UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE CIENCIAS QUÍMICAS

PROGRAMA DE POSGRADO EN BIOPROCESOS

ALIMENTO FUNCIONAL: AGENTES CO-MICROENCAPSULANTES EN LA CONSERVACIÓN DE QUERCETINA 3-D-GALACTOSIDE-BACILLUS CLAUSII

TESIS QUE PARA OBTENER EL GRADO DE

MAESTRO EN CIENCIAS EN BIOPROCESOS

PRESENTA:

L. N. Héctor Alfonso Enciso Huerta

DIRECTOR DE TESIS:
DRA. María Zenaida Saavedra Leos

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DR. Miguel Ángel Ruiz Cabrera

DRA. Claudia Álvarez Salas

SAN LUIS POTOSÍ, S. L. P. Agosto 2022

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SINODALES:

PRESIDENTE:
DRA. María Zenaida Saavedra Leos _____

SECRETARIO:
DR. Miguel Ángel Ruiz Cabrera _____

VOCAL:
DRA. Claudia Álvarez Salas _____

SAN LUIS POTOSÍ, S. L. P. AGOSTO 2022

San Luis Potosí, S.L.P.
Julio 11, 2022

Comité Académico del Posgrado
En Ciencias en bioprocesos
Facultad de Ciencias Químicas / UASLP
Presente._

Por medio de la presente comunicamos que la tesis llevada a cabo por el alumno de Maestría L.N. Héctor Alfonso Enciso Huerta, titulada "Alimento funcional: agentes co-microencapsulantes en la conservación de quercetina 3-D-Galactoside-*Bacillus calusii*", ha sido concluida y aprobada por el comité tutorial para dar inicio a los trámites correspondientes para su titulación, la cual tendrá lugar el próximo día **12 de agosto** a las **12:00** hrs. en el Auditorio Chico (G203), de la Facultad.

ATENTAMENTE

Dra. María Zenaida Saavedra Leos _____
Directora de Tesis

Dr. Miguel Ángel Ruiz Cabrera _____
Co-Director

Dra. Claudia Álvarez Salas _____
Asesora

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Contenido

Abstract

Currently, the demand for functional foods has been increasing in the public interest to improve their life expectations and general health. Food matrices containing probiotic microorganisms and active compounds encapsulated into carrier agents are essential in this context. Encapsulation by the lyophilization method is widely used because oxidation reactions affecting physicochemical and nutritional food properties are usually avoided. Encapsulated functional ingredients such as quercetin and *Bacillus clausii* using two carrier agents' matrices performed in I [inulin (I); lactose (L) and maltodextrin (MX)] and II [(Arabic (A), Guar (G) and Xanthan (X) Rubbers)] are presented in this work. A D-optimal procedure involving 59 experiments was designed to evaluate each matrix's yield, viability, and antioxidant activity (AA). Matrix I (33.3 I; 33.3 L; 33.3 MX) and matrix II (33.3 A; 33.3 G; 33.3 X) exhibited the best yield.

Keywords: functional, food, Inulin, Lactose

Abstract traducido al español

Actualmente, la demanda de alimentos funcionales ha aumentado gracias al interés del público por incrementar su expectativa de vida y mejorar su salud en general. En este contexto, son esenciales las matrices alimentarias que contengan microorganismos probióticos y compuestos activos encapsulados dentro de agentes acarreadores. La encapsulación por el método de liofilización es ampliamente utilizada debido a que usualmente se evitan las reacciones de oxidación que afectan las propiedades fisicoquímicas y nutricionales de los alimentos. En este trabajo, se presentan ingredientes funcionales encapsulados como la quercetina y el *Bacillus clausii*, con la utilización de dos matrices de agentes acarreadores formadas por: I (inulina (I); lactosa (L) y maltodextrina (MX)) y II (goma arábiga (A); goma guar (G) y goma xantana (X)). Mediante un diseño D-optimal, se condujeron 59 experimentos en los que se evaluó el rendimiento, viabilidad y actividad antioxidante (AA). La matriz I (1/3 I; 1/3 L; 1/3 MX) y la matriz II (1/3 A;1/3 G;1/3 X), exhibieron el mejor rendimiento.

Palabras clave: funcional, alimento, inulina, lactosa

Resumen

Evaluation of two active system encapsulants matrices with quercetin and 3 Bacillus claussii for functional lyophilized foods preparation

Introducción

Según el Codex alimentario (Commission, 2007) un alimento es toda sustancia elaborada, semielaborada o en bruto, que se destina al consumo humano (CXS 1-1985) que tiene un aporte nutricional. En los últimos años, derivado de la pandemia mundial del virus SARS-CoV-2 (COVID-19) la Organización Mundial de la salud (OMS) indicó consumir alimentos saludables, ya que, si bien no puede prevenir ni curar la enfermedad, es fundamental para el buen funcionamiento del sistema inmunológico y se reduce considerablemente la probabilidad de aparición de otras enfermedades como obesidad, diabetes, enfermedades cardíacas y algunos tipos de cáncer. Por lo anterior se considera que la alimentación juega un papel preponderante en la prevención de estas afecciones que tienen un impacto a nivel de salud pública. Aunado a lo anterior, un “alimento funcional”, se define según la guía (European society for clinical nutrition and metabolism) ESPEN como un alimento fortificado con ingredientes, nutrientes o componentes adicionales, con la intención de manifestar beneficios específicos para la salud. Debido a lo anterior la producción de alimentos funcionales, se ha convertido en la última década en una de las industrias biotecnológicas de alimentos más importantes, dada la creciente demanda por parte de los consumidores al buscar alargar su expectativa de vida y mejorar su salud en general, debido a la concientización sobre la prevención de enfermedades como la diabetes, el cáncer y el alzheimer. (Sarao & Arora, 2017). Comúnmente, los antioxidantes son compuestos presentes dentro de los alimentos funcionales, estos son agregados a los productos solos o en combinación con otros compuestos que permitan un sinergismo, como el que ocurre entre la vitamina C al regenerar al radical tocoferilo de la vitamina E tras su oxidación. (Addor, 2017). Los antioxidantes se añaden a los alimentos con la intención de obtener diferentes beneficios como el de suprimir la oxidación lipídica para aumentar la vida de anaquel de los productos, así como el reducir las concentraciones de radicales

libres dentro del organismo, mejorando la salud del consumidor, (Ciriminna et al., 2017). La quercetina es un antioxidante flavonoide, encontrado en manzanas, uvas, frijoles, brócoli, cebollas rojas, tomates, semillas oleaginosas, flores, hojas de té y en el Ginko biloba. La ingesta diaria en la dieta de quercetina es de entre 10 y 16 mg al día, sin embargo la concentración recomendada de quercetina en un suplemento debe ser de 1g por día (Khan et al., 2020). Entre los mecanismos por los cuales la quercetina ejerce un efecto benéfico contra las enfermedades, se encuentran: En el asma alérgica, la quercetina inhibe la expresión del gen MUC5AC en las células NCI-H292, lo cual repercute a nivel ciliar en la mucosa nasal humana, siendo un agente antisecretor, inhibiendo la secreción mucosa de las células epiteliales, mientras mantiene un movimiento ciliar normal. (Jafarinia et al., 2020). Marunaka et al. (2017) demostraron que la ingesta oral de entre 150 a 730 mg/día durante cuatro semanas de quercetina, tiene un efecto antihipertensivo, reduciendo la presión sistólica y diastólica en pacientes en la primera etapa de la hipertensión. Por otra parte, se ha observado que en pacientes con síndrome metabólico, la ingesta de 150 mg/día de quercetina durante cinco semanas, reduce significativamente la presión sistólica. Un estudio in vitro, elaborado Reyes-Farias & Carrasco-Pozo. (2019) indicó que la quercetina ha funcionado como agente antiviral contra el VIH, inhibiendo enzimas como la integrasa, la proteasa y la transcriptasa inversa, reduciendo significativamente la replicación del virus del VIH. Otro tipo de compuestos activos en los alimentos funcionales son los probióticos, estos confieren beneficios a la salud, mediante diferentes mecanismos como la producción de enzimas biliares, ácidos orgánicos, hormonas de saciedad, la modulación del sistema inmune mediante el aumento en la respuesta de los anticuerpos, la competencia por sustratos contra organismos patógenos y la interacción con la microbiota. (Sanders et al., 2019). Bacillus clausii es una bacteria aeróbica, gram-positiva utilizada como probiótico, tiene la capacidad de formar esporas y colonizar el intestino, (Paparo et al., 2020). *B. clausii* también es resistente a factores como el calor, el pH gástrico y los antibióticos, puede tolerar medios alcalinos, sus condiciones óptimas de crecimiento son a 40°C y a un pH de 9,0. (De Castro et al., 2019). Autores como De Castro et al. (2019). han reportado la utilización del B. clausii durante 7 días, para el tratamiento de la diarrea aguda

infantil, viral y asociada al uso de antibióticos, donde se logró una reducción en el tiempo de la enfermedad, los síntomas gastrointestinales y la frecuencia de evacuaciones. Recientemente en 2020, (Plomer et al., 2020), utilizaron al *B. clausii* para aminorar los efectos adversos del tratamiento para eliminar a la Helicobacter pylori,en donde por lo general se administran antibióticos que causan náusea, inflamación, vómito y diarreas, haciendo que se opte por terminar la administración del medicamento, dando como resultado el riesgo de hacer que el tratamiento falle y la Helicobacter pylori cree resistencia a los antibióticos.

Con la finalidad de mantener la actividad antioxidante y viabilidad de los productos probióticos se utilizan técnicas de microencapsulación, de cualquier ingrediente activo que se busque añadir a un alimento, son protegidos de factores ambientales como el calor, el oxígeno y la humedad. (Zuidam & Nedović, 2010). Entre las técnicas de secado más empleadas para la producción de alimentos probióticos, se encuentran el secado por aspersión y la liofilización, este último permite preservar no sólo las cualidades nutricionales de los alimentos, sino también sus aromas y sabores, además de poder ser rehidratados tras el proceso, gracias a la porosidad del producto final. (Muñoz-López et al., 2018). La liofilización es ampliamente utilizada en la industria de alimentos, debido a que, a diferencia de los procesos de secado con calor, esta no cataliza reacciones de oxidación que afectan las propiedades fisicoquímicas y nutricionales de los alimentos. (Caballero et al., 2017). El secado por liofilización utiliza la sublimación y puede ser utilizado para la encapsulación de aromas, esencias, fármacos y cualquier material termosensible en general. Tomando en consideración la importancia de los antioxidantes y probióticos en la salud, en esta investigación se planteó obtener un ingrediente funcional que contenga *B. clausii* y quercetina microencapsulado por liofilización.

Objetivos

Objetivo general

- Elaborar y caracterizar un ingrediente funcional microencapsulado de B. clausii/quercetina mediante liofilización.

Objetivos específicos

- Evaluar el efecto de inulina, lactosa y maltodextrina, sobre el rendimiento, la viabilidad de B. clausii y la actividad antioxidante de la quercetina.
- Evaluar el efecto de la goma arábiga, xantana y guar sobre el rendimiento, la viabilidad de B. clausii y la actividad antioxidante de la quercetina.
- Evaluar el efecto sinérgico de agentes acarreadores y gomas sobre el rendimiento, la viabilidad de B. clausii y la actividad antioxidante de la quercetina.

