Mariana Assis de Queiroz Cancian

ABORDAGEM QUIMIOMÉTRICA DE PARÂMETROS FÍSICOS-BIOQUÍMICOS DA *Curcuma longa* L E SÍNTESE DE CELULOSE BACTERIANA COM INCORPORAÇÃO DE CURCUMINA

Londrina/PR
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Orientador: Profa. Dra. Wilma Aparecida Spinosa

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À Deus, sempre em primeiro lugar.

À minha avó Zilda Braga Queiroz (*in memorian*),
pelo exemplo de vida.

Dedico.

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"*Verba colant, scripta manet*".

(Citação em latim que significa: "As palavras voam, os escritos permanecem").

QUEIROZ-CANCIAN, Mariana Assis. **CHEMOMETRIC APPROACH OF PHYSICAL-BIOCHEMICAL PARAMETERS OF Curcuma longa L AND SYNTHESIS OF BACTERIAL CELLULOSE WITH INCORPORATION OF CURCUMIN.** 2023. 138 pgs. Tese (Doutorado em Ciência de Alimentos) – Universidade Estadual de Londrina, Londrina, 2023.

RESUMO GERAL

A cúrcuma é um produto alimentar de alto valor amplamente utilizado nas indústrias de temperos, condimentos e corantes, tornando-se um dos produtos vegetais naturais mais populares. Simultaneamente, os alimentos orgânicos têm despertado crescente interesse dos consumidores, o que tem impulsionado seu comércio e consumo, em parte devido à atenção da mídia e publicações que destacam seus potenciais benefícios como suplemento dietético. A curcumina, principal composto fenólico encontrado na cúrcuma apresenta diversos potenciais benéficos para a saúde, incluindo propriedades antioxidantes. As complexas interações entre polifenóis, como a curcumina, e componentes alimentares, especialmente o amido, desempenham um papel crítico na modulação do conteúdo e atividade desses compostos fenólicos. Em sua composição química, a cúrcuma apresenta em seus rizomas mais de 40% de amido. A conversão do amido em açúcares pela hidrólise enzimática gera um produto com alto teor de dextrose usado como fonte de açúcar fermentável em produtos fermentados, como a celulose bacteriana (BC). BC é um biopolímero sintetizado por bactérias ácido acéticas, sendo promissor devido às suas propriedades específicas. Neste contexto, o objetivo desse trabalho foi dividido em: (1) análise do efeito dos sistemas agrícolas orgânicos e convencionais nos parâmetros físico-químicos, conteúdo de compostos bioativos e na atividade antioxidante *in vitro* da cúrcuma obtida de diferentes origens geográficas através de aplicação de técnicas estatísticas multivariadas; (2) estudar as interações polifenol-amido (curcumina-amido) presentes na cúrcuma e o efeito da hidrólise enzimática na disponibilidade da curcumina em meio aquoso; (3) síntese de celulose bacteriana (*in situ*) utilizando como fonte de carbono açucares proveniente de hidrólise enzimática do amido de cúrcuma e incorporação da curcumina na celulose bacteriana (*ex situ*). Uma análise comparativa entre sistemas agrícolas orgânicos (ORG) e convencionais (CONV) de diferentes regiões geográficas (Brasil, Índia, Estados Unidos e Suécia) foi conduzida em rizomas de cúrcuma. Foram analisadas 66 amostras comerciais quanto à composição fenólica, atividade antioxidante *in vitro*, atributos de cor e conteúdo de curcumina. Abordagens quimiométricas não supervisionadas, incluindo análise hierárquica de cluster (HCA), mapa de calor e análise de componentes principais (PCA), revelaram que os sistemas de cultivo agrícola e a origem geográfica não tiveram influência significativa no conteúdo de curcumina, na atividade antioxidante ou nos parâmetros físico-químicos básicos. A síntese de celulose bacteriana atingiu o rendimento de 1,38 $g.L^{-1}$ com o xarope de cúrcuma como fonte de carbono, com condições de cultura que incluem 10 dias de tempo de incubação, 5% (v:v) de inóculo, 0,5 $g.L^{-1}$ de extrato de levedura, 0,5 $g.L^{-1}$ de peptona, 0,115 $g.L^{-1}$ de ácido cítrico, 0,27 $g.L^{-1}$ de Na_2HPO_4 e 1% de etanol (v:v), além de 1,5% de glicose proveniente de hidrolisado de cúrcuma ($g.L^{-1}$), com uma temperatura de incubação de 30°C. A membrana de BC foi carregada separadamente com extrato etanólico de cúrcuma (BC/CURC) e apresentou atividade antioxidante. O

xarope de cúrcuma libera a curcumina da matriz de amido e demonstra um CC (conteúdo de curcumina) semelhante ao da amostra de cúrcuma, garantindo a dispersão e estabilidade da curcumina em meio aquoso.

Palavras-chave: Alimento funcional, bactérias do ácido acético, atividade antioxidante, análise multivariada.

QUEIROZ-CANCIAN, Mariana Assis. **Açafrão (*Curcuma longa* L.): síntese de celulose bacteriana, incorporação de curcumina e estudo quimiométrico entre compostos fenólicos e parâmetros físico-químico.** 2023. 138 pgs. Thesis (Doctoral degree in Food Science) – State University of Londrina, Londrina, 2023.

ABSTRACT

This study aimed to investigate the properties that turmeric (*Curcuma longa* L.) can assume in different aspects of food science and biotechnology. Turmeric is a high-value food product widely employed in the spice, condiment, and dye industry. Turmeric has become one of the most popular natural plant products. Concomitantly, organic food has gained increasing consumer interest, promoting its trade and consumption. In common, increased consumption of turmeric and organic food has been largely due to widespread media attention and several publications, including articles on its many potential benefits as a dietary supplement. Curcumin, the main phenolic compound found in turmeric, has several potential health benefits, including antioxidant properties. The complex interactions between polyphenols, such as curcumin, and food components, especially starch, perform a critical role in modulating the content and activity of these phenolic compounds. In its chemical composition, turmeric has more than 40% starch in its rhizomes. The conversion of starch into sugars through enzymatic hydrolysis generates a product with a high dextrose content used as a source of fermentable sugar for fermented products, like bacterial cellulose (BC). BC is a promising biopolymer synthesized by acetic acid bacteria due to its specificity and properties. In this context, the objective of this work was divided into: (1) analysis of the effect of organic and conventional agricultural systems on physicochemical parameters, content of bioactive compounds and *in vitro* antioxidant activity of turmeric obtained in different geographic origins through the application of multivariate statistical techniques; (2) study the polyphenol-starch (curcumin-starch) interactions present in turmeric and the effect of enzymatic hydrolysis on the availability of curcumin in aqueous media; (3) synthesis of bacterial cellulose (*in situ*) using sugars as a carbon source from enzymatic hydrolysis of turmeric starch and incorporation of curcumin into bacterial cellulose (*ex situ*). A comparative analysis of organic (ORG) and conventional (CONV) agricultural systems in different geographic regions (Brazil, India, United States and Sweden) was conducted on turmeric rhizomes. Sixty-six

commercial samples were analyzed for phenolic composition, *in vitro* antioxidant activity, color attributes and curcumin content. Unsupervised chemometric approaches, including hierarchical cluster analysis (HCA), heatmap, and principal component analysis (PCA), revealed that agricultural cropping systems and geographic origin had no significant influence on curcumin content, antioxidant activity, or basic physical chemistry parameters. BC can be produced with a yield of 1.38 $g.L^{-1}$ using TS as alternative carbon source, with culture conditions of 10 days of incubation time, 5% (v:v) inoculum, 0.5 $g.L^{-1}$ yeast extract, 0.5 $g.L^{-1}$ peptone, 0.115 $g.L^{-1}$ citric acid, 0.27 $g.L^{-1}$ Na_2HPO_4 and 1% ethanol (v:v) and 1.5% of glucose from TS ($g.L^{-1}$) at 30 °C incubation temperature. The BC membrane was separately loaded with turmeric ethanolic extract (BC/CURC) and showed antioxidant activity. Turmeric syrup released curcumin from the starch matrix and showed the same CC in the turmeric sample, ensuring curcumin aqueous dispersibility and stability.

Keywords: Functional Food, Acetic Acid Bacteria, Bioactive Compounds, Antioxidant Activity, Multivariate Analysis.

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CAPÍTULO I

1. Introdução e Objetivos

Diferentes matrizes alimentares são investigadas quanto a composição química e bioatividade, principalmente a capacidade antioxidante, como chás, vinhos, vinagres, especiarias, ervas, frutas e produtos alimentícios derivados (ZABOT *et al.*, 2022; ZIELINSKI *et al.*, 2014). Desses estudos uma conclusão comum pode ser observada: alimentos ricos em compostos fenólicos demonstram forte atividade antioxidante. A *Curcuma longa* L., conhecida como cúrcuma ou açafrão, é uma planta cujo interesse econômico e alimentar tem-se no seu rizoma e sua atividade anticancerígena, antibacteriana, antiinflamatória e antioxidante amplamente estuda e comprovada na literatura (FULORIA *et al.*, 2022).

Os diversos estudos *in vitro* e *in vivo* atribuem as propriedades biológicas do rizoma do açafrão principalmente aos curcuminóides: curcumina e seus derivados demetoxicurcumina e bisdemetoxicurcumina. A composição química do açafrão apresenta entre 0,3 a 5,4% de curcumina, seu principal metabolito secundário (ESATBEYOGLU *et al.*, 2012; FULORIA *et al.*, 2022; LIU, YUEYUE; MA; YUAN, 2023). A atividade antioxidante da curcumina é relacionada com a capacidade antioxidante dos grupos fenólicos da molécula, a estrutura química consiste em dois anéis aromáticos, contendo um radical metoxi e um grupamento hidroxila, interconectados simetricamente em conjugação por uma fração β-dicetona (ESATBEYOGLU *et al.*, 2012).

A cúrcuma apresenta mais de 40% de amido na sua composição centesimal. O amido é a fonte de reserva mais importante dos vegetais e na dieta humana é a principal fonte de carboidrato. As interações que ocorrem entre polifenóis e macro e

micronutrientes, no nível químico-físico, envolvem, por exemplo, ligações covalentes, iônicas e de hidrogênio, bem como ligações hidrofóbicas e hidrofílicas. Além disso, os carboidratos podem interagir com os polifenóis (CIANCIOSI *et al.*, 2022; JAKOBEK, 2015).

Uma de suas aplicações do amido na indústria alimentar é como fonte de açucares para produção de adoçantes e hidrolisados, com aplicação em processos fermentativos. A hidrólise dos biopolímeros de amido cliva as ligações glicosídicas progressivamente. O processo de hidrólise enzimática do amido é realizado em duas etapas: a liquefação e a sacarificação, com a atuação das enzimas α-amilase e amiloglucosidase na clivagem de ligações (α-1→4) e (α-1→6), respectivamente. Como resultado é formado uma solução de sacarídeos de baixa massa molecular, predominantemente glicose e maltose (LIN *et al.*, 2016).

A celulose bacteriana (BC) é um polissacarídeo extracelular constituído por de uma cadeia de unidades de D-glicose ligadas a β-(1→4), secretadas de diferentes espécies de bactérias. A cepa *Komagataeibacter xylinus* é uma das mais interessantes comercialmente, devido ao seu alto rendimento. A cepa tem capacidade de produzir grandes quantidades de celulose e consume como fonte de carbono diferentes açúcares. O interesse nas propriedades e características incomuns do BC tem crescido, principalmente por apresentar pureza quando comparada a celulose sintetizada pelas plantas, pois a BC é livre de lignina, hemicelulose, pectina e proteínas, e é altamente organizado e cristalino (GOMES; IDA; SPINOSA, 2022). Fontes tradicionais de carbono para fermentação microbiana são açúcares como glicose, frutose e sacarose. Nos últimos anos, muito interesse se desenvolveu na

produção de BC em diferentes meios, devido ao alto custo envolvido na produção de BC (BILGI *et al.*, 2016a; EL-GENDI *et al.*, 2022).

Diante do exposto, o objetivo deste trabalho foi dividido em três pontos principais:

1. Utilizar técnicas estatísticas multivariadas no estudo dos compostos fenólicos, atividade antioxidante *in vitro* e composição físico-química de cúrcuma de diferentes origens geográficas e diferentes cultivos;
2. Estudar as interações polifenol-amido (curcumina-amido) presentes na cúrcuma e o efeito da hidrólise enzimática na disponibilidade da curcumina em meio aquoso;
3. Estudar os parâmetros para síntese de celulose bacteriana (*in situ*) utilizando como fonte de carbono açucares proveniente de hidrólise enzimática do amido de cúrcuma;
4. Estudar a influência da incorporação da curcumina na celulose bacteriana (*ex situ*).

2. Estrutura da Tese

A tese foi estruturada de forma que os capítulos possam ser lidos independentemente uns dos outros, embora alguns conceitos fundamentais estejam interconectados e adequadamente referenciados.

O conteúdo desta tese está organizado em seis capítulos. O capítulo I tem caráter introdutório acerca do conteúdo estudado, os objetivos e a estrutura da tese. No capítulo II encontra-se uma meticulosa e atual revisão bibliográfica sobre o assunto em estudo. Nos capítulos III, IV e V, os resultados obtidos foram organizados em forma de artigo científico. O capítulo VI apresenta as conclusões gerais, bem como sugestões para trabalhos futuros.

3. Metodologia de Trabalho

Os estudos foram desenvolvidos nos laboratórios de pesquisa do Departamento de Ciência e Tecnologia de Alimentos, laboratório de Desenvolvimento de Instrumentação e Automação Analítica, Laboratório de Espectroscopia e Laboratório de Microscopia Eletrônica e de Microanálise da Universidade Estadual de Londrina (Londrina, PR).

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CAPÍTULO II – REVISÃO BIBLIOGRÁFICA

1. Cúrcuma

Curcuma longa Linn., popularmente conhecida como cúrcuma, é uma planta monocotiledônea perene pertencente à família Zingiberaceae. e possui um longo histórico de possuir propriedades curativas contra diversas doenças. De acordo com a Farmacopéia Brasileira, a parte do vegetal utilizada na culinária é o rizoma, de forma a conferir cor e sabor aos alimentos. De origem asiática (Índia e Indonésia) suas características sensoriais são odor fracamente aromático e sabor picante, levemente amargo, cultivado em maior expressão na Ásia, especificamente na Índia e na China (ANVISA, 2017; FULORIA et al., 2022; JYOTIRMAYEE; MAHALIK, 2022).

