9% relative decrease at 24°C in caffeine concentration (respect to the time 0) after 60 days. Data on these experiments can be found in tables S2 and S3. Since bioactives were quite stable for the two months, degradation kinetic trends could not be determined in this time interval and neither zero-, first- or second-order plots could be fitted to the experimental data, $R2 \leq 0.05$ (see figures S4 and S5). We have also included the ANOVA results (Tables S4 and S5) with two factors (time and temperature) to prove that differences between stability at the tested temperatures were not significant ($p=0.83$-0.93). Differences with time were significant ($p \leq 0.0001$) but the decrease was minor in two months as mentioned before. Our research group has previously found an unusual stability for astaxanthin in similar supramolecular assemblies.[15]

3.5. Physico-chemical parameters

Finally, although SUPRAS are here proposed to valorize CWW before conventional treatments, the influence of SUPRAS-based extraction on wastewater quality parameters was investigated. Table 2 shows the values found for typical quality standard parameters in CWW, before and after extraction with SUPRAS synthesized from hexanol and decanoic acid. Likewise, Table 2 includes the permitted or recommended values or ranges for these quality standard parameters in surface waters,[28] effluents discharged into inland surface waters[29] and occasional discharge of CWW in surface waters.[30]

As it was expected, major components in the raw CWW were organics, with BOD (120 mg $O_2.L^{-1}$) and COD (1255 mg $O_2.L^{-1}$) values in line with previous reports (87 – 4800 mg $O_2.L^{-1}$ for BOD, and 142 – 9130 mg $O_2.L^{-1}$ for COD [1,3]. Oxygen, consumed from water during decomposition of organic matter by chemical and biological processes, was 3.6 mg $O_2.L^{-1}$, also in line with previous reports (0.17 – 7.01 mg $O_2.L^{-1}$) .[1,3] The acid pH of the raw CWW (i.e. pH 3.7) was the consequence of the fermentation of sugars to ethanol that it is quickly converted to acetic acid.

It can be clearly seen from data in Table 2 that raw CWW requires reduction of COD, total suspended solids (TSS) and pH adjustment before dumping it occasionally into surface waters according to the Colombia legislation. Also, raw CWW is far from EU and US-EPA quality standards for surface waters and discharge of environmental pollutants to inland surface waters, respectively, in terms of COD, BOD, TSS and pH, which means that water bodies located downstream of traditional wet coffee processing plants represent an significant risk for ecological systems and that CWW treatment is mandatory.[1] Etiégni et al.[31] reported a decrease in BDO in the range 78-88% and of COD from 83 to 91% by using electrocoagulation. COD reductions between 55 and 60% were reported by Zayas Péerez et al.[4] with the use of conventional coagulation-flocculation processes. The use of alternative coagulants (*Moringa oleifera* seed extracts) allowed to reduce the total solids from 8% to 54% with a COD removal yield from 1% to 25%.[2] The rest of parameters (e.g. conductivity, nitrates and phosphates) were within the permitted limits in the legislations referred in Table 2.

The SUPRAS treatment (with both amphiphiles at optimal modelled values) generated a positive and significant effect in BOD, TSS and conductivity ($p<0.05$) and improved the values for pH and DO.

Table 2. Water quality parameter values in the untreated and valorized coffee wastewater (CWW) and recommended or mandatory quality standard parameters for surface waters.

Parameter	Raw CWW	CWW after Hexanol-based SUPRAS extraction	CWW after Decanoic acid-based SUPRAS extraction	[a]EU quality standards for surface waters	[b]US-EPA standards for discharge of pollutants into inland surface waters	[c]Colombian quality standards for occasional discharge of CWW
DO (mg L^{-1})	3.6 ± 0.4	4.10 ± 0.01	4.0 ± 0.2	≥ 2-5	-	-
BOD (mg L^{-1})	120 ± 1	13 ± 1	54 ± 2	≤ 1-10	≤ 30	≤ 400
COD (mg L^{-1})	1255 ± 70	1826 ± 60	2500 ± 82	≤ 1-40	≤ 250	≤ 650
pH	3.70 ± 0.02	4.10 ± 0.06	3.91 ± 0.05	5.5-9.0	5.5-9.0	5-9.0
TSS (mg L^{-1})	2279 ± 210	1139 ± 97	1238 ± 98	≤ 50	≤ 100	≤ 400
Conductivity (μS/cm)	444 ± 16	145 ± 10	149 ± 1	≤ 1000	-	-
Phosphates (mg L^{-1})	< 1	< 1	< 1	0.5-0.7	≤ 10	Analysis and reporting
Nitrates (mg L^{-1})	< 10	< 10	< 10	≤ 50	≤ 5	Analysis and reporting

Sources: [a-c]See section 3.5. [a]Concentration ranges for quality parameters depend on the category for surface water according to the treatment method required for transformation in drinking water.

Thus, extraction of bioactive compounds from CWW with hexanol-based SUPRAS reduced BOD in 89%, TSS in 50% and conductivity in 67%, whereas using decanoic acid-based SUPRAS the reductions were 55% for BOD, 45% for TSS and 66% for conductivity. TSS gives a measure of the turbidity of the water and may lead to negative impacts in the ecosystem, because the light penetration is reduced, as a consequence the photosynthesis also do it and the primary production reduces food availability for aquatic organisms.[1] On the other hand, the pH and DO of the untreated CWW slightly increased up to 3.9-4.1 and 4.0-4.1, respectively, under SUPRAS-based extraction. COD was the only parameter that increased for the raw CWW after extraction with SUPRAS, the increase being much higher for decanoic acid (99%) than for hexanol (45%). This increase was the consequence of the partition of the amphiphile and ethanol to the CWW.

4. Conclusions

Despite the vast amount of CWW being continuously produced during harvesting/processing periods, there are still not many approaches to valorize and treat these effluents and to prevent the consequent pollution. In this study we proved the suitability of supramolecular solvents to valorize the CWW by the recovery of bioactives, such as caffeine and antioxidants compounds. A major advantage of using SUPRAS for this purpose is that their components can be selected to be food authorized ingredients which facilitates the application for the development of nutraceutical products. On the other hand, SUPRAS extracts were stable against time (2 months) and temperature (4-24 °C) and the procedure was simple and could be carried out in an energy-less procedure by just mixing the wastewater with the SUPRAS ingredients. Thus, phase separation occurred spontaneously (SUPRAS staying at the top because of its lower density) and a passive extraction time of 20 min was enough to reach the equilibrium. Additionally, SUPRAS extraction improved most of the CWW quality parameters (e.g. BDO, DO, pH TSS and conductivity), which can help to reduce the effort in the subsequent mandatory CWW treatment. Finally, SUPRAS efficiency is not expected to be influenced by matrix components[14] so that this procedure would be easily transferrable to any coffee wastewater.

The two SUPRAS investigated were quite similar in extraction yields and antioxidant activity, however, because of the higher concentration of ingredients used for the decanoic acid-based SUPRAS under optimal extraction conditions, less improvements in water quality parameter were obtained compared to the hexanol-based SUPRAS (see Table 2). On the other hand, removal of

SUPRAS ingredients (i.e. hexanol and ethanol) in the extract containing the bioactives is simpler in the hexanol-based SUPRAS.

Further research should focus on the reduction of the SUPRAS ingredients partitioning into the CWW. In this respect, recent developments of SUPRAS made up of chemically stable nanostructures, which minimize the presence of solvent residues in the treated water[32] are very promising.

Acknowledgment

Authors gratefully acknowledge financial support from Spanish MINECO (Project CTQ2017-83823-R). A. Ballesteros-Gómez acknowledges the funding from Spanish Ministry of Science, Innovation and Universities for a Ramón y Cajal contract (RYC-2015-18482). L.S. Torres-Valenzuela thanks AUIP for her doctoral fellowship.

References

1. D. Dadi, E. Mengistie, G. Terefe, T. Getahun, A. Haddis, W. Birke, A. Beyene, P. Luis and B. Van der Bruggen, Assessment of the effluent quality of wet coffee processing wastewater and its influence on downstream water quality. *Ecohydrology & Hydrobiology*, 2018, **18**, 201–211.

2. W. K. Garde, S. G. Buchberger, D. Wendell and M. J. Kupferle, Application of Moringa Oleifera seed extract to treat coffee fermentation wastewater.*Journal of Hazardous Materials*, 2017, **329**, 102–109.

3. L. L. Aguiar, L. F. Andrade-Vieira and J. A. de Oliveira David, Evaluation of the toxic potential of coffee wastewater on seeds, roots and meristematic cells of Lactuca sativa L. *Ecotoxicology and Environmental Safety*, 2016, **133**, 366–372.

4. T. Zayas Péerez, G. Geissler and F. Hernandez, Chemical oxygen demand reduction in coffee wastewater through chemical flocculation and advanced oxidation processes. *Journal of Environmental Sciences*, 2007, **19**, 300–305.

5. P. M. B. Chagas, J. A. Torres, M. C. Silva and A. D. Corrêa, Immobilized soybean hull peroxidase for the oxidation of phenolic compounds in coffee processing wastewater. *International Journal of Biological Macromolecules*, 2015, **81**, 568–575.

6. E. Novita, Biodegradability Simulation of Coffee Wastewater Using Instant Coffee. *Agriculture and Agricultural Science Procedia*, 2016, **9**, 217–229.

7. S. Rattan, A. K. Parande, V. D. Nagaraju and G. K. Ghiwari, A comprehensive review on

utilization of wastewater from coffee processing. *Environ Sci Pollut Res*, 2015, **22**, 6461–6472.

8. I. Anastopoulos, M. Karamesouti, A. C. Mitropoulos and G. Z. Kyzas, A review for coffee adsorbents. *Journal of Molecular Liquids*, 2017, **229**, 555–565.

9. W. A. Botello Suárez, J. da Silva Vantini, R. M. Duda, P. F. Giachetto, L. C. Cintra, M. I. Tiraboschi Ferro and R. A. de Oliveira, Predominance of syntrophic bacteria, Methanosaeta and Methanoculleus in a two-stage up-flow anaerobic sludge blanket reactor treating coffee processing wastewater at high organic loading rate. *Bioresource Technology*, 2018, **268**, 158–168.

