Universidad Autónoma del Estado de México

Maestría y Doctorado en Ciencias Agropecuarias y Recursos Naturales

CINÉTICA DE LIBERACIÓN DE COMPUESTOS ANTIOXIDANTES DEL CÁLIZ DE JAMAICA (*Hibiscus sabdariffa* L.) ENCAPSULADOS EN DIFERENTES POLÍMEROS COMESTIBLES

T E S I S

QUE PARA OBTENER EL GRADO DE

DOCTOR EN CIENCIAS AGROPECUARIAS Y RECURSOS NATURALES

P R E S E N T A :

DANIEL DÍAZ BANDERA

El Cerrillo Piedras Blancas, Toluca, Estado de México. Junio, 2013.

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RESUMEN

CINÉTICA DE LIBERACIÓN DE COMPUESTOS ANTIOXIDANTES DEL CÁLIZ DE JAMAICA (*Hibiscus sabdariffa* L.) ENCAPSULADOS EN DIFERENTES POLÍMEROS COMESTIBLES

Daniel Díaz Bandera. Maestría y Doctorado en Ciencias Agropecuarias y Recursos Naturales. Universidad Autónoma del Estado de México.

El cáliz de la jamaica contiene cantidades significativas de compuestos antioxidantes cuya ingesta habitual, a través de una infusión, previene y controla enfermedades crónico-degenerativas. Sin embargo, se ha documentado su baja biodisponibilidad en el cuerpo humano, debido a la inestabilidad de sus compuestos fenólicos durante su trayecto a través del tracto digestivo. En el presente trabajo se propuso preservar la actividad antioxidante de los compuestos fenólicos de este producto vegetal para lograr una mayor bioaccesibilidad empleando polímeros comestibles con dos tecnologías de encapsulación, a) secado por aspersión y b) coacervación. En el primer caso se mezcló el extracto de la jamaica con los polímeros: maltodextrina, pectina, gelatina, carboximetilcelulosa, proteína de suero, carragenina y goma arábiga obteniendo polvos para su análisis. Los coacervados se elaboraron con grenetina y se encapsularon con una y dos coberturas de alginato de sodio gelificado por inmersión en una solución de $CaCl_2$ durante 10, 20, 30 y 40 minutos. Se evaluó la cinética de liberación de los productos obtenidos y se ajustó, en todos los casos, a una ecuación de Weibull-Prmer Orden. La constante de velocidad de liberación (k_s) de los coacervados disminuyó linealmente con el número de coberturas de alginato y con el aumento en el tiempo de inmersión en la solución de $CaCl_2$. Los resultados obtenidos muestran que la liberación de los compuestos fenólicos de la jamaica puede controlarse en función del número de coberturas de alginato y del incremento en el tiempo de inmersión en la solución de $CaCl_2$. Los polímeros utilizados en el proceso de secado por aspersión mostraron diferentes parámetros cinéticos. Derivado de la cinética de liberación, se propuso un método para estimar el tiempo de disolución de las partículas obtenidas.

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Palabras clave: *Hibiscus sabdariffa* L.; antioxidantes; gelatina; compuestos fenólicos; coacervados; cinética de liberación; secado por aspersión.

ABSTRACT

RELEASE KINETICS OF THE ANTIOXIDANT COMPOUNDS OF ROSELLE'S CALYX (*Hibiscus Sabdariffa* L.) ENCAPSULATED IN DIFFERENT EDIBLE POLYMERS

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Roselle's calyx contains significant amounts of antioxidant compounds and the regular intake of the aqueous extract prevents and controls chronic degenerative diseases. However it has been documented a low bioavailability of those compounds in the human body due to their instability during its way through the digestive tract. The present research aimed to preserve the antioxidant activity of Roselle's phenolic compounds in order to achieve a higher bioaccesibility by using edible polymers with two encapsulation technologies, a) spray drying and b)coacervation. In the first case, Roselle's extract was mixed with the following polymers: maltodextrin, pectin, gelatin, carboxymethyl-cellulose, whey protein, carrageenan and Arabic gum and their spray dried powders were obtained and analysed. The coacervates were prepared with gelatin and were encapsulated with one and two alginate coats with 10, 20, 30 or 40 min of immersion time in a $CaCl_2$ solution. The release kinetics of the obtained products was evaluated and fitted to a Weibull-First Order equation. The release rate constant (k_s) of the coacervates decreased linearly with the number of alginate coats and with the increase in immersion time in the $CaCl_2$ solution. The results obtained showed that Roselle's phenolic compounds release can be controlled as a function of the number of coats and with the immersion time in the $CaCl_2$ solution. The polymers used in the spray drying process, showed different kinetic parameters. Derived from the release kinetics study a method to estimate the dissolution time of the obtained particles was proposed.

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Key words: *Hibiscus sabdariffa* L.; *in vitro* digestion; polyphenols; antioxidant activity; confectionery products; gelatin.

INTRODUCCIÓN

La jamaica (*Hibiscus sabdariffa* L.) es una planta que pertenece a la familia Malvaceae, cuyos cálices deshidratados contienen cantidades significativas de compuestos fenólicos con capacidad antioxidante. Estos se utilizan en la obtención de extractos concentrados que se emplean principalmente para la elaboración de bebidas refrescantes o infusiones calientes, también se preparan jarabes, jaleas, mermeladas, salsas, dulces, vinos de mesa y como colorante alimentario, entre otros. Su estudio es de gran importancia porque diversas investigaciones científicas han demostrado que sus propiedades antioxidantes tienen efectos inversos en la incidencia en enfermedades crónico degenerativas, tales como como la hipertensión, diabetes y distintos problemas de cáncer entre otras, las cuales trascienden negativamente en el desarrollo de la sociedad.

La actividad antioxidante de los polifenoles, presenta serias limitaciones de biodisponibilidad en el cuerpo humano porque después de su ingesta y paso a través del sistema digestivo, alcanza muy bajos niveles de concentración en el plasma. Estos compuestos son muy lábiles, debido a las reacciones de degradación, hidrolisis y oxidación en el tracto digestivo, además de ser afectados por las condiciones ambientales de luz, pH y temperatura entre otras.

Derivado de las observaciones anteriores, el propósito del presente trabajo de investigación fue preservar la actividad antioxidante de los compuestos fenólicos extraídos del cáliz de la jamaica, para incrementar su bioaccesibilidad, desarrollando productos encapsulados empleando polímeros comestibles, con las tecnologías de secado por aspersión y coacervación-encapsulación.

El desarrollo experimental contempló una extracción acuosa eficiente de los compuestos fenólicos mediante una metodología ya reportada, seguida del concentrado del extracto. Se realizaron diversos ensayos preliminares, para la selección de los polímeros compatibles con el extracto ácido de jamaica, en el proceso de coacervación, y se establecieron los tiempos de gelificación con alginato de sodio. Para el encapsulado mediante secado por aspersión se establecieron las proporciones de polímeros y extracto

de acuerdo a otros diseños experimentales, así mismo, se establecieron las condiciones operativas del proceso para reducir la pérdida de la capacidad antioxidante por efecto térmico. Finalmente, mediante modelos matemáticos se analizaron las cinéticas de liberación de los antioxidantes y se estimaron los tiempos de disolución respectivamente en función de los polifenoles liberados de los encapsulados.

II. REVISIÓN DE LITERATURA

2.1. La jamaica

La jamaica (*Hibiscus sabdariffa* L), también conocida como rosa de Abisinia, Roselle o Karkadé, pertenece a la familia Malvaceae, es originaria de África tropical, su cultivo se extiende por Sudán, Tailandia, China, Egipto, Senegal, Tanzania, México, América Central y del sur y el Sureste asiático. (Morton, 1987; Domínguez-López *et al.*, 2008).

Los cálices se usan como colorantes alimentarios, para fabricar jarabes y mermeladas, para la preparación de una bebida ligeramente ácida y refrescante de color rojo intenso conocida como "sobo" en Nigeria, "karkade" en Egipto, o "agua de jamaica" en México (Farombi, 2003; Morton, 1987; Herrera-Arellano, Flores-Romero, Chávez-Soto, Tortoriello, (2004); Sáyago-Ayerdi, Arranz, Serrano, Goñi, (2007); y Hirunpanish, *et al.*, 2006).

El componente mayoritario de los cálices de la jamaica, es la fibra dietética con un 33.9% (Sáyago-Ayerdi *et al*., 2007), además de acuerdo con Wong *et al*. (2002), Nnam y Onyeke, (2003), los cálices contienen aproximadamente, 3.3 g/100 g, de diversos ácidos orgánicos como succínico, oxálico, tartárico y málico, una acidez total de 2.42 g/100 g, expresado como ácido málico, contienen en promedio, 1.88 mg/100 g de β-caroteno, 833 mg/100 g de hierro y 141.1 mg/100 g de ácido ascórbico. Sáyago-Ayerdi *et al*. (2007) reportaron que los cálices de jamaica contienen 2.17g GAE/100 g materia seca.

El contenido fenólico en la jamaica de acuerdo a Tsai y Huang (2004); Rodríguez-Medina, *et al*., (2009) y Ramírez-Rodrígues, Azeredo, Balaban y Marshall (2011) está compuesto por polifenoles extraíbles, principalmente antocianinas como la delfinidin-3-xilosilglucósido y la cianidin-3-xilosilglucósido y sus respectivos glucósidos delfinidin-3-glucósido y cianidin-3-glucósido.

En el Cuadro 1 se indican las cantidades contenidas de los compuestos fenólicos en los cálices de la jamaica, el total aproximado es alrededor del 6 % en base seca. Así mismo

se observa también que la mayor parte, cerca del 4%, corresponden a polifenoles no extraíbles y el resto son principalmente antocianidinas, ácidos hidroxicinámicos y ácidos hidroxibenzóicos.

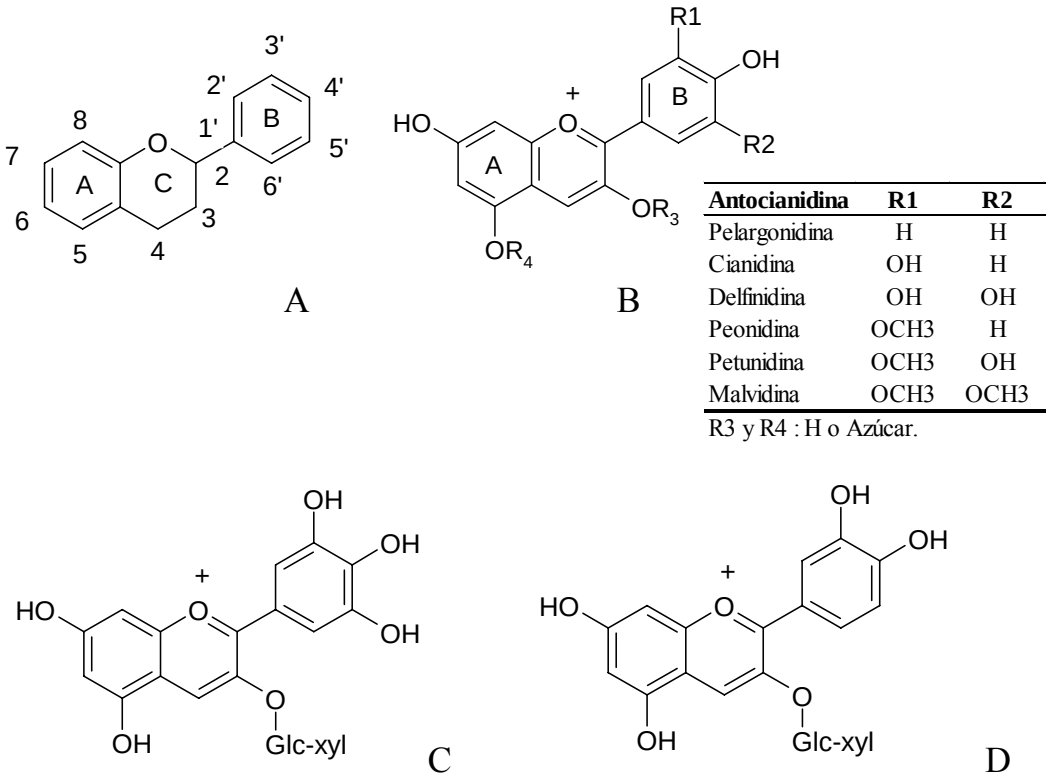
Cuadro 1. Contenido de compuestos fenólicos en los cálices de la jamaica (*Hibiscus sabdariffa* L.).

Compuestos contenidos	(g/100 g de materia seca)
Polifenoles extraíbles	2.17 ± 0.04
Ácidos Hidroxibenzóicos	32.6
Ácidos Hidroxicinámicos	30.6
Antocianidinas	30.8
Flavonoles	5.87
Polifenoles no extraíbles	
Proantocianidinas (taninos condensados)	3.38 ± 0.06
Polifenoles hidrolizables	0.58 ± 0.03

Referencia: Sáyago-Ayerdi *et al.* (2007).

2.2. Estructura básica de los polifenoles

Manach *et al*. (2004) reportaron que la estructura básica de los compuestos fenólicos consta de dos grupos fenilo (A y B) unidos por un puente de tres carbonos que forma un anillo heterocíclico oxigenado (anillo C). Los átomos de carbono presentes en los anillos C y A se numeran del 2 al 8 y los del anillo B desde el 1' al 6' (Harborne y Baxter, 1999; Pérez-Trueba, 2003). Esta estructura básica permite una multitud de patrones de sustitución y variaciones en el anillo C (Figura 1.)



Antocianidina	R1	R2
Pelargonidina	H	H
Cianidina	OH	H
Delfinidina	OH	OH
Peonidina	OCH3	H
Petunidina	OCH3	OH
Malvidina	OCH3	OCH3

R3 y R4 : H o Azúcar.

Figura 1. Estructura básica de las antocianinas. A: Estructura básica de los flavonoides y su sistema de numeración; B: Estructura básica de las antocianidinas; C: Delfinidin-3-xilosil glucósido y D: Cianidin-3-xilosil glucósido (ambas antocianinas con R3 y R4 formados por H y un azúcar, respectivamente) (Bravo, 1998).

Esta estructura básica de los compuestos fenólicos, es la que le confiere la actividad antioxidante, por su facilidad de ceder átomos de hidrógeno de sus grupos hidroxilo hacia algún radical libre y su estabilidad se debe a la deslocalización de cargas en el sistema de los dobles enlaces del anillo, también conocido como efecto resonante (Duthie *et al*., 2003).

La capacidad antioxidante de estos compuestos depende del grado de metoxilación y del número de grupos hidroxilo existente en la molécula. (Tomás-Barberán, y Espín, (2001).

La concentración de estos compuestos fenólicos puede variar considerablemente dentro de la misma especie vegetal, debido a factores genéticos y ambientales (Bravo, 1998; Scalbert y Williamson, 2000). Estos compuestos son además, responsables de las

propiedades organolépticas de los alimentos de origen vegetal (Tomás-Barberán y Espín, 2001). Un grupo muy importante de estos pigmentos son las antocianinas, responsables de los tonos rojos, azules y violáceos característicos de muchas frutas, hortalizas y del vino tinto, otro grupo de interés son los flavonoles presentes en frutas y hortalizas, los cuales les confieren un color crema-amarillento. Algunos polifenoles como las flavononas de los cítricos, aportan un sabor amargo, otros en cambio confieren astringencia a los frutos, como los taninos hidrolizables. Son responsables de la pigmentación de los pétalos en las flores de tonos amarillos, rojos y naranjas, que atraen a los agentes polinizadores. También tienen la capacidad de proteger a las plantas del daño provocado por los rayos UV y del ataque de insectos y patógenos (Waterhouse, 2002; Kong *et al*., 2003; Manach, *et al*., 2004; Yajun, 2005).

2.3. Propiedades biológicas de los compuestos fenólicos

Los compuestos fenólicos presentan una eficiente capacidad biológica antioxidante, es decir que al estar presentes en concentraciones menores a la concentración de algún sustrato oxidable, retardan o previenen significativamente su oxidación (Halliwell, 1990).

Los radicales libres y especies reactivas de oxígeno, se generan durante el metabolismo normal del cuerpo humano, o bien son inducidos o generados por factores exógenos y presentan un riesgo potencial para las células y tejidos (Nishikawa *et al*., 2000).

Estas especies reactivas pueden ser controladas con la ingesta habitual de cantidades significativas de compuestos fenólicos de origen vegetal debido a sus propiedades para atrapar radicales libres. La actividad antioxidante de los compuestos fenólicos en los alimentos se puede evaluar por varios métodos químicos ya sea *in vitro* o *in vivo*, éste último nos da tan sólo una idea aproximada de lo que ocurre en situaciones complejas *in vivo*, no obstante es un indicativo de dicha actividad (Scalbert y Williamson, 2000).