Materiales y métodos

1.1 Materiales

Maltodextrina (MX) comercial de almidón de maíz de INGREDION México (Guadalajara, México). Con equivalentes de dextrosa (DE) de 10, peso molecular de 1625 g/mol y un grado de polimerización (DP) de 2-16 unidades de glucosa. Inulina (IN) obtenida de INGREDION Mexico (Guadalajara, Mexico). α-Lactosa monohidratada (L), (Lα·H2O, pureza ≥ 99,9 %) adquirida de Sigma Aldrich Chemical Co., Metanol, (MeOH, pureza ≥99,8) de J.T. Baker. Gomas Arábiga (A), Guar (G) y Xantana (X) de INGREDION México (Guadalajara, México). Cepas de (*B. clausii*) en solución de sinuberase fue obtenido de Sanofi-Aventis México, S.A. de C.V. (Coyoacán, Ciudad de México, México). Quercetina 3-D-Galactósido (pureza ≥99%) adquirida de Química Farmacéutica Esteroidal S.A de C.V.., (Tláhuac, Ciudad de México, México). Agar tripticasa de soya adquirido de Dickinson de México S.A de C.V. (Ciudad de México, México) y 2,2-difenil-1-picrilhidracilo (DPPH) grado analítico de Sigma–Aldrich Chemical Co

1.2 Preparación de liofilizados

Con base al diseño de experimentos, se prepararon 100g de muestras p/p, con 10 g de la matriz I elaborada con I, L y MX y 1g de matriz II formada por A, G y X. Cada fracción de masa para la matriz I y II fue establecida de acuerdo con el diseño de experimentos. Los componentes de cada matriz se pasaron por un tamiz de 1mm y se añadieron 87 g de agua desionizada, seguida de agitación magnética a 35°C por 5 minutos.

Se añadió 1g de quercetina y 1g de solución de *B. clausii* a la mezcla para obtener un volumen final de la solución de 100g (p/p). Las muestras fueron almacenadas en oscuridad a -80 °C. El proceso de microencapsulación se llevó a cabo por sublimación en un liofilizador (IlShinbiobase® Modelo TFD8501, Gyeonggi-do, Corea del sur) con una presión de vacío de 5 mTorr a -65 °C por aproximadamente 120h.

1.3 Determinación de la viabilidad

Se determinó la viabilidad de *B. clausii* antes y después del proceso de encapsulación, suspendiendo 1g de micropartículas obtenidas en 9 ml de solución salina (NaCl, 0.9% p/v). Con la finalidad de romper las microcápsulas, la suspensión se agitó durante 10 minutos en un vortex y calentado a baño maría por 10 minutos a 50 °C. Las células viables se analizaron de acuerdo con el método descrito por (Miles et al., 1938). Brevemente, se realizaron diluciones desde 1x10-3 a 1x10-9 en solución salina y se sembraron en agar tripticasa de soya, posteriormente se incubaron a 35 °C por 24 h. La evaluación se realizó por triplicado y se reporta como unidades formadoras de colonia por gramo (CFU/g), usando la ecuación 1

$$\frac{UFC}{g} = \left[\frac{N\,°\,de\ colonias\ en\ caja * factor\ de\ dilución}{mL\ de\ muestra\ sembrada}\right] \quad (1)$$

1.4 Actividad antioxidante (AA)

La actividad antioxidante de la quercetina se determinó mediante el método descrito por (Brand-Williams et al., 1995). Brevemente, 1.7 mL de solución alcohólica de DPPH (0,1 mmol DPPH/L) se mezcló con 1.7 mL de suspensión del microencapsulado, donde las concentraciones del microencapsulado fueron de 2.5, 5 y 15 µg/mL. La mezcla se dejó en oscuridad por 30 minutos y se midió la absorbancia a 537 nm usando un espectrofotómetro UV-Vis Evolution 220 (Thermo Scientific, Walthman, MA. USA). El porcentaje de actividad an tioxidante se calculó usando la ecuación 2:

$$Actividad\ antioxidante(\%DPPH) = \frac{A0-A30}{A0} x100 \quad (2)$$

Donde *A0* representa la absorbancia de la solución blanco (DPPH y etanol sin microencapsulado), *A30* representa la absorbancia de la solución de DPPH y etanol con microcápsulas después de 30 minutos. La actividad antioxidante se determinó por triplicado para cada muestra.

1.5 Diseño de experimentos y análisis estadístico

Se evaluaron dos mezclas independientes: Matriz I, consistía en inulina (IN), lactosa (L) y maltodextrina (MX), mientras que la Matriz II consistía en las gomas Arábiga (A), guar (G) y xantana (X). Los niveles superior e inferior de estas variables estuvieron entre 0 y 100 (%p), y la suma de componentes en cada mezcla fue de 100% para cada prueba. Las variables de respuesta fueron rendimiento (%), Bc (Log10 CFU/g), y actividad antioxidante (con DPPH a concentraciones de 5, 10, y 30 µg/g)). De esta manera, un diseño de experimentos combinado con dos matrices para un modelo especial cúbico x especial cúbico, fue seleccionado para evaluar el efecto de cada factor sobre las variables de respuesta. La tabla 1 muestra los resultados de 59 ensayos realizados en el laboratorio en orden aleatorio.

Se llevó a cabo un análisis de varianza (ANOVA), para cada variable de respuesta (Rendimiento, Bc y actividad antioxidante), usado el software Design-Expert® versión 12 (versión de prueba) a un nivel de significancia de 0.05. El modelo Scheffe analizado, especial cúbico x especial cúbico se muestra en la escuación 3:

$$Y = (\alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_4 AB + \alpha_5 AC + \alpha_6 BC + \alpha_7 ABC) \times (\kappa_1 D + \kappa_2 E + \kappa_3 F + \kappa_4 DE + \kappa_5 DF + \kappa_6 EF + \kappa_7 DEF) \quad (3)$$

Que es una forma expandida que da como resultado 49 parámetros ajustables.

Tabla 1. Diseño experimental de dos matrices para un modelo especial cúbico x especial cúbico.

		Matrix I			Matrix II		
No	**Run**	**IN**	**L**	**MX**	**A**	**G**	**X**
1	44	100.0	0.0	0.0	0.0	50.0	50.0
2	15	0.0	100.0	0.0	100.0	0.0	0.0
3	51	0.0	100.0	0.0	0.0	100.0	0.0
4	3	0.0	100.0	0.0	0.0	0.0	100.0
5	20	0.0	100.0	0.0	50.0	50.0	0.0
6	57	0.0	100.0	0.0	50.0	0.0	50.0
7	48	0.0	100.0	0.0	0.0	50.0	50.0
8	32	0.0	0.0	100.0	100.0	0.0	0.0
9	35	0.0	0.0	100.0	0.0	100.0	0.0
10	41	0.0	0.0	100.0	0.0	0.0	100.0
11	11	0.0	0.0	100.0	50.0	50.0	0.0
12	18	0.0	0.0	100.0	50.0	0.0	50.0
13	39	0.0	0.0	100.0	0.0	50.0	50.0
14	25	50.0	50.0	0.0	100.0	0.0	0.0
15	43	50.0	50.0	0.0	0.0	100.0	0.0
16	47	50.0	50.0	0.0	0.0	0.0	100.0
17	8	50.0	50.0	0.0	50.0	50.0	0.0
18	40	50.0	50.0	0.0	50.0	0.0	50.0
19	27	50.0	50.0	0.0	0.0	50.0	50.0
20	19	50.0	0.0	50.0	100.0	0.0	0.0
21	1	50.0	0.0	50.0	0.0	100.0	0.0
22	23	50.0	0.0	50.0	0.0	0.0	100.0
23	7	50.0	0.0	50.0	50.0	50.0	0.0
24	34	50.0	0.0	50.0	50.0	0.0	50.0
25	55	50.0	0.0	50.0	0.0	50.0	50.0
26	45	0.0	50.0	50.0	100.0	0.0	0.0
27	37	0.0	50.0	50.0	0.0	100.0	0.0

28	2	0.0	50.0	50.0	0.0	0.0	100.0
29	30	0.0	50.0	50.0	50.0	50.0	0.0
30	54	0.0	50.0	50.0	50.0	0.0	50.0
31	58	0.0	50.0	50.0	0.0	50.0	50.0
32	16	33.3	33.3	33.3	33.3	33.3	33.3
33	5	100.0	0.0	0.0	33.3	33.3	33.3
34	56	0.0	100.0	0.0	33.3	33.3	33.3
35	24	0.0	0.0	100.0	33.3	33.3	33.3
36	6	50.0	50.0	0.0	33.3	33.3	33.3
37	28	50.0	0.0	50.0	33.3	33.3	33.3
38	29	0.0	50.0	50.0	33.3	33.3	33.3
39	31	33.3	33.3	33.3	100.0	0.0	0.0
40	38	33.3	33.3	33.3	0.0	100.0	0.0
41	26	33.3	33.3	33.3	0.0	0.0	100.0
42	14	33.3	33.3	33.3	50.0	50.0	0.0
43	33	33.3	33.3	33.3	50.0	0.0	50.0
44	42	33.3	33.3	33.3	0.0	50.0	50.0
45	52	100.0	0.0	0.0	100.0	0.0	0.0
46	36	100.0	0.0	0.0	0.0	100.0	0.0
47	53	100.0	0.0	0.0	0.0	0.0	100.0
48	59	100.0	0.0	0.0	50.0	50.0	0.0
49	13	100.0	0.0	0.0	50.0	0.0	50.0
50	22	66.7	16.7	16.7	66.7	16.7	16.7
51	12	66.7	16.7	16.7	16.7	66.7	16.7
52	17	66.7	16.7	16.7	16.7	16.7	66.7
53	4	16.7	66.7	16.7	66.7	16.7	16.7
54	50	16.7	66.7	16.7	16.7	66.7	16.7
55	21	0.0	0.0	100.0	0.0	0.0	100.0
56	10	0.0	0.0	100.0	100.0	0.0	0.0
57	46	0.0	0.0	100.0	0.0	100.0	0.0
58	9	0.0	0.0	100.0	50.0	0.0	50.0
59	49	0.0	0.0	100.0	0.0	50.0	50.0

Resultados y discusión

En este trabajo se evaluaron dos diferentes matrices de encapsulado para dos sistemas activos (antioxidante y microorganismo), para obtener un alimento

funcional por liofilización. En la matriz I formada por IN, L y MX, se obtuvo un rendimiento de 93.7% cuando IN está presente en un 66.71%, como ocurre en el experimento 54 (66.7IN; 16.7L; 16.7MX) y 87.6% cuando la IN está al 100% como es el caso del experimento 49 (100IN; 0L; 0MX). La matriz II que contiene gomas Arábiga (A), Guar (G), y Xantana (X), muestra un mejor rendimiento, correspondiente al 88.08% cuando las gomas A; G, y X están presentes en la misma proporción, por ejemplo en el experimento 32 (33.3A; 33.3G; 33.3X). Los resultados con respecto al rendimiento contribuyen a una amplia gama de respuestas, por ejemplo: I) el mayor rendimiento se obtiene cuando el agente acarreador en la matriz I se utiliza IN cercano a 100 y de la matriz II en una relación igual de A; G; X., II) El menor rendimiento al utilizar MX a 100 y de la matriz II al utilizar G a 100, III) Como utilizar estos encapsulantes matriz I (IN; L; MX) y la matriz II (A; G; X).