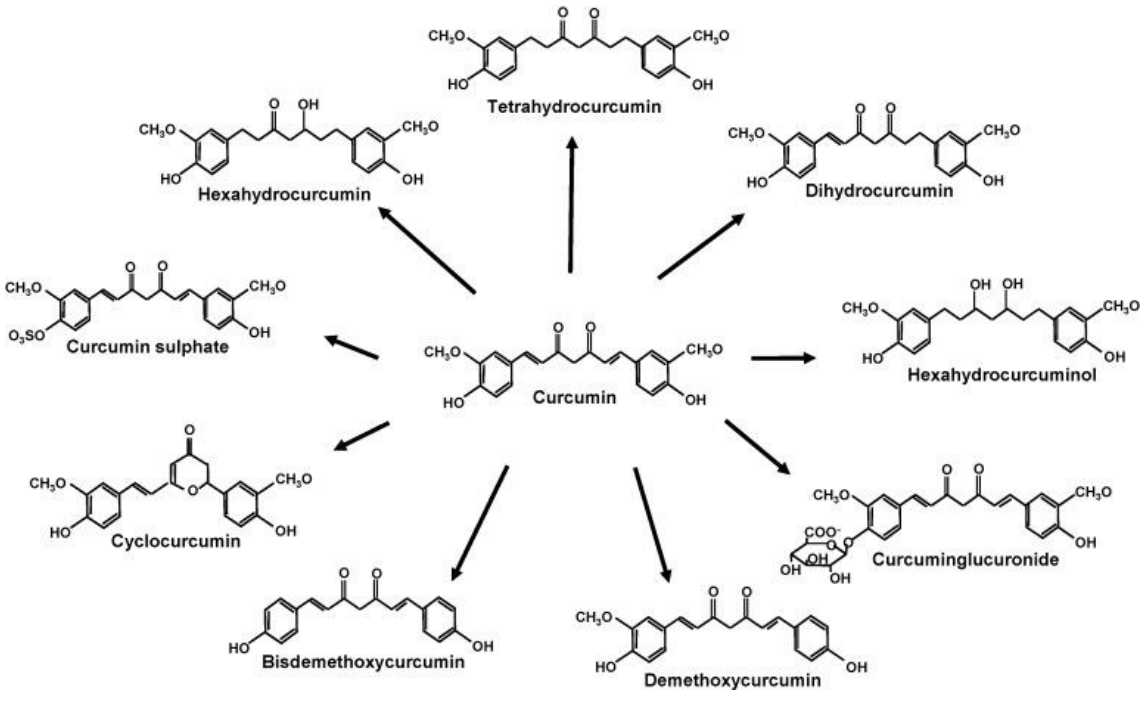
A cúrcuma, ou em certos casos a curcumina, é classificada como aditivo alimentar (INS 100i) no Compêndio da Legislação Brasileira de Aditivos Alimentares. De acordo com a Organizações das Nações Unidas para a Agricultura e Alimentação (FAO) a sua Ingestão Diária Aceitável (IDA) é estabelecida em 0 – 3 mg/kg peso corpóreo e sua classificação é corante natural. Sua aplicação tecnológica é como corante em diversos alimentos, entre eles queijos, iogurte, bala, goma de mascar, gelatinas, vinhos, conservas cárneas, produtos de panificação, sopas, sorvete, mostarda, manteiga, biscoitos e maionese (ANVISA, 2017). Em âmbito internacional, a cúrcuma é usada em formulações de pratos típicos da Índia e países asiáticos, principalmente no curry indiano (JYOTIRMAYEE; MAHALIK, 2022).

Dentre os constituintes de sua composição química, a cúrcuma tem em maior proporção o amido e em menor quantidade proteínas, lipídeos e fibras, além dos metabólitos secundários, como os pigmentos curcuminoides e os óleos essenciais. Estudos prévios do nosso grupo de pesquisa quantificaram o amido como o maior

componente da farinha do rizoma de cúrcuma (59,23 g $100g^{-1}$), qualificando o rizoma como matéria-prima com potencial para a extração e aplicação do seu amido (QUEIROZ-CANCIAN *et al.*, 2018).

A farinha de cúrcuma pode conter entre 0,02 a 10,9% do peso seco em curcumina, principal polifenol hidrofóbico derivado do rizoma (ESATBEYOGLU *et al.*, 2012). A estrutura química da molécula de curcumina, seus análogos naturais e seus importantes metabólitos (Figura 1) consistem em dois anéis aromáticos, contendo um radical metoxi e um grupamento hidroxila, interconectados simetricamente em conjugação por uma fração β-dicetona.

Figura 1 - *Análogos naturais de metabólitos da curcumina.*



Fonte: ANAND et al., 2008

Vários experimentos *in vitro* e *in vivo* mostraram que a curcumina tem vários efeitos farmacológicos, como regulação da imunidade, antioxidação, inibição da

inflamação, antitumoral, antiangiogênese, anticoagulação e eliminação de radicais livres.

Seja na forma de pó, extrato ou composto isolado, o açafrão-da-terra tem demonstrado em estudos possuir uma ampla gama de atividade farmacológica com poucos efeitos adversos. Diferentemente de outros fito-antioxidantes, a curcumina é segura e não tóxica. Na medicina tradicional chinesa e indiana, a cúrcuma tem sido utilizada como medicamento anti-inflamatório e no auxílio na cicatrização de feridas. Enquanto a administração oral de curcumina é eficaz no tratamento de diabetes, câncer, problemas gastrointestinais e distúrbios neurológicos (JYOTIRMAYEE; MAHALIK, 2022).

2. Celulose Bacteriana

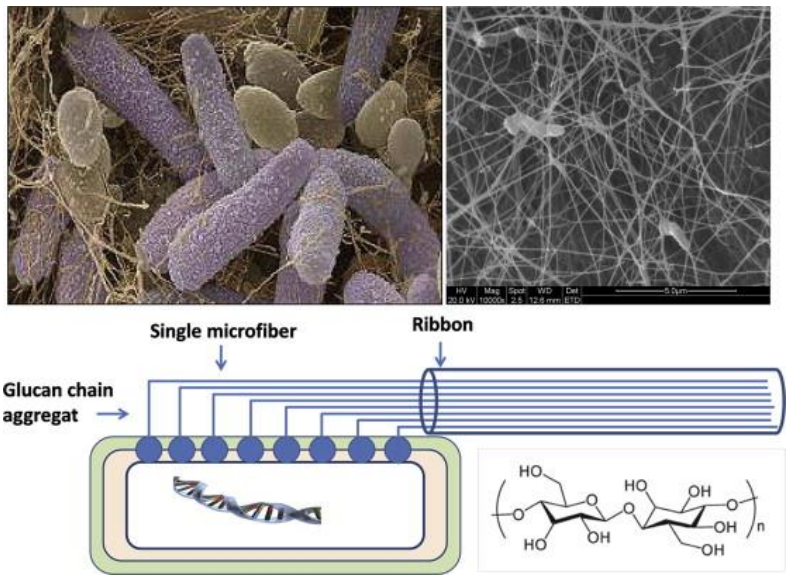
A celulose bacteriana (BC) é um biopolímero de grande importância para as indústrias médica, farmacêutica e alimentícia. Produzido por uma variedade de espécies bacterianas, o BC apresenta propriedades mecânicas, térmicas e biocompatíveis excepcionais, diferenciando-o da celulose de origem vegetal.

Com o crescente número de estudos ao longo dos anos, diversas aplicações para o uso do BC foram desenvolvidas. Eles estão divididos em diferentes áreas, sendo as principais biomédica, alimentícia, farmacêutica, cosmética e bioengenharia. Fernandes *et al.* (2020) apontam um estudo, produzido por *Pro Market Research* e o *Decision Database*, que quantifica o valor de $ 250 milhões para o mercado global de BC entre 2017 e 2019. O valor relaciona o volume de vendas de BC para suas principais aplicações: materiais compostos, produtos alimentícios, papel e outros. Os provedores globais de relatórios de pesquisa de negócios estimam que o mercado

global de BC atingirá US$ 570 milhões em 2024 e US$ 680 milhões até o final de 2025 (FERNANDES *et al.*, 2020).

O primeiro relato de celulose produzida a partir de bactérias foi em 1886 por Brown, o artigo relata a formação de uma membrana gelatinosa produzida por cepas de *Acetobacter* na superfície de um caldo de fermentação de vinagre. BC é classificada como um biopolímero não ramificado com nanofibrilas, composto por unidades repetidas de β-glicopiranosil de (1 → 4) unidades de glicose (Figura 2). Essas cadeias de glucana lineares formam ligações de hidrogênio intra e inter-moleculares altamente regulares (ANDRIANI; APRIYANA; KARINA, 2020; FERNANDES *et al.*, 2020; SHI *et al.*, 2014).

Figure 2 - *Acetobacter xylinus, rede tridimensional e a formação da celulose bacteriana.*



Fonte: SHI et al., 2014

Ao contrário da celulose vegetal, a BC é produzida como um polímero extracelular, o que facilita sua extração com alta pureza, totalmente livre de lignina e hemicelulose. O polímero BC também é caracterizado por seu alto grau de

polimerização e cristalinidade com uma rede de fibras única no tamanho micro ou nano, o que aumenta sua superfície em relação ao volume, sendo uma característica única em relação a outras fontes de celulose (EL-GENDI *et al.*, 2022).

Muitas espécies de bactérias, tais como as dos gêneros *Acetobacter, Agrobacterium, Achromobacter, Aerobacter, Azotobacter, Sarcina ventriculi, Salmonella, Escherichia* e *Rhizobium*, produzem celulose extracelular sólida. As celuloses produzidas por diferentes bactérias possuem diferentes morfologia, estrutura, propriedades e aplicações. Atualmente, cepas da bactéria *Acetobacter xylinus* (Figura 2) têm sido utilizadas para a produção de BC comercialmente, devido à sua alta produtividade (CAMPANO *et al.*, 2016; SHI *et al.*, 2014; WANG, JING; TAVAKOLI; TANG, 2019).

Além da fonte bacteriana, a síntese de BC é influenciada pela composição do meio de cultura. De um modo geral, a produção de BC depende principalmente da disponibilidade dos componentes nutricionais do meio de produção, principalmente o carbono. A fonte de carbono utilizadas pelas bactérias é um dos principais componentes que afetam o rendimento. Diferentes fontes de carbono, simples e complexas, como glicose, frutose, sacarose, manitol, amido e glicerol, são utilizadas para a produção de BC (FERNANDES *et al.*, 2020).

A fonte de nitrogênio também é determinante para a produção máxima de BC. A produção de BC é comumente suportada com fontes complexas de nitrogênio orgânico, como extrato de levedura e peptona. Numerosos aditivos foram rastreados e avaliados quanto ao seu efeito. Diferentes estudos relataram que o etanol influência as enzimas envolvidas na via de síntese de BC, melhorando assim o rendimento de

ATP (ANDRIANI; APRIYANA; KARINA, 2020; EL-GENDI *et al.*, 2022; FERNÁNDEZ MAURA *et al.*, 2016).

A influência de diferentes fontes de carbono e meios de cultivo foram e são investigados no rendimento de BC, no entanto, o meio Hestrin-Schramm (HS) continua sendo o mais usado na produção de BC. Cerca de 30% do valor total da produção de BC são atribuídos ao meio de cultivo. O alto custo de produção representa um grande desafio para atender aos requisitos de fabricação comercial (EL-GENDI *et al.*, 2022).

A otimização da produção da CB surgiu com o propósito de reduzir custos, encurtar os tempos de produção e aumentar a produtividade em escala industrial. Essa otimização pode ser realizada por meio de métodos convencionais ou através de modelos estatísticos, que buscam identificar um conjunto de parâmetros fundamentais para alcançar as condições ideais de produção da CB. O processo de otimização pode abranger a seleção do meio nutriente, dos microrganismos, a análise das condições de fermentação e a comparação desses parâmetros quanto ao seu impacto na produtividade/rendimento da BC. Além disso, também inclui a avaliação de técnicas para modificar a CB, como a adição de moléculas exógenas, visando desenvolver matrizes com características distintas para atender a diversas aplicações (ANDRIANI; APRIYANA; KARINA, 2020; FERNANDES *et al.*, 2020).

3. Métodos estatísticos em Ciência e Tecnologia de Alimentos

A quimiometria é um campo de pesquisa interdisciplinar que envolve estatística multivariada, modelagem matemática e computação, especialmente aplicada a dados químicos (SOUZA et al., 2011). O uso de técnicas estatísticas multivariada é crescente na engenharia, ciência e tecnologia de alimentos, pois tais análises estatísticas

extraem uma boa quantidade de informação de dados químicos e instrumentais (GRANATO; UCHIDA KATAYAMA; DE CASTRO, 2012). Exemplos do uso de técnicas multivariadas em ciência de alimentos são observados no controle de qualidade dos alimentos para analisar a autenticidade de alimentos, rastrear a origem geográfica do alimento, verificação o sistema de produção, autenticidade do rótulo e de adulterações (GRANATO et al., 2018).

A análise multivariada envolve a avaliação de dados resultantes de variáveis medidas a partir de um determinado conjunto de amostras. O objetivo é determinar todas as variações no estudo da matriz de dados. Assim, se pode verficar as relações entre as amostras e as variáveis, reduzindo a dimensionalidade em um determinado conjunto de dados com a finalidade de agrupa-las ou classifica-las viabilizando uma melhor interpratação do cojunto de dados (KUMAR et al., 2014).

Entre as técnicas multivariadas destacam-se os métodos não supervisionados de reconhecimento de padrões, como análise de componente principais (PCA) e a análise hierárquica de agrupamentos (HCA).

3.1. Análise de componentes principais- PCA

A análise de componentes principais - PCA (do inglês - *Principal Component Analysis*) é uma técnica da estatística multivariada que reduz a dimensionalidade da matriz dos dados originais a um novo sistema de eixos (componentes principais - CP) que retem a quantidade máxima de variabilidade do conjunto de dados (porcentagem da variância explicada). A metodologia permite a visualização do arranjo original das amostras em um espaço n-dimensional reduzido (usualmente bi ou tridimensional, representada pelos escores, que expressam as relações entre as amostras e os pesos a relação entre as variáveis). Tal fato, proporciona um melhor entendimento da

similaridade e diferença entre as amostras em um espaço dimensional reduzido sem a perca da informação do conjunto de dados original. Cada componente principal (CP) é uma combinação linear entre as variáveis originais e os CPs são ortogonais entre si, isto é, a informação são únicas em cada PC (GRANATO et al., 2018; ZIELINSKI et al., 2014). O PCA foi aplicado para separar as amostras de acordo com a teor de compostos fenólicos, flavonoides totais, curcumina e atividade antioxidante.

3.2. Análise de Agrupamento Herárquicos (HCA) e Mapa de Calor (Heat Map)

O HCA é uma técnica multivariada que organiza dos dados com base em suas características. Ele agrupa os dados considerando a sua semelhança no espaço multidimensional, como resultado, cada agrupamento (cluster) exibe alta homogeneidade no intergrupo e heterogeneidade entre os diferentes grupos. Esse fundamento fornece a base dos métodos de agrupamentos, permitindo verificar a similaridade/dissimilaridade entre grupos (KUMAR et al., 2014).