10. M. Selvamurugan, P. Doraisamy and M. Maheswari, An integrated treatment system for coffee processing wastewater using anaerobic and aerobic process. *Ecological Engineering*, 2010, **36**, 1686–1690.

11. M. Rossmann, A. T. de Matos, E. C. Abreu, F. F. e Silva and A. C. Borges, Performance of constructed wetlands in the treatment of aerated coffee processing wastewater: Removal of nutrients and phenolic compounds. *Ecological Engineering*, 2012, **49**, 264–269.

12. M. Rossmann, A. T. Matos, E. C. Abreu, F. F. Silva and A. C. Borges, Effect of influent aeration on removal of organic matter from coffee processing wastewater in constructed wetlands. *Journal of Environmental Management*, 2013, **128**, 912–919.

13. H. N. Ibarra-Taquez, E. GilPavas, E. R. Blatchley, M.-Á. Gómez-García and I. Dobrosz-Gómez, Integrated electrocoagulation-electrooxidation process for the treatment of soluble coffee effluent: Optimization of COD degradation and operation time analysis. *Journal of Environmental Management*, 2017, **200**, 530–538.

14. C. Caballo, M. D. Sicilia and S. Rubio, Supramolecular Solvents for Green Chemistry in *The Application of Green Solvents in Separation Processes*, eds. F. Pena-Pereira and M. Tobiszewski, Elsevier, 2017, pp. 111–137.

15. J. A. Salatti-Dorado, D. García-Gómez, V. Rodriguez-Ruiz, V. Gueguen, G. Pavon-Djavid and S. Rubio, Multifunctional green supramolecular solvents for cost-effective production of highly stable astaxanthin-rich formulations from Haematococcus pluvialis. *Food Chemistry*, 2019, **279**, 294–302.

16. L. S. Torres-Valenzuela, A. Ballesteros-Gómez, A. Sanin and S. Rubio, Valorization of spent coffee grounds by supramolecular solvent extraction. *Separation and Purification Technology*, 2019, **228**, 115759.

17. L. S. Torres-Valenzuela, J. A. Serna-Jiménez and K. Martínez, Coffee by-Products: Nowadays and Perspectives in *Coffee*, Intechopen, London, 2019.

18. A. Ballesteros-Gómez and S. Rubio, Environment-responsive alkanol-based supramolecular

solvents: characterization and potential as restricted access property and mixed-mode extractants. *Anal. Chem.*, 2012, **84**, 342–349.

19. F.-J. Ruiz, S. Rubio and D. Pérez-Bendito, Water-induced coacervation of alkyl carboxylic acid reverse micelles: phenomenon description and potential for the extraction of organic compounds. *Anal. Chem.*, 2007, **79**, 7473–7484.

20. P. Régnier, J. Bastias, V. Rodriguez-Ruiz, N. Caballero-Casero, C. Caballo, D. Sicilia, A. Fuentes, M. Maire, M. Crepin, D. Letourneur, V. Gueguen, S. Rubio and G. Pavon-Djavid, Astaxanthin from Haematococcus pluvialis Prevents Oxidative Stress on Human Endothelial Cells without Toxicity. *Mar Drugs*, 2015, **13**, 2857–2874.

21. European Parliament and of the Council, Regulation (EC) No. 1333/2008 of the European Parliament and of the Council - Food aditives [Internet]. 2008 [cited 2019 Aug 20]. Available from: https://eur-lex.europa.eu/eli/reg/2008/1333/oj

22. U.S. Food & Drug Administration, Food Additive Status List, http://www.fda.gov/food/food-additives-petitions/food-additive-status-list, (accessed August 20, 2019).

23. European Parliament and of the Council, Directive 2004/39/Ec of the European Parliament and of the Council. On the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredientes. Official Journal of the European Union [Internet]. 2009 [cited 2019 Aug 20];L 141(3). Available from: http://link.springer.com/10.1007/978-1-137-54507-7_21

24. D. F. Evans and H. Wennerström, *The Colloidal Domain: Where Physics, Chemistry, Biology, and Technology Meet,* Wiley-VCH, New York, 2nd edition., 1999.

25. A. Ballesteros-Gómez, M. D. Sicilia and S. Rubio, Supramolecular solvents in the extraction of organic compounds. A review. *Analytica Chimica Acta*, 2010, **677**, 108–130.

26. J. del R. Olvera and J. I. Gutiérrez, Biodegradación anaerobia de las aguas generadas en el despulpado del café. *Revista Colombiana de Biotecnología*, 2010, **12**, 230–239.

27. J. Á. Salatti-Dorado, N. Caballero-Casero, M. D. Sicilia, M. L. Lunar and S. Rubio, The use of a restricted access volatile supramolecular solvent for the LC/MS-MS assay of bisphenol A in urine with a significant reduction of phospholipid-based matrix effects. *Anal. Chim. Acta*, 2017, **950**, 71–79.

28. Environmental protection agency, *Parameters of water quality. Interpretation and standards*, Environmental protection agency, Wexford, Ireland, 2001.

29. United Stated Environmental Protection Agency, *Standars for discharge of environmental pollulants to Inland Surface waters*, Washington (DC), USA, 1989.

30. Ministry of Environment and Sustainable Development, *Resolución 631 de 2015*, Bogotá,

Colombia, 2015.

31. L. Etiégni, B. O. Orori, K. Senelwa, M. M. Mwamburi and B. K. Balozi, Ash leachate used as supporting electrolyte during wastewater treatment by electrocoagulation. *Geophysical Research Abstracts*, 2011, **13**, 2.

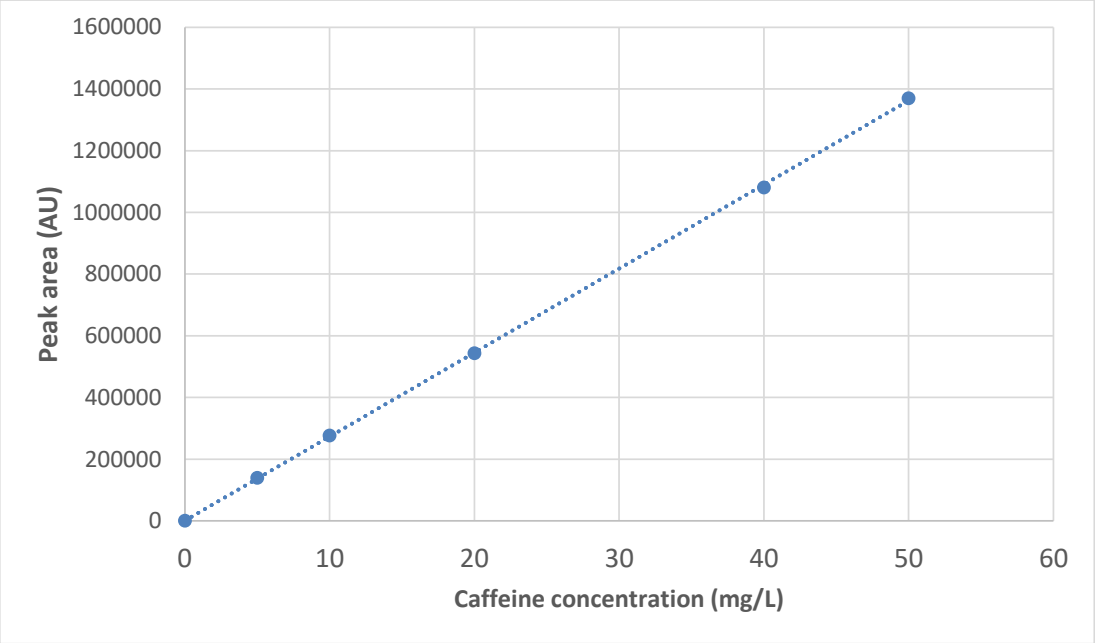
32. A. Ballesteros-Gómez, N. Caballero-Casero, S. García-Fonseca, L. Lunar and S. Rubio, Multifunctional vesicular coacervates as engineered supramolecular solvents for wastewater treatment. *Chemosphere*, 2019, **223**, 569–576.

SUPPLEMENTARY INFORMATION

Supramolecular solvents for the valorization of coffee wastewater

Laura Sofia Torres-Valenzuela [a, b], Ana Ballesteros-Gomez [a], Johanna Serna [b], Andrea Arango [b], Soledad Rubio [a]

Figure S1. Standard curve for caffeine (LC-UV, 254 nm)



Calibration curve statistics; y= 27,219 +1110: R^2= 0.9998

	Coefficients	*Standard Error*	*Lower 95%*	*Upper 95%*
Intercept	1109.9	3946.1	-9846.3	12066.2
Slope	27219.5	142.1	26824.9	27614.1

Statistics

ANOVA

	df	*SS*	*MS*	*F*	***Significance F***
Regression	1	1.49724E+12	1.49724E+12	36675.4	**4.45988E-09**
Residual	4	163296573.5	40824143.36		
Total	5	1.4974E+12			

df: degrees of freedom; SS: sum of squares; MS: mean squares; F: calculated F for the null hypothesis (linear regression not significant) as MSreg/MSres; Significance F: associated p-value (linear regression significant for Significance F ≤0.05)

Figure S2. LC-UV chromatograms of A) a standard of caffeine (10 mg/L), B) the coffee wastewater (CWW), C) hexanol-SUPRAS extract of CWW, optimal conditions and D) decanoic acid-SUPRAS extract of CWW, optimal conditions. Caffeine peak appearing at 6.5 min. B, C and D were diluted 12 times before injection.