La viabilidad de *B. clausii* se midió mediante el método de unidades formadoras de colonia por gramo (CFU/g)). Los experimentos del 1 al 59 presentaron una disminución a 9.3 a 9.9 log10 CFU/g, en comparación con el control 11.30 log10 CFU/g. p. ej. para el experimento 40, con una proporción de 33.3IN; 33.3L; 33.3MX, se determinó una viabilidad de 9.85 log10 CFU/g. Sin embargo se observó la viabilidad más alta de *B. clausii*, 9.8 log10 CFU/g cuando se utilizó lactosa cerca de la unidad, como ocurre en el experimento 34 (0IN; 100L; 0MX). Al combinar 50 L; 50 MX, la viabilidad de *B. clausii,* fue de 9.37 log10 CFU/g, correspondiente al experimento 27 (0 IN; 50 L; 50 MX). En la matriz I cuando los agentes acarreadores están presentes en la misma proporción, como ocurre en el experimento 32 (33.3IN; 33.3L; 33.3MX), la viabilidad fue de 9.75 log10 CFU/g. Los resultados demuestran que el usar más de un agente acarreador mejora la supervivencia de *B. clausii*. Cabe mencionar que todas las muestras presentadas en este diseño de experimentos de tres componentes Scheffe especial cúbico x especial cúbico, sobrepasan el valor mínimo en viabilidad de 6.0 log10 UFC/g recomendado por la FAO/OMS (2002) para ser considerado probiótico. Para la matriz II se encontró la viabilidad más alta de *B. clausii* en dos condiciones, al utilizar la unidad de G y al emplear una mezcla de G y X, por ejemplo en el experimento 31 con una composición de 0 A; 50G; 50X y el experimento 3 (0A; 100G; 0X). Los resultados

obtenidos, permiten indicar que: I) La viabilidad más alta se obtienen cuando los agentes acarreadores utilizados en la matriz I son L y/o mezcla de IN, L y MX en una relación igual y en la matriz II al utilizar G cerca de la unidad y/o G y X en la misma proporción. II) La viabilidad más baja se observa al utilizar L y MX en proporciones iguales en la matriz I y X cercana a la unidad en la matriz II III) Qué combinación de los agentes acarreadores y gomas permite una mejor preservación de la viabilidad, IV) se obtuvo un alimento funcional ya que el valor mínimo de viabilidad 6.0 log10 UFC/g recomendado por la FAO/OMS (2002) para ser considerado probiótico se determinó por arriba de este valor en todos los estándares de esta investigación.

Se determinó la capacidad antioxidante por la inhibición del radical 2,2 difenil-1-picrilhidrazil (DPPH) a concentraciones de 5, 10 y 30 µg/ml (muestra), para la matriz I formada por IN, L y MX. La mayor AA se determinó cuando el agente acarreador está cerca a la unidad de inulina y corresponde al experimento 33 (100IN; 0L; 0MX) presenta una AA de 56.05 a una concentración de 5 µg/ml, de 58.75 para 10 µg/ml y 84.35 con 30 µg/ml. Sin embargo, cuando la matriz está compuesta por 50 IN; 50 L, que corresponde al experimento 15 (50IN;50L;0MX), la AA fue de 52.88 (5 µg/ml); 57.95 (10 µg/ml) y 81.51 (30 µg/ml). Las muestras que presentaron menos AA fueron las que están cerca a la unidad de MX, con una actividad 38.58 a 5 µg/ml; 43.27 a 10µg/ml y 63.1 para 30µg/ml. Para la matriz II compuesta por G; X; A, en la concentración de 5, 10 y 30µg/ml los resultados más favorables se obtienen al utilizar las G y X en proporciones iguales y sin presencia de A, por ejemplo, para el experimento 49 (0 A; 50 G; 50 X). Sin embargo, al estar presente la A cerca a la unidad la AA es menor por ejemplo para el experimento 20 (100 A; 0 G; 0 X) que corresponde a una AA 48.36 (5 µg/ml); 52.06(10 µg/ml) y 81.93(30 µg/ml). Los resultados obtenidos permiten identificar: I) Qué combinación de los agentes acarreadores y gomas permite un mayor porcentaje de actividad antioxidante, II) La actividad antioxidante más alta se obtienen cuando el agente acarreador utilizado en la matriz I es la inulina y en la matriz II G y X en la misma proporción para las concentraciones de 5 y 10 µg/ml, III) La actividad antioxidante más baja se observa al utilizar únicamente maltodextrina en la matriz I y G cercana a la unidad en la

matriz II. Este último aporte científico coincide con resultados reportados por los investigadores

Conclusiones

Se preparó un ingrediente funcional formado por B. clausii y quercetina para dos matrices: Matriz I formada por I; L; MX y la matriz II formada por A; G; X mediante un diseño de experimentos D-optimal. Con respecto al rendimiento, al utilizar los tres componentes en una proporción igual tanto para la matriz I como para la matriz II se obtuvo el mayor rendimiento (88.08%), para el experimento 32 (1/3 I; 1/3 L; 1/3 MX) y (1/3 A;1/3 G;1/3 X). Para la matriz I se observa la viabilidad más alta 9.8 log10 CFU/g al usar lactosa cerca a la unidad, correspondiente al experimento 34 (0IN; 100L; 0MX). La AA más alta cuando se usa la inulina cerca a la unidad, correspondiente al experimento 33 (100IN; 0L; 0MX), presentando una AA de 56.05 a una concentración de 5 µg/ml, 8.75 a 10 µg/ml y 84.35 con 30 µg/ml. Para la matriz II se presentó la viabilidad más alta en dos casos, al usar G cerca de la unidad y al emplear mezclas de G y X. por ejemplo en el experimento 31 con 9.9 log10 CFU/g (0 A; 50G; 50X) y el experimento 3 con 9.8 log10 CFU/g (0A; 100G; 0X). La AA más alta se obtuvo al utilizar G y X en proporciones iguales, correspondiendo al experimento 49 (0 A; 50 G; 50 X). con AA de 54.85 a una concentración de 5 µg/ml, 55.44 a 10 µg/ml y 67.09 con 30 µg/ml. El sinergismo entre las dos matrices ocurre al usar la proporción más alta de I y G, correspondiendo al experimento 46, con un rendimiento de 86.5%, viabilidad de 9.52 log10 UFC/g y una AA de 54.85 a una concentración de 5 µg/ml, 55.44 a 10 µg/ml y 67.09 con 30 µg/ml. Cabe mencionar que todas las muestras sobrepasan el valor mínimo en viabilidad de 6.0 log10 UFC/g recomendado por la FAO/OMS (2002) para ser considerado probiótico y contribuyen a la ingesta diaria de 10-16 mg/día de quercetina y en el caso de las muestras con inulina, a la ingesta de 10-20 g/día de este carbohidrato para obtener beneficios a la salud y de 25-38g de fibra por día

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Evaluation of two active system encapsulants matrices with quercetin and Bacillus claussii for functional foods

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Corresponding Author:	María Zenaida Saavedra Leos, Ph Universiada Autonoma de San Luis Potosí Matehuala, San Luis Potosí MEXICO
First Author:	Héctor Alfonso Enciso-Huerta
Order of Authors:	Héctor Alfonso Enciso-Huerta
	Miguel Ángel Ruiz-Cabrera
	Laura Araceli Lopez-Martinez
	Raúl Gonzalez-Carcia
	Fidel Martinez-Gutierrez
	María Zenaida Saavedra Leos, Ph
Abstract:	Currently, the demand for functional foods has been increasing in the public interest to improve their life expectations and general health. Food matrices containing probiotic microorganisms and active compounds encapsulated into carrier agents are essential in this context. Encapsulation by the lyophilization method is widely used because oxidation reactions affecting physicochemical and nutritional food properties are usually avoided. Encapsulated functional ingredients such as quercetin and Bacillus claussii using two carrier agents' matrices performed in I [inulin (I); lactose (L) and maltodextrin (MX)] and II [(Arabic (A), Guar (G) and Xanthan (X) Rubbers)] are presented in this work. A D-optimal procedure involving 59 experiments was designed to evaluate each matrix's yield, viability, and antioxidant activity (AA). Matrix I (33.3 I; 33.3 L; 33.3 MX) and matrix II (33.3 A; 33.3 G; 33.3 X) exhibited the best yield.
Suggested Reviewers:	Roberto Berni Canani, Doctor 1, University of Naples Federico II berni@unina.it
	Camelia Vizireanu, Doctor 2, Dunarea de Jos University of Galati Faculty of Medicine and Pharmacy camelia.vizireanu@ugal.ro
	Saber Amiri, Doctor 3, Urmia University m.rezazadehbari@urmia.ac.ir

Evaluation of two active system encapsulants matrices with quercetin and *Bacillus claussii* for functional lyophilized foods preparation

Héctor Alfonso Enciso-Huerta[1], Miguel Ángel Ruiz-Cabrera[1], Laura Araceli Lopez-Martinez[2], Raúl Gonzalez-Carcia[1], Fidel Martinez-Gutierrez[1] and María Zenaida Saavedra-Leos[3*]

[1] Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, Av. Dr. Manuel Nava 6, San Luis Potosí, S.L.P. C.P. 78210, México.

[2] Coordinación Académica Región Altiplano Oeste, Universidad Autónoma de San Luis Potosí, Salinas de Hidalgo S.L.P. C.P. 78600, México.

[3] Coordinación Académica Región Altiplano, Universidad Autónoma de San Luis Potosí, 11 Carretera Cedral Km, 5+600 Ejido San José de las Trojes Matehuala, S.L.P. C.P. 78700, México.

* Corresponding Author. E-mail address: zenaida.saavedra@uaslp.mx

Abstract

Currently, the demand for functional foods has been increasing in the public interest to improve their life expectations and general health. Food matrices containing probiotic microorganisms and active compounds encapsulated into carrier agents are essential in this context. Encapsulation by the lyophilization method is widely used because oxidation reactions affecting physicochemical and nutritional food properties are usually avoided. Encapsulated functional ingredients such as quercetin and *Bacillus claussii* using two carrier agents' matrices performed in I [inulin (I); lactose (L) and maltodextrin (MX)] and II [(Arabic (A), Guar (G) and Xanthan (X) Rubbers)] are presented in this work. A D-optimal procedure involving 59 experiments was designed to evaluate each matrix's yield, viability, and antioxidant activity (AA). Matrix I (33.3 I; 33.3 L; 33.3 MX) and matrix II (33.3 A; 33.3 G; 33.3 X) exhibited the best yield.