A HCA é método bem estabelecido, e atualmente diferentes possibilidade de utlização dela estão disponíveis, permitindo que os usuários possam utilizar diferentes métricas para calcular as distâncias entre as amostras (distância Euclideana, Manhattan, Mahalanobis, entre outras), e ainda métodos de agrupamento (método do vizinho mais próximos, mais distante, média, centróide e Ward).

A HCA é uma técnica aglomerativa que envolve um conjunto de métodos estatísticos que apresentam similaridade ou mostram características similares (GIACOMINO et al., 2011). No método de agrupamentos, todas as amostras dentro de um grupo (cluster) são consideradas apresentando características similares. O primeiro objetivo da HCA é mostrar os dados de tal maneira que os grupos naturais e padrões possam ser mostrados em um espaço 2D (também chamado dendrograma).

Esta representação gráfica permite a visualização dos grupos entre qualquer amostra ou variáveis simultaneamente (SOUZA et al., 2011).

O mapa de calor revela simultaneamente a estrutura de cluster hierárquica de linha e coluna em uma matriz de dados. Sua apresentação gráfica consiste em um ladrilho retangular em uma escala de cores para representar o valor do elemento correspondente da matriz de dados. De acordo com Zhao et al. (2014), mapas de calor e agrupamento são usados frequentemente em estudos de análise de expressão para visualização de dados e controle de qualidade.

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CAPÍTULO III

SCIENTIFIC ARTICLE 1

Organic versus conventional turmeric: Application of Multivariate Analysis in the Study of Phenolic Compounds, *In Vitro* Antioxidant Activity, Color, and Curcumin Content

Abstract

The effect of organic and conventional agricultural systems on the physicochemical parameters, bioactive compounds content, and *in vitro* antioxidant activity of turmeric was studied. Turmeric (*Curcuma longa* L.) is a high-value food product widely employed in the spice, condiment and dye industry. This study aims to differentiate turmeric rhizomes and explore similarities based on their agricultural crop systems, organic (ORG) or conventional (CONV) and geographical origin (Brazil, India, United States and Sweden). Sixty-six commercial samples were analyzed for total phenolic composition (TPC), *in vitro* antioxidant activity (AA) using DPPH and FRAP assays, color attributes (CIELAB) and curcumin content through ultra-performance liquid chromatography -diode array detection (UPLC/DAD). The data were processed using a chemometric approach involving hierarchical cluster analysis (HCA) with heatmap and principal component analysis (PCA). The results of curcumin content, antioxidant activity, and basic physicochemical parameters combined with chemometric analysis revealed that the two cropping systems had no different, as same for geographic origin.

Keywords: *Curcuma longa* L., Bioactive Compounds, Chemometrics, Crop System, Principal Component Analysis.

1. Introduction

Different food matrices are investigated regarding their chemical composition and bioactivity, mainly antioxidant capacity, such as teas, wines, vinegars, spices, herbs, fruits, and derived food products. These studies show a common conclusion: foods rich in phenolic compounds demonstrate strong antioxidant activity. The investigation of species from *Curcuma longa* L., turmeric, a spice native to South Asia, indicates a high presence of bioactive compounds that demonstrably exhibit biological properties such as antioxidant, antimicrobial, anti-inflammatory, and anti-cancer effects (DÁVALOS; BARTOLOMÉ; GÓMEZ-CORDOVÉS, 2005; LAN, YONGLI *et al.*, 2017; ZABOT *et al.*, 2022; ZIELINSKI *et al.*, 2014).

The biological properties of turmeric are associated with the presence and activity of phytochemical compounds, especially curcumin, active polyphenol derived from turmeric (Liu et al., 2023; Salem et al., 2022). Thus, evaluating the total and individual quantification of polyphenolic compounds is essential to establish a correlation with the biological properties of the rhizome.

The research that confirms the antioxidant activity of turmeric rhizomes shows isolated results without an association and/or correlation between antioxidant responses and chemical composition. Akter et al. (2019) studied five varieties of turmeric (*Curcuma* spp), and the results demonstrated that there are significant variations in the antioxidant activity and total phenolic content (TPC) among different varieties of turmeric. In a systematic review and meta-analysis of sixty-six studies, Dehzad et al. (2023) reported that the antioxidant effects of curcumin/turmeric supplementation in adults positively impact markers of systematic oxidative stress. Likewise, Malik Al-Rubaei et al. (2014) showed that curcumin supplementation could

hamper the oxidative damage artificially induced by H_2O_2 in rats. Furthermore, these studies only address a single sample of the species (*Curcuma longa* L.) and do not assess potential variations among different commercial brands or method cultivation (organic and conventional forms).

Organic food has gained increasing consumer interest, promoting its trade and consumption. The difference between organic and conventional agriculture was synthetic chemical agents' use. The literature was inconclusive about influence that agricultural crop systems (organic and conventional) in nutritional and antioxidant profile (PEREIRA; DE ANGELIS-PEREIRA, 2022).

The objective of this work was to explore similarities and hidden patterns among samples of turmeric rhizomes according to geographical origin and crop system organic (ORG) or conventional (CONV), employing analytical methods for the analysis of phenolic compounds, antioxidant activity, and physicochemical characterization, in conjunction with chemometric techniques.

2. Material and methods

2.1 Chemicals

The standard reagent used for chromatography was curcumin, with a purity level exceeding 98%, from the Sigma-Aldrich brand (United States). Other reagents employed were of analytical grade (P.A.).

2.2 Turmeric Samples

Sixty-six commercial samples of turmeric rhizomes originating from Brazil (n = 54), India (n=3), the United States (n=3), and Sweden (n=6), cultivated using different systems: organic (n = 36) and conventional (n = 30), were analyzed. For samples of

67 turmeric from organic cultivation from Londrina-PR (Brazil) (n = 12), the rhizomes were
68 harvested in July 2020 at the State University of Londrina (UEL), with geographical
69 coordinates 23°19'42.5"S; 51°12'15.2"W.

70 **2.3 Extraction procedure**

71 The curcumin extracts from turmeric were prepared according to Roy & Rhim
72 (2020), with modifications, to determine curcumin content (CC) by ultra-performance
73 liquid chromatography (UPLC). The samples were weighed and then put in constant
74 agitation for 12 hours at 120 rpm with acetonitrile (ACN). After, the samples were
75 treated with 60 min ultrasonic and then centrifuged at 11 000 rpm for 50 min. The
76 supernatant was diluted with ACN 50:50 (v:v) and them filtered through a 0.22 µm
77 PVDF membrane filter before the injection (1 µL) into the ultra-performance liquid
78 chromatography (UPLC®) Waters (Acquity UPLC System, Waters, USA).

79 **2.4 Amount of Curcumin**

80 The UPLC was equipped with a UV-Vis detector and a C18 column (180×4.6
81 mm, 5 µm; Shiseido, Japan). The curcumin was quantified following Cheng et al.
82 (2010) recommendations with modifications. The mobile phase consisting of a mixture
83 of 0.05% aqueous phosphoric acid and acetonitrile in the ratio of 50:50 (v:v), isocratic
84 with the flow rate of 0.3 $ml.min^{-1}$ was employed at 30 °C. The detection wavelength
85 was set at 420 nm. The calibration curve was prepared using curcumin standard in the
86 concentration range 0.10 – 2.5 µg/mL, obtaining a correlation coefficient (R^2) of 0.999.
87 The injections were made in triplicate, and the results were expressed as mg CC/g dry
88 weight of the sample.

2.5 Color attributes

The color parameters L* (lightness), a* (red - green), and b* (yellow - blue) were determined using a colorimeter (Konica Minolta CR 400, Japan) with the following specifications: CIE D 65 illuminant and CIE 10º standard observer. The parameters' chroma (C*) was estimated as $C^* = (a^{*2} + b^{*2})^{1/2}$ and hue angle (h^*) was calculated by: $h^* = \tan^{-1}(b^* / a^*) + 180°$ when $a^* > 0$.

2.6 Total phenolic content (TPC)

The total phenolic content (TPC) in the extracts' samples was estimated using the Folin-Ciocalteu reagent with a microplate adaptation BOBO-GARCÍA et al. (2015). Briefly, for the reaction, 20 µL of each extract, 100 µL of Folin-Ciocalteau reagent (diluted 1:4 in H_2O), and 75 µL of 10% sodium carbonate solution were mixed. After thorough mixing, the samples were kept at room temperature with light protection for 2 hours. Absorbance was measured at a wavelength of 750 nm using a UV-VIS spectrophotometer. A standard curve (R^2 = 0.999) was plotted using different concentrations of gallic acid (4 - 24 µg mL^{-1}). The results were expressed in milligrams of gallic acid equivalent per gram of sample (mg GAE g^{-1}).

2.7 Measurement of *in vitro* antioxidant activity

The free-radical scavenging activity of the samples was determined through the reduction of the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical. The reduction of the free DPPH radical was determined as described Brand-Williams et al. (1995) with adaptations by Bobo-García et al. (2015). The assay measures the antioxidant activity observed by the colorimetric change measured at 517 nm. The reaction was carried out in microplates, where 20 µL of the sample and 180 µL of a 150 µM ethanolic DPPH

112 solution were homogenized and kept protected from light for 40 minutes.
113 Subsequently, absorbances were read using a spectrophotometer UV-VIS at 517 nm.
114 The positive control (Ac) was prepared without the sample, using 20 µL of extractor
115 solvent, and represents the maximum electron donation from the DPPH radical. The
116 blank was prepared by mixing without the DPPH solution (180 µL of water:ethanol
117 solution), without the sample, and with 20 µL of the extraction solvent used. The radical
118 scavenging activity of each sample against the DPPH radical was calculated using the
119 Equation:

120 $$DDPH \text{ scavenging activity (AA\%)} = \left(1 - \frac{As}{Ac}\right) \times 100$$

121 where Ac and As were the absorbance of DPPH of the control and test samples,
122 respectively.

123 The Ferric Reducing Antioxidant Power (FRAP) assay, which evaluates the
124 sample's ability to reduce ferric ions in an aqueous medium, was performed according
125 to the method described by Benzie & Strain (1996) adapted for microplates. The FRAP
126 stock solution contained 25 mL of acetate buffer (0.3 mM, pH 3.6), 2.5 mL of 10 mM
127 TPTZ in 40 mM HCl, and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ solution. Before use, the stock
128 solution was incubated at 37 °C for 30 minutes. For the reaction, 30 µL of the extract,
129 90 µL of ultrapure water (Millipore), and 900 µL of the FRAP solution were mixed, and
130 the solution was kept in darkness at 37 °C for 30 minutes before absorbance readings
131 were taken at 595 nm. The standard curve was linear for ethanol solutions of Trolox
132 ranging from 50 to 600 µM. Results were expressed as milligrams of Trolox equivalents
133 per gram of the sample (mg $TE.g^{-1}$).

2.3 Statistical analysis and chemometrics analysis

A chemometric approach composed of heatmap, principal component analysis (PCA) and hierarchical cluster analysis (HCA) was used to analyze the parameters. Before the chemometrics application, all variables were autoscaled (transformation into z-scores) to standardize the statistical importance of all responses. Then, a matrix of samples (n = 66) and response variables (n = 10) was built, in which samples were adopted as rows and variables as columns, totaling 660 data points.

The Pearson correlation coefficient (r) and P-value were used to evaluate the correlation between cropping systems (organic and conventional) and phenolic composition, *in vitro* antioxidant activity using DPPH and FRAP assays, color attributes and curcumin content. Probability values of $p < 0.05$ were considered statistically significant. All analyses were performed using the statistical software R (R Core Team, 2023) with the FactoMineR, phheatmap and factoextra packages (KASSAMBARA; MUNDT, 2017; LÊ; JOSSE; HUSSON, 2008)(Lê et al., 2008).

2.3.1 Hierarchical cluster analysis (HCA) and heatmap

A heatmap of results shows their general pattern at a glance while retaining their precise identification. It shows the aspects of the response in one graph. A heatmap was created from a matrix of samples and response variables for a more comprehensive overview and comparison of variables studied in the samples.

Hierarchical cluster analysis (HCA) is an initial approach for exploring datasets for inherent groupings among samples defined by a set of measured features. Due to its unsupervised nature, HCA functions as a pattern recognition technique capable of unveiling the underlying structure within a dataset (ZIELINSKI *et al.*, 2014). In this analysis sample similarities computed based on squared Euclidean distance and the

Ward hierarchical agglomerative method employed for cluster formation. A dendrogram depicting sample relationships was constructed using software R. This dendrogram establishes a hierarchy of similarities, providing a two-dimensional visualization of the overall similarity or dissimilarity among all samples utilized in the study.

2.3.2 Principal Component Analysis (PCA)

PCA is employed as an unsupervised method, serving as a technique for analyzing and simplifying datasets to eliminate chemical noise. It is frequently utilized for reducing the dimensionality of datasets. The primary principle involves transforming informative variables containing redundant data into linearly uncorrelated variables via orthogonal transformations. This approach retains most of the original data, enabling the utilization of a limited set of principal components for sample description (LIU, SHUANGSHUANG *et al.*, 2024).

PCA analysis was conducted using the geographical origin and agricultural crop systems (organic and conventional) as samples. Then, a matrix of samples and variables was built, in which samples were adopted as rows and variables as columns, totaling 660 data points. Eigenvalues higher than 1.0 were adopted to explain the projection of the samples on the factor plane. Statistical significance was set at $P < 0.05$. PCA was applied to the dataset to discriminate and pattern recognition of the samples according to their total phenolics compounds, $L*$, $a*$, $b*$, $C*$, $h*$, moisture, curcumin content, DPPH and FRAP.