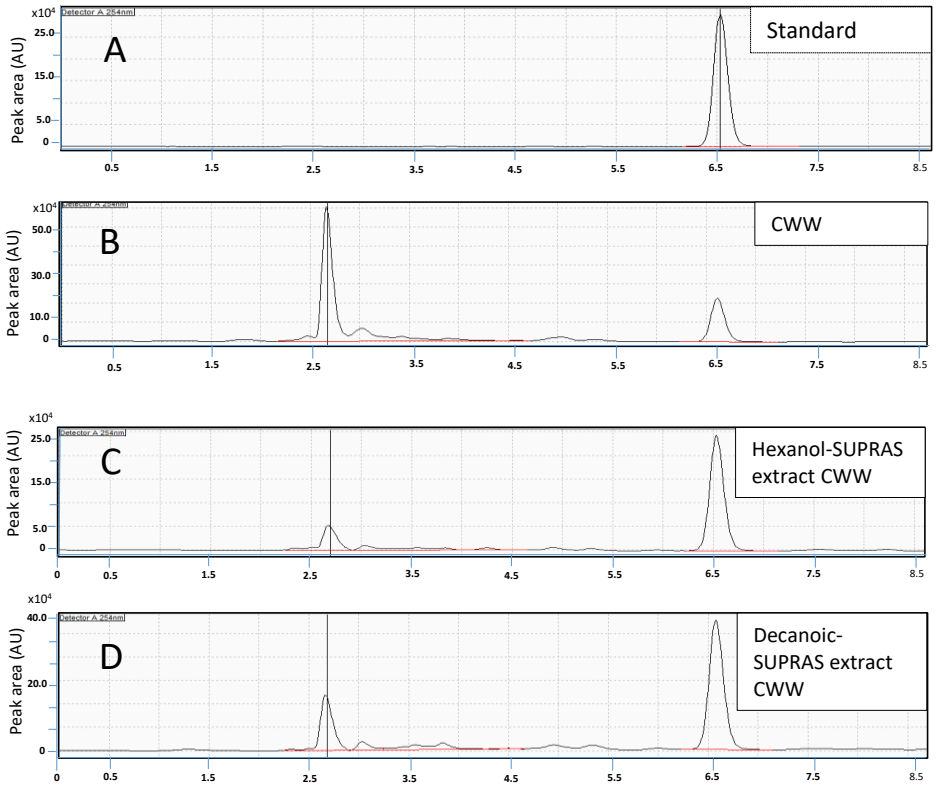


Figure S3. Extraction of caffeine by SUPRAS from CWW under resting conditions, expressed as mg of recovered caffeine per liter of wastewater against time (3.5-30 min).

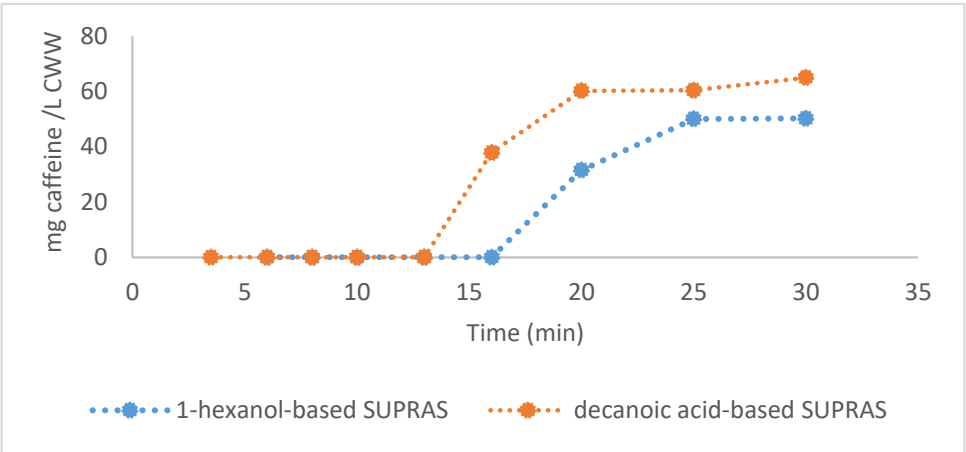


Table S1. Absorbance values for $ABTS^{\bullet+}$ antioxidant activity (AA) of SUPRAS extracts. $AA(\%)= Abs_0-Abs_{30}/Abs_0*100$, where Abs_0 is the absorbance of $ABTS^{+}$ reagent solution at time zero and Abs_{30} is the absorbance of the reagent in the presence of SUPRAS extracts (previously diluted in 1:10 with methanol) at 30 minutes of reaction.

	Abs0	**Abs30 (hexanol SUPRAS extract)**	**Abs30 (decanoic acid SUPRAS extract)**	**AA % (hexanol SUPRAS extract)**	**AA % (decanoic acid extract)**
Replicate 1	0.709	0.321	0.411	54.7	42.03
Replicate 2	0.703	0.353	0.416	50.2	41.33
Replicate 3	0.710	0.320	0.415	54.6	42.00
Average				**53.2**	**41.8**
SD				**2.6**	**0.4**
SDR				**4.8**	**0.9**

Table S2. Degradation kinetics (zero-order, first-order and second-order) of caffeine in 1-hexanol-based SUPRAS at different temperatures. CA: caffeine concentration in SUPRAS extract (mg/L)

T = 4 °C				**T = 14 °C**				**T = 24 °C**			
Days	CA	lnCA	1/CA	Days	CA	lnCA	1/CA	Days	CA	lnCA	1/CA
0	122	4.80	0.008	0	122	4.80	0.008	0	122	4.80	0.008
1	112	4.72	0.009	1	103	4.64	0.010	1	110	4.70	0.009
2	115	4.75	0.009	2	117	4.77	0.009	2	117	4.76	0.009
6	105	4.66	0.009	6	104	4.65	0.010	6	105	4.65	0.010
9	108	4.68	0.009	9	99	4.60	0.010	9	106	4.67	0.009
16	112	4.72	0.009	16	115	4.74	0.009	16	116	4.75	0.009
23	106	4.67	0.009	23	107	4.67	0.009	23	104	4.64	0.010
30	109	4.69	0.009	30	115	4.75	0.009	30	112	4.72	0.009
50	113	4.73	0.009	50	116	4.75	0.009	50	109	4.69	0.009
60	113	4.73	0.009	60	113	4.73	0.009	60	111	4.71	0.009

Table S3. Degradation kinetics (zero-order, first-order and second-order) of caffeine in decanoic acid-based SUPRAS at different temperatures. CA: caffeine concentration in SUPRAS extract (mg/L)

T = 4 °C				**T = 14 °C**				**T = 24 °C**			
Days	CA	lnCA	1/CA	Days	CA	lnCA	1/CA	Days	CA	lnCA	1/CA
0	196	5.28	0.005	0	196	5.28	0.005	0	196	5.28	0.005
1	184	5.21	0.005	1	190	5.25	0.005	1	179	5.19	0.006
2	180	5.19	0.006	2	176	5.17	0.006	2	185	5.22	0.005
6	172	5.15	0.006	6	176	5.17	0.006	6	172	5.15	0.006
9	170	5.14	0.006	9	160	5.08	0.006	9	170	5.14	0.006
16	187	5.23	0.005	16	181	5.20	0.006	16	185	5.22	0.005
23	167	5.12	0.006	23	170	5.14	0.006	23	170	5.14	0.006
30	170	5.14	0.006	30	188	5.24	0.005	30	181	5.20	0.006
50	186	5.23	0.005	50	188	5.24	0.005	50	175	5.16	0.006
60	185	5.22	0.005	60	180	5.19	0.005	60	178	5.18	0.006

Figure S4. Degradation kinetics of caffeine in 1-hexanol-based SUPRAS. CA: caffeine concentration in SUPRAS extract (mg/L). CA (zero-order plot), lnCA (first-order plot) and 1/CA (second-order plot) are plotted for each temperature against time (0-60 days).