Keywords: functional, food, Inulin, Lactose

1. Introduction

According to the Codex alimentarius (Commission, 2007), food is any processed, semi-processed, or raw non-processed substance intended for human consumption (CXS 1-1985) with nutritional support. In the last years, derived from the SARS-CoV-2 (COVID-19) worldwide pandemic, the World Health Organization (WHO) suggested consuming healthy and essential foods for immunologic system function to prevent and considerably reduce the probability of appearance of diseases such as obesity, diabetes, cardiac, and some cancer types. For the reasons above, food plays a significant role in disease prevention, impacting public health. A “functional food” (FF) is defined by the European Society for Clinical Nutrition and Metabolism (ESPEN) guide as an enriched food with ingredients, nutrients, or additional compounds to manifest specifically benefits to health. In the last decade, FF production has become an important biotechnology industry given the growing consumer interest in improving their life expectancy and healthy due to awareness-raising about the prevention of certain diseases such as diabetes, cancer, and Alzheimer's (Sarao & Arora, 2017). Commonly, antioxidants are compounds of FF, added alone or mixed with other compounds to allow synergism, i.e. vitamin C to regenerate vitamin E tocopheryl radical after its oxidation (Addor, 2017). Antioxidants are added, obtaining different benefits, such as suppressing lipidic oxidation, increasing products' shelf life, and reducing the free radical concentrations inside of organism, consequently improving consumers' healthy (Ciriminna et al., 2017). Quercetin is an antioxidant flavonoid found in apples, grapes, beans, broccoli, red onion, tomatoes, oilseeds, flowers, tea leaves, and Ginko Biloba. Between 10-16 mg is ingested daily, although the recommended concentration is 1g daily (Khan et al., 2020). Different quercetin mechanisms had been reported in various diseases, i.e., in allergic asthma, the compound showed the inhibition of MUC5AC gene expression in NCI-H292 cells, triggered nasal mucosa human being anti-secretory agent preventing

50 the mucosa secretion in epithelial cells while maintaining a normal ciliary movement (Jafarinia et al.,
51 2020).
52 Marunaka et al. (2017). demonstrated that oral quercetin ingests of 150-730 mg daily for four weeks had
53 antihypertensive, reducing systolic and diastolic pressure in patients in the first stage of hypertension.
54 On the other hand, patients with metabolic syndrome who had consumed 150 mg daily of quercetin for
55 five weeks significantly reduced their systolic pressure. An in vitro study by Reyes-Farias & Carrasco-
56 Pozo. (2019) showed that quercetin functions as an antiviral agent against HIV, inhibiting the integrase,
57 protease, and inverse transcriptase enzymes reducing HIV replication significantly.
58 Probiotics are other compounds present in FF, and their use confers benefits to health through the
59 production of biliary enzymes, organic acid, satiety hormones, and immune system modulation through
60 the improvement of antibodies response, the competence of substrates against pathogenic organisms,
61 and the interaction with the microbiota (Sanders et al., 2019). Bacillus *clausii* is an anaerobic bacterium,
62 gram-positive, used as a probiotic capable of generating spores and intestinal colonizers (Paparo et al.,
63 2020). B. clausii is also resistant to heat, gastric pH, and antibiotics and can tolerate alkaline conditions.
64 Nevertheless, its optimal conditions are 40°C at the pH of 9,0 (De Castro et al., 2019). De Castro et al.
65 (2019). reported using it for seven days to treat acute infant diarrhea by viral cause or associated with
66 antibiotics, showing the reduction of disease time, gastrointestinal symptoms, and evacuation frequency.
67 Recently, in 2020 (Plomer et al., 2020). used B. *clausii* to reduce the adverse effects of Helicobacter
68 pylori treatment, generally treated by antibiotics causing nausea, inflammation, vomit, and diarrheas
69 triggering treatment failure and bacterial resistance.
70 Microencapsulation techniques are used to conserve any active ingredient sought to be added to food
71 to preserve the antioxidant activity and viability of probiotic products against heat, oxygen, and humidity
72 (Zuidam & Nedović, 2010). Drying by aspersion and lyophilization are most employer techniques for
73 probiotic foods' production. Lyophilization preserves not only the nutritional foods' qualities but also their
74 aromas and flavors, permitting their rehydration after the process by the final product porosity (Muñoz-

López et al., 2018). The Lyophilization technique is widely used in the food industry because, unlike the heat drying process, it does not catalyze oxidation reactions that can affect the properties of physicochemical and nutritional food (Caballero et al., 2017). Lyophilization drying uses sublimation of water and can be used to encapsulate aromas, scents, drugs, and in general, any thermosensitive material. Taking into consideration the antioxidants and probiotics for health, this research was planted to obtain a functional ingredient containing B. c*lausii* and quercetin microencapsulated by lyophilization.

2. Materials and methods

2.1 Materials

Commercial Maltodextrin (MX) extracted from maize starch was acquired from INGREDION Mexico (Guadalajara, Mexico). Dextrose (DE) equivalent of MX was 10; with a molecular weight of 1625 g/mol and polymerization grade (DP) of 2-16 glucose units. Inulin (IN) was purchase from INGREDION Mexico (Guadalajara, Mexico). α-lactose monohydrate (L), (Lα·H2O, purity ≥ 99,9 %) purchased from Sigma-Aldrich Chemical Co., Methanol, (MeOH, purity ≥99,8) was obtained from J.T. Baker. Rubbers Arabic (A), Guar (G) and Xanthan (X) from INGREDION Mexico (Guadalajara, Mexico). *Bacillus* strain (*B. clausii*) in sinuberase solution was purchased from Sanofi-Aventis Mexico, S.A. de C.V. (Coyocan, Mexico City, Mexico). Quercetin 3-D-Galactose (purity ≥99%) was acquired from Química Farmacéutica Esteroidal S.A de C.V.., (Tlahuac, Mexico City, Mexico). Trypticase Soy Agar was obtained from Dickinson de México S.A de C.V. (Mexico City, Mexico). And 2,2-diphenyl-1-picrilhidrazile (DPPH) analytical grade from Sigma–Aldrich Chemical Co.

2.2 Lyophilizes preparation

Based on the mix's experiments, was prepared 100 g of samples (p/p), 10 g of matrix I, performed with IN, L, and MX, and 1 g of Matrix II of rubbers containing: A, G, and X. Each mass fraction for matrix I and II were established according to the experimental design. The compounds of each matrix were passed

by a 1 mm sieve and added to 87 g of deionized water, followed by magnetic agitation at 35 °C for 5 minutes.

1 g of quercetin and 1g of B. clausii solution for 100 g final volume of solution (p/p) was added. Samples were stored in darkness at -80 °C. The micro-encapsulation process was performed by sublimation in a lyophilizer (IlShinbiobase® Model TFD8501, Gyeonggi-do, South Korea) with a vacuum pressure of 5 mTorr -65 °C for approximately 120 h.

2.3 Determination of microbial viability

The viability of B. clausii before and after the encapsulation process was determined, resuspending 1 g of the microparticles obtained in 9 ml of saline solution (NaCl, 0.9% w/v). To break microcapsules, the suspension was agitated for 10 minutes with a vortex and incubated in a water bath for 10 minutes at 50 °C. The viable cells were analyzed according to the method described by Miles et al. (1938). Briefly, dilutions of $1x10^{-3}$ a $1x10^{-9}$ performed in saline solution were sown on trypticase soy agar and incubated at 35 °C for 24 h. The evaluation was performed in triplicate and reported in colony-forming units per gram (CFU/g), using equation 1:

$$Viability = \left[\frac{Number\ of\ colonies\ in\ box * dilution\ factor}{mL\ of\ sample\ sown}\right] \quad (1)$$

2.4 Antioxidant activity (AA)

Quercetin antioxidant capacity was determinate according to the method described by Brand-Williams et al. (1995). Briefly, 1.7 mL of alcoholic solution of DPPH (0,1 mmol DPPH/L) were mixed with 1,7 mL of microencapsulated suspension, where the concentration of the microencapsulated varied from 2.5, 5, and 15 µg/mL. The mixture was left to stand in darkness for 30 minutes, and the absorbance at 537 nm was measured using a spectrophotometer UV-Vis Evolution 220 (Thermo Scientific, Walthman, MA. USA). The sweep percentage was calculated using equation 2:

$$AA\ (\%DPPH) = \frac{A0-A30}{A0} x100 \qquad (2)$$

Where A_o represent the absorbance of blank solution (DPPH mixture and ethanol without microencapsulates), A_{30} represent the absorbance of DPPH solution and ethanol with microencapsulates after 30 minutes. The sweep activity was determined by triplicate for each sample.

2.5 Design of experiments and statistical analysis

Two independent mixtures were tested: Matrix I consisted of inulin (IN), lactose (L), and maltodextrin (MX), while Matrix II consisted of rubbers Arabic (A), guar (G), and xanthan (X). The lower and upper levels of these variables were between 0 and 100 (wt %), and the sum of the components in each mixture was 100% for each trial. The response variables were yield (%), *Bc* (Log10 CFU/g), and Antioxidant activity (the DPPH at concentrations of 5, 10, and 30 (µg/g)). In this manner, a combined experimental design of two matrices for a model Special Cubic x Special Cubic was selected to evaluate the effect of each factor over each response variable. Table 1 shows the resulting 59 trials performed at the laboratory in random order.

An analysis of variance (ANOVA), was performed for each response (yield*, Bc*, Antioxidant activity) using Design-Expert® Version 12 Software (trial version) at the significance level of 0.05. The analyzed Scheffe model, special cubic x special cubic, was written as Equation 3:

$$Y = (\alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_4 AB + \alpha_5 AC + \alpha_6 BC + \alpha_7 ABC) \times (\kappa_1 D + \kappa_2 E + \kappa_3 F + \kappa_4 DE + \kappa_5 DF + \kappa_6 EF + \kappa_7 DEF) \qquad (3)$$

which is an expanded way that results in 49 adjustable parameters.

Table 1. Experimental design of two matrices for a model special cubic x special cubic.