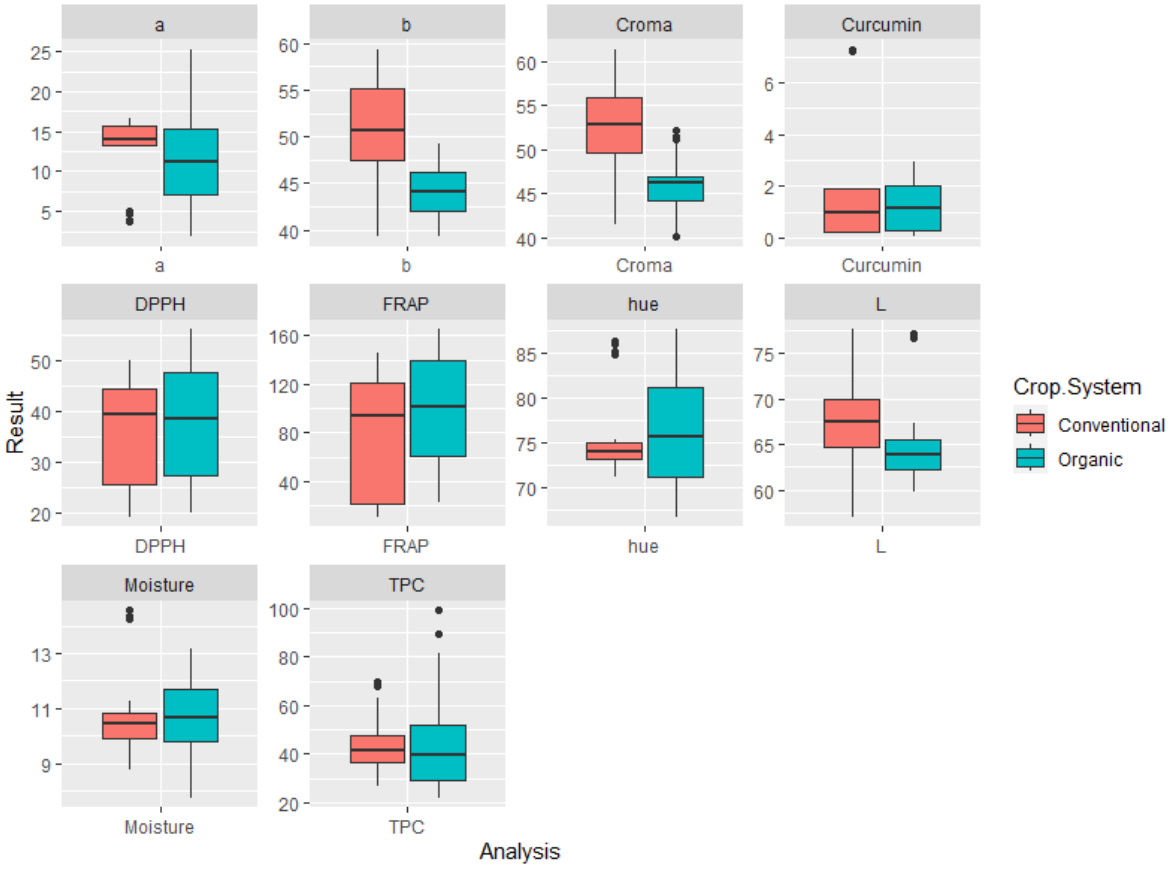
3. Results and discussion

3.2 Exploratory data analysis

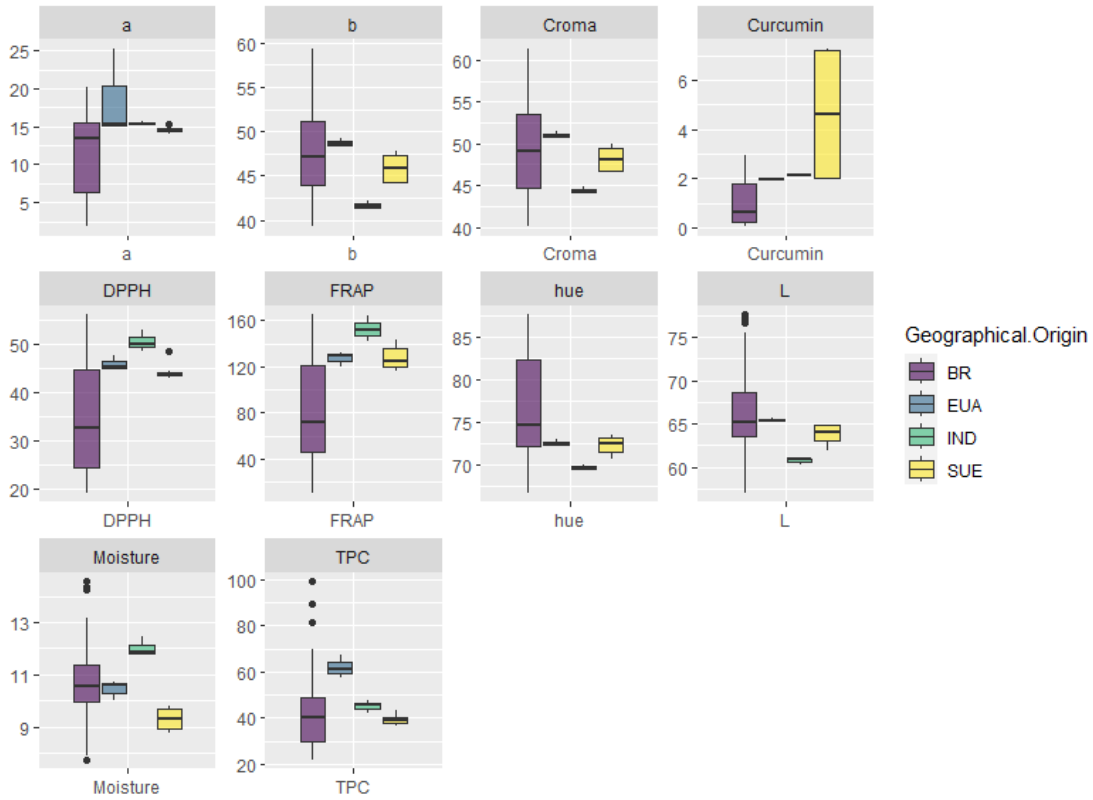
Table 1 and boxplot in Figure 1 shows the results of all chemical, physicochemical analyses and antioxidant activity. It is possible to observe that a considerable variability within the same system crop, the data shows a range that is in agreement with literature (CAS; GHIDONI, 2019; ESATBEYOGLU *et al.*, 2012; NÚÑEZ *et al.*, 2020b).

Figure 1 - Boxplot depicting the distribution of data points for turmeric samples across chemical, color and antioxidant properties for crop system (a) and geographical origin (b)

a)



b)



190

191 The chromaticity coordinates a* of the analyzed samples ranged from 3.79 to
192 19.87. According to the literature, positive and higher values of a* may be related to a
193 greater amount of curcumin. As for the chromaticity coordinate b*, which represents
194 the variation from blue (-) to yellow (+), there was a range from 39.75 to 59.13. The L*
195 component ranged from 57.23 to 77.03, with higher L* values indicating lighter colors,
196 as this parameter measures the brightness of the sample. According to the boxplot,
197 the plots showed a range for *a* and *hue* were high for the results of the organic crop
198 system, whereas for the conventional system, this behavior is observed for *b*, chroma
199 and *L*. In general, the means for the evaluated color parameters differed between the
200 two cultivation systems. When the results of the color attributes are assessed while
201 distinguishing the geographical origin, the amplitude is more pronounced for the
202 Brazilian samples.

Table 1 - Chemical, color and antioxidant properties of Brazilian, American, Indian and Swedish turmeric samples in organic and conventional crop system.

Turmeric Sample	Crop System	Geographical origin	*L* (lightness)*	*a* (redness)*	*b* (yellowness)*	*C* (color intensity)*	*h* (hue angle)*	Moisture	DPPH (% reduction)	FRAP (mg TE.g^{-1})	TPC (mg GAE g^{-1})	Curcumina (mg CC.g^{-1})
1	Organic	Brazil	66.92	5.27	44.41	44.72	83.23	7.76	21.38	43.71	26.58	0.15
2	Organic	Brazil	67.26	4.82	44.34	44.60	83.80	7.76	21.75	33.71	23.24	0.15
3	Organic	Brazil	66.58	5.36	43.89	44.22	83.04	7.90	22.88	39.96	24.91	0.15
4	Organic	Brazil	77.13	1.79	41.31	41.35	87.52	7.88	20.13	22.88	22.33	0.05
5	Organic	Brazil	77.21	2.02	40.89	40.94	87.18	8.17	20.38	29.13	24.45	0.05
6	Organic	Brazil	76.75	1.83	40.88	40.92	87.44	8.47	20.30	100.79	49.91	0.03
7	Organic	Brazil	67.20	15.50	43.87	46.52	70.54	10.76	56.00	158.71	99.30	2.95
8	Organic	Brazil	66.69	15.26	46.05	48.52	71.67	10.74	50.50	160.79	81.58	2.94
9	Organic	Brazil	64.20	16.51	48.11	50.87	71.06	11.41	54.25	164.96	89.45	2.92
10	Organic	Brazil	64.07	19.49	47.36	51.21	67.63	11.58	48.00	137.04	68.70	2.48
11	Organic	Brazil	63.47	20.27	47.11	51.28	66.72	11.70	49.25	145.38	66.88	2.43
12	Organic	Brazil	64.39	19.86	48.19	52.13	67.60	11.48	46.50	143.71	65.97	2.47
13	Organic	Brazil	61.39	7.93	40.84	41.60	79.01	9.51	29.38	51.63	24.00	0.43
14	Organic	Brazil	59.85	8.02	39.23	40.04	78.44	10.11	28.13	64.54	29.00	0.40

15	Organic	Brazil	60.00	8.17	39.38	40.22	78.28	9.82	26.13	59.13	29.00	0.43
16	Organic	Brazil	60.77	7.91	41.22	41.97	79.17	12.22	28.13	58.71	34.45	0.30
17	Organic	Brazil	62.63	7.11	43.72	44.29	80.76	11.86	27.50	62.04	39.15	0.31
18	Organic	Brazil	61.64	7.30	42.44	43.06	80.23	11.76	27.13	67.88	38.39	0.30
19	Organic	Brazil	63.94	7.36	45.91	46.50	80.89	10.55	28.50	70.79	34.91	0.29
20	Organic	Brazil	62.80	7.49	44.49	45.11	80.45	10.91	27.13	72.46	36.88	0.27
21	Organic	Brazil	62.26	7.57	43.98	44.62	80.24	10.38	28.13	77.04	42.33	0.27
22	Organic	Brazil	64.44	6.67	46.37	46.84	81.82	10.54	29.75	58.71	23.24	0.33
23	Organic	Brazil	64.91	6.23	46.80	47.21	82.41	10.26	31.25	67.46	28.70	0.32
24	Organic	Brazil	65.49	5.51	46.20	46.53	83.20	11.51	36.13	66.63	21.88	0.32
25	Organic	Brazil	63.56	14.92	43.94	46.40	71.24	12.59	44.75	101.21	36.58	1.81
26	Organic	Brazil	63.98	14.31	44.36	46.61	72.12	12.44	40.63	102.88	48.85	1.80
27	Organic	Brazil	63.54	14.75	43.62	46.05	71.31	13.14	43.13	104.96	42.64	1.82
28	Conventional	Brazil	77.58	4.98	54.88	55.10	84.82	10.86	30.00	21.21	27.64	0.24
29	Conventional	Brazil	75.23	4.78	54.20	54.41	84.96	11.19	24.00	22.46	29.61	0.23
30	Conventional	Brazil	75.49	4.59	54.42	54.61	85.18	10.86	20.88	16.21	26.88	0.24
31	Conventional	Brazil	63.41	13.59	47.16	49.08	73.92	10.12	33.75	62.46	35.97	0.82
32	Conventional	Brazil	63.57	13.26	47.40	49.22	74.37	9.92	32.38	68.29	41.88	0.80

33	Conventional	Brazil	63.54	13.38	47.29	49.15	74.20	9.72	33.13	63.71	42.03	0.83
34	Conventional	Brazil	68.10	15.77	55.49	57.69	74.13	10.57	45.75	122.04	45.36	1.88
35	Conventional	Brazil	67.73	16.12	55.02	57.33	73.67	10.13	45.50	123.29	41.88	1.87
36	Conventional	Brazil	67.88	15.96	55.17	57.44	73.86	11.17	45.63	127.04	43.55	1.89
37	Conventional	Brazil	64.35	16.68	50.13	52.83	71.59	10.49	48.50	129.96	63.24	1.88
38	Conventional	Brazil	64.82	16.55	51.06	53.67	72.04	10.63	49.88	142.46	52.64	1.91
39	Conventional	Brazil	64.90	16.28	51.61	54.12	72.49	11.26	48.25	145.79	48.55	1.92
40	Conventional	Brazil	57.23	13.14	39.26	41.40	71.49	14.37	40.88	121.21	55.67	0.78
41	Conventional	Brazil	57.00	13.47	39.54	41.77	71.18	14.57	40.00	128.29	69.91	0.78
42	Conventional	Brazil	57.47	13.53	40.46	42.66	71.51	14.26	43.25	118.71	67.79	0.77
43	Conventional	Brazil	68.71	16.02	58.76	60.90	74.75	10.44	38.75	85.38	38.39	1.12
44	Conventional	Brazil	68.88	15.87	59.26	61.34	75.01	10.68	36.25	94.13	41.12	1.20
45	Conventional	Brazil	68.70	15.60	59.37	61.38	75.28	10.15	38.88	87.04	45.21	1.19
46	Conventional	Brazil	67.04	13.99	50.44	52.35	74.50	10.22	46.63	94.54	47.94	1.70
47	Conventional	Brazil	67.13	14.01	51.08	52.97	74.66	10.76	44.63	116.21	49.76	1.70
48	Conventional	Brazil	66.58	14.11	50.09	52.03	74.27	10.74	43.38	104.54	46.12	1.67
49	Conventional	Brazil	70.06	14.90	49.89	52.07	73.37	10.45	20.75	16.63	40.97	0.16
50	Conventional	Brazil	70.26	14.69	50.14	52.24	73.67	9.91	19.13	12.46	30.52	0.15

51	Conventional	Brazil	69.88	14.67	49.82	51.93	73.59	10.22	19.63	12.88	35.21	0.15
52	Conventional	Brazil	74.28	3.95	55.91	56.05	85.96	9.61	19.75	12.04	35.36	0.16
53	Conventional	Brazil	74.41	3.66	55.41	55.53	86.22	9.40	20.75	13.29	36.27	0.14
54	Conventional	Brazil	74.50	3.76	55.79	55.92	86.14	9.16	20.38	10.38	37.18	0.14
55	Organic	EUA	65.43	25.29	48.52	50.88	72.51	10.61	45.13	119.96	67.03	1.94
56	Organic	EUA	65.64	15.05	49.22	51.47	72.99	10.02	47.63	132.88	61.12	1.96
57	Organic	EUA	65.17	15.41	48.37	50.76	72.33	10.75	44.63	129.54	57.64	1.95
58	Organic	India	60.86	15.31	41.49	44.22	69.75	11.85	49.88	141.63	47.33	2.11
59	Organic	India	60.25	15.62	41.40	44.25	69.33	11.79	48.63	164.13	45.52	2.14
60	Organic	India	60.96	15.32	42.12	44.82	70.01	12.45	52.88	152.46	42.18	2.12
61	Organic	Sweden	63.36	14.54	44.57	46.88	71.94	9.72	48.63	138.71	37.48	1.99
62	Organic	Sweden	61.90	15.41	44.06	46.68	70.72	9.56	42.88	128.29	40.21	1.98
63	Organic	Sweden	62.95	14.87	44.23	46.67	71.42	9.80	44.38	143.29	43.55	2.01
64	Conventional	Sweden	64.95	13.95	47.28	49.30	73.56	8.90	43.63	119.54	36.73	7.24
65	Conventional	Sweden	64.68	14.50	47.25	49.43	72.94	9.06	43.75	116.63	40.06	7.29
66	Conventional	Sweden	64.87	14.41	47.81	49.94	73.23	8.75	43.38	120.79	38.09	7.23

205

206 The total phenolic compound content in the saffron samples ranged from 24.61
207 mg GAE g^{-1} to 90.11 mg GAE g^{-1}. The variability in the results reflects the presence of
208 polyphenolic compounds at different levels. Consequently, the composition and quality
209 of commercial turmeric products can exhibit significant variation. This variation in
210 phenolic composition can have a notable impact on the quality of the samples, both in
211 terms of flavor and potential health benefits. Examining both sets of data in the boxplot,
212 it is possible to infer that, in general, the values found for the crop system are similar,
213 whereas, for different geographical origins, higher values are observed for the samples
214 from the USA.