Diversas evidencias científicas indican que estos compuestos antioxidantes reducen la incidencia y/o severidad, de un amplio rango de enfermedades, porque pueden actuar como agentes terapéuticos frente a una amplia variedad de enfermedades, actuando

como antibióticos, antiinflamatorios, antihemorrágicos, vasodilatadores, antineoplásicos, antivirales, antibacterianos, antialérgicos y hepatoprotectores entre otros (Renaud y de Lorgeril, 1992; Pietta, 2000; Middleton, *et al*., 2000; Sun, *et al*., 2002). Algunos de los mecanismos que explican los efectos de los polifenoles en pro de la salud se encuentran, la quelación de metales, la inhibición enzimática y la regulación génica atribuidos a su capacidad para neutralizar o secuestrar radicales libres (Erlejman *et al*., 2004).

Estudios recientes evidencian que los flavonoides regulan también ciertas actividades enzimáticas celulares debido a su capacidad para alterar la estructura de la membrana plasmática (Caturla *et al*., 2003). Este efecto, permite actuar a los flavonoides en la apoptosis y en la funcionalidad de las mitocondrias (Schroeder *et al*., 2008). Su consumo frecuente forma parte del denominado sistema de defensa antioxidante exógeno del organismo, es decir, aquellas defensas que se adquieren a través de la dieta y se relaciona con la prevención y control de diversas enfermedades crónico degenerativas. (Manach, *et al*., 2004; Scalbert, *et al*., 2005; Moreno, *et al.,* 2008; Olvera-García, 2008).

2.4. Bioaccesibilidad y biodisponibilidad de los compuestos fenólicos

La bioaccesibilidad se define como la cantidad ingerida de un nutrimento que está disponible para ser absorbida en el intestino después de la digestión (Hedren *et al*., 2002), y la biodisponibilidad se define como la cantidad de sustancias activas o principios terapéuticos contenidos en un medicamento que son absorbidos para estar disponibles directamente en el sitio de acción (FDA, 2000).

En la actualidad aún no se conocen al detalle los mecanismos implicados en la absorción y biodisponibilidad de los compuestos fenólicos (Hendrich, 2002). Las estructuras químicas en las que se encuentran contenidos en los alimentos son sustancialmente determinantes de su bioaccesibilidad y biodisponibilidad, conjuntamente los procesos digestivos inciden a nivel físico, químico y enzimático sobre la biodisponibilidad final (Parada y Aguilera, 2007).

Se cree que, la presencia de algunos grupos sulfatados y glicosilados en las estructuras fenólicas, facilitan su eliminación antes de ser absorbidos; los flavonoides son escindidos para dar, por una parte su aglicona y por otra su glicósido. Este último compuesto al tener mayor solubilidad en agua, se absorbe rápidamente, sin embargo la aglicona puede tardar hasta tres horas en ser absorbida. Por este motivo, el promedio de las concentraciones pico de los compuestos fenólicos se da tras 1.75 horas de la ingesta (Wittemer, *et al*., 2005; Laranjinha, 2002).

Los compuestos fenólicos después de su absorción se distribuyen homogéneamente en todos los tejidos corporales, logrando incluso atravesar la barrera hematoencefálica (Abrahamse, *et al*., 2005; Youdim, *et al*., 2004).

Los flavonoides inician su proceso de metabolización desde el tracto digestivo, y sus metabolitos no obstante sean absorbidos, ya no tienen la misma funcionalidad (Abrahamse *et al*., 2005), alcanzando una biodisponibilidad tan solo del 1.5% en comparación con la cantidad inicial administrada (Jiménez *et al*., 2001). Lo anterior sugiere que hay un proceso de biotransformación intensivo en diversas partes del organismo, incluyendo al hígado, donde se adicionan o eliminan grupos polares, (Abrahamse, *et al*., 2005; Youdim, *et al*., 2004; Rimm, *et al*., 1996).

Los compuestos fenólicos después de ser metabolizados total o parcialmente, son excretados por dos vías: Los compuestos insolubles en agua se excretan junto con la bilis al duodeno, y los compuestos hidrosolubles a las vías urinarias con la orina, siendo esta última la salida predominante (Abrahamse *et al*., 2005). Es importante conocer la estructura química de los flavonoides pues de ello depende la ruta que seguirán en su proceso metabólico, por tanto, si un flavonoide sólo es glucuronidado se excretará por vía renal como la catequina, pero si es metilado y sulfatado será excretado por la vía hepática como la quercetina (Manach *et al*., 1999).

Manach *et al*. (2005) indican, que la biodisponibilidad de los polifenoles en humanos depende de la forma en la cual se encuentren dentro de la matriz alimenticia. Passamonti *et al*. (2003) en un estudio proponen la absorción gástrica como una posible explicación a la rápida cinética de aparición de antocianinas en plasma, atribuyendo su facilidad de

absorción a un transportador de membrana: una bilitranslocasa. Posteriormente Passamonti, *et al*. (2009) incluyen una propuesta para explicar este mecanismo de absorción, el cual se basa en la carga mostrada y en la configuración espacial de las antocianinas en el C4 del anillo C en donde si este grupo funcional es un azúcar, se establece la coincidencia con el sitio activo de la bilitranslocasa, la cual se encuentra tanto en el estómago como en el intestino delgado. Por otro lado, estos mismos autores, hacen referencia a la necesidad de hacer llegar este tipo de antioxidantes a las porciones bajas del tracto digestivo, colon específicamente, dado que es un sitio donde la producción de radicales libres es altamente significativa.

A pesar de la actividad antioxidante expresada por los compuestos bioactivos de la jamaica, se reporta una biodisponibilidad limitada con bajos niveles circulantes en el plasma después del consumo del extracto acuoso de este cultivo (van Duynhoven *et al*., 2011). Las limitaciones en los procesos de bioaccesibilidad y biodisponibilidad observadas para los compuestos fenólicos ya mencionados, sugieren la búsqueda de alternativas viables y de factibilidad tecnológica, para incrementar la bioaccesibilidad y por ende la biodisponibilidad.

2.5. Ingesta de compuestos fenólicos

En la actualidad se desconocen a detalle los procesos de absorción y metabolismo de los fitoquímicos. El Comité Permanente Sobre La Evaluación Científica y su panel en dietas antioxidantes y compuestos relacionados y la junta de alimentación y nutrición en el Instituto de Medicina de Estados Unidos, optó por no crear una ingesta dietética de referencia o dosis diaria recomendada (DRI) para estos compuestos (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1998).

Por lo tanto, no existe actualmente una dieta diaria recomendada para el consumo de fitoquímicos, hoy en día muchas autoridades de salud como la Sociedad Americana del Cáncer y La Asociación Americana del Corazón recomiendan consumir una dieta alta en frutas y vegetales para asegurar así una ingesta individual adecuada de compuestos fitoquímicos antioxidantes. Considerando que no existe una referencia para el consumo de polifenoles, la Organización Mundial de la Salud (OMS) (2003) indica que la

recomendación en el consumo de frutas es de 400 g (5 porciones de 80 g cada una) al día por persona y que solamente una pequeña minoría de la población mundial logra llegar a esta cifra por lo que esta misma organización promueve el consumo de frutas y verduras, sobre todo en países en vías de desarrollo enfocando un mayor esfuerzo en zonas marginales de las comunidades urbanas.

De acuerdo a Williamson y Holst, (2008) debido a la falta de información para establecer un DRI para los polifenoles y otros fitoquímicos el valor referido de 5 porciones al día por la OMS es el más utilizado. También es importante y necesario establecer la dosis de los niveles tóxicos, antes de que pueda establecerse una DRI.

El pretender cumplir con la ingesta de 5 porciones diarias se ve limitado por fuertes factores socioculturales y económicos, en México un estudio llevado a cabo por Pérez-Lizaur *et al*. (2008) sobre el consumo de frutas en escuelas primarias en la ciudad de México demostró que el promedio de ingesta era de solo una porción al día de fruta y solo el 11% reportó un consumo mayor o igual a 3 porciones por día, un estudio similar llevado cabo en 2005 por Ortiz-Hernández y Gómez-Tello, (2008) con adolescentes entre 12 y 19 años, reveló que sólo el 39.1% y el 36.4% consumía frutas y vegetales una vez al día.

2.6. La salud, alimentos funcionales, nutracéuticos y fitoquímicos

En los últimos años la preocupación por la salud y la prevención de enfermedades crónico degenerativas ha ido en incremento, la tendencia al consumos de alimentos saludables y la adopción de cambio en los hábitos básicos alimenticios, es el indicativo de una inquietud que busca la satisfacción hacia una mejor expectativa de vida. La industria alimentaria ha estudiado el potencial de mercado de este tipo de alimentos y se ha iniciado a nivel mundial una intensa actividad de investigación en esta área.

Los alimentos promotores de la salud pueden ser clasificados como funcionales, nutracéuticos y fitoquímicos, realmente existe una línea muy estrecha entre sus definiciones, lo cierto es que todos convienen en incidir a favor de la salud.

Los alimentos funcionales de acuerdo a Rafter (2002), se definen como alimentos o ingredientes que mejoran el estado general de salud y/o reducen el riesgo de enfermedad.

Los efectos promotores de la salud deben demostrarse científicamente para validar sus efectos y poder emitir declaraciones nutricionales en su etiqueta, así queda ya establecido en el proyecto titulado "Proceso de evaluación de apoyo científico de declaraciones en los alimentos" por sus siglas en inglés (PASSCLAIM) (Roberfroid, 2002; Aggett, *et al*., 2005).

Una de las áreas más prometedoras en el desarrollo de alimentos funcionales se fundamenta en la posibilidad de modular los sistemas redox y antioxidante en el cuerpo humano (Roberfroid, 2000). Por esta razón, en la actualidad muchos alimentos funcionales tienen como finalidad incrementar el aporte de antioxidantes naturales en la dieta. En este contexto, la adición de extractos vegetales ricos en compuestos fenólicos ha sido propuesta como una estrategia factible para el desarrollo de alimentos (Larrosa *et al*., 2002).

La importancia del reconocimiento de los beneficios aportados por los alimentos a la salud desde hace tiempo fue plasmada por Hipócrates quién citó hace ya 2500 años "Que la comida sea tu medicina y la medicina sea tu alimento". Esta frase marca la línea entre el alimento y los medicamentos (Adelaja y Schilling, 1999).

Para la definición de nutracéutico Dureja *et al*. (2003) refieren a Stephen DeFelice quien en 1979 lo definió por vez primera como: "un alimento o parte de un alimento que proporciona un beneficio médico o en la salud incluyendo la prevención y tratamiento de una enfermedad".

Los fitoquímicos fueron definidos de acuerdo a Zhao, (2007) como metabolitos vegetales que promueven la salud humana, mientras que Wahlqvist y Wattanapenpaiboon (1998) los definen como componentes de origen vegetal que pueden no ser esenciales en el sentido estricto y que es posible que tampoco sean necesarios para mantener la vida, pero que parecen contribuir a la obtención de una salud óptima.

Los alimentos funcionales pueden contener fitoquímicos como ingredientes promotores de la salud, dentro de estos ingredientes están los compuestos fenólicos que contribuyen al aporte de antioxidantes al cuerpo humano (Heo, *et al*., 2007; Cooper 2004; Sun y Tanumihardjo, 2007).

2.7. Polímeros portadores de los antioxidantes de la jamaica

Los polímeros portadores son ampliamente utilizados para proteger moléculas de interés biológico durante su paso a través del tracto digestivo de las condiciones ácidas y enzimáticas del estómago, ácido-neutras del intestino delgado y alcalinas del colon donde se lleva a cabo su liberación y absorción (Luykx *et al*., 2008). Los polímeros pueden tener varias funciones, algunos se usan para aportar volumen (ciclodextrinas), otros son espesantes (almidones) y gelificantes (pectinas), por lo que su uso como encapsulantes también ha sido utilizado. Por mencionar otros portadores de los más empleados está el suero de leche, proteína de soya, grenetina, carragenina, carboximetilcelulosa, goma arábiga, pectina, goma xantana, quitosano, alginato de sodio, caseinatos, ceras, almidón de trigo, maíz, arroz y papa, también algunas grasas y aceites (Shepherd, *et al*., 2000; Maltais, *et al*., 2005; Sanguansri y Augustin, 2006).

Los polímeros portadores se usan como encapsulantes, entre ellos están las proteínas y los polisacáridos (Bennion, 1980), por su fuente de origen en el área de alimentos se les conoce también como biopolímeros portadores y cumplen con pertenecer a la clasificación GRAS (Generalmente Reconocidos como Seguros) de acuerdo a la FDA. Las proteínas se usan como emulsificantes, estabilizantes y gelificantes sirviendo así como portadores o vehículos de moléculas de interés biológico (Chen *et al*., 2006). Los polímeros de carbohidratos o polisacáridos pueden encontrarse formando largas cadenas que difieren en el tipo de azúcar que los forma y en el tipo de enlace que los une y pueden encontrarse tanto en plantas, como en animales, en algas, y como metabolitos de algunos microorganismos como el dextrano (Kosaraju *et al*., 2005).

2.8. Técnicas de encapsulación

Las diversas tecnologías de encapsulación de compuestos bioactivos, tienen diferentes objetivos, como lo son: proteger el material de la degradación del medio ambiente por factores como, la luz, humedad, y oxígeno, disminuir el nivel de evaporación, modificar las características físicas del material original para facilitar su transporte, controlar la liberación con respecto al tiempo o a un tiempo particular, para enmascarar sabores o aromas indeseados, para diluir o concentrar el material, incrementando al mismo tiempo su vida útil (Shahidi y Han, 1993; Desai y Dark, 2005).

Las formas del encapsulado dependen de varios factores, algunos de ellos son: el requerimiento específico, el tipo de proceso de encapsulación, los materiales de carga y el tipo de encapsulante entre otros (Fang y Bhandari, 2010).

Las tecnologías de encapsulación de compuestos con actividad biológica más empleadas en alimentos son: el secado por aspersión, extrusión, coacervación, liofilización, inclusión, atrapamiento en liposomas, complejación, co-cristalización, nano-encapsulación y encapsulación en levaduras (Fang y Bhandari, 2010).

Las técnicas de encapsulación se pueden dividir en dos tipos de procesos: químicos y mecánicos. Los procesos químicos se dividen en las técnicas de coacervación, co-cristalización, polimerización interfacial, gelificación iónica, incompatibilidad polimérica, atrapamiento por liposomas e inclusión molecular. Dentro de los procesos mecánicos están las técnicas de secado por aspersión, secado por congelamiento/enfriamiento y extrusión (Madene, Scher y Desobry, 2006; Yañez *et al*., 2002).

La tecnología de micro encapsulación consiste en el desarrollo de micro partículas conformadas por una membrana polimérica porosa, contenedora de una sustancia activa, donde el material o mezclas de materiales a encapsular puede ser cubierto o atrapado dentro de otro material o sistema que consiste de una membrana semipermeable de forma irregular, a veces esférica, delgada y fuerte alrededor de un centro solido/líquido (Yañez, *et al*., 2002; Montes, De Paula y Ortega, 2007).

Dentro de las técnicas utilizadas para micro encapsular, se encuentran el secado por aspersión. Las sustancias que se micro encapsulan pueden ser vitaminas, minerales, colorantes, prebióticos, probióticos, sabores, nutracéuticos, antioxidantes, compuestos aromáticos, aceites, enzimas, bacterias, perfumes, drogas, lípidos, sabores e incluso fertilizantes (Murúa, Beristain y Martínez, 2009; Ferreira, Rocha y Coelho, 2007; Sozer y Kokini, 2009; Bastos, Araujo y Leao, 2009; Ranadheera, Baines y Adams, 2010).

El secado por aspersión es una de las operaciones más económicas e importantes en la industria alimentaria, puede producir partículas sólidas menores a 100 μm y una de las grandes ventajas de este método es que es apropiado para materiales sensibles al calor ya que el tiempo de exposición a temperaturas elevadas es muy corto de 5 a 30 segundos (Dziezak, 1998; Casper, 2003).

La utilización de polímeros comestibles en microcápsulas ha sido propuesta por Shimizu y Hachimura (2011) con el fin de preservar la capacidad antioxidante de los compuestos fenólicos y controlar su cinética de liberación en el sitio objetivo de absorción p.ej. el colon, logrando así también una nueva forma de administración concentrada que puede ser incluida en otros alimentos y a su vez enmascar el sabor astringente y/o amargo indeseado de este tipo de compuestos. Kosaraju *et al.* (2008) encapsularon antioxidantes de compuestos fenólicos de diferente origen en una emulsión proteína-lípido, la cual fue secada por aspersión con éxito, encontrando una retención altamente significativa de actividad antioxidante después del proceso.

La encapsulación por coacervación es una de las técnicas más caras pero muy útil, Saravanan y Panduranga (2010) desarrollaron encapsulados con pectina y grenetina por la técnica de coacervación usando alginato. La grenetina tiene la capacidad de producir hidrogeles firmes en un rango amplio de pH y puede reaccionar con los polifenoles generando una matriz compleja, probablemente los polifenoles actúan como puentes de unión y la concentración de la solución de cloruro de calcio determina la rigidez del gel de alginato, conociéndose este proceso como gelificación ionotrópica. El tiempo de inmersión influye directamente en la rigidez y en la porosidad del alginato (Rowe, Sheskey, y Quinn, 2009).