		Matrix I			Matrix II		
No	**Run**	**IN**	**L**	**MX**	**A**	**G**	**X**
1	44	100.0	0.0	0.0	0.0	50.0	50.0
2	15	0.0	100.0	0.0	100.0	0.0	0.0

3	51	0.0	100.0	0.0	0.0	100.0	0.0
4	3	0.0	100.0	0.0	0.0	0.0	100.0
5	20	0.0	100.0	0.0	50.0	50.0	0.0
6	57	0.0	100.0	0.0	50.0	0.0	50.0
7	48	0.0	100.0	0.0	0.0	50.0	50.0
8	32	0.0	0.0	100.0	100.0	0.0	0.0
9	35	0.0	0.0	100.0	0.0	100.0	0.0
10	41	0.0	0.0	100.0	0.0	0.0	100.0
11	11	0.0	0.0	100.0	50.0	50.0	0.0
12	18	0.0	0.0	100.0	50.0	0.0	50.0
13	39	0.0	0.0	100.0	0.0	50.0	50.0
14	25	50.0	50.0	0.0	100.0	0.0	0.0
15	43	50.0	50.0	0.0	0.0	100.0	0.0
16	47	50.0	50.0	0.0	0.0	0.0	100.0
17	8	50.0	50.0	0.0	50.0	50.0	0.0
18	40	50.0	50.0	0.0	50.0	0.0	50.0
19	27	50.0	50.0	0.0	0.0	50.0	50.0
20	19	50.0	0.0	50.0	100.0	0.0	0.0
21	1	50.0	0.0	50.0	0.0	100.0	0.0
22	23	50.0	0.0	50.0	0.0	0.0	100.0
23	7	50.0	0.0	50.0	50.0	50.0	0.0
24	34	50.0	0.0	50.0	50.0	0.0	50.0
25	55	50.0	0.0	50.0	0.0	50.0	50.0
26	45	0.0	50.0	50.0	100.0	0.0	0.0
27	37	0.0	50.0	50.0	0.0	100.0	0.0
28	2	0.0	50.0	50.0	0.0	0.0	100.0
29	30	0.0	50.0	50.0	50.0	50.0	0.0
30	54	0.0	50.0	50.0	50.0	0.0	50.0
31	58	0.0	50.0	50.0	0.0	50.0	50.0
32	16	33.3	33.3	33.3	33.3	33.3	33.3
33	5	100.0	0.0	0.0	33.3	33.3	33.3
34	56	0.0	100.0	0.0	33.3	33.3	33.3
35	24	0.0	0.0	100.0	33.3	33.3	33.3
36	6	50.0	50.0	0.0	33.3	33.3	33.3
37	28	50.0	0.0	50.0	33.3	33.3	33.3
38	29	0.0	50.0	50.0	33.3	33.3	33.3
39	31	33.3	33.3	33.3	100.0	0.0	0.0
40	38	33.3	33.3	33.3	0.0	100.0	0.0
41	26	33.3	33.3	33.3	0.0	0.0	100.0
42	14	33.3	33.3	33.3	50.0	50.0	0.0
43	33	33.3	33.3	33.3	50.0	0.0	50.0
44	42	33.3	33.3	33.3	0.0	50.0	50.0

45	52	100.0	0.0	0.0	100.0	0.0	0.0
46	36	100.0	0.0	0.0	0.0	100.0	0.0
47	53	100.0	0.0	0.0	0.0	0.0	100.0
48	59	100.0	0.0	0.0	50.0	50.0	0.0
49	13	100.0	0.0	0.0	50.0	0.0	50.0
50	22	66.7	16.7	16.7	66.7	16.7	16.7
51	12	66.7	16.7	16.7	16.7	66.7	16.7
52	17	66.7	16.7	16.7	16.7	16.7	66.7
53	4	16.7	66.7	16.7	66.7	16.7	16.7
54	50	16.7	66.7	16.7	16.7	66.7	16.7
55	21	0.0	0.0	100.0	0.0	0.0	100.0
56	10	0.0	0.0	100.0	100.0	0.0	0.0
57	46	0.0	0.0	100.0	0.0	100.0	0.0
58	9	0.0	0.0	100.0	50.0	0.0	50.0
59	49	0.0	0.0	100.0	0.0	50.0	50.0

3. Results and discussion

3.1 Microencapsulation performance

The microencapsulation technique comprises the coating of small particles to form capsules with unique properties and different morphologies, which can reach diameters between nanometres to millimeters, protect bioactive ingredients against adverse reactions, and improve their functionality and bioavailability. (Zambrano et al., 2019). Lyophilization is a microencapsulation process comprising the product's previous freezing (-40°C) to be lyophilized, to after ice to sublime at sub-atmospheric pressures.

This work evaluated two different encapsulating matrices for two actives systems (antioxidant and microorganism) to get functional lyophilized food. Matrix I containing IN, L and MX, as shown in Figure 1a, an efficiency of 93.7% when IN is present at 66.71%, for example experiment 54 (66.7IN; 16.7L; 16.7MX) and 87.6% for IN at 100% as is the case for experiment 49 (100IN; 0L; 0MX). Matrix II, containing Arabic (A), Guar (G), and Xanthan (X) rubbers, shows better efficiency, corresponding to 88.08% when A; G, and X rubbers are present in the same proportion, e. g. the 32 experiment (33.3A;

33.3G; 33.3X), as shown in figure 1b. These results are consistent with (Enache et al. (2020) they employed to dry lyophilisation method to blackcurrant (*Ribes nigrum*) extract anthocyanins and encapsulate with bacteria (*Akkermansia muciniphila and Lactobacillus plantarum*) micro-encapsulate efficiency employing inulin and chitosan as carrier agent, reporting 95.46% ± 1,30% and 87,38% ± 0,48% efficiency, respectively. Pudziuvelyte et al. (2020), reported the ethanolic extract of *Elsholtzia ciliata*, microencapsulate lyophilisation yield with 6 carrier agents at 20% concentration and its mixes at 10% concentration: they employed Arabic rubbers (GUM_E), maltodextrin (MALTO_E), resistant maltodextrin (RES_E), skimmed milk (SKIM_E), sodium caseinate (SOD_CAS_E), beta-cyclodextrin (BETA_CYCL_E), the authors indicated that higher yield was observable when they employed SKIM_E and MALTO_E showed 100% and 95% efficiency, respectively, for mixtures of their observed 100% yield under two situations, use SKIM_E;MALTO_E and GUM_E; BETA_CYCL_E. Sharifi et al. (2021) performed co-microencapsulate Lactobacillus Plantarum and phytosterols mixtures formed by β-sitosterol (49.54%), campesterol (26.12%), stigmasterol (19.1%), Brassicasterol (1.48%). Researchers used Arabic rubber (GA), 2.25% w/v, and whey protein isolate (WPI), 5% w/v encapsulating agents. They formed coacervate followed by two dry process techniques: aspersion dry and lyophilization, obtaining 58.62±2.01% and 65.23±0.51% yield, respectively. These results demonstrate improved performance when using a dry freeze-drying process. The yield results contribute to a range of responses, for example: (i) higher yields are obtained when the carrier agent used in matrix I is IN closer to 100, for matrix II the A; G; X in the same ratio, (ii) lower yields when using only MX at 100, in the case of matrix II when using G 100.

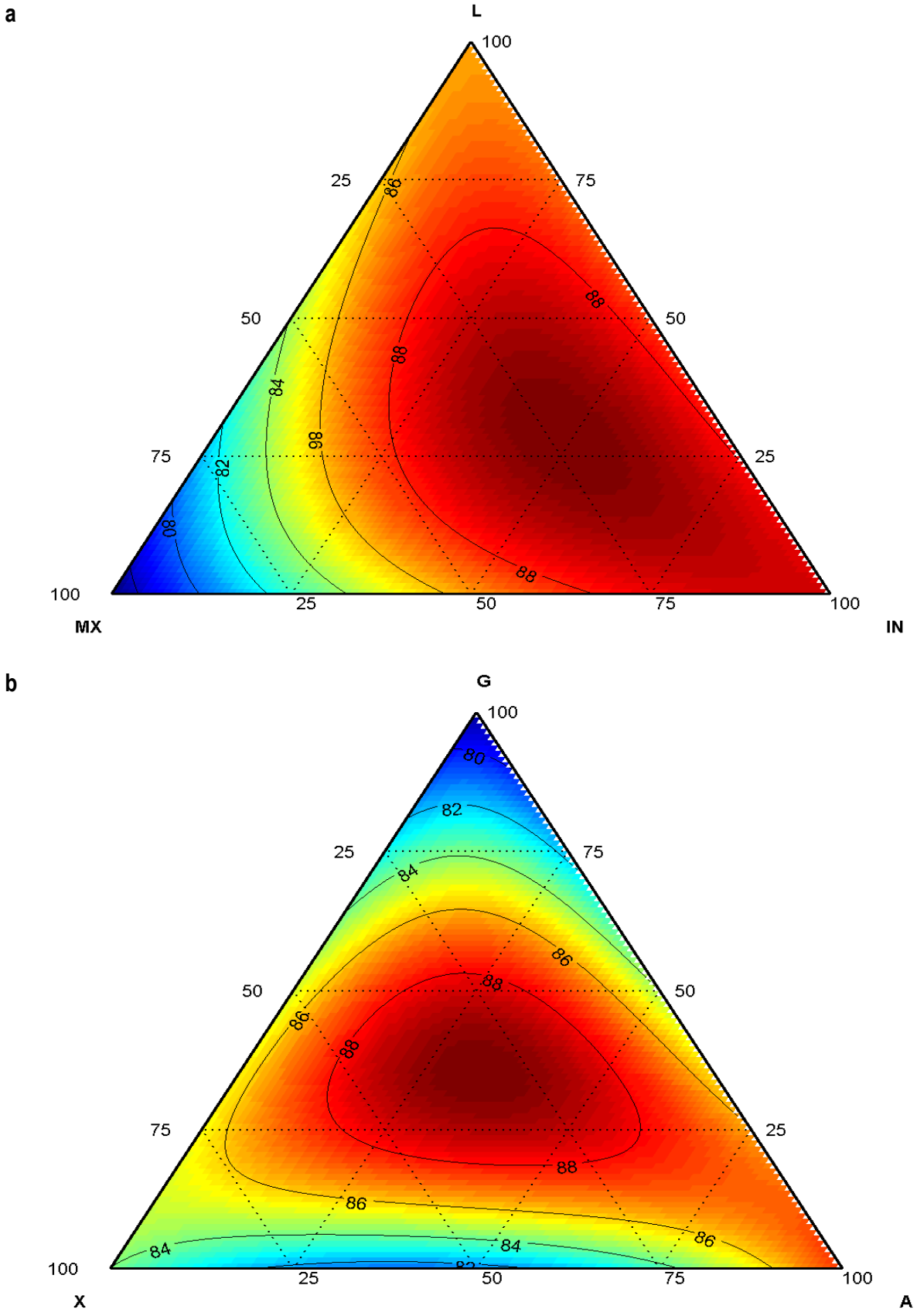

Figure 1. Yield surface graphic. (a) matrix I IN;L;MX, (b) Matrix II A; G; X

3.2. Viability of B. clausii microencapsulated

The viability of *B. clausii* was measured by the method Colony Forming Unit (CFU/g)). The results shown in figure 2a were determined concerning the viability in matrix I. Experiments 1 to 59 had viability diminution at 9.3 to 9.9 log10 CFU/g rate against control 11.30 log10 CFU/g. E.g., For experiment 40, with the proportion of 33.3IN; 33.3L; 33.3MX, a viability value of 9.85 log10 CFU/g was determined. Notwithstanding, we observed the higher viability of B. clausii 9.8 log10 CFU/g when we used L closer to the unit, as experiment 34 shows (0IN; 100L; 0MX). Using a 50 L; 50 MX ratio, B. clausii viability was 9.37 log10 CFU/g, corresponding to experiment 27 (0 IN; 50 L; 50 MX). In matrix I, when the carrier agents were present in the same proportion, matching with experiment 32 (33.3IN; 33.3L; 33.3MX the viability was 9.75 log10 CFU/g. Results show that using more than encapsulating agent improves the B. clausii survivance. It is worth mentioning that the standards presented in these experimental designs, particular three components Scheffe Special cubic x Special cubic, overpass the 6.0 log10 CFU/g minimum value recommended by FAO/OMS (2002) to be considered probiotic and correlated with previous reports as Enache et al. (2020), who performed the co-microencapsulate *Lactobacillus casei* and the blackcurrant (Ribes nigrum) extract anthocyanin dried by lyophilization. They employed whey protein isolate (WPI), chitosan, and inulin at a 2:1:1 rate as carrier agents. The authors reported that the viability powder was 11 log10 UFC/g as started value, after storage for 90 days at 4 °C it reduced to 8.13-6.35 log10 UFC/g. Showing the stability of carrier agents' mixtures, Milea et al. (2020), reported the viability of co-microencapsulate via flavonoids lyophilization, got of yellow onion peelings (Allium cepa) and Lactobacillus casei employing whey protein isolate (WPI), inulin (I), and maltodextrin (MD) as carrier agents (2:1:1 proportion). The got encapsulated by lyophilization at 1% and, 2% concentrations were probed into food one (cream cheese). They reported results after storage of 21 days at 4 °C, showing a 6.6 and 7.41 log10 UFC/g viability at concentrations mentioned above. Cayra et al. (2017) suggest protection provided by L, IN, and MX materials to cellular structures of microorganisms by the formation of crystals and water molecules replacement at the polar groups of cellular membrane lipids. For the

Matrix II, we find the higher *B. clausii* viability value in two conditions, to use the unit of G and to employ a G and X mixture. E.g., the 31 experiment with 0 A; 50G; 50X composition and the 3 experiment (0A; 100G; 0X). Got results showed I) higher viability was obtained when I and L were employed as a carrier agent in the matrix I and/or IN, L and MX at the same proportion, by the matrix II to use G closer to the unit and/or G and X at the same ratio. II) Lower viability was observed to use L and MX at the same rate in matrix I, and X closer to the unit in the matrix II. III) The better combination of carrier agents and rubbers that allows a better viability preservation. IV) We obtained one functional food, since the viability minimum value 6.0 log10 CFU/g recommended by the FAO/OMS (2002) to be considerate as probiotic was determined over cross the value in all experiment of this research.