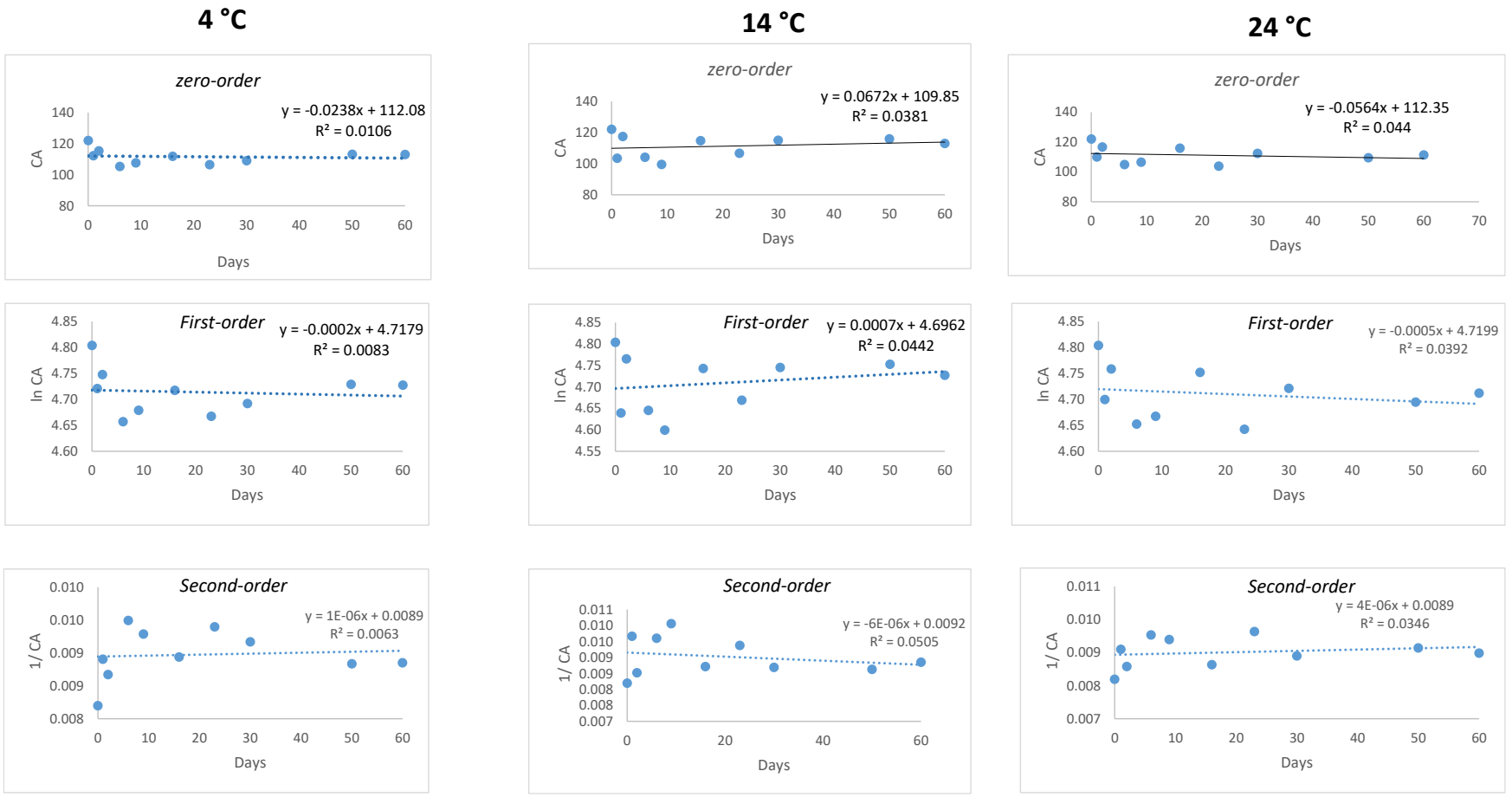


Figure S5. Degradation kinetics of caffeine in decanoic acid-based SUPRAS. CA: caffeine concentration in SUPRAS extract (mg/L). CA (zero-order plot), lnCA (first-order plot) and 1/CA (second-order plot) are plotted for each temperature against time (0-60 days).

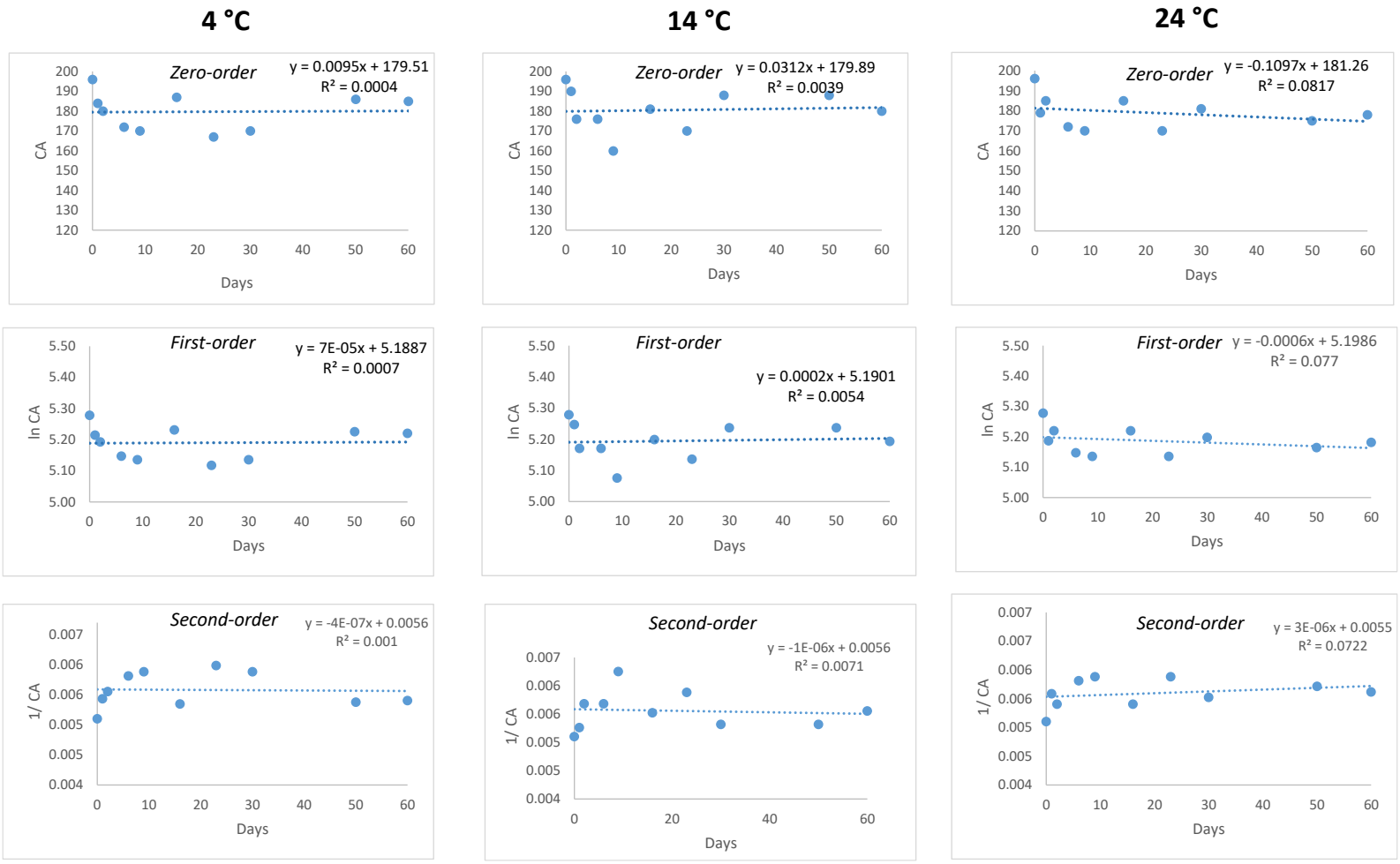


Table S4. ANOVA (two factors) for caffeine content in 1-hexanol-based SUPRAS. Time: 0-60 days, temperature: 4, 14 and 24 °C

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Time	2182.895	9	242.543	12.289	5.21806E-06	2.456
Temperature	2.911	2	1.455	0.073	**0.929**	3.554
Error	355.236	18	19.735			
	2541.043	29				
Total	2182.895	9	242.543	12.289	5.21806E-06	2.456

SS: sum of squares; df: degrees of freedom; MS: mean squares; F: calculated F for the null hypothesis (not significance) as MSreg/MSres; Significance F: associated p-value (Significance F ≤0.05)

Table S5. ANOVA (two factors) for caffeine content in decanoic acid-based SUPRAS. Time: 0-60 days, temperature: 4, 14 and 24 °C

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Time	1939.367	9	215.485	8.044	0.0001	2.456
Temperature	9.866	2	4.933	0.184	**0.833**	3.554
Error	482.133	18	26.785			
Total	2431.367	29				

SS: sum of squares; df: degrees of freedom; MS: mean squares; F: calculated F for the null hypothesis (not significance) as MSreg/MSres; Significance F: associated p-value (Significance F ≤0.05)

CONCLUSIONES

CONCLUSIONES

La investigación desarrollada en esta Tesis Doctoral ha tenido como objetivo el diseño y aplicación de disolventes supramoleculares a procesos de extracción de componentes bioactivos (polifenoles, alcaloides y antimicrobianos) de residuos agrícolas. En concreto estos residuos proceden de la producción de café especial de Colombia. Se proponen así estrategias de valorización como alternativa de generación de valor agregado y/o tratamiento de residuos en la agrocadena del café.

Los disolventes evaluados fueron producidos a partir de sustancias anfifílicas utilizando el autoensamblaje como ruta de síntesis espontánea y económica y el agua como agente coacervante biocompatible. Se abordaron tres tipos de residuos; borras, pulpa y aguas residuales. La tabla 1 resume las características más relevantes de las metodologías propuestas así como la composición y propiedades beneficiosas de los extractos SUPRAS obtenidos.

Todas las metodologías desarrolladas presentaron elevada eficiencia para la extracción de compuestos bioactivos de interés, fueron simples y rápidas y se desarrollaron a temperatura ambiente, todo lo cual las convierten en idóneas para su aplicación a nivel industrial.

Los SUPRAS desarrollados cumplen con los principios de la química verde, ya que son aptos para aplicaciones alimentarias por el uso de reactivos (anfifilos y disolventes) clasificados como *"Generalmente Reconocidos como Seguros"* (*GRAS*) y permiten la valorización de residuos en un sector productivo de importancia a nivel mundial, con prevalencia en países en vía de desarrollo, como es la producción de café especial en Colombia.

Existe una escasa aplicación de los SUPRAS en este ámbito hasta la fecha, por lo cual el desarrollo de esta Tesis Doctoral abre nuevas líneas de investigación con impactos significativos a nivel económico, social y ambiental.

Tabla 9. Resumen de las metodologías desarrolladas

	Borras	Pulpa	Aguas residuales
Origen del residuo de café	Preparación de la bebida	Tratamiento primario, vía húmeda	Tratamiento primario, vía húmeda
Compuestos bioactivos en el extracto de SUPRAS (peso seco)	Cafeína: 3.32 mg g^{-1} 5-CGA: 4.3 mg g^{-1} Polifenoles totales : 60.1 mg 5-CGA g^{-1} (5-CGA, *n-O*-dicaffeoyl quinic acids, *n-O*-feruloylquinic acids, *n-O*-caffeoylquinic acids, *n-O*-feruloylquinic lactones, *n-O*-coumaroylquinic acids, *n-O*-caffeoylshikimic acid and *n-O*-caffeoylquinic lactones)	Cafeína: 3.6±0.3 mg g^{-1} Ácido protocatéquico: 0.9±0.1 mg g^{-1} Ácido gálico: 0.25±0.02 mg g^{-1} 5-ácido 5-CGA: 0.13±0.01 mg g^{-1}. Rutina: 30.5±2 µg g^{-1}, 5-O- ácido feruloilquínico: 17.3±0.5 µg g-1, 3-O-ácido cumaroilquínico: 13.4±0.4 µg g^{-1}, p-ácido cumárico: 4.5±0.1 µg g^{-1}, ácido cafeíco: 2±0.2 µg g^{-1}, n-O-ácidos dicafeoilquínicos: 1.6±0.1 µg g^{-1}, 4-CGA: 1.1±0.05 µg g^{-1}, 3-CGA: 0.34±0.04 µg g^{-1} 3-O-ácido feruoilquínico: 0.4±0.03 µg g^{-1}	Cafeína: ~54-65 mg L^{-1} agua residual
Otras propiedades en el extracto de SUPRAS	Capacidad antioxidante: 21 ± 3% DPPH, 68 ± 4%, FRAP, 405 ± 6 µM TEAC/g (ABTS) Capacidad antimicrobiana: MIC 20-40 mg extract/mL for *B. cereus*, 5 mg extract/mL for *S. aureus,* 10-20 mg extract /mL for *E. Coli and* 20-60 mg extract/mL for *S. typhi.*	Capacidad antioxidante: 45±1% DPPH, 91±4% ABTS.	Capacidad antioxidante: 41.5-52.5±3% ABTS
Tipo de SUPRAS (disolución de síntesis)	24:30:46 % v/v 1-hexanol:etanol:agua *(*síntesis *in situ)*	5:24:71 % v/v ácido octanoico:etanol:agua *(*síntesis *ex situ)*	17:25:58 % v/v 1-1-hexanol:etanol:agua 17:46:37 % v/v 1-ácido decanoico:etanol:agua *(*síntesis *in situ)*