Una de las pruebas más importantes a las que son sometidos los encapsulados es la cinética de liberación que en muchas ocasiones se realiza en condiciones *in vitro*, estos resultados predicen con mayor precisión el tiempo de liberación del ingrediente activo encapsulado, es decir, su bioaccesibilidad, que por ende favorece una mayor biodisponibilidad (Parada y Aguilera, 2007).

III. JUSTIFICACIÓN, HIPÓTESIS Y OBJETIVOS

3.1. Justificación

El aprovechamiento de los recursos naturales, en beneficio de la salud de la población, con especies botánicas que han probado su incidencia en el control de enfermedades crónico-degenerativas, como lo es el cáliz de la jamaica,(*Hibiscus sabdariffa* L.) incitó el origen del presente trabajo de investigación.

Los estudios sobre la jamaica sugieren la presencia de cantidades importantes de fitoquímicos, específicamente polifenoles con capacidad antioxidante, cuya ingesta regular manifiesta efectos terapéuticos. La inestabilidad de estas moléculas es alta, pierden fácilmente su capacidad antirradical por factores como el pH, O_2, la luz y diversas reacciones químicas dentro del cuerpo humano incluyendo el tracto digestivo. Los compuestos fenólicos de la jamaica presentan una alta capacidad antioxidante *in vitro*, pero de acuerdo con Janssen *et al*. (2005), manifiestan una baja biodisponibilidad *in vivo*. Esto sugiere la necesidad de buscar métodos que protejan la actividad de estas moléculas mediante vehículos portadores que sean generalmente reconocidos como seguros y logren una mayor deposición de moléculas activas en el sitio deseado.

Existen diversos métodos de encapsulación de componentes como los polifenoles (Fang y Bhandari, 2010) cuya eficiencia se evalúa principalmente determinando el nivel actividad antioxidante retenido en las cápsulas y evaluando la cinética de liberación.

Este proyecto de investigación planteó la utilización del cáliz de la jamaica como materia prima para extraer sus compuestos antioxidantes y encapsularlos en diferentes polímeros comestibles. Las tecnologías de coacervación con cobertura de alginato y la micro encapsulación por secado por aspersión, son dos estrategias de encapsulación factibles que pueden generar productos funcionales que aumenten no solo el consumo de compuestos fenólicos si no también su bioaccesibilidad.

El proyecto pretende generar una alternativa más para la utilización del cáliz de la jamaica a nivel industrial, a su vez promoverá el cultivo para beneficio de las zonas

productoras. Los conocimientos obtenidos servirán de base para ofrecer una tecnología que permita una utilización alternativa de los cálices de la jamaica en beneficio de la salud humana y de los productores agrícolas.

3.2. Hipótesis

Los biopolímeros funcionan como agentes protectores de los compuestos antioxidantes del cáliz de jamaica.

Los polímeros de encapsulación comestibles modifican la cinética y tiempo de liberación de los compuestos antioxidantes del cáliz de jamaica.

3.3. Objetivo General

Evaluar las cinéticas de liberación de los compuestos antioxidantes del cáliz de la jamaica (*Hibiscus sabdariffa* L.) encapsulados en diferentes polímeros comestibles.

3.4. Objetivos particulares

Elaborar el encapsulado de los compuestos antioxidantes extraídos del cáliz de la jamaica utilizando polímeros portadores y aplicando las técnicas de coacervación, gelificado ionotrópico y secado por aspersión.

Caracterizar los encapsulados a través de análisis fisicoquímicos.

Realizar el proceso de disolución para determinar la capacidad antioxidante y fenoles totales liberados al medio, así como los tiempos de disolución a través de la toma periódica de muestras y el uso de análisis fisicoquímicos.

Modelar las cinéticas de liberación y estimar el tiempo de disolución aplicando modelos matemáticos.

IV. MATERIALES Y MÉTODOS

A continuación se presenta, de forma general, la metodología utilizada para llevar a cabo el trabajo experimental del presente proyecto. Los materiales y referencias a los procedimientos y metodología se describen detalladamente en los capítulos subsiguientes.

4.1. Material

La jamica se obtuvo en el municipio de Chiautla en Puebla, México. Los polímeros se adquirieron en la empresa local Aaldich S.A. de C.V y los reactivos se adquirieron con los laboratorios JT Baker, Sigma-Aldrich, Riedel-de Haën y Fluka. Los equipos empleados para el desarrollo de la investigación fueron suministrados por las Facultades de Ciencias Agrícolas y Química de la UAEM.

4.2. Métodos

El proyecto se dividió en dos fases para su desarrollo; a continuación se describe la metodología de manera general y en los siguientes capítulos se reporta al detalle.

4.2.1. Primera fase: Coacervados – encapsulados con alginato

a) Adquisición del cáliz deshidratado de jamaica, variedad "*criollo*",
b) Preparación del extracto de jamaica, de acuerdo a la metodología propuesta por Serrano-Cruz *et al*. (2013).
c) Concentrado del extracto al vacío.
d) Elaboración de los coacervados con la metodología de DeMars y Ziegler, (2001).
e) Cobertura y gelificado con alginato en $CaCl_2$ por 10, 20, 30 y 40 minutos.
f) Para los coacervados con dos coberturas se repiten los tiempos de gelificado anteriores.
g) Cinética de liberación por el método de Serrano-Cruz *et al*. (2013).
h) Los polifenoles totales de acuerdo a Stevanato *et al*. (2004)
i) DMPD por la metodología de Fogliano *et al*. (1999).
j) FRAP de acuerdo a la metodología propuesta por Benzie y Strain. (1996).

k) El análisis matemático con el programa (Table curve 2D Versión 5.01 Systat Software Inc. Chicago IL, USA).

4.2.2. Segunda fase: Micro encapsulado por secado por aspersión

Se repiten los puntos a, b y c anteriores.

a) Preparación de los biopolímeros portadores (maltodextrina, pectina, grenetina, CMC, Suero de leche, Carragenina y Goma arábiga).
b) Preparación de la solución Extracto-Biopolímero.
c) Encapsulado por secado por aspersión.
d) Determinación de: eficiencia de secado, porcentaje de recuperación, densidad, pH,
e) humedad, Fenoles totales (TPC), tiempos de disolución y modelado de las cinéticas.

V. RESULTADOS

5.1. Release kinetics of antioxidant compounds from *Hibiscus sabdariffa* L. encapsulated in gelatin beads and coated with sodium alginate.

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Release kinetics of antioxidant compounds from *Hibiscus sabdariffa* L. encapsulated in gelatin beads and coated with sodium alginate

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Summary Gelatin beads containing a concentrated extract of Roselle (*Hibiscus sabdariffa* L.) calyx rich in polyphenolic compounds were coated with sodium alginate and ionotropically gelled using $CaCl_2$. Single-coated beads and double-coated beads were obtained by this technique, and the release pattern of the loaded extract was evaluated. As a result, release pattern of these compounds fits properly to a first–order Weibull distribution equation. The release rate constant decreased linearly with the number of alginate coats and with the increase in immersion time in $CaCl_2$ and the *Lag* period increased significantly with the number of alginate coats. The release of *H. sabdariffa's* polyphenols can be well controlled manipulating the number of alginate coats and the immersion time in a $CaCl_2$ solution, allowing not only to control the gastrointestinal segment where they could be released but also to control the release rate with the certainty that the initial concentration will be completely released showing a highly significant antioxidant activity as well.

Keywords Antioxidants, gelatin, *Hibiscus sabdariffa* L., ionotropic gelation, polyphenolic compounds, release kinetics, Roselle.

Introduction

Roselle (*Hibiscus sabdariffa* L. Malvaceae) also called Karkadeh is an annual shrub whose flowers yield a fleshy red calyx used to prepare a light, astringent and sour aqueous infusion. Geographical regions with dry tropical weather like Sudan, Thailand, China, Mexico, Egypt, Senegal and Tanzania are among the main commercial producers of this herbal tea (Domínguez-López *et al*., 2008). In an earlier study, Du & Francis (1973) reported that this calyx contained a significant amount of polyphenols mainly anthocyanins (delphinidin-3-xylosyl-glucoside and cyanidin-3-xylosyl-glucoside) and in a lower quantity the glycosides of the same molecules (delphinidin-3-glucoside and cyanidin-3 glycoside). This finding was recently confirmed by Rodríguez-Medina *et al*. (2009) and later by, Ramirez-Rodrigues *et al*. (2011) who reported that an aqueous extract of Roselle calyx contained hibiscus acid and two derivatives, hydroxybenzoic acid, caffeoylquinic acid, and flavonols as well. According to Lin *et al*. (2011), it has been demonstrated that these bioactive compounds exert many biological effects: protection against oxidative damage induced by tert-butyl hydroperoxide in rat primary hepatocytes, atherosclerosis chemopreventive agent, hepatoprotection, inhibition of the carcinogenic action of various chemicals in different tissues of the rat, cell apoptosis especially in leukaemia and gastric cancer, etc. In general, Roselle extracts exhibit activities against atherosclerosis, liver disease, cancer, diabetes and other metabolic syndromes.

Some authors have reported high antiradical scavenging activity and high antioxidant capacity of both aqueous and ethanolic infusions due to polyphenols' content. For instance, Sáyago-Ayerdi *et al*. (2007)

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reported that Roselle's calyx contains 2.17 ± 0.04 g of extractable polyphenols (expressed in GAE/100 g of dry matter) and that the aqueous infusion (5 g of dried calyx per 100 mL water) showed an antioxidant capacity of 312 ± 7.8 μmol TROLOX/100 mL, measured by the FRAP assay. This significant antioxidant activity is probably the reason of the aforementioned chemopreventive action of this floral accession.

Despite the antioxidant activity expressed by the bioactive compounds of Roselle, the intact forms of polyphenols have limited bioavailability with low circulating levels in plasma (van Duynhoven *et al*., 2011). Frank *et al*. (2005) among other authors, who studied these compounds, reported that after the consumption of an aqueous extract of Roselle, only a low concentration (0.018% of the administered dose) was incorporated into the gastrointestinal tract (GIT) and excreted in urine. This effect could be explained by the inability of the intestine to absorb glycosylated anthocyanins being the deglycosylation a limiting step in anthocyanin absorption and bioavailability. Moreover, once ingested, the polyphenols are bio-transformed/catabolised through the GIT (deglycosylation, dehydroxylation, demethylation, etc.) and each compound derived from this process may express a specific antioxidant activity which collectively might not maintain the same level as the parent compound (van Duynhoven *et al*., 2011).

To preserve the antioxidant function of these bioactive compounds and to control their release in a target site of the GIT, that is, the colon (Shimizu & Hachimura, 2011), protection by natural edible polymer microcapsules has been studied (Fang & Bhandari, 2010). Therefore, the encapsulated polyphenols may be incorporated into conventional foods for a better administration and to avoid the unpleasant taste of high concentration of some molecules (astringency and/or bitterness) as well.

Microcapsules are small vesicles or particles that may range from submicron to several millimetres in size (Dziezak, 1998). According to Fang & Bhandari (2010), many shapes can be produced from the encapsulation, but two major morphologies are commonly seen: mononuclear capsules, which have a single core surrounded by a shell, and aggregates, with many cores embedded in a matrix. Microcapsule's specific shape is determined by the type of technology of the process and by the core and wall materials. Microencapsulation technologies tend to enhance the protection of the core material from adverse environmental conditions, such as undesirable effects of light, moisture and oxygen, thereby contributing to an increase in the shelf life of the product, promoting a better and controlled release of the active molecule (Shahidi & Han, 1993). Some antioxidant compounds' encapsulation technologies include spray drying, emulsion forming, liposome entrapment, inclusion complexation, cocrystallisation, nanoencapsulation, freeze-drying, yeast encapsulation and coacervation.

Saravanan & Panduranga Rao (2010) developed microcapsules employing pectin and alginate by complex coacervation with gelatin and reported that alginate was better than pectin for coacervation with gelatin in terms of less aggregation, smaller particle size and easy dispersion. Both compounds are inexpensive, nontoxic natural polymers and have been used as food additives and as thickening and gelling agents as well. Furthermore, gelatin produces a relatively firm hydrogel in a wide pH range and reacts with polyphenols generating a complex matrix where probably polyphenols act as cross-linkers (Zhang *et al*., 2011), while different concentrations of calcium solutions promote and determine alginate's gel strength (Repka & Singh, 2009). In the area of controlled release, microparticles of indomethacin were coacervated in an alginate-gelatin system to control the release of the active molecule (Joseph & Venkataram, 1995). Alginic acid has also been shown to be beneficial in the development of alginate gel encapsulated, chitosan-coated nanocores, where the alginate acts as a protective agent for sensitive macromolecules such as proteins and peptides to be released in a controlled manner (Rawat *et al*., 2008).

Considering the above-mentioned, the aim of this study was to elaborate gelatin-Roselle extract beads controlling the release rate of Roselle's encapsulated polyphenols by modifying with calcium the strength of multiple layers of alginate surrounding the gelatin beads. Gelatin-Roselle beads have potentially functional qualities to act as nutraceutical ingredients in food products.

Materials and methods

Materials

Roselle dehydrated calyx, 'Criollo' cultivar was purchased from a co-operative of Roselle growers from Chiautla de Tapia, Puebla, Mexico, ground in a blade mill (IKA-Werke, M-20 S3, IKA Works, Inc. Wilmington, NC, USA) and sieved into 0.248–0.418 mm particle size with a #60 mesh. Dehydrated and grounded calyx was stored in hermetically sealed plastic bags until used.

All reagents used for total polyphenols quantification and antioxidant activity determinations (FRAP and DMPD assays) were provided as follows: Folin-Ciocalteu reagent, sodium carbonate, sodium acetate, acetic acid, ferric chloride, ferrous sulphate, sodium chloride were purchased from Sigma-Aldrich (St. Louis, MO. USA). Sodium bicarbonate, hydrochloric acid and methanol were purchased from JT Baker;

2,4,6-tripyridyl-S-triazine (TPTZ) from Fluka; gallic acid from Riedel-de Haën; N,N-Dimethyl-p-phenylenediamine dihydrochloride (DMPD) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Aldrich Chem. Co.; gelatin powder of 280, 290 and 300 Bloom strength trademarks Wilson®, Duche® and Pilsac®, respectively, and sodium alginate were purchased from a local supplier. Spectrometric measurements were done in an UV-Vis spectrophotometer (Genesys 10S UV-Vis Thermo Spectronic; Thermo Fisher Scientific Inc. Waltham, MA, USA)., pH adjustment was done with a Thermo Orion Model 520 (Thermo Fisher Scientific Inc.), and samples were taken using a 5000 μL pipette with an Ankom filter adapted to the tip (ANKOM Technology, Macedon, NY, USA) according to Villanueva-Carvajal *et al.* (2012). Disposable UV-grade methacrylate 1.5 mL cuvettes (Plastibrand®) were used to measure absorbance, and deionised water was used to prepare all solutions.

Methods

Preparation of the Roselle extract

Roselle extract was prepared according to the methodology proposed by Serrano-Cruz *et al.* (2013): 100 g of dehydrated and powdered Roselle calyx was subjected to extraction in 1000 mL of distilled water for 30 min at 50 °C on a hot plate with stirrer. The extract was then filtered and concentrated on a rotary evaporator (R-205V800. Büchi Labortech ink AG. CH-9230. Flawil, Switzerland) until obtaining 24 ± 0.2 g/100 mL. The concentrated product was stored in amber glass bottles at 4 °C until required (24–48 h).

Preparation of gelatin beads

Bead solution was prepared by agitating until total dissolution, 33.25 g of gelatin in 100 mL of distilled water at 80 °C, according to an adapted methodology of DeMars & Ziegler (2001). Subsequently, the obtained solution was cooled to 48 °C and added to 55.3 g of Roselle extract under gentle agitation during 10 min, so that the final concentration of the bead solution was 41.5 g/100 g. To remove air bubbles, the obtained solution was degassed in a Büchi-Vac V-500 equipment (Büchi Corp. New Castle, DE, USA) at 48 °C and 120 mPa pressure, until a translucent liquid was observed (~45 min). To shape the beads, the Roselle-gelatin solution was dropped into canola oil at 4 °C and the drop-shaped beads were maintained at the bottom of the container for 5 min. After this, the beads were drained to remove excess oil, washed in an aqueous solution of ethanol (60 mL/100 mL) agitating them for 10 min and finally drained to remove excess of ethanol at room temperature during 30 min. The obtained beads measured 4.64 ± 0.13, 4.76 ± 0.17 and 4.89 ± 0.26 mm of diameter and weighted 0.061 ± 0.003, 0.069 ± 0.003 and 0.070 ± 0.002 g for 280, 290 and 300 Bloom strength gelatins, respectively.