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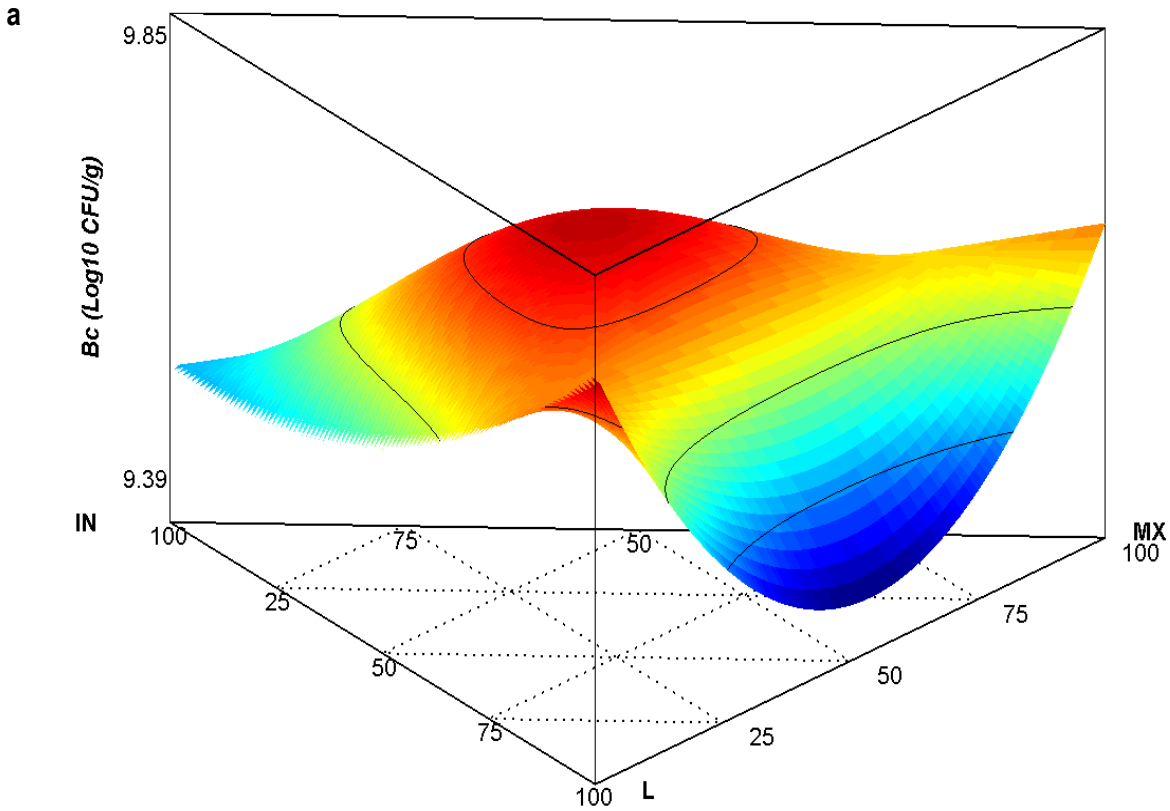
a
9.85
9.39
Bc (Log10 CFU/g)
IN
100
25
50
75
100
L
100
75
50
25
MX
100
75
50
25

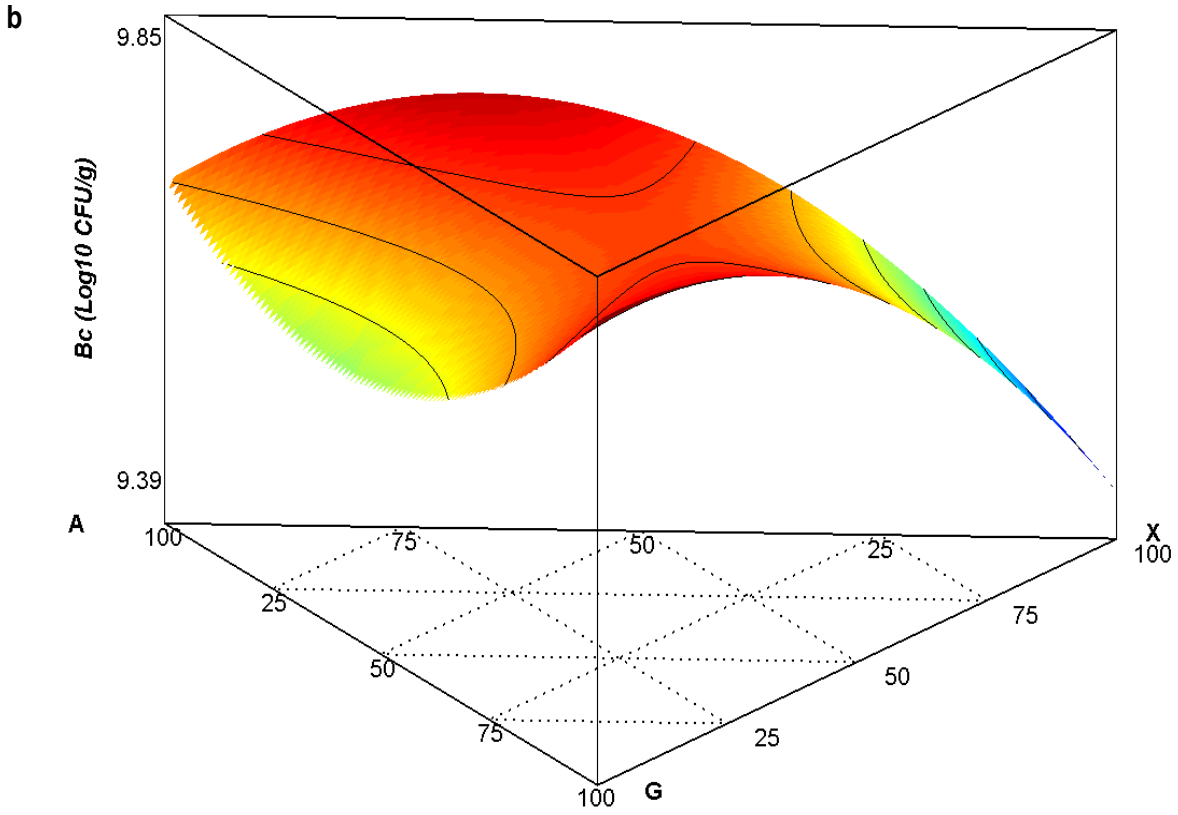

Figure 2. Graphics of surface responses to *B. clausii* viability dried by lyophilization expressed at $\log_{10}$ (CFU/g). (a) Matrix I IN; L; MX, (b) Matrix II A; G; X.

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235 *3.3. Antioxidant capacity determination*

236 Antioxidant capacity was determined by 2,2 diphenyl-1-picrilhidrazil (DPPH) radical inhibition using 5,10
237 and 30 µg/ml (sample) as concentrations, as can be observed in figures 3 a), b) and c) the matrix I formed
238 by IN, L and MX, higher antioxidant activity (AA) was determinate when the carrier agent is closer to inulin
239 the unit and correspond to the 33 experiment (100IN; 0L; 0MX) presenting a 56.06 AA to 5 µg/ml
240 concentration, 58.75 to 10 µg/ml, and 84.35 with 30 µg/ml. Nevertheless, when the matrix is compound
241 by 50 IN; 50 L, corresponding to the 15 experiment (50IN;50L;0MX) AA was 52.88 (5 µg/ml); 57.95 (10
242 µg/ml) and 81.51 (30 µg/ml). Samples that presented less AA were those that were closer to the MX unit,
243 as shown in figures 3a;b;c; with an activity of 38.58 at 5 µg/ml; 43.27 at 10 µg/ml y 63.1 to 30µg/ml.
244 These results correlate with observations reported by other authors such as Martins et al. (2021), who
245 performed a lemongrass (*Cymbopogon citratus* DC. Stapf) essential oil micro-encapsulation study, the
246 object of this work was to evaluate development, characterization and produced particle antioxidant

potential. Generated three different formulations for essential oil encapsulate, called M1 (5% of essential oil), M2 (10% of essential oil) and M3 (15% of essential oil), each mixture used maltodextrin MD (DE 20) and gelatine (GEL) at 4:1 (w/w) ratio as encapsulates agents. Result emulsions were lyophilized under 0.011 mbar and −60 °C conditions for 48 h. Concerning antioxidant activity, authors reported in general way variety of the presence of bioactive compounds, functional groups and polarities such as part of essential oil, the lemongrass essential oil antioxidant effect had a start value of antioxidant capacity of 22.16±0.04 mg TE/g measured by the DPPH method, after their lyophilization, samples presented as the antioxidant potential for M1 2.46±0.12 mg TE/g, M2 7.74±0.05 mg TE/g and M3 12.10±0.30 mg TE/g. Results showed that the MD (DE 20) use influences the antioxidant capacity. Azarpazhooh et al. (2019), evaluated pomegranate (Punica granatum L.) grinds extracted by the DPPH method antioxidant capacity (RSA). The process consisted of the use of Maltodextrin (MDX) in three proportions 5, 10 and 15% as carrier agent, and calcium alginate at 0.1/ (w/w), at 1:5 proportion. Researchers reported lower inhibitory concentration (IC50) 0.56 mg/ml at MDX to 15% sample. Against the 0.86 mg/ml IC50 observed in MDX at a 5% sample, their results indicate the influence MDX concentration over RSA increase. Notwithstanding, the obtained results could have been influenced by anthocyanins and polyphenols presents in the sample.