Relación SUPRAS (mL):muestra (g, peso seco)	11:1	4:1	0.32-0.44 (% v/v)
Tiempo de extracción por muestra	1 min (3,000 rpm)	5 min (3,000 rpm)	1 min (3,000 rpm) or 30 min (0 rpm)
Precio (EUR)/muestra (Kg o L)	18	5	0.2

Abreviaturas: TEAC: trolox equivalent antioxidant capacity; CGA: ácido clorogénico; MIC: capacidad mínima inhibitoria; ensayos de capacidad antioxidante: DPPH (técnica basada en el radical 2,2-Difenil-1-Picrilhidrazilo), ABTS (técnica basada en el radical 2,2'-azinobis (3-etilbenzotiazolina-6-sulfonato), FRAP (técnica de El Potencial Reductor Férrico).
[a]Precios estimados según plataformas online de venta de productos químicos industriales de calidad alimentaria para pedidos de al menos 1 tonelada.

Las conclusiones específicas más relevantes relacionadas con las actividades desarrolladas en cada uno de los capítulos incluidos en la Memoria de la Tesis se comentan a continuación.

Capítulo I: Disolventes verdes para la extracción de compuestos de alto valor añadido a partir de residuos agroalimentarios

- La revisión bibliográfica sobre los disolventes verdes empleados en la extracción de compuestos bioactivos de residuos agroindustriales evidencia la importancia de esta línea de investigación a nivel mundial.
- Existe una necesidad urgente de valorizar los altos volúmenes de residuos agrícolas generados a escala global utilizando alternativas rápidas, económicas y con bajos (o nulos) impactos ambientales.
- La comparación crítica de las principales características, ventajas y desventajas de los distintos disolventes verdes (fluidos supercríticos, líquidos iónicos, líquidos eutécticos, bio-disolventes y SUPRAS), con particular énfasis en su aplicabilidad industrial (costos, toxicidad, etc.), pone de manifiesto el alto potencial de los SUPRAS para la valorización de residuos mediante la extracción de compuestos bioactivos, debido a la alta eficiencia proporcionada, y bajo costo de operación.

Capítulo II: Valorización de borras de café mediante la extracción de compuestos bioactivos con disolventes supramoleculares

La valorización de borras de café, uno de los subproductos más abundantes en el procesamiento del café (se generan alrededor de 6 millones de toneladas anualmente) se investigó utilizando varios tipos de SUPRAS sintetizados a partir de dos anfifilos de diferente grupo funcional y aceptados como aditivos alimentarios: un alcohol (1-hexanol) y un ácido carboxílico (ácido decanoico). El proceso de coacervación se realizó adicionando agua a suspensiones coloidales de los anfifilos en etanol (disolvente prótico) y THF (disolvente aprótico). Las conclusiones más relevantes obtenidas en este estudio son:

- Los SUPRAS constituidos por 1-hexanol presentaron mejores rendimientos de extracción para los compuestos bioactivos considerados mayoritarios en borras de café (cafeína y ácido 5-clorogénico), comparado con los constituidos por ácido decanoico. Este comportamiento puede ser atribuido a que los enlaces por puente de hidrógeno son más energéticos para 1-hexanol debido a la menor longitud de su cadena hidrocarbonada.
- El medio utilizado para el proceso de coacervación del compuesto anfifilico también influyó en el rendimiento de extracción de los compuestos bioactivos seleccionados; fue mayor en mezclas etanol-agua comparada con mezclas THF-agua. Este comportamiento se explica en base a la mayor capacidad enlazante del etanol (aceptor/donador de puentes de hidrógeno) comparada con la de THF (aceptor de puentes de hidrógeno).
- Los extractos de SUPRAS obtenidos fueron biocompatibles y recuperaron a partir de las borras hasta 3.32 mg cafeína g^{-1}, 4.3 mg ácido clorogénico g^{-1} y 60.1 mg de polifenoles totales g^{-1}, expresados como CGA.
- El proceso de valoración fue muy simple y económico (agitación de la muestra con el SUPRAS durante 1 min y centrifugación para la separación del extracto).
- Los extractos mostraron un alto poder antioxidante por medio de varios ensayos (DPPH, ABTS y FRAP) y cierta capacidad antimicrobiana respecto a microorganismos de importancia en la industria alimentaria como *S. aureus*, *B. cereu*s, *S. enterica* y *P. putida.*

Capítulo III: Extracción de compuestos bioactivos de pulpa de café empleando disolventes supramoleculares

En las investigaciones incluidas en este Capítulo se evaluó la extracción de compuestos bioactivos a partir de *cáscara* seca de café (*coffee cherry pulp*), otro de los principales residuos del procesamiento del café, empleando SUPRAS formados por ácidos carboxílicos de diferente longitud de cadena (C_8 y C_{10}) en mezclas de etanol:agua. La extracción se llevó a cabo adicionando a la muestra el volumen adecuado de SUPRAS y de la disolución de equilibrio generada durante el proceso de síntesis. Las conclusiones más relevantes de este estudio son:

- El rendimiento de extracción para las sustancias bioactivas mayoritarias en el residuo (cafeína y ácido protocatéquico) fue superior para SUPRAS constituidos por ácido octanoico debido a la mayor energía de los puentes de hidrógeno establecidos con los solutos comparada con ácido decanoico.
- El uso de la disolución de equilibrio en la fase extractante permitió la humectación de la muestra y por tanto, la reducción del volumen de SUPRAS requerido para la extracción.
- Los extractos de SUPRAS obtenidos fueron biocompatibles y recuperaron a partir de las cáscaras hasta 3.6±0.3 mg cafeína g^{-1} y 0.9±0.1 mg ácido protocatéquico g^{-1}.
- El rendimiento de extracción obtenido fue entre 7 y 11 veces superior al obtenido con disolventes orgánicos convencionales, incluyendo etanol, metanol, acetona y acetonitrilo.
- Los extractos de SUPRAS, evaluada mediante dos métodos, presentaron una elevada actividad antioxidante (45% para DPPH y 91% para ABTS).

Capítulo IV: Disolventes supramoleculares para la extracción y pre-tratamiento de aguas residuales de la transformación primaria del café

En las investigaciones desarrolladas en este Capítulo, se aplicaron SUPRAS de agregados hexagonales inversos de 1-hexanol y ácido decanoico, producidos espontáneamente en mezclas etanol-agua residual, para la valorización y tratamiento de las aguas residuales procedentes del procesamiento húmedo de café especial de Colombia. Se estima que se

generan alrededor de 40-45 L de agua residual por kg de café procesado. Las conclusiones más relevantes de este estudio son:

- Los dos tipos de SUPRAS investigados mostraron una eficacia de extracción similar para cafeína, permitiendo la recuperación de 53-64 mg por litro de agua residual.
- La cafeína fue estable en los extractos de SUPRAS durante al menos 2 meses en el intervalo de temperaturas investigado (4-14 ºC).
- Los extractos de SUPRAS mostraron una buena capacidad antioxidante por el ensayo ABTS (de hasta 52%).
- El procedimiento de extracción puede realizarse mediante simple agitación, sin necesidad de centrifugación para separar el SUPRAS del agua residual, lo cual favorece el escalamiento del mismo a nivel industrial.
- En el proceso de extracción se mejoraron algunos parámetros de calidad del agua tales como demanda biológica de oxígeno, sólidos en suspensión y conductividad, lo que supone un pretratamiento simultáneo de los residuos.
- Los parámetros de calidad del agua residual después del proceso de valorización, fueron mejores para el SUPRAS sintetizado a partir de hexanol.

Como perspectivas futuras, proponemos las siguientes líneas de investigación a continuar en base a los resultados de esta Tesis y que están especialmente orientadas a posibilitar la implantación industrial de estos desarrollos preliminares:

- Estudio en mayor extensión de la capacidad de los SUPRAS para la estabilización y almacenamiento de compuestos bioactivos poco estables, como los carotenoides, propensos a la oxidación.
- Desarrollo de técnicas posteriores a la extracción para el enriquecimiento/preconcentración de los compuestos bioactivos en los extractos de SUPRAS.
- Desarrollo de técnicas de encapsulación y estabilización basadas en oleorresinas y “lipid nanocarriers” a partir de los extractos de SUPRAS para su posterior implementación industrial.
- Estudios de toxicidad, biocompatibilidad y absorción intestinal de los productos finales a comercializar.