Coating of the gelatin beads

The gelatin-loaded beads previously obtained were coated with one or two layers of sodium alginate as follows: the beads were dispersed in an aqueous solution of sodium alginate (2 g/100 mL) for 30 s and then immersed into a 0.05 M calcium chloride solution during 10, 20, 30 or 40 min. The coated beads were filtered, dried at room temperature for 3 h and stored in amber glass bottles at 4 °C until polyphenols' release kinetics assays were carried out. The obtained beads measured 4.76 ± 0.12, 5.18 ± 0.14 and 5.2 ± 0.16 mm of diameter and weighted 0.085 ± 0.005, 0.096 ± 0.004 and 0.088 ± 0.003 g for 280, 290 and 300 Bloom strength gelatin, respectively. Separately, coated beads which had been dispersed for 10 min in the solution of calcium chloride were redispersed a second time into the sodium alginate solution for 30 s and then re-immersed in the solution of calcium chloride for 10, 20, 30 or 40 min. The double-coated beads were filtered, dried at room temperature for 3 h and stored in amber glass bottles at 4 °C until polyphenols' release kinetics assays were carried out. Double-coated beads measured 5.05 ± 0.13, 5.79 ± 0.23 and 5.63 ± 0.19 mm of diameter and weighted 0.097 ± 0.004, 0.110 ± 0.006 and 0.116 ± 0.004 g for 280, 290 and 300 Bloom strength gelatin, respectively. Control-beads were prepared according to the previously described methodology using distilled water instead of concentrated Roselle extract.

Polyphenols' release kinetics

Triplicate samples (ten beads) of experimental beads were placed into a beaker containing 50 mL of an aqueous solution of NaCl (0.93 g/100 mL) adjusted to pH 2.0 and heated to 37 °C (Serrano-Cruz *et al.*, 2013). Maintaining constant agitation in a magnetic stirrer, 1 mL samples were collected from the medium at regular intervals (every 10 min until steady state) and were put directly into 1.5 mL spectrometer cuvettes. After doing a visible wavelength scan (not shown), the highest readings were obtained between 519 and 521 nm (λ_{max} = 520 nm, according to Du & Francis, 1973), so colour increase was monitored by measuring absorbance at 520 nm with the UV-Vis spectrophotometer described above. After the colour measurement, samples were poured back into the release medium. Simultaneously and to assess antioxidant activity and total polyphenols release, another triplicate of the same beads was subjected to the same experiment, but in this case, samples were collected from the medium, without adding fresh isotonic solution back to the beaker, and stored in Eppendorf®

vials at 4 °C, until further analysis. Total polyphenols' release was quantified according to an adjusted methodology done by Stevanato *et al.* (2004) using gallic acid aqueous solutions in the range of 0.01–0.1 mg mL^{-1} for calibration, and results were reported as gallic acid equivalents (mg GAE mL^{-1}). Antioxidant activity was calculated by the DMPD method proposed by Fogliano *et al.* (1999) using Trolox methanolic solutions in the range of 12.5–250 mg mL^{-1} for calibration, and results were reported in TEAC (Trolox Equivalents of Antioxidant Capacity) in mmol Trolox mL^{-1}. The FRAP assay was carried out according to the method reported by Benzie & Strain (1996) using heptahydrated ferrous sulphate aqueous solutions in the range of 0.25–2.50 mmol L^{-1}, and results were reported in FRAP equivalents (mmol $FeSO_4$ mL^{-1}). All determinations were done in triplicate.

Data analysis

Experimental absorbance, GAE, FRAP and DMPD data were used to calculate polyphenols' release kinetics fitted by least squares curve implemented in the Table curve 2D software (Table curve 2D Version 5.01 Systat Software Inc. Chicago IL, USA). Parameter estimates of the model proposed in the eqn 1 were calculated using the Levenberg-Marquardt algorithm. The coefficient of determination (R^2) and uncertainty statistics were obtained by regression analysis.

Results and discussion

Polyphenols' release kinetics as a function of gelatin strength

Figure 1 shows the release kinetics of total phenolic content (TPC) as a function of time caused by the disintegration of gelatin beads elaborated with three different Bloom strengths (280, 290 and 300). To model the relationship between the residence time of the beads in the isotonic solution and the release of phenolic compounds, a modified first-order kinetics model was used (eqn 1). This model which is related to the Weibull statistical distribution has been proposed by Papadopoulou *et al.* (2006) to describe the release kinetics of diltiazem and diclofenac and to evaluate the kinetics of hydration of foodstuffs as a function of their porosity and time as well (Marabi *et al.*, 2003; Saguy & Marabi, 2005).

$$\beta_t = \beta_0 + \beta_\infty (1 - e^{-k_s t})^{k_L} \quad (1)$$

Parameter estimates β in eqn (1) represent absorbance at 520 nm or the concentration of each variable quantified (TPC, FRAP or DMPD) per mL at kinetics starting point (β_0), at the steady state or maximum release (β_∞) and at a t time (β_t) in minutes; k_s is the release rate constant for each variable per min, and k_L is the *Lag* period (in minutes). This last coefficient implies that all the kinetics may start with an 'initial burst effect' or conversely, by a significant delay. In the kinetics release models proposed by Rothstein *et al.* (2009), Lao *et al.* (2011), Siepmann *et al.* (1999) and by Siepmann & Peppas (2000), k_s and k_L coefficients have been described and used as numerical estimates of the degree of deformation of the particles (swelling, dissolution, erosion, among other phenomena) and the migration of each loaded compound into the liquid medium during the release process.

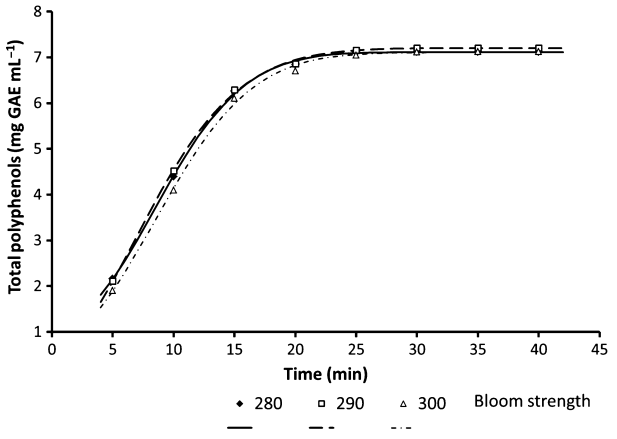

Figure 1 Total phenolic content release profiles of uncoated gelatin beads of three Bloom strengths.

To evaluate whether different gelatin strengths would show different release behaviour, the kinetic parameters were calculated. Table 1 shows the parameter estimates for gelatin beads using eqn (1), standard errors and coefficients of determination (R^2) for gelatin different strengths, demonstrating that the release process follows the Weibull kinetic model. As it can be seen, the three strengths of gelatin showed a similar behaviour: TPC at the steady state (β_∞) occurs at 25 min approximately, the *Lag* period lasts 2.011 min average and the rate constant ranged between 0.087 and 0.095 mg GAE mL^{-1} by minute. An analysis of variance (not shown) of the parameter estimates demonstrates that there are no significant differences between the different Bloom strengths, thus all gelatin Bloom strengths were used as replicates for further comparisons and analyses being not necessary to evaluate different Bloom strengths during the rest of the study.

Bloom strength is essentially the rigidity of a gelatin gel formed and measured under standard conditions. Rao & Singh (2002) conducted a study to determine how sensitivity of cross-linked gelatin materials varied with respect to their bloom strength, type and source and reported that the dissolution time of gelatin in an

Table 1 Parameter estimates, coefficients of determination and standard error of the estimates of the TPC (mg GAE) kinetic release model (eqn 1) for the uncoated gelatin beads of three Bloom strengths

Bloom strength	R^2	β_0	SE β_0	β_∞	SE β_∞	k_s	SE k_s	k_L	SE k_L
280	0.9995	1.191	0.195	5.923	0.205	0.089	0.246	2.144	0.143
290	0.9995	0.627	0.280	6.576	0.291	0.095	0.322	1.832	0.130
300	0.9987	0.862	0.312	6.244	0.329	0.087	0.395	2.058	0.204
Mean	0.9992	0.893	0.262	6.248	0.275	0.090	0.321	2.011	0.159

SE, Standard error of the estimate; β_0, kinetics starting point; β_∞, maximum release at the steady state; k_s, release rate constant; k_L, *Lag* period (min).

aqueous medium increases with the Bloom strength. According to the results reported by these authors, the dissolution time of minimum 250 Bloom strength gelatins was higher than 60 min (40 °C) and the increase in time per Bloom strength unit was 0.16 ± 0.02 min. Therefore, a 280 to 300 Bloom strength gelatins exhibit close dissolution times. This could explain the similarity of the gelatins studied here.

Highly significant linear correlations were obtained when absorbance of the liquid medium, FRAP and DMPD experimental observations against TPC were correlated. Table 2 shows the R^2 coefficients, the standard error of the estimates (SEE), and the intercepts and slopes for each experimental sample. The high correlations obtained show that, as expected, the increase in absorbance and then in the antioxidant activity measured by the FRAP and DMPD assays responds to the incorporation of polyphenols into the isotonic solution. FRAP method quantifies the power to reduce oxidised species, and DMPD quantifies the inhibition of a radical cation, both methods are used to measure antioxidant activity of foodstuffs (Singh & Singh, 2008). In 2003, Nielsen *et al.* reported a good correlation between results of FRAP and DMPD assays in black currant anthocyanins and more recently Fu *et al.* (2010) reported a high correlation between antioxidant capacity (measured by the FRAP assay) and TPC in Chinese wild fruits. Villanueva-Carvajal *et al.* (2012) reported high correlations between TPC, FRAP and DMPD methods during an *in vitro digestion* of Roselle calyx as well. According to these results, monitoring the colour release measured as absorbance of the liquid medium could be an adequate indicator of the release profile of the antioxidant compounds from the beads.

Table 2 Parameter estimates, coefficients of determination and standard error of the estimates for linear regression between the Absorbance, FRAP, DMPD and TPC release profile for different strength's gelatin beads

Variables					
Independent	**Dependent**	R^2	**Intercept**[a]	**Slope**	**SE**
TPC	Absorbance	0.9983	−0.0090	0.0558	0.0046
TPC	FRAP	0.9866	−4.2459	19.6184	4.5905
TPC	DMPD	0.9994	−18.6580	77.3016	3.8855
FRAP	Absorbance	0.9942	0.0059	0.0028	0.0085
FRAP	DMPD	0.9868	3.8761	3.8891	17.8567
DMPD	Absorbance	0.9984	0.0046	0.0007	0.0045

SE, Standard error of the estimate.
[a]All Intercept coefficients were not significant ($P > 0.05$).

Colour release kinetics as a function of alginate coats and ionotropic gelation

The use of loaded particles coated with different polymers (other than the composition of the same particle) has been investigated to control the release kinetics of the loaded compounds (Coppi *et al.*, 1998; Pasparakis & Bouropoulos, 2006; Sriamornsak & Kennedy, 2006). These heterogeneous particles could be considered as reservoir systems, according to the classification pattern for primarily diffusion controlled drug delivery systems proposed by Siepmann & Siepmann (2008).

To obtain particles of optimum strength so that the delay in the release process could be increased, single- and double-coated gelatin beads were subjected to four different dispersion times in $CaCl_2$ solution, increasing with this procedure the concentration of calcium in the bead's coating. Figure 2 shows the observed and predicted release profile of all the different beads, by monitoring the absorbance. It could be observed that for the one-coated beads an increase from 10 to 30 min of immersion time could slow down the release time from 40 to 80 min approximately, so the time to reach the maximum release increased significantly with the dispersion time. This effect is enhanced when another coat is added so the double-coated beads can increase the release time from 120 to 220 min just adding 20 min of immersion time. Interestingly, the release of polyphenols was also constant during the whole process, and there were no significantly differences between the maximum amounts reached, concluding that the number of coats and immersion time does not affect the final concentration of polyphenols but that those variables could effectively control the release rate. Table 3 displays the parameter estimates of this

behaviour and the uncertainty statistics of the kinetics model proposed in eqn 1. This table highlights the effects of dispersion time in $CaCl_2$ and the number of coats on the rate constant k_s. For the uncoated beads, this parameter was 0.087 min^{-1}, while for the single-coat beads at 10 min of dispersion time, it can be seen a decrease to 0.043 min^{-1} (50.6%). When a second coat and 10 min were added, k_s decreased to 0.011 min^{-1}, which means a decrease of 87.2% of the original k_s value. The decrease in the k_s value as a function of the dispersion time was significantly linear ($P < 0.01$) with a slope of −0.0007 ($R^2 = 0.9302$) for the single-coated beads and −0.0002 ($R^2 = 0.9934$) for the double-coated beads. The alginate coat acts as a porous barrier through which the liquid medium is migrating causing swelling, erosion and cracks, among other phenomena, and then the loaded compound is transferred to the medium (Siepmann & Siepmann, 2008). Therefore, swelling is critical in the release of active molecules from or through hydrogels (Sriamornsak & Kennedy, 2006). According to Crcarevska *et al.* (2008), a greater extent of calcium chloride limits the swelling ratio of beads, thus swelling and diffusion processes could explain the results obtained.

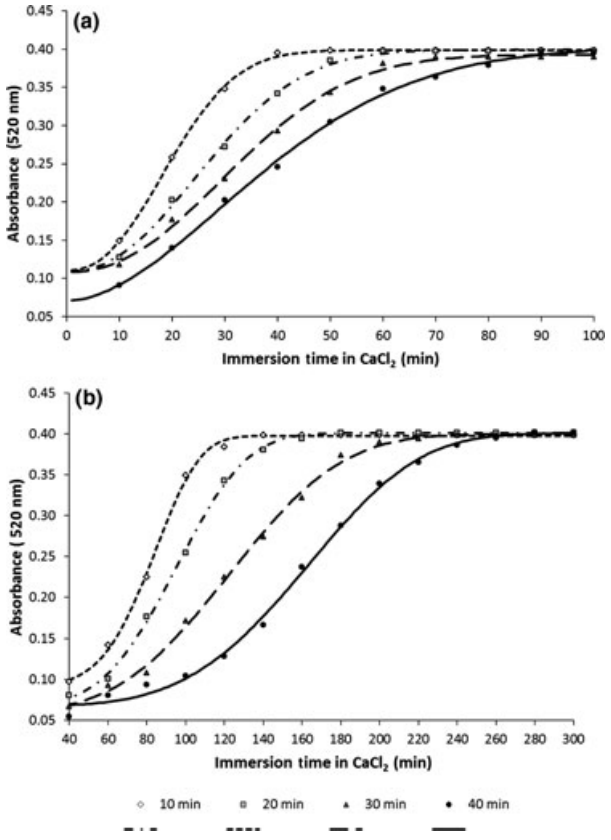

Figure 2 Predicted and observed colour release kinetics as a function of dispersion time and number of coats. (a) single-coated beads, (b) double-coated beads.

On the other hand, the multilayer coating structure of the beads contributes in decreasing the release rate of the polyphenols as it is also increased the dispersion time in the calcium chloride solution, but none of those variables stop the release rate at all, showing the same behaviour of the uncoated beads but with a better control of the release. In alginates, Ca^{2+} is known to produce a gelled network by establishing interactions between calcium ions and the alginate structure when there are two or more neighbouring guluronic acid residues, creating the so-called 'egg-box' structures (Perez-Moral *et al.*, 2013). In other words, addition of calcium chloride causes cross-linking of the sodium alginate. Therefore, mechanical properties, water and drug permeability and swelling behaviour of the resulted cross-linked polymer will vary with the degree of cross-linking (Remuñán-López & Bodmeier, 1997).

These results show correspondence with those obtained by Sriamornsak & Kennedy (2006) when

Table 3 Parameter estimates, coefficients of determination and standard error of the estimates of the absorbance (520 nm) kinetics release model (eqn 1) for the uncoated and coated gelatin beads

Coats	DT	R^2	β_0	SE β_0	β_∞	SE β_∞	k_s	SE k_s	k_L	SE k_L
0	Mean	0.999	0.044	0.004	0.349	0.004	0.087	0.001	1.934	0.035
1	10	0.999	0.110	0.008	0.288	0.008	0.043	0.001	2.313	0.135
1	20	0.998	0.109	0.009	0.290	0.010	0.031	0.001	2.228	0.184
1	30	0.997	0.108	0.010	0.285	0.011	0.026	0.001	2.214	0.204
1	40	0.998	0.071	0.011	0.332	0.016	0.022	0.001	1.814	0.162
2	10	0.999	0.093	0.006	0.305	0.006	0.011	0.000	4.908	0.295
2	20	0.999	0.067	0.005	0.333	0.005	0.010	0.000	3.926	0.162
2	30	0.998	0.062	0.007	0.338	0.008	0.007	0.000	3.161	0.189
2	40	0.997	0.064	0.006	0.351	0.012	0.005	0.000	3.187	0.223

DT, Dispersion time in $CaCl_2$; SE, Standard error of the estimate; β_0, kinetics starting point; β_∞, maximum release at the steady state; k_s, release rate constant; k_L, *Lag* period (min).

developed calcium alginate gel-coated pellets for oral administration. According to these authors, increased calcium content, represented by increasing residence time in calcium chloride solution in the present study, reflects an increased level of cross-linking, which increases the barrier for the free diffusion of drugs. Presumably, this is due to an increase in molecular crowding and possibly a reduction in the void volume within the gels.