215 Different techniques have been employed for determining the antioxidant
216 activity of biologically active substances with distinct principles, mechanisms of action,
217 ways to express results and diverse applications. In this work, two assays were
218 employed to assess the antioxidant activity of turmeric. DPPH has been widely utilized
219 for assessing free-radical scavenging capacity. Antioxidants, upon interaction with the
220 DPPH radical, induce a color change from purple to yellow and a reduction in
221 absorbance at a wavelength of 517 nm. The results demonstrated that DPPH inhibition
222 ranged from 19.83% to 53.585% reduction. FRAP assay was employed to assess the
223 reducing abilities linked to the presence of compounds that act by disrupting the free-
224 radical chain through the donation of a hydrogen atom (BENZIE; STRAIN, 1996). In
225 this study, the FRAP results show average content ranged from 11.90 to 161.49 mg
226 TE.g^{-1}.

227 For DPPH, FRAP and TPC, the boxplot infer that, in general, the values for
228 media found for the crop system are similar. Whereas for different geographical origins,
229 higher values are observed for the samples from the USA in TPC results and India

samples for DPPH and FRAP. Furthermore, the Brazilian samples exhibit lower values and a wide range for the three analyses.

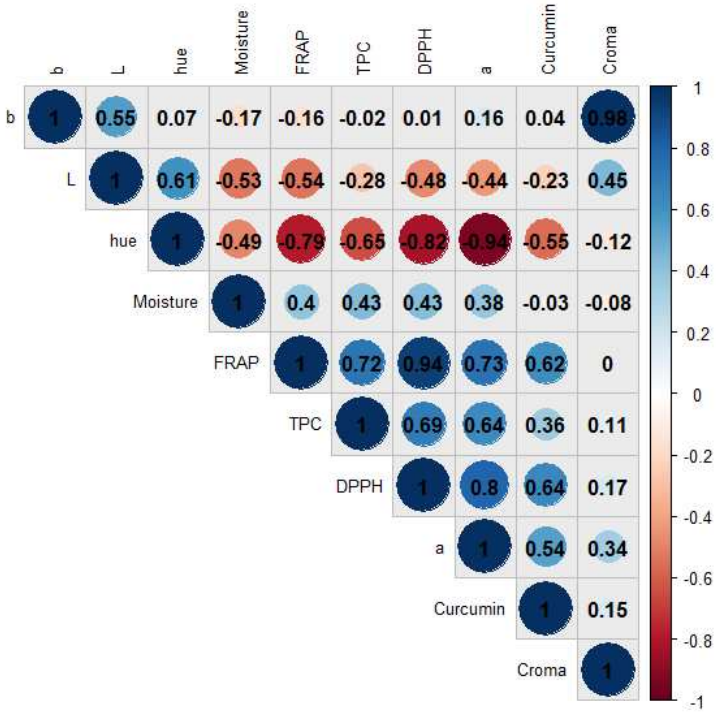
Curcuminoids are compounds orange-yellow, the presence of curcumin serves as a marker compound for the quality control of rhizomes (CAS; GHIDONI, 2019). The results obtained in this study for curcumin content are in accordance with what has been reported in the literature for bulk commercial products (MANIGLIA *et al.*, 2015). Its average content ranged from 0.04% to 7.25%. According to the boxplot, the distribution of data for the two cultivation systems exhibits similar behavior, including the observed mean and data range. However, when comparing geographical origins, the Swedish samples show a high content, the samples from the USA and India exhibit similar content, and the Brazilian samples have lower content.

Variations in the levels of phenolic compounds in turmeric (*Curcuma longa* L.) can be influenced by several factors that directly impact the quality of the product. Among these factors, the plant's geographic origin, cultivation conditions, climate, soil, and the stage of maturity of turmeric roots play a crucial role. Gururani et al. (2022) assessed variations in the chemical composition and biological activities of essential oil from local cultivars of Curcuma longa rhizomes collected at different altitudes in India. In another study, Chumroenphat et al. (2021) investigated various drying methods that affect the chemical components and microstructure of turmeric. Both researches have demonstrated that factors in studies differ in the ways in which they affect bioactive compounds in turmeric, as well as its antioxidant activities.

The Pearson correlation coefficient (r) is used to express the strength between two continuous variables related to each other. The values for the Pearson Coefficient are shown in Figure 2. For DPPH and FRAP, the r value with TPC is 0.69 and 0.72,

254 respectively ($p < 0.05$). The correlation between the curcumin content and the total
255 polyphenol contents ($r = 0.36$), DPPH ($r = 0.64$), and FRAP ($r = 0.62$) indicates a
256 significant positive correlation considering ($p < 0.05$). No research experiment reported
257 in the literature has so far compared the organic and conventional system crops for
258 turmeric (*Curcuma longa* L.). There is no scientific consensus regarding the correlation
259 between the chemical composition and antioxidant activity of various vegetables
260 cultivated under conventional or organic crop systems. The results found in this study
261 indicate that curcumin acts in a similar mechanism to phenolic compounds; however,
262 its action is not exclusive, suggesting that its antioxidant action and that of other
263 phenolic compounds (quantified by TPC) are based on electron transfer and hydrogen
264 atom transfer.

265 **Figure 2 -** Pearson's correlations: Visualization of relationships between chemical,
266 color and antioxidant properties of turmeric samples, showing the degree of
267 association.



268

While numerous studies highlight disparities between organic and conventional foods, a consensus on which is nutritionally superior remains elusive. Several reports suggest that organic food may exhibit enhanced nutritional and nutraceutical qualities, characterized by higher levels of macronutrients, phytochemicals, and antioxidant properties. In contrast, other investigations have found no significant distinctions in nutrient and nutraceutical components between organic and conventional products (HURTADO-BARROSO *et al.*, 2019; PALMA *et al.*, 2023).

In this aspect, it is essential to understand how the antioxidant activity of turmeric is linked to its chemical composition. In this context, heatmap analysis, HCA and PCA were applied.

3.3 HCA heatmap analysis

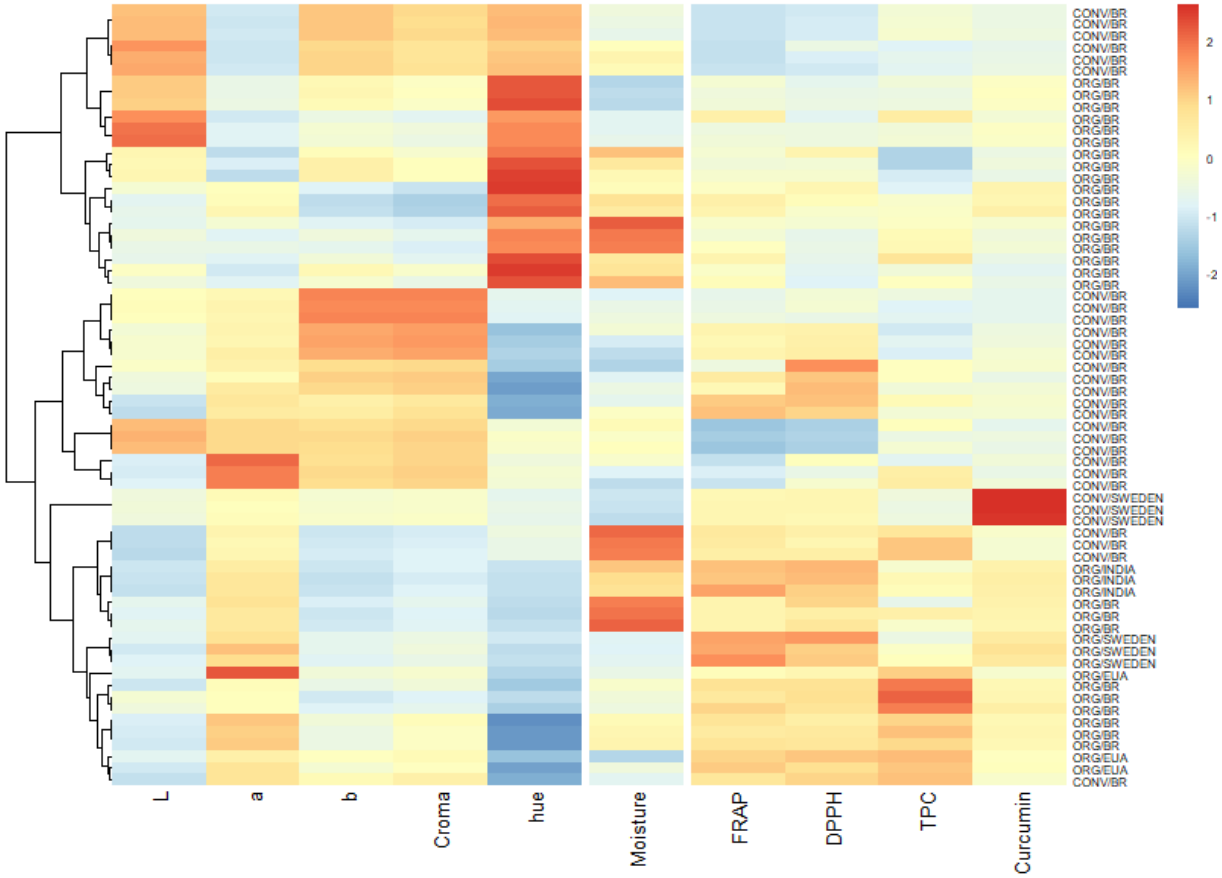
The results of all chemical, physicochemical analyses, phenolic compounds, and antioxidant analyses in samples of turmeric were further visualized with a clustered heatmap (Figure 3.a). A heatmap generated a more comprehensive overview and comparison, and this method is suitable for presenting all flows of matter at once while retaining their precise identification. Comparison of variations in color intensity in heatmap among the samples did not revealed differences in the system crop studied the results were the same as those obtained using the horizontal dendrogram.

The visualization of the heatmap for instrumental color based on redness (a* coordinate), yellowness (b* coordinate), lightness (L*), and a combination of these parameters (chroma - C* and hue angle - h*), the results show that changes influence the variation in the color of the turmeric samples in the values of a* and b*.

Curcumin, the main curcuminoid found in turmeric, influences the powdered sample's yellow and red coloration. In the heatmap, it is possible to differentiate a

cluster with distinct values for a*, b*, L*, chroma, and hue angle (°hue). This cluster consists of 8 samples, including 2 organic and 6 conventional ones of Brazilian origin. For these samples, it is possible that other metabolites belonging to different phytochemical classes may have interfered with the coloration and antioxidant activity.

Figure 3 - Heatmap of the chemical, color and antioxidant properties for crop system in 66 turmeric samples and dendrogram for turmeric samples obtained from the hierarchical cluster analysis.



That is not possible to correlate high curcumin content with stronger colors for yellow (color attribute b*), red (color attribute a*), and hue angle. Furthermore, all analyzed values indicate that the yellow color is more pronounced in the samples (66.7 to 87.5).

Using the hierarchical cluster analysis applied to the samples, the clusters suggested did not separate the samples distinct in organic and conventional, the same conclusion is observed in classified origin crops. The clustering distance for Sweden's samples is more significant than India's or EUA samples. The dendrogram imposes a hierarchy on samples and affords a two-dimensional vision of the similarity of the entire set of samples used in the study.

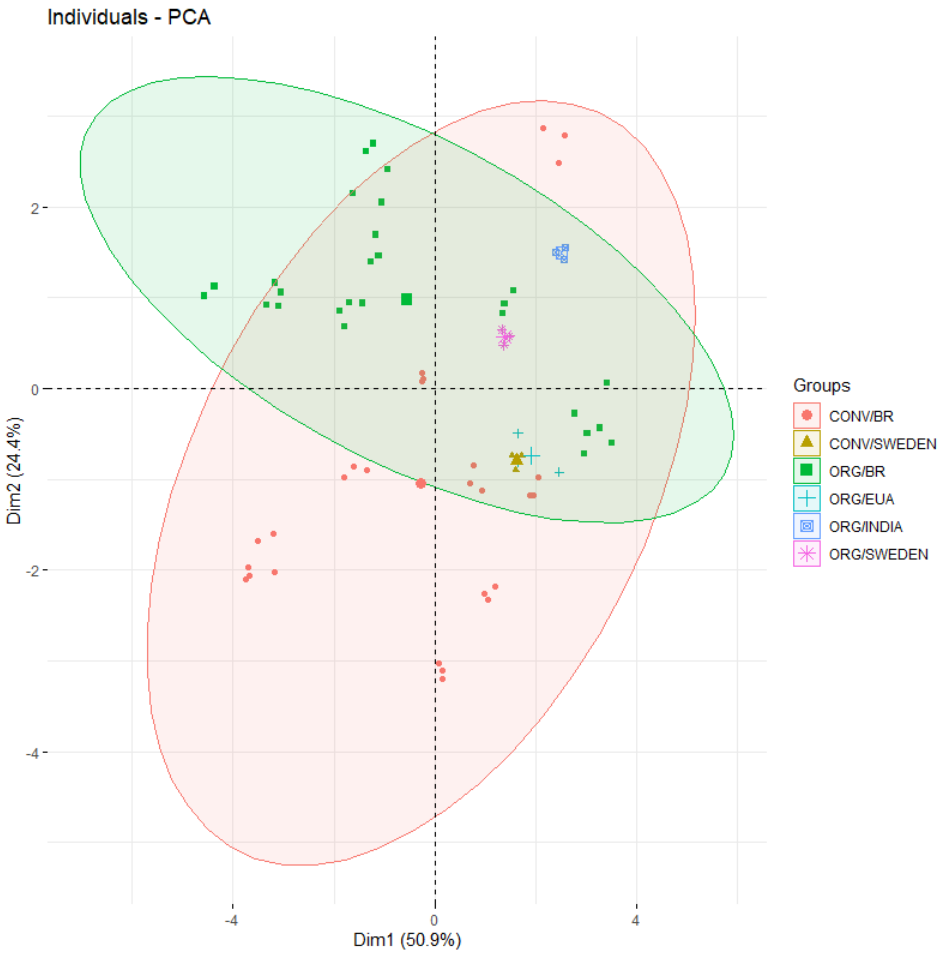
3.4 Exploratory analysis by PCA

PCA was applied to discriminate the samples according to the phenolic composition, color, and antioxidant activity. A data matrix with a dimension of 66 (sample) × 10 (phenolic composition, color attribute, moisture, curcumin contend and antioxidant activity) was obtained. Data was autoscaled to provide similar weights to all the variables. Principal Component 1 (PC1) accounted for 50.92% of the total variance of the original variables, while PC2 accounted for 24.35%. Together, these two components explained 75.27% of the total variance with having eigenvalues exceeding 1. Therefore, the first two principal components effectively summarize the total sample variance and can be employed to study the dataset.