APÉNDICE A:
PUBLICACIONES CIENTÍFICAS DERIVADAS DE ESTA TESIS

Publicaciones científicas derivadas de la Tesis doctoral con índices de calidad

Laura Sofía Torres-Valenzuela, Ana Ballesteros, Soledad Rubio. Green solvents for the extraction of high added-value compounds from agri-food waste. Food Engineering Reviews **2019,** https://doi.org/10.1007/s12393-019-09206-y.
Factor de impacto: 4.217 (JCR, 2018)
Área temática en la Base de Datos de referencia: Food Science and Technology
Cuartil de la revista Q1 (primer decil) (12/135, JCR, 2018)

Laura Sofía Torres-Valenzuela, Ana Ballesteros, Alejandra Sanin, Soledad Rubio. Valorization of spent coffee grounds by supramolecular solvent extraction. *Separation and purification technology*, 228, **2019,** *115759.*
Factor de impacto: 5.107 (JCR, 2018)
Área temática en la Base de Datos de referencia: Engineering, Chemical
Cuartil de la revista Q1 (14/138, JCR, 2018)

Laura Sofía Torres-Valenzuela, Ana Ballesteros, Alejandra Sanin, Soledad Rubio. Supramolecular solvent extraction of bioactives from coffee cherry pulp. *Journal of Food Engineering (2020): In press*
Factor de impacto: 3.625 (JCR, 2018)
Área temática en la Base de Datos de referencia: Engineering, Chemical
Cuartil de la revista Q1 (28/138, JCR, 2018)

Laura Sofía Torres-Valenzuela, Ana Ballesteros, Johanna Serna, Andrea Arango, Soledad Rubio. Supramolecular solvents for the valorization of coffee wastewater. *Environmental Science Water Research & Technology (2020), DOI: 10.1039/c9ew01095e*
Factor de impacto: 4.195 (JCR, 2018)
Área temática en la Base de Datos de referencia: Water resources, Science
Cuartil de la revista Q1 (primer decil) (7/91, JCR, 2018)

APÉNDICE B:
COMUNICACIONES A CONGRESOS DERIVADAS DE ESTA TESIS

PÓSTER INTERNACIONAL

Supramolecular solvent extraction for valorization of coffee husks

L.S. Torres-Valenzuela[1,2], A. Ballesteros-Gómez[1*], S. Rubio[1]

[1]Departamento de Química Analítica, Instituto Universitario de Química Fina y Nanoquímica IUNAN, Universidad de Córdoba, Campus de Rabanales, Edificio Marie Curie (anexo), E-14071 Córdoba, España
[2]Universidad La Gran Colombia Seccional Armenia, Ciudadela del Saber La Santa María, km 7 vía Armenia – La Tebaida, Armenia, Colombia
*corresponding author: ana.ballesteros@uco.es

Abstract

The agricultural world production is continuously increasing every year as a result of the rising global demand for food and generates billion tons of by-products each year. The disposal of agri-waste is a growing issue that can cause phytosanitary problems and cross-contamination in food industries. As a consequence, new strategies to manage or benefit from agri-waste are urgently needed. One of the most promising options is to valorize the bioactive components presents in the by-products that may have applicability in functional foods and nutraceutical developments. Among raw materials, coffee stand outs as one of the most valuable primary products in world trade because of the high consumption of coffee beverage. The coffee industry generates about 2 billion tons of agri-waste which represent a great pollution hazard (Iriondo-DeHond et al., 2018). Depending on the processing method, either wet or dry, coffee pulp and husks are the first by-products and account for 29% and 12% of the overall coffee cherry (Janissen et al, 2018). Coffee by-products have been of interest as substrates for biofuels, composting, extraction of bioactive compounds such as caffeine, tannins, cyanidins and polyphenols and to obtain extracts with prebiotic, antimicrobial and antioxidant properties.

Supramolecular solvents (SUPRAS) are green solvents made up of assembled aggregates of amphiphiles dispersed in a continuous phase (aqueous or hydro-organic phase) (Caballo et al, 2017). The synthesis is made by a simple two-step self-assembly and coacervation process. First, amphiphiles spontaneously assemble into tridimensional individual aggregates (mainly micelles and/or vesicles). The second stage generates a new highly packed phase by the assembly of the aggregates into a nano- or micro-structured liquid (SUPRAS phase). This second phase is triggered by an external stimuli or coacervating agent (change of pH, temperature, addition of salt or addition of a poor solvent for the amphiphile) that diminishes the repulsion among the aggregates and promotes their assembly. The SUPRAS phase remains in equilibrium with the bulk solution, which contains the amphiphile at a low critical aggregation concentration. SUPRAS can be extracted and stored if required (keeping its structure and properties) for application to solid samples or stabilization of compounds or applied together with the equilibrium solution, which acts as dispersion phase for the matrix and as sink of interferents. SUPRAS have shown a wide applicability to a variety of compounds of very different nature and polarity, given the multiple binding interactions capacity that is conferred and tuned by switching the functional groups of the amphiphile and the dispersion solvent (ionic, anionic, hydrogen bonds, dispersion interactions, etc.). Their special internal structure creates also microenvironments of different polarity and restricted access properties for the exclusion of macromolecules, which are many time interferents in extraction processes. The extraction of high-value bioactive compounds using SUPRAS constitutes an economically attractive alternative for the valorization of coffee by-products due to the potential of high extraction efficiency for a variety of compounds, rapidity, simplicity and low-cost synthesis (room temperature, low consumption of cheap reagents). SUPRAS can be also considered green solvents due to their low volatility, flammability and low toxicity. In this study,we investigate the suitability of SUPRAS for the valorization of dry coffee husks in terms of caffeine content, total polyphenolic content and antioxidant activity.

Keywords: supramolecular solvents, green solvents,coffee,by-productsantioxidant, valorization

462

PÓSTER NACIONAL

SEQA 2019

XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA

VALLADOLID 17, 18 Y 19 DE JULIO

LIBRO DE RESÚMENES

SEQA Sociedad Española de Química Analítica

UVa

ALI-P04

VALORIZACIÓN DE BORRAS DE CAFÉ CON DISOLVENTES SUPRAMOLECULARES

L.S. Torres-Valenzuela, A. Ballesteros-Gómez, S. Rubio

Departamento de Química Analítica, Facultad de Ciencias, Universidad de Córdoba Edificio Marie Curie, Campus de Rabanales, 14014, Córdoba (España)
ana.ballesteros@uco.es

El café es la segunda bebida más consumida a nivel mundial. Se cultiva en alrededor de 80 países, de los cuales el 90% son países en vías de desarrollo. Para producir una taza de café se genera un 95% de biomasa residual. En estos residuos se encuentran sustancias bioactivas que pueden ser de interés para nuevos procesos y/o productos, permitiendo de esta manera disminuir los impactos negativos del desecho de residuos y generando un valor añadido a la agrocadena del café. La extracción con fluidos supercríticos o extracción con disolventes orgánicos asistida por ultrasonidos o microondas o utilizando elevadas presiones o temperaturas han sido las técnicas más investigadas para su valorización. En este estudio se emplean disolventes supramoleculares (SUPRAS) [1] como un alternativa sostenible, económica y eficiente para la valorización de los residuos de café. Los SUPRAS son líquidos nanoestructurados que ofrecen múltiples interacciones y sitios de unión para la extracción de compuestos en una amplia polaridad y que se caracterizan por baja volatilidad, inflamabilidad y toxicidad.

Entre los compuestos con interés funcional presentes en residuos de café, la cafeína y los ácidos clorogénicos son los de mayor interés. La cafeína tiene efecto estimulante del sistema nervioso central y los ácidos clorogénicos (CGAs) son compuestos con elevada capacidad antioxidante. Estos tipos de compuestos bioactivos se seleccionaron como representativos para la optimización del proceso de extracción de borras de café húmedas.

En este estudio se investigaron SUPRAS formados espontáneamente a través de autoensamblaje y coacervación de agregados de 1-hexanol o ácido decanoico (8-24% v/v) en una fase hidro-orgánica de etanol o THF (20-40 % v/v) y agua (36-72 %) para la extracción de compuestos bioactivos de borras de café húmedas procedentes de la variedad *Castillo* cosechada en Colombia. Los SUPRAS se sintetizaron in situ en presencia del residuo de café y se investigó la capacidad de extracción de los mismos para cafeína y ácido clorogénico. El SUPRAS que produjo la máxima eficiencia de extracción fue el sintetizado a partir de 24% v/v 1-hexanol, 30% etanol v/v y 46% agua v/v. El proceso consistió en agitación de la mezcla durante 1 min a 3,000 rpm y centrifugación para la separación del residuo del extracto de SUPRAS. Estos extractos mostraron altos contenidos en cafeína (3.3 $mg.g^{-1}$) y 5-CGA (4.3 $mg.g^{-1}$), elevado valor en polifenoles totales (60 mg/g), así como alta actividad antioxidante (evaluada con diferente métodos: DPPH, FRAP, ABTS) y cierta actividad antimicrobiana, especialmente ante bacterias gram-negativas (*S. enterica* and *P. putida.*).

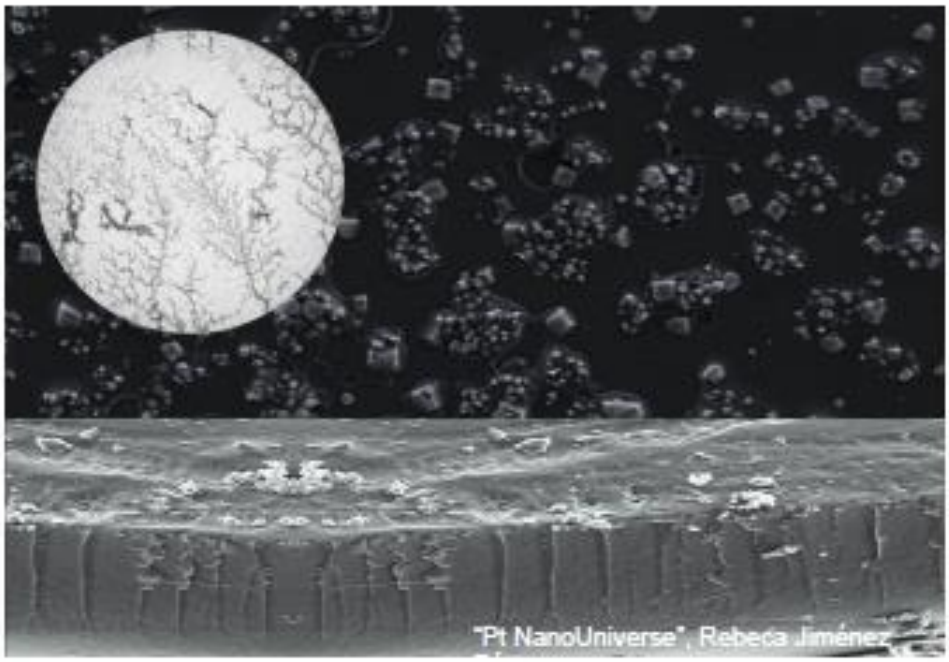
En base a estos resultados podemos concluir que los SUPRAS presentan una alternativa verde y viable para la valorización de borras de café.