Figure 3 shows the theoretically predicted and experimentally verified release profile of the beads uncoated and covered with one or two alginate coats by monitoring the TPC of the isotonic solution using the model described by eqn 1, adding evidence to the conclusions shown above. Table 4 shows the parameter estimates and uncertainty statistics of this mathematical model for beads with different number of coats. As it can be seen, the rate constant (k_s) declines, while the k_L parameter enhances as the number of coats increases. Furthermore, the β_0 and β_∞ parameters showed no significant differences between beads although β_∞ parameter was slightly higher for the uncoated samples. The time needed to achieve the steady state concentration was higher in the case of the double-coat beads, but reached the same maximum concentration of polyphenols released as the uncoated and single-coated beads. Although a slightly decrease in FRAP and DMPD is shown for single- and double-coated beads, there are no significant differences.

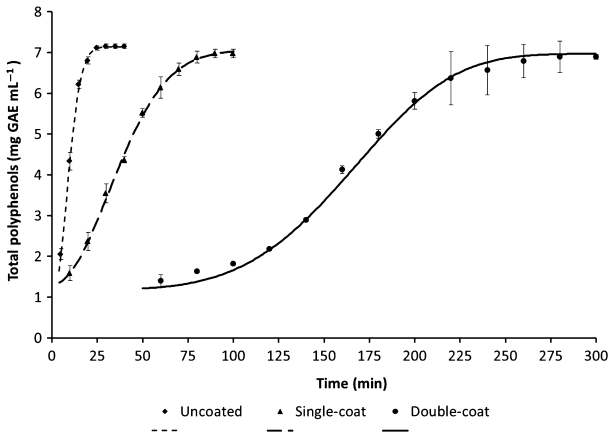

Figure 3 Predicted and observed total phenolic content release profiles as a function of the number of bead's coats (immersion time: 40 min)

Conclusion

Gelatin beads containing a concentrated extract of *H. sabdariffa's* calyx rich in phenolic compounds were coated with sodium alginate and gelled with calcium chloride by ionotropic gelation. Single-coated beads and double-coated beads were obtained by this technique, and the release pattern of the loaded extract was evaluated. Results obtained showed that gelatin Bloom strength has no influence on the release of phenolic compounds from the bead's matrix. The release pattern of these compounds fits properly to a first-order Weibull distribution, and the parameter estimates of this kinetic model were used to evaluate the effect of dispersion time in calcium chloride and of the number of coats on the release pattern of polyphenols. For coated beads, the release rate constant of the kinetic model decreased linearly with the increase in dispersion time in calcium chloride and with the number of coats. On the other hand, the *Lag* period increased significantly with the number of alginate coats and with the dispersion time in calcium chloride. The release of *H. sabdariffa*'s polyphenols can be well controlled manipulating the number of alginate coats and the immersion time in a $CaCl_2$ solution, allowing not only to control the gastrointestinal segment where they could be released (by controlling the release time) but also to control the release rate of these phenolic

Table 4 Parameter estimates, coefficients of determination and standard error of the estimates of the TPC, FRAP and DMPD kinetics release model for the uncoated and coated gelatin beads

Assay	Coats	R^2	β_0	SE β_0	β_∞	SEβ_∞	k_s	SE k_s	k_L	SE k_L
TPC	0	0.9992	0.893	0.262	6.248	0.275	0.090	0.321	2.011	0.159
	1	0.9974	1.310	0.187	5.748	0.249	0.023	1.280	2.000	0.180
	2	0.9963	1.187	0.111	5.783	0.198	0.006	3.278	4.142	0.335
FRAP	0	0.9971	14.808	10.237	123.938	10.944	0.083	0.732	1.783	0.266
	1	0.9983	26.887	2.915	110.579	4.155	0.022	1.112	2.009	0.145
	2	0.9981	24.239	1.703	107.795	2.662	0.006	2.009	4.731	0.267
DMPD	0	0.9993	77.932	15.449	455.317	16.313	0.086	0.263	2.275	0.161
	1	0.9979	96.089	13.069	438.524	17.963	0.022	1.203	1.938	0.162
	2	0.9977	96.964	7.504	422.555	10.784	0.006	2.091	4.555	0.299

SE, Standard error of the estimate; β_0, kinetics starting point; β_∞, maximum release at the steady state; k_s, release rate constant; k_L, *Lag* period (min).

compounds with the certainty that the initial concentration will be completely released showing a highly significant antioxidant activity as well.

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References

Benzie, I.F. & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": the FRAP assay. *Analytical Biochemistry*, **239**, 70–76.

Coppi, G., Lannuccelli, V. & Cameroni, R. (1998). Polysaccharide film-coating for freely swellable hydrogels. *Pharmaceutical Development and Technology*, **3**, 347–353.

Crcarevska, M.S., Dodov, M.G. & Goracinova, K. (2008). Chitosan coated Ca-alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa. *European Journal of Pharmaceutics and Biopharmaceutics*, **68**, 565–578.

DeMars, L.L. & Ziegler, G.R. (2001). Texture and structure of gelatin/pectin-based gummy confections. *Food Hydrocoloids*, **15**, 643–653.

Domínguez-López, A., Remondetto, G.E. & Navarro-Galindo, S. (2008). Thermal kinetic degradation of anthocyanins in a roselle (*Hibiscus sabdariffa* L. cv. "Criollo ") infusion. *International Journal of Food Science and Technology*, **43**, 322–325.

Du, C.T. & Francis, F.J. (1973). Anthocyanins of roselle (*Hibiscus sabdariffa* L.). *Journal of Food Science*, **38**, 810–812.

van Duynhoven, J., Vaughan, E.E., Jacobs, D.M. *et al.* (2011). Metabolic fate of polyphenols in the human superorganism. *Proceedings of the National Academy of Sciences of the United States of America*, **108S**(1), 4531–4538.

Dziezak, J.D. (1998). Microencapsulation and encapsulated food ingredients. *Food Technology*, **42**, 136–151.

Fang, Z. & Bhandari, B. (2010). Encapsulation of polyphenols - a review. *Trends in Food Science & Technology*, **21**, 510–523.

Fogliano, F., Verde, V., Randazzo, R. & Rietieni, A. (1999). Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *Journal of Agricultural and Food Chemistry*, **47**, 1035–1040.

Frank, T., Janssen, M., Netzel, M. *et al.* (2005). Pharmacokinetics of anthocyanidin-3-glycosides following consumption of *Hibiscus sabdariffa* L. extract. *Journal of Clinical Pharmacology*, **45**, 203–210.

Fu, L., Xu, B.T., Xu, X.R., Qin, X.S., Gan, R.Y. & Li, H.B. (2010). Antioxidant Capacities and Total Phenolic Contents of 56 Wild Fruits from South China. *Molecules*, **15**, 8602–8617.

Joseph, I. & Venkataram, S. (1995). Indomethacin sustained release from alginate-gelatin or pectin-gelatin coacervates. *International Journal of Pharmaceutics*, **125**, 161–168.

Lao, L.L., Peppas, N.A., Chiang Boey, F.Y. & Venkatramana, S.S. (2011). Modeling of drug release from bulk-degrading polymers. *International Journal of Pharmaceutics*, **418**, 28–41.

Lin, H.-H., Chen, J.-H. & Wang, C.-J. (2011). Chemopreventive properties and molecular mechanisms of the bioactive compounds in *Hibiscus sabdariffa* Linne. *Current Medicinal Chemistry*, **18**, 1245–1254.

Marabi, A., Livings, S., Jacobson, M. & Saguy, I.S. (2003). Normalized Weibull distribution for modeling rehydration of food particulates. *European Food Research & Technology*, **217**, 311–318.

Nielsen, I.L.F., Haren, G.R., Magnussen, E.L., Dragsted, L.O. & Rasmussen, S.E. (2003). Quantification of anthocyanins in commercial black currant juices by simple high-performance liquid chromatography. Investigation of their pH stability and antioxidative potency. *Journal of Agricultural and Food Chemistry*, **51**, 5861–5866.

Papadopoulou, V., Kosmidis, K., Vlachou, M. & Macheras, P. (2006). On the use of the Weibull function for the discernment of drug release mechanisms. *International Journal of Pharmaceutics*, **309**, 44–50.

Pasparakis, G. & Bouropoulos, N. (2006). Swelling studies and *in vitro* release of verapamil from calcium alginate and calcium alginate–chitosan beads. *International Journal of Pharmaceutics*, **323**, 34–42.

Perez-Moral, N., Chueca-Gonzalez, M. & Parker, R. (2013). Preparation of iron-loaded alginate gel beads and their release characteristics under simulated gastrointestinal conditions. *Food Hydrocolloids*, **31**, 114–120.

Ramirez-Rodrigues, M.M., Plaza, M.L., Azeredo, A., Balaban, M.O. & Marshall, M.R. (2011). Physicochemical and phytochemical properties of cold and hot water extraction from *Hibiscus sabdariffa*. *Journal of Food Science*, **76**, 429–435.

Rao, K.V.R. & Singh, S. (2002). Sensitivity of gelatin raw materials to cross-linking: the influence of bloom strength, type, and source. *Pharmaceutical technology*, **26**, 42–46.

Rawat, M., Singh, D., Saraf, S. & Saraf, S. (2008). Development and *in vitro* evaluation of alginate gel encapsulated, chitosan-coated ceramic nanocores for oral delivery of enzyme. *Drug Development and Industrial Pharmacy*, **34**, 181–188.

Remuñán-López, C. & Bodmeier, R. (1997). Mechanical, water uptake and permeability properties of crosslinked chitosan glutamate and alginate films. *Journal of Controlled Release*, **44**, 215–225.

Repka, M.A. & Singh, A. (2009). Alginic Acid. In: *Handbook of Pharmaceutical Excipients*, 6th edn (edited by R.C. Rowe, P.J. Sheskey & M.E. Quinn). Pp. 20–22. Grayslake, IL: Pharmaceutical Press.

Rodríguez-Medina, I.C., Beltrán-Debón, R., Micol Molina, V. *et al.* (2009). Direct characterization of aqueous extract of *Hibiscus sabdariffa* using HPLC with diode array detection coupled to ESI and ion trap MS. *Journal of Separation Science*, **32**, 3441–3448.

Rothstein, S.N., Federspiel, W.J. & Little, S.R. (2009). A unified mathematical model for the prediction of controlled release from surface and bulk eroding polymer matrices. *Biomaterials*, **30**, 1657–1664.

Saguy, I.S. & Marabi, A. (2005). Rehydration of dried food particulates. *Encyclopedia of Agricultural, Food, and Biological Engineering*, **1**, 1–9.

Saravanan, M. & Panduranga Rao, K. (2010). Pectin-gelatin and alginate-gelatin complex coacervation for controlled drug delivery: influence of anionic polysaccharides and drugs being encapsulated on physicochemical properties of microcapsules. *Carbohydrate Polymers*, **80**, 808–816.

Sáyago-Ayerdi, S.G., Arranz, S., Serrano, J. & Goñi, I. (2007). Dietary fiber content and associated antioxidant compounds in roselle flower (*Hibiscus sabdariffa* L.) beverage. *Journal of Agricultural and Food Chemistry*, **55**, 7886–7890.

Serrano-Cruz, M.R., Villanueva-Carvajal, A., Morales Rosales, E.J., Ramírez Dávila, J.F. & Dominguez-Lopez, A. (2013). Controlled release and antioxidant activity of Roselle (*Hibiscus sabdariffa* L.) extract encapsulated in mixtures of carboxymethyl cellulose, whey protein, and pectin. *LWT - Food Science and Technology*, **50**, 554–561.

Shahidi, F. & Han, X.Q. (1993). Encapsulation of food ingredients. *Critical Reviews in Food Science and Nutrition*, **33**, 501–547.

Shimizu, M. & Hachimura, S. (2011). Gut as a target for functional food. *Trends in Food Science & Technology*, **22**, 646–650.

Siepmann, J. & Peppas, N.A. (2000). Hydrophilic matrices for controlled drug delivery: an improved mathematical model to predict

the resulting drug release kinetics (the "sequential layer" model). *Pharmaceutical Research*, **17**, 1290–1298.
Siepmann, J. & Siepmann, F. (2008). Mathematical modeling of drug delivery. *International Journal of Pharmaceutics*, **364**, 328–343.
Siepmann, J., Kranz, H., Bodmeier, R. & Peppas, N.A. (1999). HPMC-matrices for controlled drug delivery: a new model combining diffusion, swelling, and dissolution mechanisms and predicting the release kinetics. *Pharmaceutical Research*, **16**, 1748–1756.
Singh, S. & Singh, R.P. (2008). *In vitro* methods of assay of antioxidants: an overview. *Critical Reviews in Food Science and Nutrition*, **48**, 649–671.
Sriamornsak, P. & Kennedy, R.A. (2006). Development of polysaccharide gel-coated pellets for oral administration. 2. Calcium alginate. *European Journal of Pharmaceutical Sciences*, **29**, 139–147.
Stevanato, R., Fabris, S. & Momo, F. (2004). New enzymatic method for the determination of total phenolic content in tea and wine. *Journal of Agricultural and Food Chemistry*, **52**, 6287–6293.
Villanueva-Carvajal, A., Bernal-Martínez, L.R., García-Gasca, M.T. & Dominguez-Lopez, A. (2012). *In vitro* gastrointestinal digestion of *Hibiscus sabdariffa* L.: the use of its natural matrix to improve the concentration of phenolic compounds in gut. *LWT-Food Science and Technology*, **51**, 260–265.
Zhang, Z.-Q., Pan, C.-H. & Chung, D. (2011). Tannic acid cross-linked gelatin–gum arabic coacervate microspheres for sustained release of allyl isothiocyanate: characterization and *in vitro* release study. *Food Research International*, **44**, 1000–1007.

5.2. Assessing release kinetics and dissolution of spray-dried Roselle (*Hibiscus sabdariffa* L.) extract encapsulated with different carrier agents

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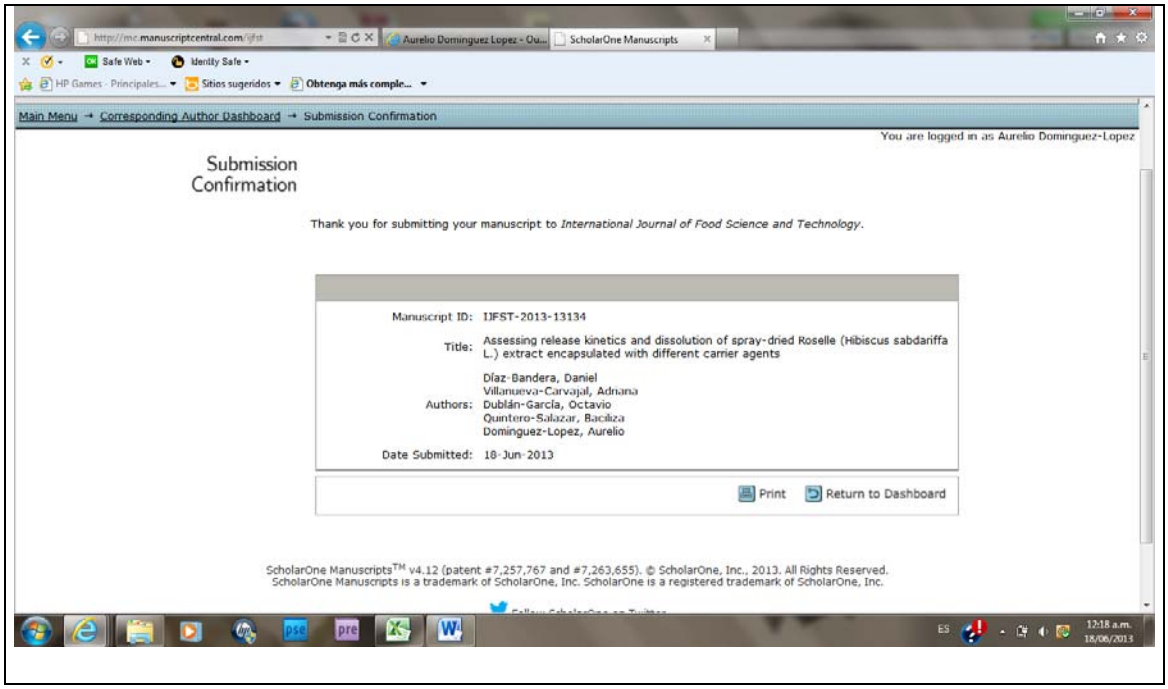

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Assessing release kinetics and dissolution of spray-dried Roselle (*Hibiscus sabdariffa* L.) extract encapsulated with different carrier agents

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Abstract

The aim of this study was to investigate the effect of different carriers (maltodextrin, pectin, gelatin, carboxymethyl cellulose, whey protein, carrageenan and arabic gum) on the release kinetics and dissolution of *H. sabdariffa*'s extract obtained through a spray drying process. All the powders obtained followed a Weibull-First Order kinetic model and a dissolution measure method has been proposed. Pectin retained the higher amount of polyphenols after the drying process (98.20%) and released the higher amount of phenolic compounds (79.48%) during a longer period of time.