Figure 4 shows the PCA scores (a) and loadings (b). When decomposed, these vectors establish a relationship with each axis, potentially correlating with each principal component. The influence of each variable on each PC can be assessed through the values of these correlations. The samples were segregated along the first principal component (PC1) due to differences observed in DPPH, FRAP, a, TPC, curcumin content, moisture, and the color attributes L and hue, with correlation coefficients of 0.936, 0.924, 0.887, 0.767, 0.631, 0.543, 0.0543, -0.628, -0.939, respectively. The second PC (PC2) separated the samples based on results for

329 chroma and the color attributes chroma, b, L, a and moisture with correlation
330 coefficients of 0.969, 0.962, 0.599, 0.268, and -0.267, respectively ($p < 0.05$).

331 **Figure 3 -** A scatter plot of PC1 versus PC2 of the main sources of variability between
332 the turmeric samples



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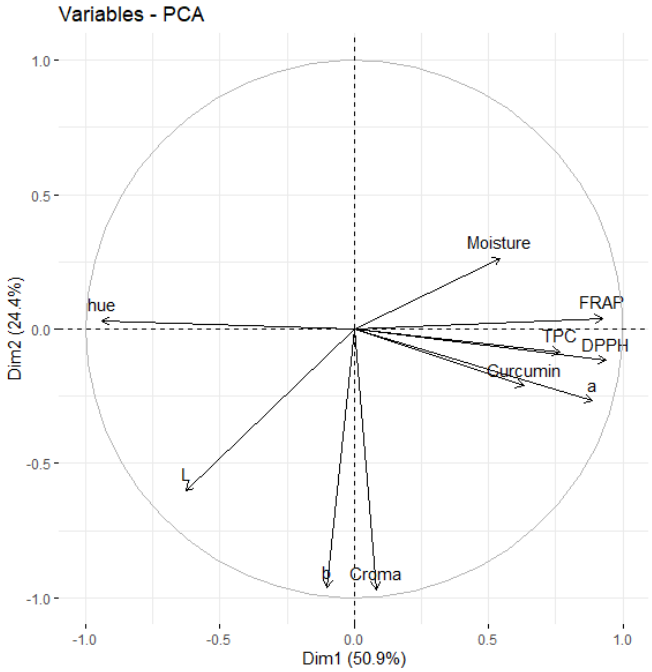

Several studies aimed to evaluate the feasibility of chemometrics to differentiate and characterize turmeric. Gad & Bouzabata (2017) studied the metabolic profiling of 30 commercial samples of turmeric from Algeria and Egypt by UV, FT-IR, ^{1}H NMR and HPLC. PCA and HCA were applied to differentiate high-quality samples from low-quality ones. Rohaeti et al. (2015) discrimination the three species *Curcuma longa, Curcuma xanthorrhiza* and *Zingiber cassumunar*, PCA was employed to discriminate the samples according to the species based on the FTIR spectra in the region, using the first two PCs which accounted for 76% of the total variance (PC1 = 59.3% and PC2 = 16.7%) and the tested samples were clustered into three different groups. Salem et al. (2022) used a PCA score plot to reveal clear segregation of the dried from fresh samples of turmeric (PC1 = 85.4 and PC2 = 4.5%), while the HCA also revealed separation of fresh and dry samples to two different clusters reflecting the metabolic composition. Contrary to the findings in the mentioned articles, our results with PCA analysis did not show differences between the two cropping systems, suggesting that the two agronomic practices can provide the crop with a similar profile.

4. Conclusion

Relatively comprehensive phytochemical analysis combined with multivariate analyses of food spices attracted considerable attention as a potential quality assessment tool. In this study, two different cropping systems, organic and conventional, and geographic origin (Brazil, India, EUA and Sweden) were studied to evaluate the effect on phenolic composition, *in vitro* antioxidant activity using DPPH and FRAP assays, color attributes and curcumin content. Chemometric results revealed that the two cropping systems had no difference, as the same for geographic origin.

To further investigate the similarity among the samples, we conducted hierarchical cluster analysis (HCA), which supported the results obtained from principal component analysis (PCA), as the samples did not form distinct clusters. In this study, no unsupervised statistical method proved effective in distinguishing between organic and conventional samples, irrespective of their origin. This suggests that the geographical origin and crop system, as considered in this study, may not be prominent determinants of the analyzed characteristics. Further investigations and the consideration of additional factors may be necessary to gain a more comprehensive understanding of the factors influencing turmeric rhizome properties.

We suggest that future research endeavors should encompass a larger sample size and explore additional potential biomarker compounds to establish an analytical platform based on chemical and statistical analysis for the classification of turmeric.

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626

CAPÍTULO IV

SCIENTIFIC ARTICLE 2

Statistical optimization of bacterial cellulose production using turmeric syrup and its *ex situ* impregnation with curcumin

Abstract

Bacterial cellulose (BC) is a biopolymer synthesized by acetic acid bacteria, its promising due to its specific and properties. The current study exploited the glucose from enzymatically hydrolyzed turmeric (TS) as the carbon source for BC production by *Komagataeibacter xylinus* (ATCC 53582). Seven parameters were screened by the Plackett–Burman design (PBD), and significant parameters were optimized by the response surface methodology using central composite rotatable design (CCRD). Optimal conditions for production of BC were found as: 2.11% turmeric syrup as alternative carbon source, 5% (v:v) inoculum, yeast extract 0.5 $g.L^{-1}$, peptone 0.5 $g.L^{-1}$, citric acid 0.115 $g.L^{-1}$, Na_2HPO_4 0.27 $g.L^{-1}$, 0.59% etanol (v:v). After optimization and validation steps, a production yield of 2.11 $g.L^{-1}$ was achieved. Membranes presented the same crystallinity as observed for BC produced in Hestrin and Schramm (HS) medium. The results showed that TS is a promising carbon source for pure BC production. The BC membrane was separately loaded with turmeric ethanolic extract (BC/CURC) to enhance its antioxidant activity for multiple applications. This was the first demonstration of the application of sugars provided by enzymatic hydrolysis of turmeric in BC production and the application of turmeric as a source of curcumin to provide modifications after BC biosynthesis and promote antioxidant activities on BC properties.

Keywords: Biomaterials, Cellulose biosynthesis, acetic acid bacteria, *Komagataeibacter xylinus*, *Curcuma longa* L.

1. Introduction

Bio-based materials with high purity, mechanical stability, and barrier properties have become a widespread choice for the research community to develop environmentally friendly systems (VERSINO *et al.*, 2023). The bacterial production of cellulose is receiving attention as an environmental-friendly polymeric material due to the singular properties and characteristics of BC, like high specific surface area and rich in multiple hydroxyl groups, providing the potential to be used in packaging materials, biomedical applications, food active, textiles, filtration membranes and biosensors (GOMES *et al.*, 2021; GREGORY *et al.*, 2021; MA *et al.*, 2020) .

Bacterial cellulose (BC) is a naturally occurring nanomaterial (chain of β-1→4 linked d-glucose units) produced by the fermentation of some species of bacteria. BC is mainly synthesized by acetic acid bacteria from the genus *Komagataeibacter*, the most common and attractive strain is *Komagataeibacter xylinus*, a member of the family *Acetobacteraceae*, due to its ability to produce large amounts of cellulose and to consume a variety of sugars and other compounds as carbon sources (GOMES; IDA; SPINOSA, 2022; SILVA *et al.*, 2021). BC has a high degree of crystallinity and mechanical properties superior to other celluloses, when interposed with wood cellulose, BC shows high purity, free from lignin and hemicellulose and very high-water content. BC has become an essential nanomaterial in many industrial processes as it is biocompatible, biodegradable, and renewable (RAUT *et al.*, 2023).

Traditional carbon sources for microbial fermentation are sugars such as glucose, fructose, and sucrose. In recent years, much interest has developed in producing BC on different mediums due to the high cost involved in BC production. About 30 % of the total BC production cost was attributed to the applied medium. The

significant factors that many researchers have investigated are the optimal medium, the culture conditions, and their interaction effects. More recently, unconventional feedstocks from renewable resources and waste streams have been investigated. Corn steep liquor, carob and haricot bean, maple syrup, enzymatically treated food waste liquids, and soybean molasses medium are examples of studies of different mediums used in the literature (BILGI *et al.*, 2016b; EL-GENDI *et al.*, 2023a; FERNANDES *et al.*, 2020; GOMES *et al.*, 2021; PARK; KIM; KIM, 2006; VAZQUEZ *et al.*, 2013; ZENG; SMALL; WAN, 2011).

Turmeric (*Curcuma longa* L.) is valued for its pharmaceutical properties and its exhibited biological activities such as antiviral, anti-inflammatory, antimicrobial, and antioxidant (DOLDOLOVA *et al.*, 2021; KHATUN *et al.*, 2021; YANG *et al.*, 2020). In a previous study in 2017, our research group developed an enzymatic turmeric hydrolyzate. This hydrolyzate produced a TS via enzymatic reached 100% conversion of starch into sugars and 2.58% curcumin content and may be used as a source of fermentable sugar for fermented, or it can be incorporated into food products to promote their antioxidant (QUEIROZ-CANCIAN *et al.*, 2018).

This study investigates the influence of turmeric syrup like carbon sources for microbial fermentation for BC in synthesis and after biosynthesis (*ex situ*). In the first moment, the production of BC, by *Komagataeibacter xylinus* ATCC 53582, using turmeric syrup as the renewable carbon source. Culture conditions affecting biomass and BC production are determined using Plackett–Burman design and optimized by the surface-response methodology (RSM) using central composite rotatable design (CCRD). In the second time, BC is immersed in a solution where it can interact with curcumin (BC/CURC), available in a turmeric ethanolic extract. In this case,

75 modifications after biosynthesis are capable of enhancing the antioxidant properties of
76 BC. The BC obtained under optimal conditions and BC/CURC were characterized
77 using FTIR and X-Ray Diffraction.

78 **2. Material and methods**

79 **2.1. Microorganisms, medium and materials**

80 *Komagataeibacter xylinus* (ATCC 53582) was obtained from the State
81 University of Londrina culture collection. The culture medium was prepared from sterile
82 mannitol agar (MYP) (composition: yeast extract (5 $g.L^{-1}$), peptone (3 $g.L^{-1}$), mannitol
83 (25 $g.L^{-1}$), agar (15 $g.L^{-1}$) and incubated for 72 h at 30 °C. To prepare the inoculum, *K.*
84 *xylinus* from an agar plate was transferred aseptically into 500 mL reagent bottles
85 containing 100 mL of HS medium and incubated at 30 °C in a shaker incubator set at
86 120 rpm.

87 TS, a turmeric hydrolysate was prepared according to Queiroz-Cancian et al.
88 (2018), with modifications. Briefing, the turmeric flour was suspended in distilled water
89 (150 g/L) for the enzymatic hydrolysis. For liquefaction, 0.23 $g.100g^{-1}$ for α-amylase
90 (204 $KNU.g^{-1}$) was added at pH 6.03, 91.5 °C at 10 minutes in a constant agitation 70
91 rpm. For saccharification, 1.60 $mL.100g^{-1}$ for amyloglucosidase was added at pH 4.5,
92 60 °C at 8 hours in a constant agitation of 150 rpm.

93 Turmeric flour was dispersed into ethanol (80:20 v/v) for produced ethanolic
94 extract of curcumin according to Queiroz-Cancian et al. (2018), the final concentration
95 was 3.14 g $curcumin.100g^{-1}$ turmeric flour.

96 All the other reagents used in this study were of analytical grade.

2.2. Screening parameters using the Plackett–Burman design

In order to improve the yield of BC, PBD was applied to screen the seven variables that affect BC production and select the most significant ones. Design parameters for PBD, % glucose from a TS like carbon source, yeast extract, peptone, Na_2HPO_4, citric acid, seed culture and ethanol, were chosen according to literature. Parameters were tested in 8-run PBD at two levels (maximum and minimum value) to determine key factors of the BC production, and the addition of a central point allowed us to verify the repeatability of the process. The actual values of the variables are given in Table 1a, where each factor is represented.

The dependent variable Y is the yield BC production ($g.L^{-1}$) ($P < 0.05$). The fermentation was performed in the static culture at 30 ºC for 10 days. The dry weight of BC was used to analyze PBD results; variables with confidence levels greater than 95% were considered to influence BC production significantly. The experimental design was generated and analyzed using Statistica 14.0 software (TIBCO Software Ins, USA).

2.3. Central Composite Rotational Design (CCRD)

CCRD experiments were performed with significant variables determined by PBD to find the optimum level for each parameter. Optimization was carried out using a response surface methodology (RSM) with CCRD to improve BC yield. The independent variable was the mass of BC produced and expressed in dry weight ($g.L^{-1}$). The dependent variables were % glucose from a TS like carbon source (X_1) and ethanol (% v:v) (X_2) concentrations. The experimental design included 11 combinations, according to CCRD 2^2, adding 4 axial points ($\alpha = \pm 1{,}41$) and 3 central points for replications (Table 2).

A second-order polynomial model given in Eq. (2) was fitted to response:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x^2{}_i + \sum_{i=1}^{k-1} \sum_{j=1}^{k} \beta_{ij} x_i x_j + \epsilon \quad (1)$$

Where, Y is the response (dependent variable), x_i , x_j are independent variables, $\beta_0, \beta_i, \beta_{ii}, \beta_{ij}$ are the regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively, and ϵ the error term in the model (MYERS; MONTGOMERY; ANDERSON-COOK, 2016).

The real and coded values for independent values were shown in Table 2. The analysis of experimental design data, optimization of yield of BC, and calculation of predicted responses was obtained with Statistica 14.0 software (TIBCO Software Ins, USA). The analysis of variance (ANOVA) was used to determine the significance level of the mathematical models and terms. The adequacies of the model were checked according to the values of R^2 and *adjusted-*R^2, as well as the insignificant lack of fit value ($p > 0.05$). Finally, BC was prepared in the optimal condition to validate the optimized process conditions obtained by Student's *t-test* ($p < 0,05$). Another experiment was carried out in HS medium to compare BC production.

2.4. BC purification

After 10 days of fermentation, the BC pellicles produced in 2.2 and 2.3 were subjected to purification steps to remove the remaining cells, culture medium, and other impurities. The samples were purified with sodium hydroxide 1M at 80 °C for 2 h and washed with water until neutral pH. At last, BC films were dried until constant weight to determine the respective yields expressed in dry weight ($g.L^{-1}$).