For matrix II consisting of G; X; A, we show the results in figures 4 a), b) and c) at the concentration of 5, 10 and 30µg/ml more favourable results were obtained using the G and X at the same proportions without A presence. E.g. for the 49 experiment (0 A; 50 G; 50 X). Notwithstanding, with A closer to the unit, AA is lower, e.g. for the 20 experiment (100 A; 0 G; 0 X) corresponds to AA of 48.36 (5 µg/ml); 52.06 (10 µg/ml) and 81.93(30 µg/ml) as shown in table 1. Mansour et al. (2020), micro-encapsulated anthocyanins (AC) extracts obtained by lyophilization from raspberries (*Rubus idaeus L.*). Evaluating three different anthocyanins concentrations, 0.025%, 0.05% and 0.075%, two encapsulating agents, soy protein isolate (SPI) and Arabic rubber (GA) at 5% concentration w/v and SPI; GA at 2.5;2.5 % w/v concentration.

Antioxidant capacity observed for these compounds at 0.025% was 25% for SPI, 45% for GA and 35% for SPI+GA mixture.

Rezende et al. (2018). Elaborated on industrial waste and pulp of steelyard (*Malpighia emarginata* DC). micro-encapsulate. They employed Arabic rubber (GA) and maltodextrin (MD) mixture in the same proportion (1:1; w/w) as carrier agents. They contrasted the antioxidant activity through the DPPH method for the industrial waste and pulp of steelyard by the dry aspersion and lyophilisation process. Antioxidant activity after the samples encapsulation process was 129.16 µM TE/g for lyophilised samples and 155.24 µM TE/g for the samples dried by aspersion. Obtained results alow to identify I) carrier agents combination and rubbers that allow the best antioxidant activity percentage, II) higher antioxidant activity was obtained when the carrier agent used in the matrix I is inulin, for matrix II G and X at the same proportion for the 5 y 10 µg/ml concentrations, III) lower antioxidant activity is observing to use only maltodextrin in the matrix I and G closer to the unit in the matrix II. The last evidence is in line with results reported by researchers.

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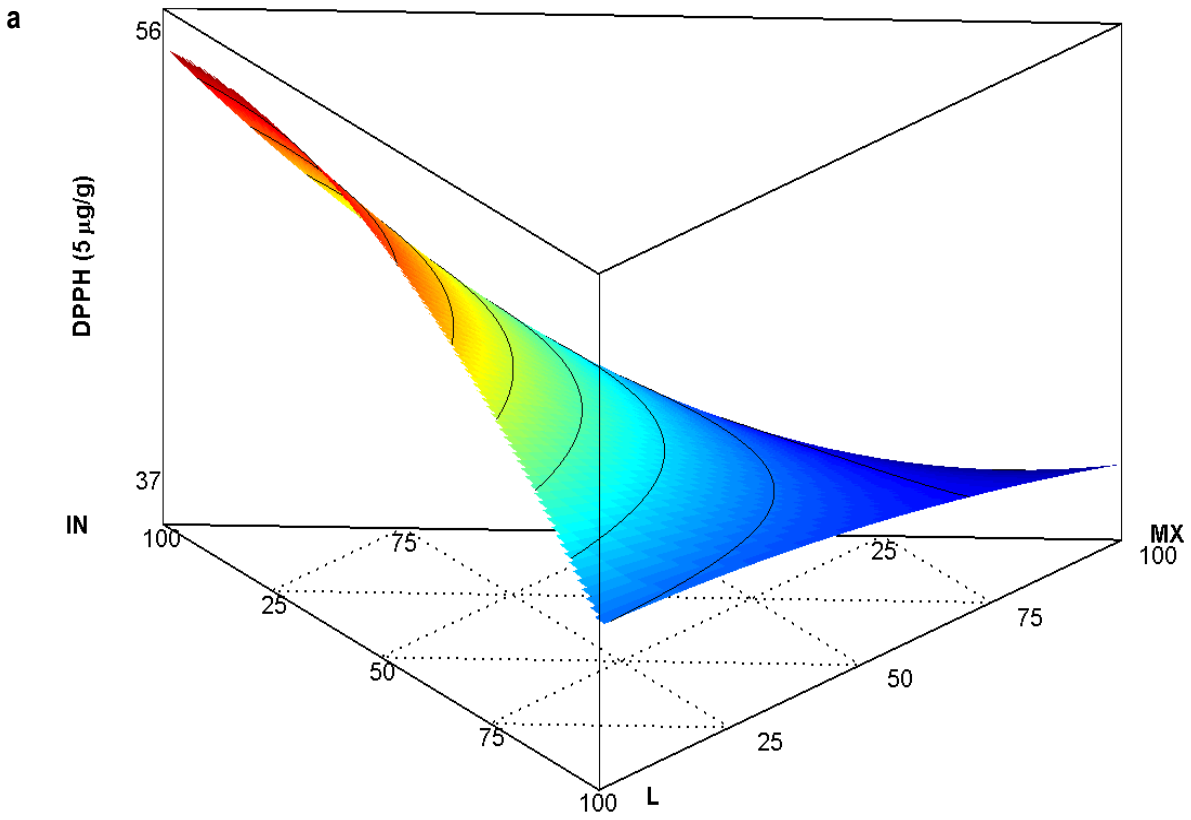
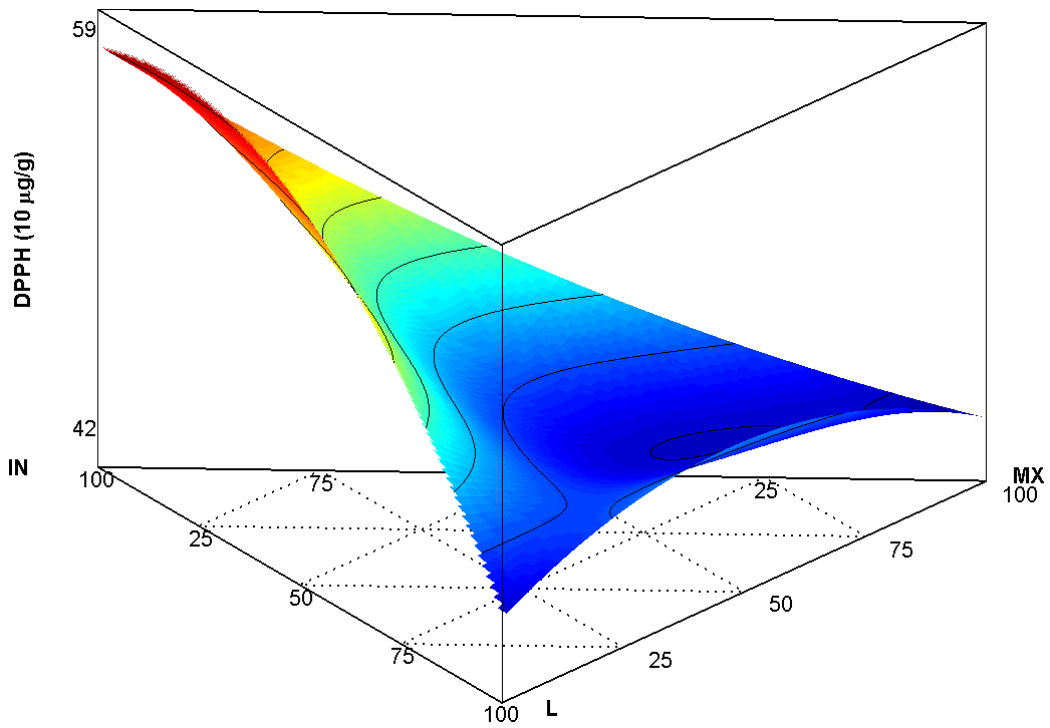


b



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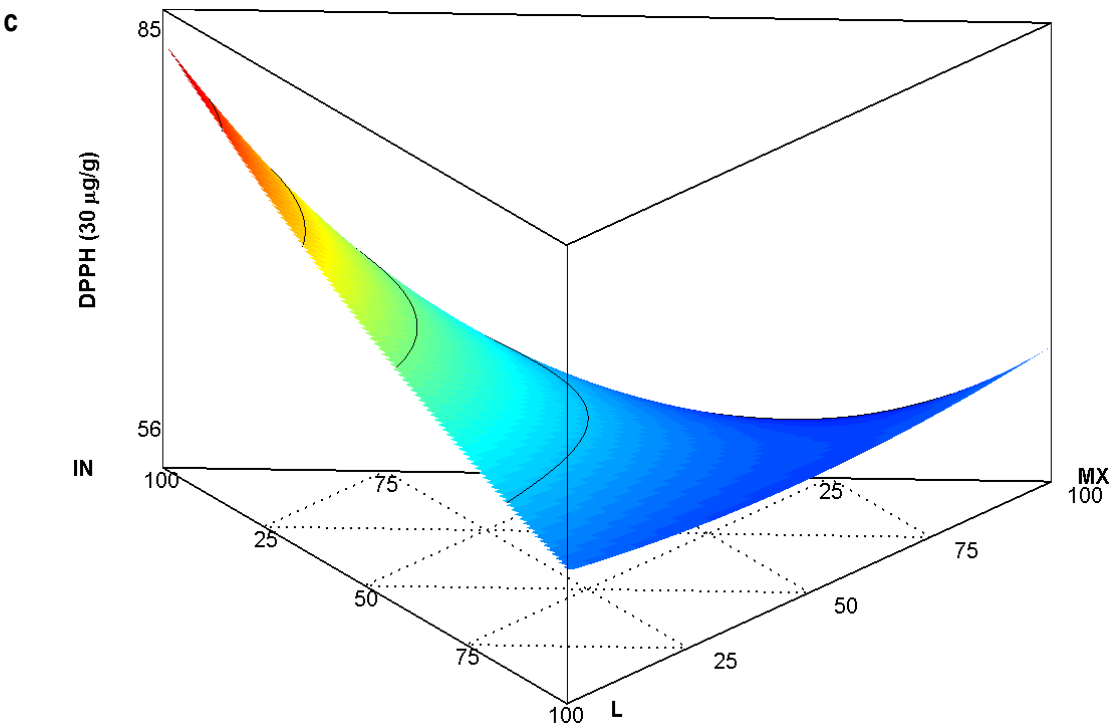
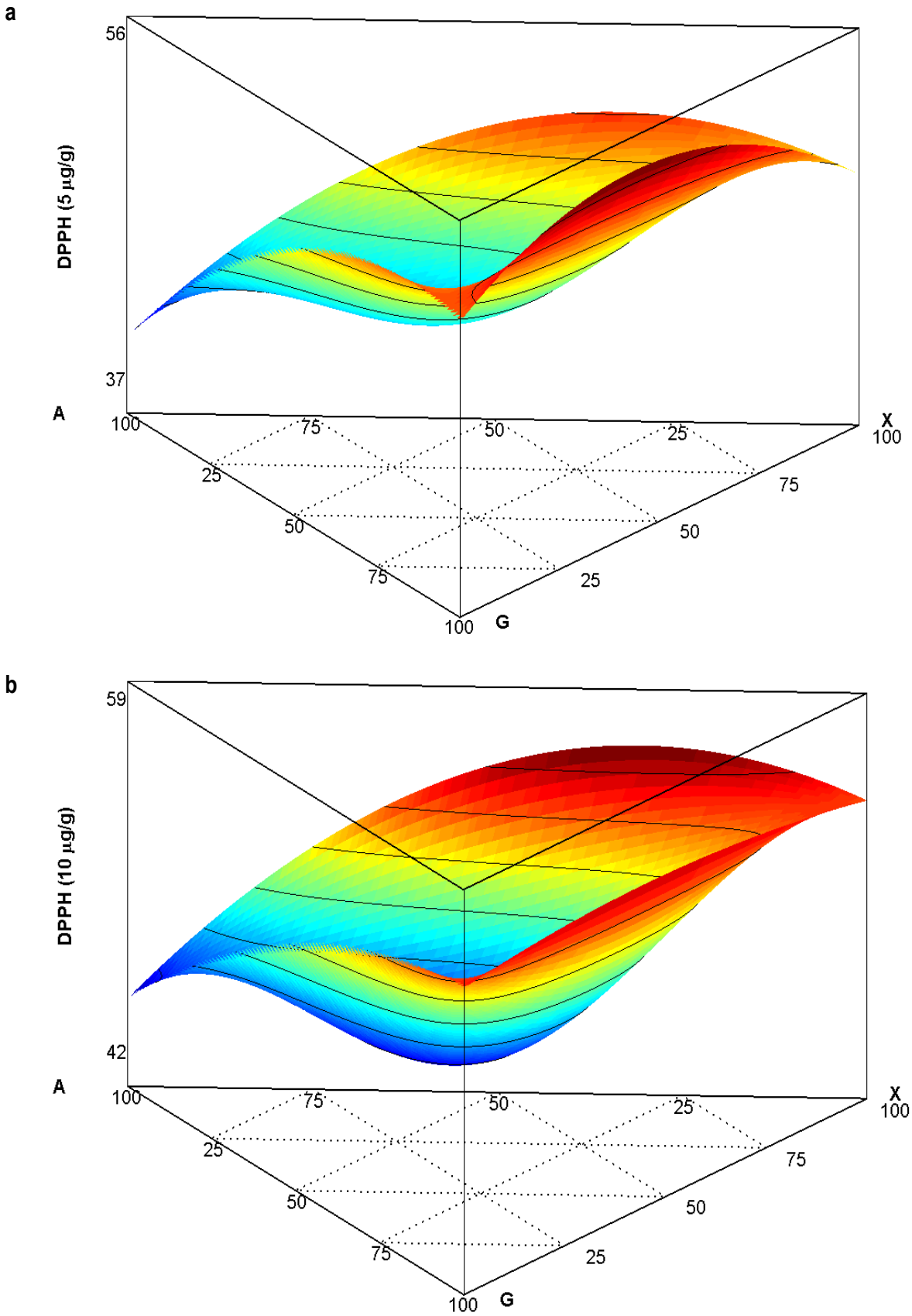