Keywords

Roselle calyx; *Hibiscus sabdariffa* L.; Spray-drying; Release kinetics; Polyphenols.

Introduction

Roselle (*Hibiscus sabdariffa* L., Malvaceae) also known as Karkadé is an annual shrub grown in tropical dry weather, native to tropical Africa that can be found in countries like Malaysia, India, Thailand, Indonesia, Saudi Arabia, Vietnam, Philippines, Sudan, Egypt and Mexico (Abu-Tarboush, Ahmed, & Al Kahtani, 1997; Chewonarin, *et al*. 1999). Roselle´s calyx are usually used to prepare an herbal beverage (Tsai, et al. 2002; Hirunpanich, *et al*. 2006) characterized by a brilliant red color, given mainly by the anthocyanins delphinidin-3-xylosil-glucoside and cyanidin-3-xylosil-glucoside (Wong, *et al*. 2002). The health or nutraceutical effects attributed to the consumption of Roselle's beverage have been extensively reported (antidiabetic, cardio protective, antihypertensive and low density lipoprotein antioxidation action) (Lin *et al*., 2011; Nnam & Onyeke, 2003).

Roselle's polyphenols have shown to be beneficial for gut's health acting as chemoprotective and chemopreventive agents against colon cancer (Athar *et al*. 2007). The effectiveness of nutraceutical products in preventing diseases depends, among other reasons, on increasing the concentration of the active compound, consequently increasing the bioaccesibility and preserving the activity of the molecule of interest. To increase the concentration of those compounds it is required to provide protection to the molecule responsible of the nutraceutical activity, from the consumption until the bioactive compound reaches the target tissue. Different kinds of materials have been used to achieve this goal acting as carriers. An interesting alternative to transfer those bio functional properties to a solid phase in food carriers is the use of the spray-drying process. This technique minimizes the loss of nutrients content in processing and storage, the powder obtained is soluble and convenient to carry anywhere, it requires less storage space and it is one of the most commonly used methods to transform a wide range of liquid food products into powder (Favaro-Trindade, *et al*, 2010). Some of the most used materials include pectin, whey protein, carrageenan, carboxymethyl cellulose (CMC), gelatin and xanthan gum among others, trying to protect the core of the particle against adverse environmental conditions, providing at the same time, an easy handling and release rate control (Sanguansri & Augustin, 2006).There are some characteristics of the carriers that must be considered when applying spray dry process, thermoplasticity, hygroscopicity and stickiness to mention some (Bhandari *et al*., 1997; Adhikari, 2004) and the performance of a spray dryer could be evaluated by the product recovery. Material loss in a spray drying system is due mostly to the adhesion of droplets to the wall of the system (Maa *et al*. 1998). According to Freudig, Hogekamp, and Schubert (1999) the reconstitution process in water depends on the wetting, which is defined as the ability of a bulk powder to imbibe a liquid,

and depends on some physicochemical parameters as, density, porosity, surface charge, surface area and particle size, this last parameter is very important as it influences the hydration. The major determinant of hydration kinetics is the particle size, which reflects the changes in surface area exposed to water. Galet *et al*. (2004) have been described four steps in the reconstitution process: wetting, submersion, dispersion, and dissolving. The wetting and dissolution processes of powders have been frequently described (Buckton, 1990; Hogekamp & Schubert, 2003). Faldt and Bergenstahl (1996) separately measured the re-dispersion and dissolution of spray-dried powders, but did not point out which step was rate-limiting. The aim of this study was to investigate the effect of different carriers (maltodextrin, pectin, gelatin, carboxymethyl cellulose, whey protein, carrageenan and arabic gum) on the dissolution and release kinetic of Roselle's extract and carriers-Roselle's powders obtained through a spray drying process.

2. Materials and Methods

2.1 Materials

H. sabdariffa's dehydrated calyx, "Criollo" cultivar, was purchased from a co-operative of Roselle's growers located in Chiautla de Tapia, Puebla, Mexico, grounded in a blades-mill (IKA-Werke, M-20 S3, IKA Works, Inc. Wilmington, NC, USA) and sieved into 0.248-0.418 mm particle size with a #60 mesh. Dehydrated and grounded calyxes were stored in hermetic sealed plastic bags until used. Folin-Ciocalteu reagent and arabic gum (ARAB) were purchased from Sigma-Adrich and sodium bicarbonate from Aldrich Chem. Co. Gelatin powder (GELA) (Duche®), maltodextrin (MALTO), pectin (PECT), carboxymethyl cellulose (CMC), whey protein (WHEY) and carrageenan gum (CARR)

were purchased from a local supplier. Spectrometric measurements were done in an UV-Vis spectrophotometer (Genesys 10S UV-Vis Thermo Spectronic, Thermo Fisher Scientific Inc. Waltham, MA, USA). Dissolution test was carried out by mixing in a Vortex Mixer (Barnstead/Thermolyne Maxi-Mix II, M63215, Iowa U.S.A), pH measurements were done with a glass electrode pH-meter (Thermo Orion Model 520, Thermo Fisher Scientific Inc. Waltham, MA, USA), and total soluble solids contents (°Brix) were measured using a refractometer (N1 Brix 0~32%, Atago, Tokyo, Japan). Samples were taken out using a 5000 µL pipette with an Ankom filter adapted to the tip (ANKOM Technology, Macedon, NY, USA). Disposable UV-grade methacrylate 1.5 mL cuvettes (Plastibrand®) were used to measure absorbance and deionized water was used to prepare all solutions.

2.2 Methods

2.2.1 Roselle's concentrated extract elaboration process

Roselle's extract was prepared according to the methodology proposed by Serrano-Cruz *et al*. (2013): 100 g of Roselle's calyxes (#60 mesh) were subjected to extraction in 1000 mL of distilled water for 30 min at 50 °C. The extract was then filtered (# 2) and concentrated on a rotary evaporator (R-205V800, Büchi Labortech ink AG. CH-9230, Flawil, Switzerland) until a final concentration of 6 ± 0.2g /100 mL. The concentrated extract was stored in amber glass bottles at 4° C until required.

2.2.2 Carrier's solutions elaboration process

Carrier's solutions (MALTO, PECT, GELA, CMC, WHEY, CARR and ARAB) were prepared according to the following concentrations (g 100 mL^{-1}): 7.5, 3.0, 3.0, 0.5, 4.0, 1.0,

and 4.0 respectively. These concentrations were selected based on each carrier dissolution properties according to preliminary trials (not shown).

2.3 Carrier-Roselle mixture elaboration process

Maintaining constant agitation, carrier agents' solutions were added to Roselle's concentrated extract (1:1.25 v/v) until a final volume of 900 mL. Roselle-carrier's mixtures were filtered (# 2) and maintained at 30 ± 0.5 °C prior to be subjected to the spray drying procedure.

2.4 Spray drying procedure

The spray drying procedure was performed in a pilot scale spray dryer (assembled at Faculty of Chemistry at Universidad Autónoma del Estado de México) under the following conditions: nozzle diameter 0.5 mm, compressor air pressure 4.0 ± 0.5 bar, feed flow rate 7.5 mL min^{-1}, and inlet and outlet air temperature 155 ± 4.1 and 55 ± 2 °C, respectively. The feed was pulverized in a rotary atomizer with an airflow produced by a blower with an air flow rate at 56 ± 2 $m^3 h^{-1}$. Samples from the collection vessel were recovered, weighed, sealed in amber glass flasks and stored in dark at 4 °C until further analysis. Drying efficiency and powder recovery (Fang & Bhandari, 2011) were calculated. Particles deposited on the dryer chamber were discarded. All processes and analyses were carried out in triplicate.

2.5 Analysis of carrier-Roselle's powders

The spray-dried powders were characterized according to their bulk density, pH, moisture content, total phenolic compounds content (TPC) and dissolution kinetics, as described below. All determinations were carried out in triplicate.

2.5.1 Bulk density

Bulk density (g/mL) was determined by gently adding 2 g of powder sample into an empty 10 mL graduated cylinder and holding the cylinder on a vortex vibrator for 1 min and was calculated according to Goula, Adamopoulos, and Kazakis, (2004).

2.5.2 pH determination

Carrier-Roselle's powder pH was determined in a sample-deionized water 1:5 (w/v) solution at 20 °C, according to the method described by Tuyen *et al*. (2010).

2.5.3 Moisture determination

The moisture content was determined according to the Tuyen *et al*., (2010). Triplicate samples of Roselle extract and carriers-Roselle's powders (20 mg each) were dried in a vacuum oven (Thermoline Scientific, Australia) at 70°C for 24 hours and 500 mbar.

2.5.4 Total Phenolic Compounds (TPC)

Total phenolic compounds were quantified according to a modified spectrophotometric method reported by Villanueva-Carvajal *et al*., (2013) using gallic acid solutions (0.01-0.1 mg mL^{-1}) for calibration. 50 mg of spray-dried samples were diluted in 25 mL deionized water, 0.5 mL of carrier-Roselles' solutions and Roselle's liquid extract was diluted in 10 mL deionized water for an adequate measurement. Results are expressed in mg of gallic

acid equivalents (GAE) per g of dry material. TPC retention after spray drying was also calculated.

2.5.5 Release kinetics and dissolution test

The release kinetics and the dissolution test were carried out according to the methods described by Díaz-Bandera *et al*. (2013) and Quek, Chok, and Swedlund (2007) respectively. 50 mg of spray dried Roselle concentrated extract or carrier-Roselle's samples were dissolved in 50 mL of deionized water, mixed immediately at half speed using a Vortex Mixer. Samples of 500 µL, with replacement, were taken every 3 seconds until total dissolution and were analyzed at 520nm. Measurements were done in triplicate.

3. Results and discussion

3.1 Characterization of the carrier-Roselle powders obtained

Table 1 shows a preliminary characterization of the powders obtained after the spray drying process of Roselle's extract using different carriers. It is important to mention that total amount of solids obtained were different because different concentrations of carriers were used due to their different viscosity properties (preliminary studies not shown). From this Table 1 it could be highlighted that the bulk density for CARR-Roselle powder was the highest while GELA-Roselle powder exhibits the lower value. Retention of polyphenols' after the drying process could be an indicative of the capability of different polymers to protect the structure and the function of these active molecules thru the drying process. Roselle's extract subjected to drying conditions without any polymer to protect its antioxidant compounds retained 72.06 g/100 g of polyphenols while the mixtures CARR-Roselle and PECT-Roselle retained 94.3 and 98.2 g/100 g respectively.

3.5.9. Polyphenols' release kinetics as a function of the carrier

Figure 1 shows the release kinetics of total phenolic content (TPC) as a function of time caused by the disintegration of the carrier-Roselle powders. In order to model the relationship between the residence time of the powders in the aqueous solution and the release of phenolic compounds (measured by the absorbance increase at 520 nm), a modified first order kinetics model was used (Equation 1). This model which is related to the Weibull statistical distribution has been proposed by Papadopoulou *et al.* (2006) to describe the release kinetics of diltiazem and diclofenac, and to evaluate the kinetics of hydration of foodstuffs as a function of their porosity and time as well (Marabi *et al.*, 2003). Recently it has been used by Díaz-Bandera *et al*. (2013) to evaluate the release kinetics of antioxidant compounds from *H. sabdariffa* encapsulated in gelatin beads and coated with sodium alginate.

$$\frac{\beta_t}{\beta_\infty} = (1 - e^{-k_s t})^{k_L} \qquad (1)$$

Parameter estimates β in Equation 1 represent Absorbance at 520 nm at the steady state or maximum release (β_∞) and at a *t* time (β_t) in seconds; k_s is the release rate constant for this variable per second, and k_L is the *Lag* period (in seconds). This last coefficient implies that all the kinetics may start with an "initial burst effect" or conversely, by a significant delay. In the kinetics release models proposed by Rothstein *et al.* (2009), Lao *et al.* (2011), Siepmann *et al.* (1999) and by Siepmann and Peppas, (2000), k_s and k_L coefficients have been described and used as numerical estimates of the degree of deformation of the particles (swelling, dissolution, erosion, among other phenomena) and the migration of each loaded compound into the liquid medium during the release process.

In order to evaluate if different carrier agents would show different release behavior, the kinetic parameter estimates (β and *k* values) were calculated using Equation 1 and reported in Table 2. The small standard error values and high coefficients of determination (R^2) for each carrier show that the release process follows the Weibull-First Order kinetic model. Results shown in Table 2 indicate significant differences between the behaviour of the mixtures carrier-Roselle during the release of the polyphenols, besides same Table 1 shows that the carrier-Roselle mixtures are grouped as a function of the Least Significant Difference (LSD) for each parameter estimate defined in Equation 1. Regarding the release of the non-encapsulated extract, it could be seen that at the steady state (β_∞), the absorbance reached a maximum of 0.624 due to its dissolution in the liquid medium. Taking this into account, it was also observed that all the carriers, expect WHEY, presented a lower absorbance than the non-encapsulated extract at the steady state (β_∞), showing their capability of retaining Roselle´s polyphenols. CARR presented the highest retention of polyphenols that is, the lowest β_∞ value, following by CMC, GELA, ARAB and with the lowest retention ability the group formed by MALTO and PECT. Regarding the rate constant, it was observed that PECT presented the lowest value for this constant, followed by a statistically similar group formed by GELA, CMC and CARR. This group releases the polyphenols at the same release rate that shows the non-encapsulated extract. ARAB's rate constant is significantly higher, followed by the group formed by MALTO and WHEY with higher liberation rates. Concerning the lag period, there were no differences between carriers and as an average all of them presented 0.299 time units except for WP whose value was significantly higher (1.5 time units).

An additional application of Equation 1 parameter estimates is the assessment of the dissolution time (t_D) of the carrier-Roselle mixtures, that is, the moment when the powder

(particles) have been dissolved, releasing all the loaded molecules and producing, as a consequence, the highest value of the measured variable, β_∞, in this case, the absorbance. Theoretically, this value is reached at an infinite time, but a convenient way to estimate the dissolution time is to establish previously a value of the rate β_t / β_∞ near 1.0 that should be inserted in the dependent variable of Equation 1. The proposed value in the present study was 0.9999, so that the variable measured at this t time represents the 99.99 % of β_∞; subsequently it is necessary to obtain equation 2 from equation 1:

$$t_D = \frac{Ln(1 - 0.9999^{\frac{1}{k_L}})}{-k_s} \qquad (2)$$

Table 3 shows dissolution times experimentally measured and dissolution times estimated by the equation proposed (Equation 2). The regular method to determine dissolution times is dependent on the precision of the equipment and/or the observation of the analyst but employing Equation 2 these sources of variability are eliminated assuming that if 99.99 % of the active molecule has been liberated, the whole particle should be completely disintegrated.

Conclusions

The release rate of phenolic compounds sprayed-dried with different carriers (maltodextrin, pectin, gelatin, carboxymethyl cellulose, whey protein, carrageenan and arabic gum) followed a Weibull-First Order kinetic model. Interestingly whey powder released the higher amount of Roselle's antioxidant compounds with no significant differences with Roselles' extract but in a shorter time (25.56 s). Carrageenan gum, arabic gum, pectin and CMC showed the lowest polyphenol release values at the steady state. There were

significant differences between release rate constants (k_s) and pectin showed the lowest value. Pectin retained the higher amount of polyphenols after the drying process (98.20%) and released 79.48% of the phenolic compounds released by Roselle's extract but in a higher period of time (353.42 s). The carriers used showed different protection characteristics against spray-drying process and displayed different kinetic parameters allowing the design of Roselles' polyphenols spray-dried particles with controlled release. The method proposed to calculate the dissolution time proved to be more reliable than the regularly used observation method.

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References

Abu-Tarboush, H.M., Ahmed, S.A.B., & Al Kahtani, H.A. (1997). Some nutritional properties of karkade (*Hibiscus Sabdariffa*) seed products. *Cereal Chemestry*, **74**, 352-355.

Adhikari, B., Howes, T., Bhandari, B.R., & Troung, V. (2004). Effect of addition of maltodextrin on drying kinetics and stickiness of sugar and acid-rich foods during convective drying: experiments and modeling. *Journal Food Engineering*, **62**, 53-68.

Athar, M., Back, J.H., Tang, X., Kim, K.H., Kopelovich, L. & Bickers, D.R. (2007). Resveratrol: a review of preclinical studies for human cancer prevention. *Toxicology and Applied Pharmacology*, **224**, 274-83.

Bhandari, B. R., Datta, N., Crooks, R., Howes, T., & Rigby, S. (1997). A semi-empirical approach to optimize the quantity of drying aids required to spray dry sugar rich foods. *Drying Technology*, **15**, 2509–2525.

Bhandari, B.R., Datta, N., & Howes, T., (1997). Problems associated with spray drying of sugar-rich foods. *Drying Technology*, **15**, 671-684.

Buckton, G., (1990). The role of compensation analysis in the study of wettability, solubility, disintegration, and dissolution. *International Journal of Pharmaceutics*, **66**, 175-182.