2.5. Preparation of biocellulose/curcumin

Biocellulose/curcumin BC (BC/CURC) was prepared according to Luo et al. (2012), with modifications. The membranes obtained in the 2.3 were separately loaded with turmeric ethanolic extract. The BC was added to 50 mL of ethanolic extracts (80:20 v:v) and stirred at 30 °C for 24 hours on a rotary shaker (120 rpm). The BC/CURC obtained after the incorporation of curcumin was dried until constant weight and kept in a desiccator prior to characterization. Furthermore, control films without the incorporation of curcumin were prepared with the standard HS culture medium.

2.5.1. Amount Of Curcumin

The curcumin extracts from BC/CURC were prepared according to Roy & Rhim (2020), with modifications, to determine curcumin content (CC) by ultra-performance liquid chromatography (UPLC). The samples were cut into small circles (6 mm) and weighed, then put in constant agitation at 21 hours at 120 rpm with acetonitrile (ACN). After, the samples were treated with 60 min ultrasonic and then centrifuged at 11 000 rpm for 50 min. The supernatant was filtered through a 0.22 µm PVDF membrane filter before injection (1 µL) into the ultra-performance liquid chromatography (UPLC®) Waters (Acquity UPLC System, Waters, USA) equipped with a UV-Vis detector and a C18 column (180×4.6 mm, 5 µm; Shiseido, Japan). The curcumin was quantified according to Cheng et al. (2010), with modifications. The mobile phase consisting of a mixture of 0.05% aqueous phosphoric acid and acetonitrile in the ratio of 50:50 (v/v), isocratic with the flow rate of 0.3ml.min^{-1} was employed at 30 °C. The detection wavelength was set at 420 nm.

The chromatographic peaks were identified by comparison with the retention time of standard curcumin. The calibration curve was prepared using curcumin

standard in the concentration range 0.10 – 2.5 µg/mL, obtaining a correlation coefficient (R^2) of 0.999. The injections were made in triplicate, and the results were expressed as µg CC/g dry weight of the sample.

2.5.2. Antioxidant activity

The antioxidant activity of BC and BC/CURC samples was determined using 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) (BRAND-WILLIAMS; CUVELIER; BERSET, 1995; ROY; RHIM, 2020). For the DPPH analysis, 50 mg of the film sample was placed in a 10 mL DPPH solution and incubated at room temperature for 30 min. The DPPH solution without film was used as the control. After incubation, the absorbance was determined using a UV-Vis spectrophotometer (Mecasys Optizen POP Series UV/Vis spectrophotometer, Seoul, Korea) at 517 nm, and the antioxidant activity of the film was calculated as follows:

$$DDPH \text{ scavenging activity } = \left(1 - \frac{As}{Ac}\right) \times 100 \quad (2)$$

where Ac and As were the absorbance of DPPH of the control and test film, respectively.

2.5.3. Optical properties

The apparent color of the BC/curcumin was evaluated using a colorimeter (Konica Minolta CR 400, Japan). The color parameters L* (lightness), a* (red - green), and b* (yellow - blue) were determined with the following specifications: CIE D 65 illuminant and CIE 10º standard observer. The parameters' chroma (C*) was estimated as $C^* = (a^{*2} + b^{*2})^{1/2}$ and hue angle (h^*) was calculated by: $h^* = \tan^{-1}(b^* / a^*) + 180°$ when $a^* > 0$.

2.6. Characterization and properties of the film

2.6.1. FT-IR Spectroscopy

Infrared spectra were recorded to determine the main bands that characterize the bacterial cellulose polymer. Dried BC and BC/CURC samples were incorporated into potassium bromide and pressed into tablets under high pressure. The spectra were recorded on a Shimadzu IR-Prestige-21 spectrometer (Brazil) in the region of 4000 to 400 cm^{-1} at a resolution of 1 cm^{-1}.

2.6.2. X-Ray Diffraction

The XRD patterns of the BC and BC/CURC membranes were obtained using an X-ray diffractometer (Panalytical X'Pert Pro MPD; Malvern, Almelo, Netherlands) and Cu-Kα radiation (λ=1.5418 Å) at 40 kV and 30 mA. All assays were performed with a scan speed of 1 °/min, analyzing the range of 5–40° (2θ). The degree of crystallinity was determined for cellulose I as described by Segal et al. (1959).

$$Crystallinity\ Index(\%) = \frac{(I_{002} - I_{am})}{I_{002}}\ x\ 100 \quad (3)$$

where I_{002} is the maximum peak intensity corresponding to the 002 lattice diffraction at angle 2 = 22.8°, and I_{am} is the intensity of diffraction corresponding to the amorphous background at angle 2 = 18°.

3. Results and discussion

3.1. Plackett-Burman design

The Plackett-Burman design (PBD) is mathematical modeling for identifying the critical factors that mainly affect the response. PBD is a valuable method to screen many parameters reliably and practically eliminate ineffective parameters by

investigating the effects of each parameter without doing numerous experiments (PLACKETT; BURMAN, 1946).

In the present study, turmeric syrup was successfully used for BC production. Table 1 shows the Plackett and Burman experimental design containing actual values of independent variables and the response variable corresponding to the mass of BC obtained in each of the 11 experimental runs.

Table 1 - Plackett–Burman Design matrix and results.

Run	Code independent variables							BC Production ($g.L^{-1}$)
	x_1	x_2	x_3	x_4	x_5	x_6	x_7	
1	0	0.25	0.25	0.27	0.115	6.0	0	0.917
2	2.0	0.25	0.25	0.09	0.038	6.0	3.0	0.476
3	0	0.75	0.25	0.09	0.115	1.0	3.0	0.590
4	2.0	0.75	0.25	0.27	0.038	1.0	0	0.655
5	0	0.25	0.75	0.27	0.038	1.0	3.0	0.658
6	2.0	0.25	0.75	0.09	0.115	1.0	0	0.505
7	0	0.75	0.75	0.09	0.038	6.0	0	0.597
8	2.0	0.75	0.75	0.27	0.115	6.0	3.0	0.404
9	1.0	0.50	0.50	0.18	0.080	3.5	1.5	0.585
10	1.0	0.50	0.50	0.18	0.080	3.5	1.5	0.610
11	1.0	0.50	0.50	0.18	0.080	3.5	1.5	0.696

Legend: x_1: Glucose from TS ($g.L^{-1}$). x_2: Yeast extract ($g.L^{-1}$). x_3: Peptone ($g.L^{-1}$). x_4: Na_2HPO_4 ($g.L^{-1}$). x_5: Citric acid ($g.L^{-1}$). x_6: Seed culture (% v:v). x_7: Ethanol (% v: v)

Table 1b - Plackett–Burman Design % contribution of the parameters.

	Code independent variables						
	x_1	x_2	x_3	x_4	x_5	x_6	x_7
p-Value	**0.0180***	0.1359	0.0534	0.0556	0.8571	0.9329	**0.0376***

Legend: x_1: Glucose from TS (g.L^{-1}). x_2: Yeast extract (g.L^{-1}). x_3: Peptona (g.L^{-1}). x_4: Na_2HPO_4 (g.L^{-1}). x_5: Citric acid (g.L^{-1}). x_6: Seed culture (% v:v). x_7: Ethanol (% v: v). *Statistically significant at $p < 0.05$.

The response of this PBD produced BC with dry weights varying between 0.404 and 0.917 g.L^{-1}. Table 1B shows the *p-value* of parameters for BC production, the results were detected as glucose from a TS (g.L^{-1}) and ethanol (% v:v) with significant parameters at a confidence level $p < 0.05$. The coefficient of determination (R^2) was equal to 0.95084, indicating that the model can explain 95.08% of the variability in the results. Adjusted R^2 was equal to 0.83614.

The PBD useful to select the most significant variables. Seed culture was set at 5% (v:v). Na_2HPO_4, one of the most common salts used in the culture medium, and citric acid is organic acid, are among the frequently used organic nutrients in BC production, and the concentration was set at 0.27 and 0.115 g.L^{-1}, respectively. Nitrogen sources were indispensable for the cell. The literature describes these two components with co-fundamental substrates in cell construction and growth of microorganisms. The values in media were set in 0.5 g.L^{-1} for yeast extract and peptone, and the values were set based on HS medium values (ANDRIANI; APRIYANA; KARINA, 2020; FERNANDES *et al.*, 2020; GOMES; IDA; SPINOSA, 2022).

The PBD was useful in selecting the most significant variables. For ethanol, the significance was connected with the influence of these chemical compounds in the enzyme involved in the BC synthesis pathway. Ethanol is known to improve the

242 production of adenosine triphosphate (ATP) used in the BC synthesis pathway.
243 Furthermore, Yunoki et al. (2004) suggested that the addition of ethanol to the culture
244 medium would improve BC production by functioning as an energy source instead of
245 glucose. The results of glucose from a TS ($g.L^{-1}$) follow the literature since carbon
246 source is considered one of the most important factors for BC production. Different
247 kinds of carbon sources have been tested for the production of BC, and the
248 development of the most suitable medium for production is critical because cultivation
249 parameters can significantly affect this production (ANDRIANI; APRIYANA; KARINA,
250 2020; FERNANDES *et al.*, 2020; MOHAMMADKAZEMI; AZIN; ASHORI, 2015).

251 **3.2. Experimental Design**

252 Optimizing cultivation conditions is essential for diminishing the demands on the
253 medium and operations, thereby minimizing supplementary expenses in the production
254 process. Presently, the utilization of mathematical and statistical techniques to design
255 of experiments (DoE) for process optimization has demonstrated itself as a
256 dependable and expedient approach, capable of assessing the impacts of individual
257 and interacting variables on end products, thus showing the most probable optimal
258 conditions (EL-GENDI *et al.*, 2023b).

259 The mathematical quadratic model (non-significant terms removed) is a
260 canonical equation fitted to the experimental data (Eq. (4)). The dependent variable Y
261 is ($P < 0.05$).

262 $$Y = 1.070 + 0.081\,X_1 - 0.166\,X_2 - 0.187{X_2}^2 \quad (4)$$

263 where Y represents BC production ($g.L^{-1}$), X_1 and X_2 are Glucose from TS ($g.L^{-1}$)
264 and Ethanol % (v:v), respectively.

265 Table 2 summarizes the results of the 2^2 CCRD (star configuration, α= ±1.41).

266 **Table 2** - Uncoded and coded levels of independent and dependent variables used in
267 the RSM design for enzymatic hydrolysis.

Experiments	Code independent variables		Uncode independent variables		Dependent Variable
	x_1	x_2	Glucose from TS ($g.L^{-1}$)	Ethanol % (v:v)	BC Production ($g.L^{-1}$)
1	-1	-1	0.5	0.44	0.980
2	-1	1	0.5	2.56	0.601
3	1	-1	1.5	0.44	1.203
4	1	1	1.5	2.56	0.820
5	-1.41421	0	0.29	1.5	0.953
6	1.41421	0	1.71	1.5	1.100
7	0	-1.41421	1.0	0	0.880
8	0	1.41421	1.0	1.7071	0.478
9	0	0	1.0	1.5	1.067
10	0	0	1.0	1.5	1.142
11	0	0	1.0	1.5	1.053

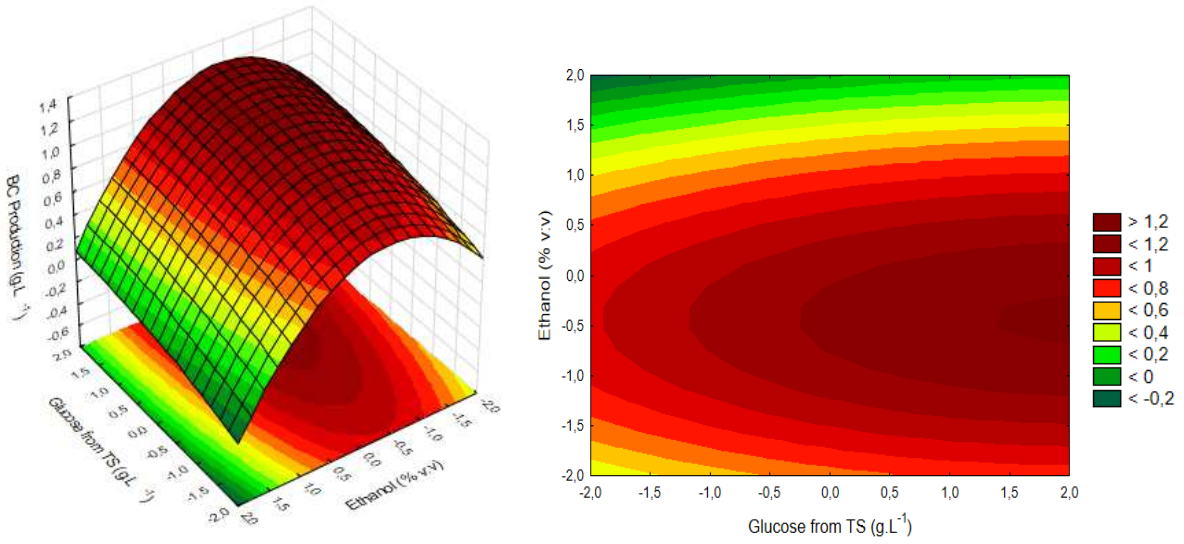
268

269 According to Eq. (4), the linear terms for X_1 and X_2 and the quadratic term for X_2
270 were significant ($P < 0.05$). The linear term for the X_1 variable is positively influenced
271 the response Y (BC Production ($g.L^{-1}$), while the coefficients for linear and quadratic
272 terms for the X_2 variable influenced it negatively. The coefficient of determination (R^2
273 = 0.9091) was satisfactory, indicating that 90.91% of the data variability can be
274 explained by the predicted model. Adjusted R^2 was slightly lower than regular R^2

(0.8069). The lack-of-fit test was not significant ($p > 0.10$), indicating that the predicted model was well-adjusted to the data.

The quality of the model was verified through ANOVA. The model calculated for BC Production ($g.L^{-1}$) was statistically significant and predictive at a confidence level of 95% (*P < 0.05*). The lack of fit was nonsignificant (*P=0.433*).

Figure 1 Response surfaces and contour curves for BC Production ($g.L^{-1}$) (Y) with concentration of Glucose from TS ($g.L^{-1}$) (X_1) and Ethanol (% v:v) (X_2) independent variables.