[1] Ballesteros-Gómez A, Sicilia MD, Rubio S. Supramolecular solvents in the extraction of organic compounds. A review, Anal. Chim. Acta. 2010; 677:108–130. doi:10.1016/j.aca.2010.07.027.

PÓSTER NACIONAL

INSTITUTO UNIVERSITARIO DE NANOQUÍMICA

UNIVERSIDAD D CÓRDOBA



LIBRO DE RESÚMENES

NANOUCO VII

Encuentro sobre Nanociencia y Nanotecnología

Rectorado de la Universidad de Córdoba
21 y 22 de Enero de 2019

P59-AG

DISOLVENTES SUPRAMOLECULARES: UNA ALTERNATIVA VERDE PARA EL APROVECHAMIENTO DE RESIDUOS DEL CAFÉ

Laura Sofia Torres-Valenzuela[a,b] **Ana Ballesteros-Gomez**[a], **Soledad Rubio**[a]
[a]*Departamento de Química Analítica, Facultad de Ciencias, Universidad de Córdoba, Edificio Anexo Marie Curie, Campus de Rabanales, 14071, Córdoba (España), ana.ballesteros@uco.es*
[b]*Universidad La Gran Colombia Seccional Armenia, Ciudadela del Saber La Santa María, km 7 vía Armenia – La Tebaida, Armenia, Colombia*

El café es un producto de importancia a nivel mundial, puesto que es la segunda bebida más consumida y es cultivado en cerca de 80 países (90% en vías de desarrollo). Para producir una taza de café se genera un 95% de biomasa residual. En estos residuos se encuentran sustancias bioactivas que pueden ser de interés para nuevos procesos y/o productos, permitiendo de esta manera disminuir los impactos negativos del desecho de residuos y generando un valor añadido a la agrocadena del café.

En este estudio se emplean disolventes supramoleculares (SUPRAS) como un alternativa verde, barata y eficiente para la valorización de los residuos de café con respecto a las técnicas convencionales basadas en disolventes orgánicos o fluidos supercríticos. Los SUPRAS son líquidos nanoestructurados que ofrecen múltiples interacciones y sitios de unión para la extracción de compuestos en una amplia polaridad y que ofrecen características de baja volatilidad, inflamabilidad y toxicidad.

Entre los compuestos con interés funcional presentes en residuos de café, encontramos la cafeína y la catequina. La cafeína tiene efecto estimulante del sistema nervioso central y la catequina es un compuesto con alta capacidad antioxidante. Para la extracción de estos compuestos a partir de cáscaras de café secas se emplearon SUPRAS biocompatibles formados espontáneamente a través de autoensamblaje y coacervación de agregados inversos de ácido octanoico en una fase hidro-orgánica de etanol y agua. Adicionalmente se realizaron extracciones con los disolventes convencionales etanol, acetona, metanol y acetato de etilo, con fines comparativos.

El proceso de extracción se inició con la síntesis del SUPRAS en el cual se obtienen tras la centrifugación, dos fases, la fase superior de SUPRAS y la fase de disolución de equilibrio (DE). Ambas fueron empleadas en el proceso de extracción, la segunda con el fin de mejorar la dispersión de la muestra. Las condiciones óptimas fueron de 0.13 mL de DE y 0.67 mL de SUPRAS por 200 mg de muestra (valores escalables para procesos industriales) y 5 minutos con agitación continua en vórtex a 3000 rpm. A los extractos obtenidos se les midió el contenido en cafeína, catequina, polifenoles totales por el método de Folin-Ciocalteau y antioxidantes por ABTS. La extracción disolventes tradicionales se realizó siguiendo el mismo procedimiento.

Los valores de cafeína y catequina con SUPRAS fueron de 0.14 $mg.g^{-1}$ y 0.67 $mg.g^{-1}$, respectivamente. Los rendimientos de extracción fueron notablemente superiores que los obtenidos con disolventes tradicionales, los cuales fueron máximos para metanol (0.052 $mg.g^{-1}$, 0.301 $mg.g^{-1}$) y mínimos para acetona (0.004 $mg.g^{-1}$, n.d.). Los extractos de SUPRAS presentaron un contenido en polifenoles de 310 mg $GAE.L^{-1}$ y una capacidad antioxidante del 60%. En base a estos resultados se puede concluir que los SUPRAS son una alternativa inocua y de alta eficiencia para el aprovechamiento integral de cáscaras de café a través de la extracción de compuestos bioactivos que pueden ser empleados como aditivos en la industria farmacéutica, cosmética, alimentaria y química.

Agradecimientos: Los autores agradecen el apoyo financiero del Ministerio Español de Ciencia, Innovación y Universidades (Proyecto CTQ2017-83823R). A.B.G agradece su beca Ramón y Cajal (RYC-2015-18482)

APÉNDICE C:
OTRAS CONTRIBUCIONES EN EL APROVECHAMIENTO DE RESIDUOS DE CAFÉ

C1. PUBLICACIONES CIENTÍFICAS EN ARTÍCULOS NO INDEXADOS EN WOS

No incluidos en esta tesis

1. *Secado de Pulpa de Café: Condiciones de Proceso, Modelación Matemática y Efecto sobre Propiedades Fisicoquímicas*

   Torres-Valenzuela, Laura S.; Martínez, Katherine G.; Serna-Jimenez, Johanna A.; Hernández, María C.
   Información Tecnológica 30 (2): 189 – 200, 2019
   DOI: http://dx.doi.org/10.4067/S0718-07642019000200189

2. *Aprovechamiento de la pulpa de café como alternativa de valorización de subproductos*

   Johanna Andrea Serna-Jiménez, Laura Sofía Torres-Valenzuela, Katherine Martínez Cortínez, María Camila Hernández Sandoval
   Revista Ion 31 (1): 37 – 42, 2018
   DOI: http://dx.doi.org/10.18273/revion.v31n1-2018006

3. *Extracción asistida por ultrasonido de cafeína proveniente de café especial (Coffea arabica)*

   Johanna Andrea Serna-Jiménez, Laura Sofía Torres-Valenzuela, Luisa Fernanda Duque, Nadine Acero
   Agronomía Colombiana. 34 (1): 467 - 469, 2016

C2. CAPÍTULOS DE LIBRO

CORHUILA
CORPORACIÓN UNIVERSITARIA DEL HUILA

CORPORACIÓN UNIVERSITARIA DEL HUILA –CORHUILA
NIT. 800.107.584-2

Aplus ISO 9001

El sello editorial de la Corporación Universitaria del Huila – CORHUILA

Certifica

Que el artículo titulado **EXTRACCIÓN DE CAFEÍNA EN CAFÉ ESPECIAL (coffea arabica l. var. Colombia) por medio de microondas**, escrito por **Alejandra Sanín Villareal, en coautoría de Laura Sofía Torres Valenzuela, Johana Andrea Serna Jiménez, Diego Fernando Barreto Varón y Lizeth Juliana Jiménez Aguilar,** cumplió de forma cabal el proceso de sometimiento, revisión disciplinar, admisión y edición, lo que mereció su inclusión como capítulo de libro en el volumen compilatorio de resultados de investigación de nuevo conocimiento titulado **Saberes emergentes para la cuarta revolución industrial,** editado por el sello CORHUILA.

La presente certificación tendrá validez para todo trámite académico y administrativo y obrará como evidencia irrefutable de autenticidad y reconocimiento de autoría.

En constancia,

Marcos Fabián Herrera

Coordinador Editorial – Corhuila

IntechOpen

NOTIFICATION OF CHAPTER ACCEPTANCE

September 03, 2019, London

Dear Professor Torres Valenzuela,

It is my pleasure to inform you that the manuscript titled "Coffee by-products: Nowadays and perspectives" has been accepted for publication.

Your chapter will appear in the Open Access book, "Coffee" edited by Dr. Dalyse Castanheira

This is a huge achievement and I would like to thank you for your important contribution to the field and ensuring your research is freely available to the world.

We wish you every success with your publication.

Sincerely yours,

Anke Beck,
CEO, IntechOpen

INTECHOPEN LIMITED, Registered in England and Wales No. 11086078, The Shard 25th Floor, 32 London Bridge Street, London, United Kingdom, SE1 9SG
P: +44 (0) 20 3457 0462 | W: intechopen.com | M: info@intechopen.com

C3. OTRAS CONTRIBUCIONES A CONGRESOS NACIONALES E INTERNACIONALES

ORAL INTERNACIONAL

ORAL INTERNACIONAL

ORAL INTERNACIONAL

ORAL INTERNACIONAL

PROGRAMA DE QUÍMICA
FACULTAD DE CIENCIAS BÁSICAS Y TECNOLOGÍAS
UNIVERSIDAD DEL QUINDIO
SIQUIA

1 SIMPOSIO INTERNACIONAL DE ANÁLISIS FISICOQUÍMICO Y MICROBIOLÓGICO DE LOS ALIMENTOS

VIII SIMPOSIO DE QUÍMICA APLICADA

Certifican que

LAURA SOFIA TORRES VALENZUELA

Participó como *CONFERENCISTA* al *1 SIMPOSIO INTERNACIONAL DE ANÁLISIS FISICOQUÍMICO Y MICROBIOLÓGICO DE LOS ALIMENTOS*, en el *VIII SIMPOSIO DE QUÍMICA APLICADA*, que se desarrolló entre los días 27 al 29 de Septiembre de 2017, en Armenia - Quindío