Chewonarin, T., Kinouchi, T., Kataoka, K., Arimachi, H., Kuwahara, T., Initke-kummuen, U., & Ohnishi, Y. (1999). Effects of roselle (*Hibiscus sabdariffa* Linn), a Thai medicinal plant, on the mutagenicity of various known mutagens in Salmonella typhimurium and on formation of Aberrant Crypt Foci induced by the colon carcinogens azoxymethane and 2-amino-methyl-6-phenylimidazo (4, 5-b) pyridine in F344 rats. *Food Chemical Toxicology*, **37**, 591-601.

Díaz-Bandera, D., Villanueva-Carvajal, A., Dublán-García, O., Quintero-Salazar, B. & Dominguez-Lopez, A.(2013) Release kinetics of antioxidant compounds from Hibiscus sabdariffa L. encapsulated in gelatin beads and coated with sodium alginate. *International Journal of Food Science & Technology*. DOI: 10.1111/ijfs.12199.

Faldt, P., & Bergenstahl, B. (1995). Fat encapsulation in spray dried food powders. *Journal of American Oil Chemical Society*, **72**, 171-176.

Fang, Z. & Bhandari, B. (2011). Effect of spray drying and storage on the Stability of bayberry polyphenols. *Food Chemistry*, **129**, 1139-1147.

Favaro-Trindade, C. S., Santana, A. S., Monterrey-Quintero, E. S., Trindade, M. A., & Netto, F. M. (2010). The use of spray drying technology to reduce bitter taste of casein hydrolysate. *Food Hydrocolloids*, **24**, 336-340.

Freudig, B., Hogekamp. S., & Schubert, H. (1999). Dispersion of powders in liquids in a stirred vessel. *Chemical Engineering and Processing*, **38**, 525-532.

Galet, L., Vu, T. O., Oulahna, D., & Fages, J.(2004).The Wetting behaviour and dispersion rate of cocoa powder in water. *Food and Bioproducts Processing*, **82**, 298-303.

Goula, A. M., Adamopoulos, K. G., & Kazakis, N. A., (2004). Influence of spray drying conditions on tomato powder properties. *Drying Technology*, **22**, 1129-1151.

Hirunpanich, V., Utaipat, A., Morales, N. P., Bunyapraphatsara, N., Sato, H., Herunsale, A., & Suthisisang, C. (2006). Hypocholesterolemic and antioxidant effects of aqueous extracts form the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. *Journal of Ethno-pharmacology*, **103**, 252-260.

Hogekamp, S., & Schubert, H., (2003). Rehydration of food powders. *Food Science and Technology International*, **9**, 223–35.

Lao, L.L., Peppas, N.A., Chiang Boey, F.Y. & Venkatramana, S.S. (2011). Modeling of drug release from bulk-degrading polymers. *International Journal of Pharmaceutics*, **418**, 28-41.

Lin, H.-H., Chen, J.-H. & Wang, C.-J. (2011). Chemopreventive properties and molecular mechanisms of the bioactive compounds in Hibiscus sabdariffa Linne. *Current Medicinal Chemistry*, **18**, 1245-1254.

Maa, Y., Nguyen, P., Sit, K., & Hsu, C. C. (1998). Spray-drying performance of a bench-top spray dryer for protein aerosol powder preparation. *Biotechnology and Bioengineering*, **60**, 301-309.

Marabi, A., Livings, S., Jacobson, M. & Saguy, I.S. (2003). Normalized Weibull distribution for modeling rehydration of food particulates. *European Food Research & Technology*, **217**, 311-318.

Nnam, N. M., & Onyeke, N. G. (2003). Chemical composition of two varieties of sorrel (*Hibiscus sabdariffa* L.), calyces and the drinks made from them. *Plant Foods for Human Nutrition*, **58**, 1–7.

Papadopoulou, V., Kosmidis, K., Vlachou, M. & Macheras, P. (2006). On the use of the Weibull function for the discernment of drug release mechanisms. *International Journal of Pharmaceutics*, **309**, 44-50.

Quek, Y.S., Chok, N.K. & Swedlund, P. (2007). The physicochemical properties of spray-dried watermelon powders. *Chemical Engineering and Processing*, **46**, 386-392.

Rothstein, S.N., Federspiel, W.J. & Little, S.R. (2009). A unified mathematical model for the prediction of controlled release from surface and bulk eroding polymer matrices. *Biomaterials*, **30**, 1657-1664.

Sanguansri, L., & Augustin, M.A. (2006). Microencapsulation and delivery of omega-3 fatty acids. In: Functional Food Ingredients and Nutraceuticals: Processing Technologies. (pp. 297–327). Taylor and Francis, Boca Raton, FL: Shi, J. (ed.).

Serrano-Cruz, M. R., Villanueva-Carvajal, A., Morales Rosales, E.J., Dávila, J. F., & Domínguez-López, A. (2012). Controlled release and antioxidant activity of Roselle (*Hibiscus sabdariffa* L.) extract encapsulated in mixtures of carboxymethyl cellulose, whey protein, and pectin. *LWT - Food Science and Technology*, **50**, 554-561.

Siepmann, J. & Peppas, N.A. (2000). Hydrophilic matrices for controlled drug delivery: an improved mathematical model to predict the resulting drug release kinetics (the "sequential layer" model). *Pharmaceutical Research*, **17**, 1290-1298.

Siepmann, J., Kranz, H., Bodmeier, R. & Peppas, N.A. (1999). HPMC-matrices for controlled drug delivery: a new model combining diffusion, swelling, and dissolution mechanisms and predicting the release kinetics. *Pharmaceutical Research*, **16**, 1748-1756.

Tsai, P. J., McIntosh, J., Pearce, P., Camden, B. & Jordan, B. R. (2002). Anthocyanin and antioxidant capacity in roselle (*Hibiscus sabdariffa* L.) extract. *Food Research International*, **35**, 351- 356.

Tuyen C. Kha, Minh H. Nguyen., & Paul D. Roach. (2010). Effects of spray drying conditions on the physicochemical and antioxidant properties of the Gac (Momordica cochinchinensis) fruit aril powder. *Journal of Food Engineering*, **98**, 385–392.

Villanueva-Carvajal, A., Bernal-Martínez, L.R., García-Gasca, M.T., & Domínguez-López, A. (2013). *In vitro* gastrointestinal digestion of *Hibiscus sabdariffa* L. The use of its natural matrix to improve the concentration of phenolic compounds in gut. *LWT-Food Science and Technology*, **51**, 260-265.

Wong, P. K., Yusof, S., Ghazali, H. M., & Man, Y.B.C. (2002). Physico-chemical characteristics of roselle (*Hibiscus sabdariffa* L.). *Journal of Nutrition and Food Science*, **32**, 68-73.

Table and figure captions

Table 1. Characterization of carrier-Roselle powders obtained from spray-dry process.

Table 2. Parameter estimates, coefficients of determination, and standard error of the estimates of the absorbance (520 nm) kinetics release model (Equation 1) for the carrier-Roselle powders.

Table 3. Dissolution times for the mixtures carrier-Roselle.

Figure 1. Predicted and observed release kinetics (measured by the absorbance at 520 nm of the aqueous solution) as a function of the carrier-Roselle powders.

Table 1. Characterization of carrier-Roselle powders obtained from spray-dry process.

Mixture	Carrier-Roselle solids (g/mL)	Powder recovery (g/100 g)	Drying efficiency (%)	Bulk density (g/cm^3)	Moisture (g/100 g) (dry basis)	pH[2]	TPC[3] retention (mg GAE/ 100 g)
Roselle[1]	0.033	69.77	76.38	0.582	4.48	2.28	72.06
MALTO	0.067	85.98	79.41	0.427	3.99	2.43	77.60
PECT	0.047	62.24	76.64	0.476	4.06	2.29	98.20
GELA	0.047	50.76	75.54	0.392	4.08	2.33	88.24
CMC	0.036	54.12	74.42	0.588	4.53	2.17	84.33
WHEY	0.051	50.44	76.81	0.527	4.12	2.39	89.09
CARR	0.038	54.34	74.22	0.849	4.88	2.29	94.30
ARAB	0.051	63.05	76.34	0.557	4.42	2.22	82.01

[1]Roselle extract non-encapsulated.
[2]pH of the re-solubilised powder according to procedure described in 2.5.2.
[3]TPC- Total phenolic compounds after the drying process.
MALTO, Maltodextrin, PECT, Pectin, GELA, Gelatin, CMC, Carboxymethylcellulose, WHEY, Whey powder, CARR, Carrageenan Gum, ARAB, Arabic Gum.

Table 2. Parameter estimates, coefficients of determination, and standard error of the estimates of the absorbance (520 nm) kinetics release model (Equation 1) for the carrier-Roselle powders.

Carrier	R^2	β_∞	SE β_∞	k_s	SE k_s	k_L	SE k_L
Roselle	0.999	0.624^{D}	0.005	0.109^{AB}	0.021	0.213^{A}	0.028
WHEY	0.996	0.588^{D}	0.003	0.375^{C}	0.044	1.475^{B}	0.275
MALTO	1.000	0.496^{C}	0.000	0.384^{C}	0.017	0.346^{A}	0.022
PECT	0.993	0.477^{C}	0.038	0.023^{A}	0.010	0.359^{A}	0.040
AARAB	1.000	0.475^{BC}	0.001	0.149^{B}	0.011	0.113^{A}	0.007
GELA	0.998	0.454^{ABC}	0.003	0.101^{AB}	0.011	0.368^{A}	0.031
CMC	0.985	0.428^{AB}	0.014	0.065^{AB}	0.022	0.359^{A}	0.071
CARR	0.991	0.405^{A}	0.020	0.051^{AB}	0.022	0.336^{A}	0.060

SE: Standard error of the estimate; β_∞: maximum release at the steady state; k_s: release rate constant; k_L: *Lag* period (sec).

Table 3. Dissolution times for the mixtures carrier-Roselle

Carrier	$t_{estimated}$	$t_{observed}$
Roselle[1]	70.14	19
MALTO-Roselle	21.22	13
PECT-Roselle	353.42	35
GELA-Roselle	81.03	26
CMC-Roselle	126.63	25
WHEY-Roselle	25.56	26
CARR-Roselle	158.49	22
ARAB-Roselle	47.24	17

[1] Roselle extract non-encapsulated.

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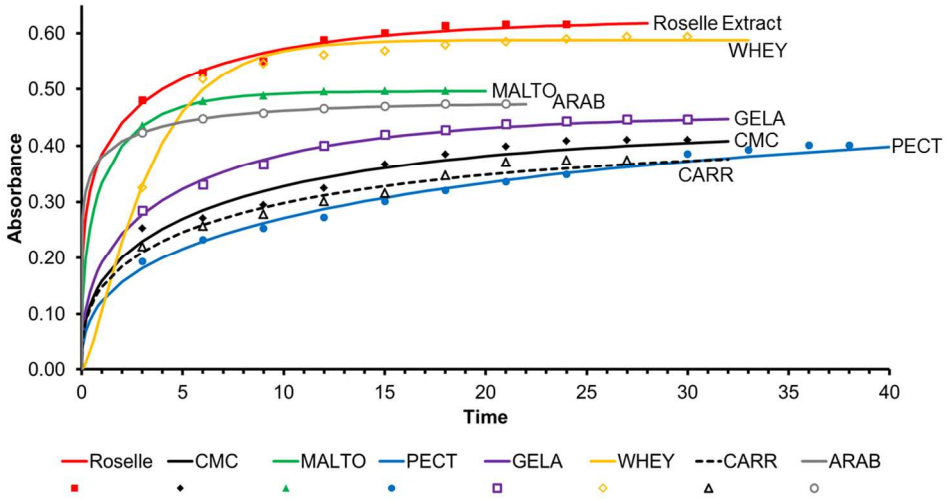

Predicted and observed release kinetics (measured by the absorbance at 520 nm of the aqueous solution) as a function of the carrier-Roselle powders.
369x200mm (96 x 96 DPI)

VI. CONCLUSIÓN GENERAL

Los ensayos preliminares de compatibilidad del extracto de jamaica con pH igual a 2 fueron determinantes para la selección de los polímeros portadores a utilizar en los procesos subsecuentes de encapsulación.

La obtención del extracto acuoso del cáliz de la jamaica (*Hibiscus sabdariffa* L.) confirmó, a través de los diferentes métodos de análisis, la presencia de compuestos con actividad antioxidante.

Al ser encapsulados los extractos del cáliz de la jamaica con diferentes polímeros comestibles y por dos métodos de encapsulación diferentes, se comprueba la eficiencia de los polímeros portadores en la formación de estructuras protectoras de compuestos lábiles como los polifenoles.

Todas las partículas obtenidas por los métodos de encapsulación estudiados se ajustaron adecuadamente a una cinética de primer orden. Los parámetros de las ecuaciones derivadas representan la velocidad de liberación, el periodo *lag,* que indica la rapidez o retraso en el inicio de la liberación de las moléculas de interés y la máxima liberación de los compuestos antioxidantes. Los encapsulados obtenidos por el método de coacervación, con una y dos coberturas de alginato, demostraron para este estudio que la velocidad cinética disminuye linealmente con el aumento del tiempo de gelificación del alginato y con el número de coberturas de éste.

Con el desarrollo del proceso de la cinética de disolución de los micro encapsulados obtenidos por secado por aspersión, fue posible comparar los resultados obtenidos de los tiempos de disolución observados directamente y los estimados con el modelo matemático propuesto, resultando ser más confiables los estimados que los obtenidos regularmente por el método de observación.

Los tiempos de liberación de los encapsulados obtenidos indican claramente que el método de coacervación controla mejor los tiempos de cinética de liberación en tiempos superiores a los 30 minutos.

De acuerdo con los resultados obtenidos, se concluye que es muy factible la inclusión de los encapsulados obtenidos por coacervación en algún alimento funcional, por ejemplo, el yogurt.

VII. REFERENCIAS BIBLIOGRÁFICAS

Abrahamse, S.L., Kloots, W.J., & Amelsvoort, J.M.M. (2005). Absorption, distribution, and secretion of epicatechin and quercetin in the rat. *Nutrion Research, 25,* 305-317.

Adelaja, A. & Schilling, B. (1999). Nutraceuticals: Blurring the Line Between Food and Drugs in the Twenty-first Century. Choices Fourth Quarter: 35-39.

Aggett, P. J., Antoine, J. M., Asp, N. G., Bellisle, F., Contor, L., Cummings, J. H., Howlett, J., Muller, D. J., Persin, C., Pijls, L. T., Rechkemmer, G., Tuijtelaars, S., & Verhagen, H. (2005). PASSCLAIM: consensus on criteria. *The European Journal of Nutrition, 44,* 5- 30.

American Cancer Society, Phytochemicals. (2007). http://www.cancer.org/docroot/ETO/content/ETO_5_3X_Pphytochemicals.asp?sitearea=ETO

Bastos, D., Araujo, K., & Leao, M. (2009). Ascorbic acid retaining using a new calcium alginate-capsul based edible film. *Journal of Microencapsulation, 26,* 97-103.

Bennion, M. (1980). *The Science of Food*. N.Y., USA: John Wiley & Sons, pp. 371.

Benzie, I. F. F.,& Strain, J. J. (1996). Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry, 239,* 70-76

Bhandari, B.R., Datta, N., & Howes, T. (1997). Problems associated with spray drying of

sugar-rich foods. *Drying Technology, 15,* 671-685.

Bravo, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews. 56,* 317-333.

Calixto, J.B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Brazilian Journal of Medical and Biological Research, 33*, 179-189.

Casper, D. (2003). GF was first in encapsulation. *Food Technology, 57,* 88-89

Caturla, N., Vera-Samper, E., Villalaín, J., Mateo, C., & Micol, V. (2003).The relationship between the antioxidant and the antibacterial properties of

galloylated catechins and the structure of phospholipid model membranes. *Free Radical, Biology and Medicine, 34,* 648-662.

Chen, L., Remondetto, G., & Subirade, M. (2006). Food protein-based materials as nutraceutical delivery systems. *Trends in Food Science and Technology, 17*, 272-283.

Cooper, D.A. (2004). Carotenoids in health and disease: recent scientific evaluations, research recommendations and the consumer. *Journal of Nutrition, 134*, 221–224.

Desai, K.G.H., & Park, H.J. (2005). Recent developments in microencapsulation of food ingredients. *Drying Technology, 23,* 1361-1394.

Demars, L. L. & Ziegler, G. R. (2001). Texture and structure of gelatin/pectin-based gummy confections. *Food Hydrocolloids, 15*, 643-653

Domínguez-López, A., Remondetto, G.E., & Navarro, S. (2008). Thermal kinetic degradation of anthocyanins in a roselle (*Hibiscus sabdariffa* L. cv. 'Criollo') infusion. *International Journal of Food Science and Technology, 43*, 322.

Dureja, H., Kaushik, D., & Kumar, V. (2003). Developments in nutraceuticals. *Indian Journal of Pharmacology, 35*, 363-372.

Duthie, G., & Crozier, A. (2000). Plant-derived phenolic antioxidants. *Current Opinion in Lipidology, 11,* 43–47.