Figure 3. Antioxidant capacity by the 2,2 difenil-1-picrilhidrazil (DPPH) radical inhibition **a)** 5 µg/ml**,** **b**) 10 µg/ml and **c**) 30 µg/ml (sample), for matrix **I** formed by IN, L and MX


a
56
DPPH (5 µg/g)
37
A
100
25
50
75
100
G
50
25
75
50
25
X
100
b
59
DPPH (10 µg/g)
42
A
100
25
50
75
100
G
50
25
75
50
25
X
100

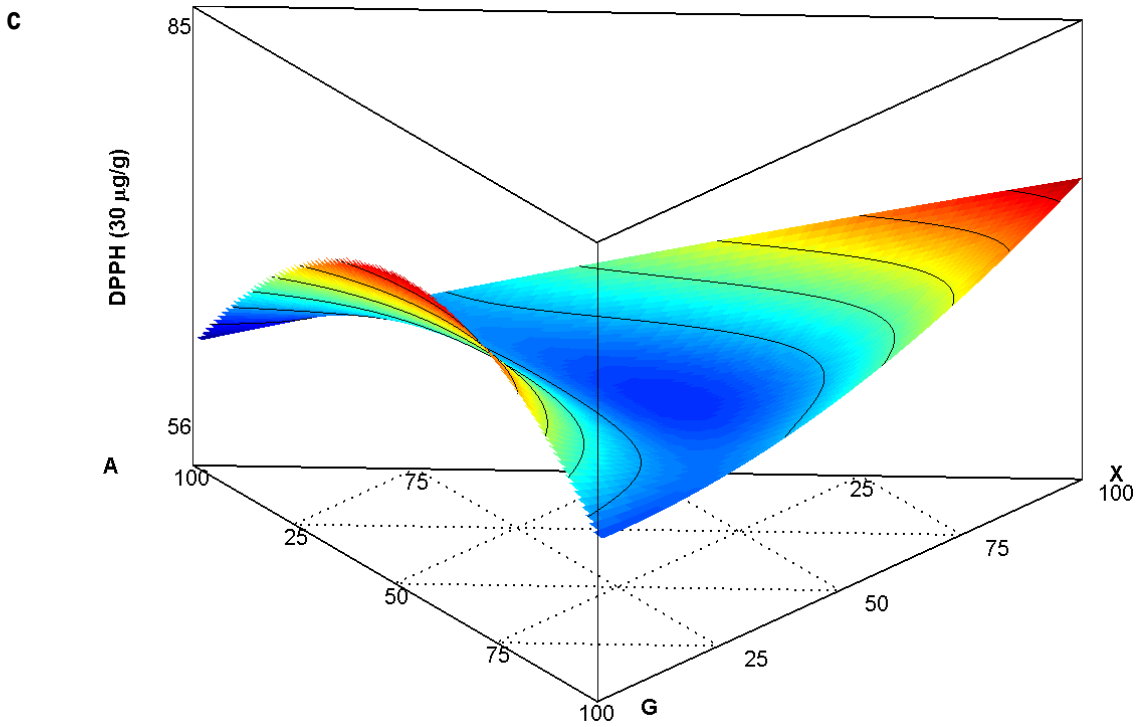


Figure 4. Antioxidant capacity by the 2,2 difenil-1-picrilhidrazil (DPPH) radical inhibition **a)** 5 µg/ml**,** **b**) 10 µg/ml and **c**) 30 µg/ml (sample), for matrix **II** formed by A, G and X

4.Conclusion

We prepared a functional ingredient formed by *B. clausii* and quercetin for two matrices: Matrix I formed by I; L; MX and matrix II formed by A; G; X through experimental designs D-optimal. With yield regard, using the three compounds with the same proportion for both matrices we obtained a higher value (88.08%), for the 32 experiment (33.3I; 33.3 L; 33.3 MX) and (33.3 A; 33.3 G; 33.3 X). We observed for matrix II, the higher viability 9.8 log10 CFU/gto use L closer to the unit corresponding to the 34 experiment (0IN; 100L; 0MX). We obtained higher AA when the inulin was used closer to the unit corresponding to the 33 experiment (100IN; 0L; 0MX) presenting an AA of 56.05 at 5 µg/ml concentration, 8.75 to 10 µg/ml y 84.35 with 30 µg/ml. For matrix II, it presented higher viability values in two cases, to use G closer to

the unit, and to employ a G and X mixture. E.g., in the 31 experiment 9.9 log10 CFU/g (0 A; 50G; 50X) and the 3 experiment 9.8 log10 CFU/g (0A; 100G; 0X). Higher AA was got to use G and X in equal proportions, corresponding to the 49 experiment (0 A; 50 G; 50 X). With AA of 54.85 to a concentration of 5 µg/ml, 55.44 to 10 µg/ml and 67.09 with 30 µg/ml. Synergism between two matrices occurs using the higher I and G proportion, corresponding to the 46 experiment, with yield 86.5%, viability of 9.52 log10 CFU/g9.52 log10 UFC/g and AA of 54.85 at 5 µg/ml concentration, 55.44 to 10 µg/ml and 67.09 with 30 µg/ml. It is worth mentioning all samples over cross minimum viability value of 6.0 log10 CFU/g recommended by FAO/OMS (2002) to be probiotic and contribute to diary inulin ingest of 10-16 mg/day recommended of quercetin and in cases of samples with inulin, to ingest of 10-20 g/day of this carbohydrate to get benefits to health and 25-38g of dietetic fibre per day.

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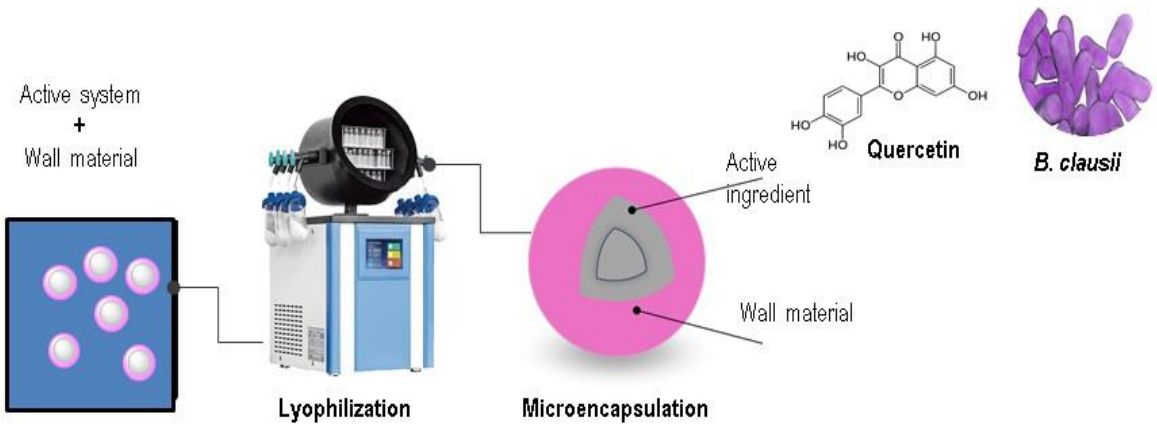
Graphical Abstract



22 July 2022

Dr. Marcello Iacomini,
Editor in Chief
Carbohydrate Polymers

We attached the original research article entitled "**Evaluation of two active system encapsulants matrices with quercetin and *Bacillus claussii* for functional lyophilized foods preparation "**which we would like to be considered for publication in the **Carbohydrate Polymers.** The present study contributes to currently, the demand for functional foods has been increasing in the public interest to improve their life expectations and general health. Food matrices containing probiotic microorganisms and active compounds encapsulated into carrier agents are essential in this context. Encapsulation by the lyophilization method is widely used because oxidation reactions affecting physicochemical and nutritional food properties are usually avoided. Encapsulated functional ingredients such as quercetin and *Bacillus claussii* using two carrier agents' matrices performed in I [inulin (I); lactose (L) and maltodextrin (MX)] and II [(Arabic (A), Guar (G) and Xanthan (X) Rubbers)] are presented in this work. A D-optimal procedure involving 59 experiments was designed to evaluate each matrix's yield, viability, and antioxidant activity (AA). Matrix I (33.3 I; 33.3 L; 33.3 MX) and matrix II (33.3 A; 33.3 G; 33.3 X) exhibited the best yield.

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PhD. María Zenaida Saavedra Leos
Full-time Professor
Coordinación Académica Región Altiplano.
Universidad Autónoma de San Luis Potosí.
Carretera Cedral Km. 5+600 Ejido San José de las Trojes
Matehuala, S.L.P. C.P. 78700. México.