En testimonio de ello se firma en el mes de Septiembre de 2017

RAMIRO GARCÍA ARIAS
DECANO FACULTAD DE CIENCIAS BÁSICAS

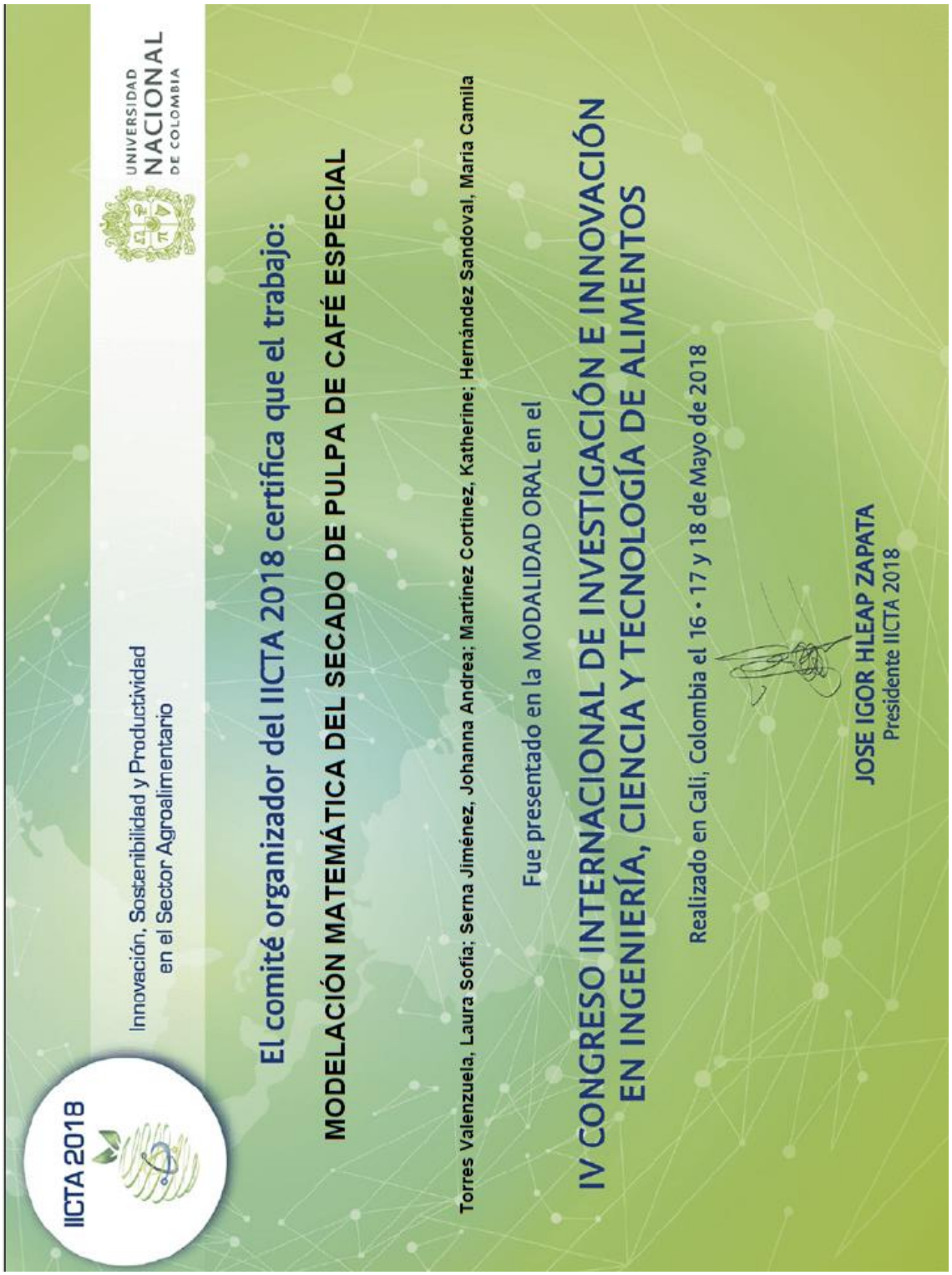
FERNANDO CUENÚ CABEZAS
DIRECTOR PROGRAMA DE QUÍMICA

CLARA MARÍA MEJÍA DORIA
COORDINADORA VIII SIQUIA

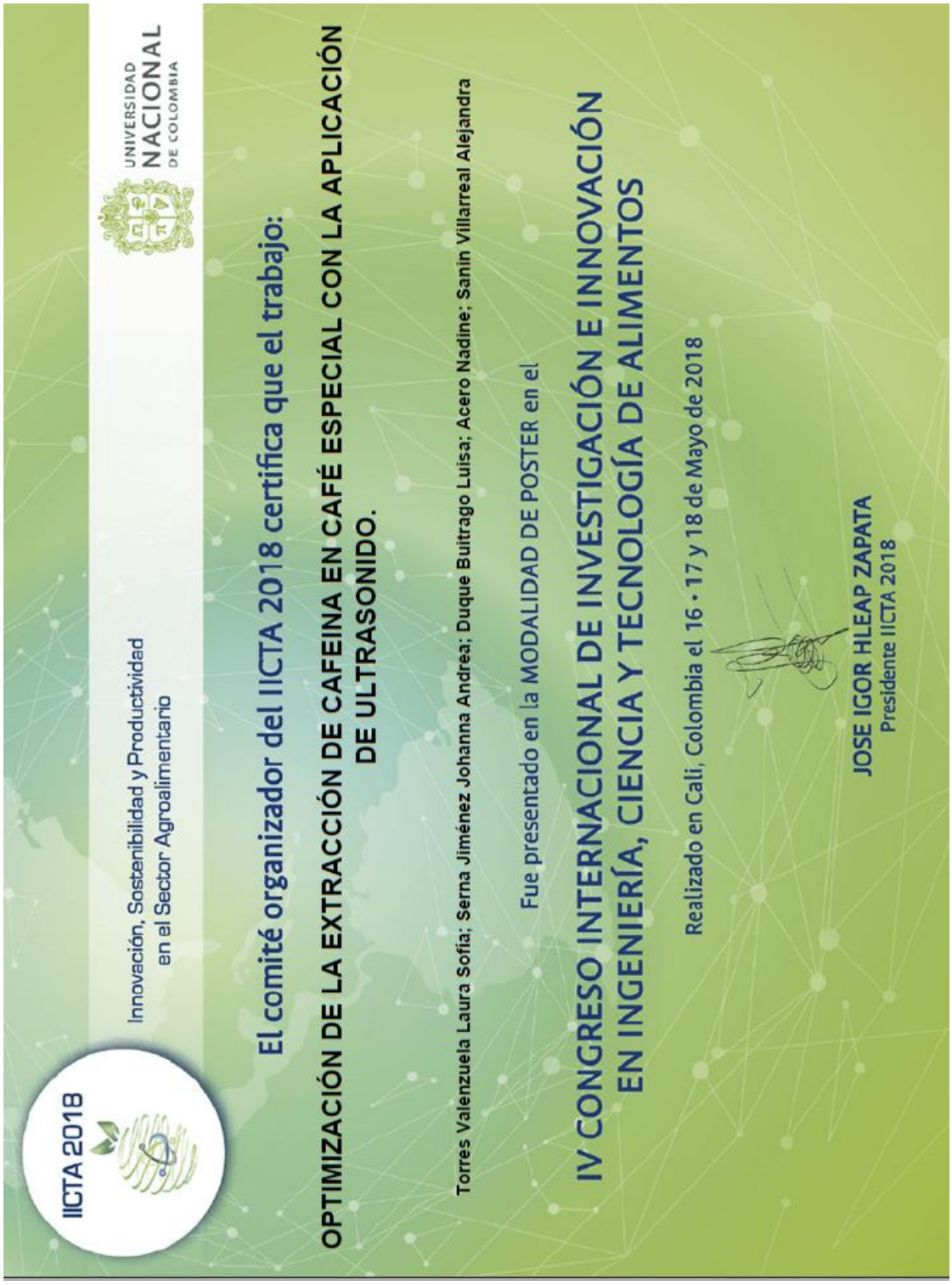
PERTINENTE CREATIVA INTEGRADORA

www.uniquindio.edu.co

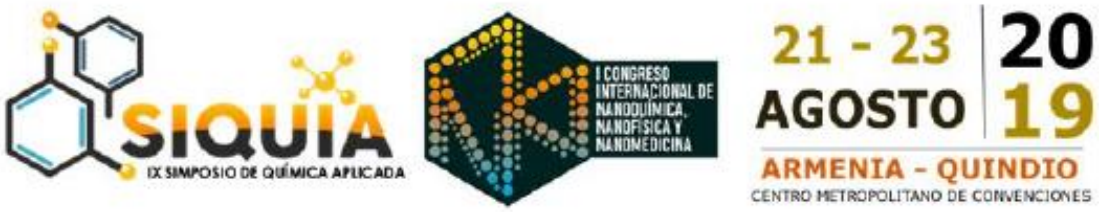
ORAL INTERNACIONAL



PÓSTER INTERNACIONAL



PÓSTER INTERNACIONAL



La Universidad del Quindío y el comité organizador del IX SIQUIA y el I CINNN 2019 certifican que:

Andrea Arango, Laura Sofía Torres-Valenzuela, Johanna Andrea Serna-Jiménez, Alejandra Sanín.

han presentado la contribución titulada:

Caracterización fisicoquímica y microbiológica de aguas mieles del beneficio del café

En modalidad:

Poster

Como presentación oral en el **IX SIMPOSIO DE QUÍMICA APLICADA (IX SIQUIA) Y I CONGRESO INTERNACIONAL DE NANOQUÍMICA, NANOFÍSICA Y NANOMEDICINA (I CINNN)**, desarrollado en Armenia, Colombia, Agosto 21 al 23, 2019.

Dr. Cristian camilo Villa
Coordinador Comité Organizador
Docente Programa de Química

Dr. Fernando Cuenu Cabezas
Director Programa de Química

M.Sc. Germán Dario Gómez Marin
Decano de la Facultad de Ciencias Básicas y Tecnologías