Duthie, G.G., Gardner, P.T., & Kyle, J.A.M. (2003). Plant polyphenols: are they the new magic bullet?. *Proceedings of the Nutrition Society Journal, 62,* 599-603.

Dutta, P., Tripathi, S., Mazutti, G., & Dutta, J. (2009). Review: Perspectives for chitosan based antimicrobial films in food applications. *Journal Food Chemistry, 114,* 1173–1182.

Dziezak, J. D. (1998). Microencapsulation and encapsulated food ingredients. *Food Technology, 42,* 136-151.

Erlejman, A.G., Verstraeten, S.V., Fraga, C.G., & Oteiza, P.I. (2004). The interaction of flavonoids with membranes: potential determinant of flavonoid antioxidant effects. *Free Radical Research, 38,* 1311-1320.

Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols - a review. *Trends in Food Science and Technology, 21,* 510-523.

Farombi, O.E. (2003). African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. *African Journal of Biotechnology, 2,* 662-671.

FDA, The Food and Drug Administration (2000) Title 21-Food and drugs chapter, part 320-Bioavailability and bioequivalence requirements, Subpart A-General Provisions, Sec. 320.1 Definitions. Code of Federal Regulations, Title 21, Volume 5, Parts 300 to 499. Pp. 185-199. http://www1.va.gov/oro/apps/compendium/Files/21CFR320.htm.

Ferreira, I., Rocha, S., & Coelho, M. (2007). Encapsulation of antioxidants by spray-drying. *Chemical Engineering Transactions, 11*, 713-717.

Fogliano, V., Verde, V., Randazzo, G., & Ritieni, A. (1999). Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *Journal of Agricultural and Food Chemistry, 47,* 1035-1040.

Halliwell, B. (1990). How to characterize a biological antioxidant. *Free Radical Research Communications, 9*, 1-32.

Harborne, J.B., & Baxter, H. (1999).The Handbook of Natural Flavonoids. Vol. 1 and 2 (Chichester: John Wiley and Sons).

Harborne, J.B., Williams, C.A. (2000). Advances in flavonoid research since 1992. *Phytochemistry, 55,* 481–504.

Hedren, E., Mulokozi, G., & Svanberg, U. (2002). In vitro accessibility of carotenes from green leafy vegetables cooked with sunflower oil or red palm oil. *International Journal of Food Science and Nutrition, 53*, 445–453.

Hendrich, S. (2002). Bioavailability of isoflavones. *Journal of Chromatography B, 777,* 203-210.

Heo, H.J., Kim, Y.J., Chung, D., & Kim, D.O. (2007). Antioxidant capacities of individual and combined phenolics in a model system. *Food Chemistry, 104,* 87–92.

Herrera, A., Flores, S., Chávez, M.A., & Tortoriello, J. (2004). Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: a controlled and randomized clinical trial. *Phytomedicine, 11,* 375-382

Janssen, F.T., Netzel, M., Strass, G., Kler, A., & Kriesl, E. (2005). Pharmacokinetics of anthocyanidin-3-glycosides following consumption of *Hibiscus sabdariffa* L. extract. *Journal of Clinical Pharmacology, 45,* 203-210.

Jimenez-Escrig, Rincón, A.M., Pulido,R & Saura-Calixto, F. (2001). Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. *Journal of Agriculture and Food Chemistry. 49*, 5489-5493.

Kong, J. M., Chia, L. S., Goh, N. K., Chia, T. F., & Brouillard, R. (2003). Analysis and biological activities of anthocyanins. *Phytochemistry, 64,* 923-933.

Kosaraju, S. L. (2005). Colon targeted delivery systems, review of polysaccharides for encapsulation and delivery. *Critical Reviews in Food Science and Nutrition, 45,* 251–258.

Kosaraju, S.L., Labbett, D., Emin, M., IzabelaKonczak, I., & Lundin, L. (2008). Delivering polyphenols for healthy ageing. *Nutrition & Dietetics, 65*, 48–52.

Laranjinha, J. (2002). Caffeic acid and related antioxidant compounds: biochemical and cellular effects. In: Cadenas E, Packer L, editors. Handbook of antioxidants. 2nd edition. Nueva York: Marcel Dekker., pp. 279-302.

Larrosa, M., Llorach, R., Espín, J.C., & Tomás, F.A. (2002). Increase of antioxidant activity of tomato juice upon functionalization with vegetable by product extracts. *Lebens.Wiss.-Technology Journal uri icon, 35,* 532-542.

Luykx, D., Peters, R., van Ruth, S., & Bouwmeester, H. (2008). A Review of Analytical Methods for the Identification and Characterization of Nano Delivery Systems in Food. *Journal of Agricultural and Food Chemistry, 56*, 8231-8247.

Madene, A., Scher, J., & Desobry, S. (2006). Flavour encapsulation and controlled release - a review. *International Journal of Food Science and Technology, 4,* 1-21

Maltais, A., Remondetto, G.E., González, R., & Subirade, M. (2005) Formation of Soy Protein Isolate Cold-set Gels: Protein and Salt Effects. *Journal of Food Science, 70*, C67-C73.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition, 79*, 727–47.

Manach, C., Texier, O., Morand, C., Crespy, V., Régérat, F., Demigné, C., & Rémésy, C. (1999). Comparison of the bioavailability of quercetin and cetequin in rats. *Free Radical Biology and Medicine, 27,* 1259-1266.

Manach, C., Williamson, G., Morand, Ch., Scalbert, A., & Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition, 81*, 230S– 42S.

Martínez, I., Periago, M.J., & Ros, G. (2000). Significado nutricional de los compuestos fenólicos en la dieta. *Archivos Latinoamericanos de Nutrición, 50,* 5-18

Middleton, E., Kandaswami, C., & Theoharides, T. (2000). The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease, and Cancer. *Pharmacological Reviews, 52,* 673-751.

Montes, E., De Paula, C., & Ortega, F. (2007). Determinación de las condiciones óptimas de encapsulamiento por co-cristalización de jugo de maracuya (Passifloraedulis). *Revista Temas Agrarios, 12,* 5-12.

Moreno, D.A., García, C., Gil, J. I., & Gil, A. (2008). Betalains in the era of global agri-food science, technology and nutritional health. *Phytochemistry Reviews, 7,* 261–280.

Morton, J.F. Roselle, *Hibiscus sabdariffa* L. on Fruits of Warm Climates. Morton, J.F. (Ed.). Miami, Fl. USA. 1987; pp: 281–286.

Murúa, B., Beristain, C., & Martínez, F. (2009). Preparation of starch derivatives using reactive extrusion and evaluation of modified starches as shell materials for encapsulation of flavoring agents by spray drying. *Journal of Food Engineering, 91,* 380–386.

Nnam, N. & M., Onyeke, N. G. (2003). Chemical composition of two varieties of sorrel (*Hibiscus sabdariffa* L.), calyces and the drinks made from them. *Plant Foods for Human Nutrition, 58*, 1-7.

Nishikawa, T., Edelstein, D., & Brownlee, M. (2000). The missing link: a single unifying mechanism for diabetic complications. *Kidney International*, *77,* 26-30.

Olvera-García, V., Castaño, E., Rezéndiz, R.I., Reynoso, R., González, E., Elizondo, G., & Loarca, G. (2008). *Hibiscus sabdariffa* L. Extracts Inhibit the Mutagenicity in

Micro suspension Assay and the Proliferation of HeLa Cells. *Journal of Food Science, 73*, T-75-T-81.

Ortiz-Hernández, L., & Gómez-Tello, B. L. (2008). Food consumption in Mexican adolescents. *Pan American Journal of Public Health, 24,* 127-135

Parada, M., & Aguilera, J.M. (2007). Food Microstructure Affects the Bioavailability of Several Nutrients. *Journal of Food Science, 72*, 21-32.

Passamonti, S., Terdoslavich, M., Franca, R., Vanzo, A., Tramer, F., Braidot, E., Petrussa, E., & Vianello, A. (2009). Bioavailability of Flavonoids: A Review of Their Membrane Transport and the Function of Bilitranslocase in Animal and Plant Organisms. *Current Drug Metabolism, 10,* 369-394.

Passamonti, S., Vrhovsekb, U., Vanzo, A., & Mattivi, F. (2003).The stomach as a site for anthocyanins absorption from food 1. *FEBS Letters, 544*, 210-213

Pérez-Lizaur, A. B., Kaufer-Horwitz, M., & Plazas, M. (2008). Environmental and personal correlates of fruit and vegetable consumption in low income, urban Mexican children. *Journal of Human Nutrition and Dietetics, 21*, 63–71.

Pérez-Trueba, G. (2003). Los flavonoides: antioxidantes o prooxidantes. Rev. Cubana. *Investigaciones Biomedicas, 22,* 48-57.

Pietta, P.G. (2000). Flavonoids as Antioxidants. *Journal of Natural Products, 63,* 1035-1042.

Rafter, J.J. (2002). Scientific basis of biomarkers and benefits of functional foods for reduction of disease risk: cancer. *British Journal of Nutrition, 88,* 219-224.

Ramírez-Rodrígues, M. M., Plaza, M. L., Azeredo, A., Balaban, M. O., & Marshall, M. R. (2011). Physicochemical and phytochemical properties of cold and hot water extraction from *Hibiscus sabdariffa*. *Journal of Food Science, 76,* 429-435.

Ranadheera, R., Baines, S., & Adams, M. (2010). Review Importance of food on probiotic efficacy. *Food Research International, 4,* 1-7.

Renaud, S., & de Lorgeril, M. (1992). Wine, alcohol, platelets and the French paradox for coronary heart disease. *Lancet, 339,* 1523-1526.

Rowe, R.C., Sheskey, P.J., & Quinn, M. E. (2009). Handbook of Pharmaceutical Excipients. Sixth Ed. Pharmaceutical Press and American Pharmacists Association . Pp. 20-22.

Rimm, E.R., Katan, M.B., Ascherio, A., Stampfer, M., & Willet, W. (1996). Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Annals of Internal Medicine, 125,* 384-389.

Roberfroid, M. B. (2000). Concepts and strategy of functional food science: the European perspective. *American Journal of Clinical Nutrition, 71,* 1660–1664.

Roberfroid, M.B. (2002). Global view on functional foods: European perspectives. *British Journal of Nutrition, 88*, 133-138.

Rodríguez, I.C., Beltrán, R., Micol, V., Alonso, C., Joven, J., & Menéndez, J.A. (2009). Direct characterization of aqueous extract of Hibiscus sabdariffa using HPLC with diode array detection coupled to ESI and ion trap MS. *Journal of Separation Science, 32,* 3441-3448.

Rodríguez-Medina, I. C., Beltrán-Debón, R., Micol Molina, V., Alonso-Villaverde, C., Joven, J., & Menéndez, J. A. (2009). Direct characterization of aqueous extract of Hibiscus sabdariffa using HPLC with diode array detection coupled to ESI and ion trap MS. *Journal of Separation Science, 32,* 3441-3448.

Sanguansri, P., & Augustin, M. A. (2006). Nanoscale materials development a food industry perspective. *Trends in Food Science and Technology, 17,* 547-556.

Saravanan, M., & Panduranga, R. K. (2010). Pectin–gelatin and alginate–gelatin complex coacervation for controlled drug delivery: Influence of anionic polysaccharides and drugs being encapsulated on physicochemical properties of microcapsules. *Carbohydrate Polymers, 80*, 808-816.

Saura, F., Serrano, J., & Goñi, I. (2007). Intake and bioaccessibility of total polyphenols in a whole diet. *Food Chemistry, 101*, 492–501.

Sáyago-Ayerdy, S.G., Arranz, S., Serrano, J., & Goñi, I. (2007). Dietary Fiber Content and Associated Antioxidant Compounds in Roselle Flower (*Hibiscus sabdariffa* L.) Beverage. *Journal of Agricultural and Food Chemistry, 55*, 7886-7890.

Scalbert, A., Manach, C., Morand, C., Remesy, C., & Jimenez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Sciences and Nutrition, 45*, 287-306.

Scalbert, A., Williamson, G. (2000). Dietary intake and bioavailability of phenols. *Journal of Nutrition, 130,* 2073-2085.

Serrano-Cruz, M. R., Villanueva-Carvajal, A., Morales, R., Edgar J., Ramírez, D., José F., Dominguez-Lopez, A. (2013). Controlled release and antioxidant activity of Roselle (Hibiscus sabdariffa L.) extract encapsulated in mixtures of carboxymethyl cellulose, whey protein, and pectin. *LWT - Food Science and Technology, 50,* 554-561.

Shepherd, R., Roberston, A., & Ofman, D. (2000). Dairy glycoconjugate emulsifiers: casein-maltodextrins. *Food hydrocolloids, 14,* 281-286

Schroeder, E.K., Kelsey, N.A., Doyle, J., Breed, E., Bouchard, R.J., Loucks, A., Harbison, A., & Linseman, D.A. (20008). Green tea epigallocatechin 3-gallate accumulates in mitochondria and displays a selective antiapoptotic effect against inducers of mitochondrial oxidative stress in neurons. *Antioxidant and Redo Signaling, 11,* 469-480.

Shahidi, F., & Han, X., (1993). Encapsulation of Food Ingredients. *Critical Reviews in Food Science and Nutrition, 33,* 501-547

Shimizu, M., & Hachimura, S. (2011). Gut as a target for functional food. *Trends in Food Science and Technology, 22*, 646-650.

Sozer, N., & Kokini, J. (2009). Nanotechnology and its applications in the food sector. *Trends in Biotechnology, 27,* 82-89.

Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and Its Panel on Dietary Antioxidants and Related Compounds. (1998). Dietary references intakes: Proposed definition and plan for review of dietary antioxidants and related compounds. Washington, DC: National Academy Press, p.p.1-3

Stevanato, R., Fabris, S., & Momo, F. (2004): New enzymatic method for the determination of phenolic content in tea and wine, *Journal of agricultural and food chemistry, 52,* 6287-6793.

Sun, J., Chu, Y.F., Wu, X., & Liu, R.H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry, 50,* 7449-7454.

Sun, T. & Tanumihardjo, S.A. (2007). An Integrated Approach to Evaluate Food Antioxidant Capacity. *Journal of Food Science, 72,* 159 –165.

Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzyme as determinants of quality in fruits and vegetables. *Journal of Agricultural and Food Chemistry, 81,* 853-876

Tsai, P.J., & Huang, H.P. (2004). Effect of polymerization on the antioxidant capacity of anthocyanins in roselle. *Food Research International, 37,* 313-318

Van Duynhoven, J., Vaughan, E.E., Jacobs, D.M., Kemperman, R.A., Van Velzen, E.J. , Gross, G., Roger, L.C., Possemiers, S., Smilde, A.K., Doré, J., Westerhuis, J.A., & Van de Wiele, T. (2011). Metabolic fate of polyphenols in the human superorganism. National *Academy of Sciences of the United States of America, 108,* 4531-4538.

Wahlqvist, M.L., & Wattanapenpaiboon, N. (1998). Phytochemical malabsorption: clinical significance. *World Journal of Gastroenterology, 4*, 469-470.

Waterhouse, A.L. (2002). Wine Phenolics. Ann. *N.Y. Academy Sciences, 95,* 21-36.

WHO/FAO (2003) Diet, Nutrition and the Prevention of Chronic Diseases.Technical Report Series 916. http://whqlibdoc.who.int/trs/who_trs_916.pdf

Williamson, G., & Holst, B. (2008). Dietary reference intake (DRI) value for dietary polyphenols: are we heading in the right direction? *British Journal of Nutrition, 99,* 55–58.

Wittemer, S.M., Ploch, M., Windeck, T., Müller, S.C., Drewelow, B., Derendorf, H., & Veit, M. (2005). Bioavailability and pharmacokinetics of caffeoylquinic acids and flavonoids after oral administration of artichoke leaf extracts in humans. *Phytomed, 12,* 28-38.

Wong, P. K., Yusof, S., Ghazali, H. M., & Che-Man, Y. B. (2002). Physico-chemical characteristics of Roselle (*Hibiscus sabdariffa* L.), *Nutrition and Food Science, 32,* 68-73.

Yañez, J., Salaza, J.A., Chaires, L., Jiménez, J., Márquez, M., & Ramos, E.G. (2002). Aplicaciones biotecnológicas de la microencapsulación. *Revista Avance y Perspectiva, 21*, 313-319.

Yajun, Y., Abhijeet, K., & Mattheos, A.G.K. (2005). Biosynthesis of Natural Flavanones in Saccharomyces cerevisiae. *Applied and Environmental Microbiology, 71,* 5610–5613.

Youdim, K.A., Qaiser, M.Z., Begley, D.J., Rice, C.A., & Abbott, N.J. (2004). Flavonoid permeability across an in situ model of the blood–brain barrier. *Free Radical Biology and Medicine, 36,* 592-604.

Zhao, J. (2007). Nutraceuticals, Nutritional Therapy, Phytonutrients and Phytotherapy for Improvement of Human Health: A Perspective on Plant Biotechnology Application. *Recent Patents on Biotechnology, 1*, *1*, 75-97.