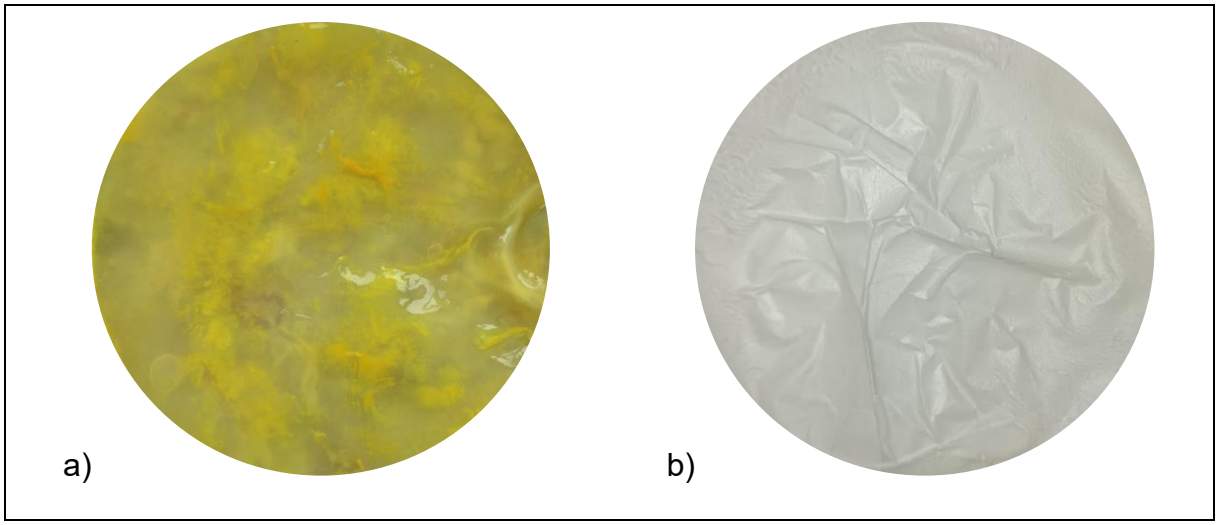


The three-dimensional response surface and the contour curves for the BC production disclosed the best values that occurred in the region with higher values for TS glucose concentration and lower values close to the central point for ethanol (Fig. 3).

From the quadratic polynomial Eq. (4) and the response surface plots, the optimal conditions for BC production are % glucose from a TS like carbon source and ethanol (% v:v) were 1.15 and 0.85, respectively, resulting in a maximum yield of 1.09 $g.L^{-1}$ of BC Production. Figure 3-2 shows their visual appearance. A BC prepared under these process conditions helped to validate the model. When the measured BC

Production ($g.L^{-1}$) was compared to the predicted value, the relative deviation values revealed that the predicted and experimental were well correlated.

Figure 2 - Membrane loaded with turmeric ethanolic extract (BC/CURC) (a) before and (b) after drying.



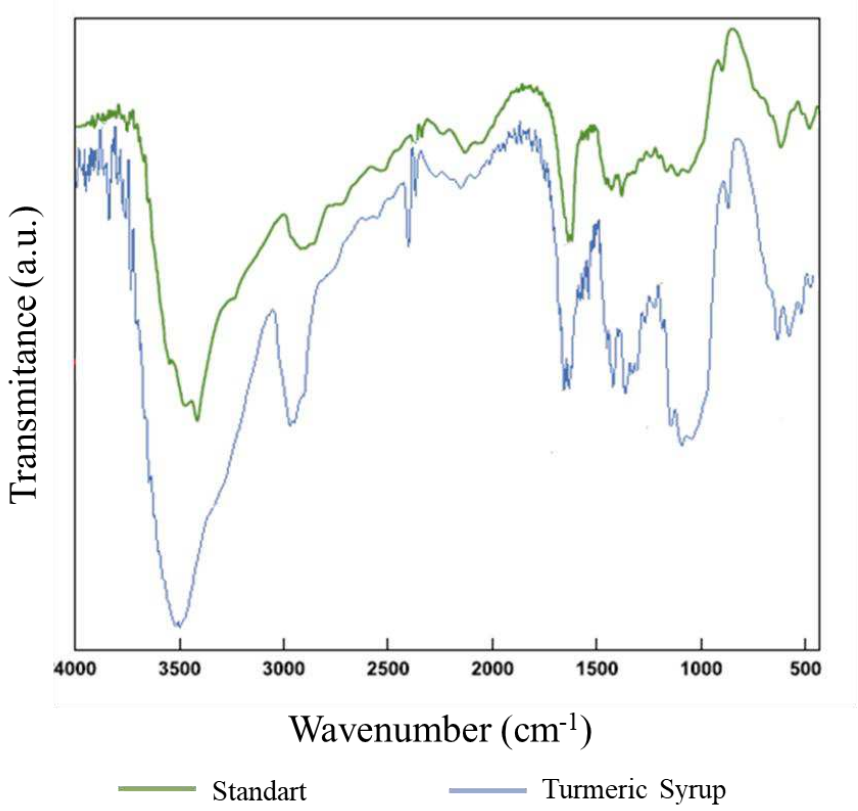
3.3. FT-IR

FT-IR spectroscopy allows the evaluation of the efficiency of the purification method and the investigation of the structure of the BC samples. This technique allows the detection of functional groups present in the structure of organic compounds, reveals the bonds in the sample structure, and characterizes molecular bonding properties using bond vibration frequency. Chemical characterization of functional groups of BC produced from the optimized medium was performed by FT-IR spectroscopy, and compared to samples produced from standard HS medium, FT-IR spectra of BC (Figure 3) exhibited typical absorption bands of cellulose.

The bands were identified as O-H stretching for pure cellulose (3400–3500 cm^{-1}), alkane C-H stretching and CH_2 asymmetric stretching vibration (2926 cm^{-1}),

308 CH_2 deformation vibration (1433 cm^{-1}), CH_3 deformation vibration (1363 cm^{-1}) and C-
309 O deformation vibration (1252–1000 cm^{-1}).

310 **Figure 3** - FT-IR spectra of bacterial cellulose produced by *Komagataeibacter xylinus*
311 in standard and optimized media



312

313 Mangolim et al. (2014) suggested that curcumin keto-enol tautomeric form from
314 curcumin can be detected by bands in FT-IR in the peaks is the most significant
315 carbonyl region (1800–1650 cm^{-1}). In this study, standard and optimized FT-IR spectra
316 showed the bands, and it was stronger in the spectra that have turmeric/curcumin in
317 the medium. Nonetheless, it is more probable that the curcumin present in the medium
318 was washed from BC pellicles in the purification process, and this band corresponded
319 to the cellulose spectrum.

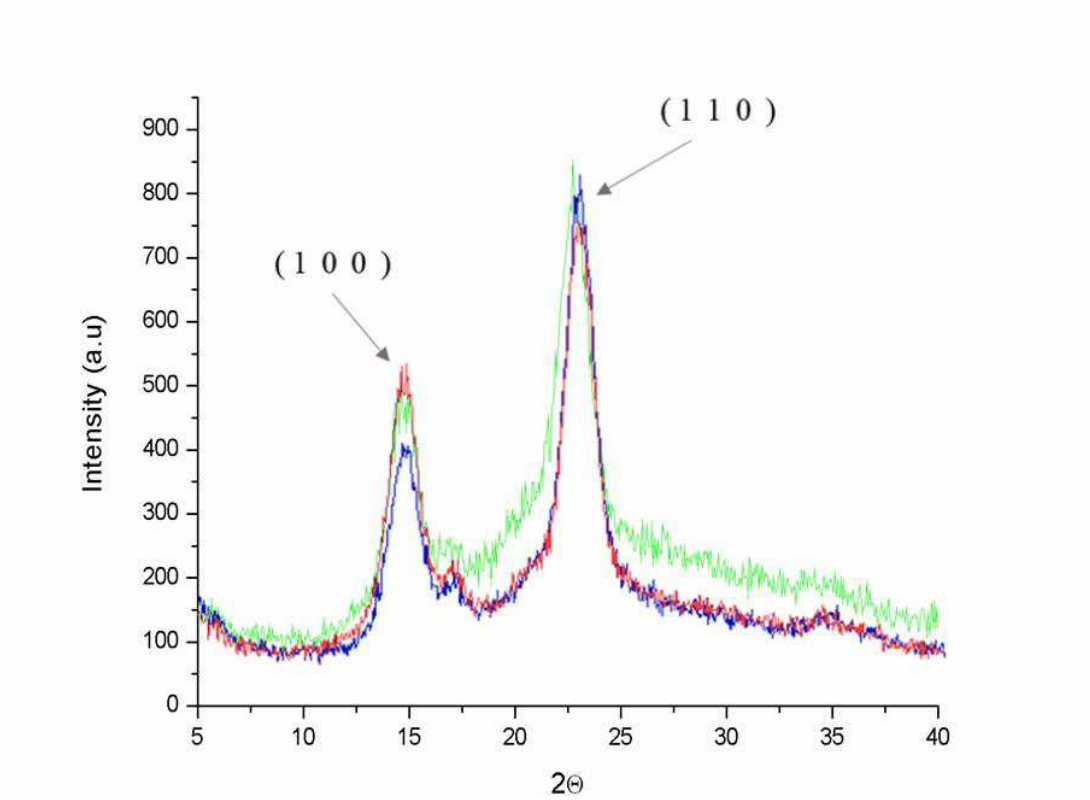
320 BC produced either with HS standard or optimized medium shows the same
321 characteristic bonds in FT-IR, and these spectra agree with studies of BC biopolymers.

Additionally, this observation signifies that the generated compounds were chemically pure bacterial cellulose.

3.4. X-ray diffraction

X-ray diffraction patterns of the BC samples and standard medium are shown in Figure 4. The results indicated that the culture medium with TS and the standard medium showed similar crystalline profiles. Both samples present two major peaks at 14.8° 22.6° and a low-intensity peak at 17.3° and correspond to diffraction planes (101), (002), and (10i), respectively, which is the most abundant form of cellulose found in nature. These peaks demonstrated that both BC samples showed typical crystalline forms of cellulose I. The calculated crystallinity indexes of TS and HS medium are 81.6% and 81.96%. From their similar crystallinity, it can be concluded that turmeric's presence does not affect BC's crystallization. The values of the crystallinity index observed in this work for the membranes produced in both medium were similar to those reported by other authors in an earlier study (BILGI *et al.*, 2016b; GOMES *et al.*, 2021).

Figure 3 - XRD patterns of the bacterial cellulose produced by *Komagataeibacter xylinus* in standard (blue), optimized media (red), and BC/CURC (green)



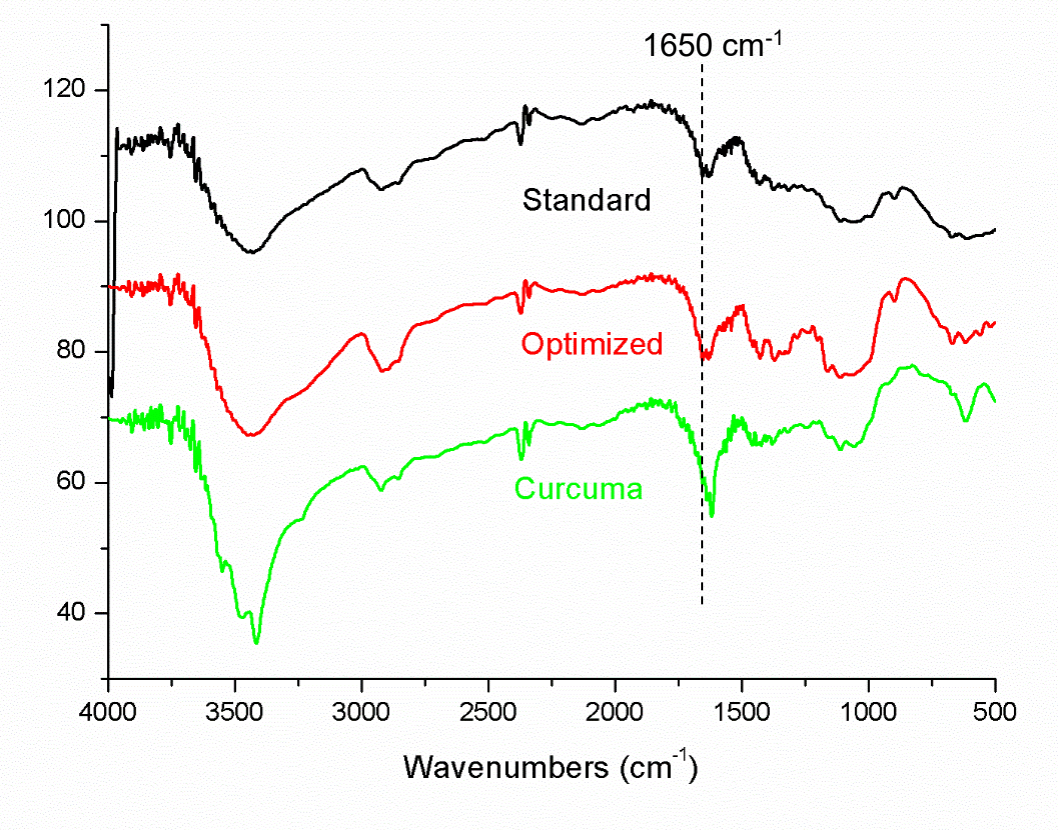
3.5. BC/curcumin

To enhance the biological activity of BC produced, the membrane was separately loaded with turmeric ethanolic extract. El-Gendi et al. (2023) loaded BC membrane with several fruit byproduct extracts to enhance its biological activity for multiple applications, the results showed BC loaded with pomegranate peel extract (BC/PPE) revealed significant broad-spectrum antimicrobial activity. Wen et al. (2021) developed an intelligent and active film based on 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-oxidized bacterial cellulose containing thymol and anthocyanin-rich purple potato extract that exhibits good antibacterial and antioxidant activities. The central idea for this studies, and other studies (ATTA *et al.*, 2022; FATIMA *et al.*, 2021) were showed BC loading with natural plant is a widely accepted approach for boosting

the BC's biological activity for several applications, in such a way that the physicochemical and mechanical properties of BC surpass production limitations, like high production cost and lack of activity.

Figure 6 shows the FTIR spectra of BC/curcumin. The spectra indicated that the curcumin was successfully absorbed into the films, the main indication is the displacement of peaks of the carbonyl group of cellulose shifted from 1650 cm^{-1} to 1640 cm^{-1}. The peak shift may be due to the interaction between cellulose and curcumin, similar results were observed in other studies (LI *et al.*, 2020; MA *et al.*, 2020). X-ray diffraction patterns of the BC/curcumin are shown in Figure 5.

Figure 4 - FT-IR spectra of bacterial cellulose produced by *Komagataeibacter xylinus* in standard, optimized media and loaded with turmeric ethanolic extract (BC/CURC)



The results indicated that similar crystalline profiles, the major peaks at 14.67° and 22.82° and a low-intensity 16.7° peak correspond to diffraction planes (101), (002),

and (10i), respectively. These peaks demonstrated that both BC samples showed typical crystalline forms of cellulose I. From their similar crystallinity, it can be concluded that turmeric's presence does not affect BC's crystallization. However, the sharp diffraction peaks of curcumin are absent in the BC/curcumin, which indicates that the curcumin exists in films as an amorphous structure or that its presence shows a concentration that makes it difficult to detect any crystalline curcumin, as observed by Ma *et al.* (2020).

Table 3 - Optical properties, amount of curcumin and antioxidant activity for BC/curcumin

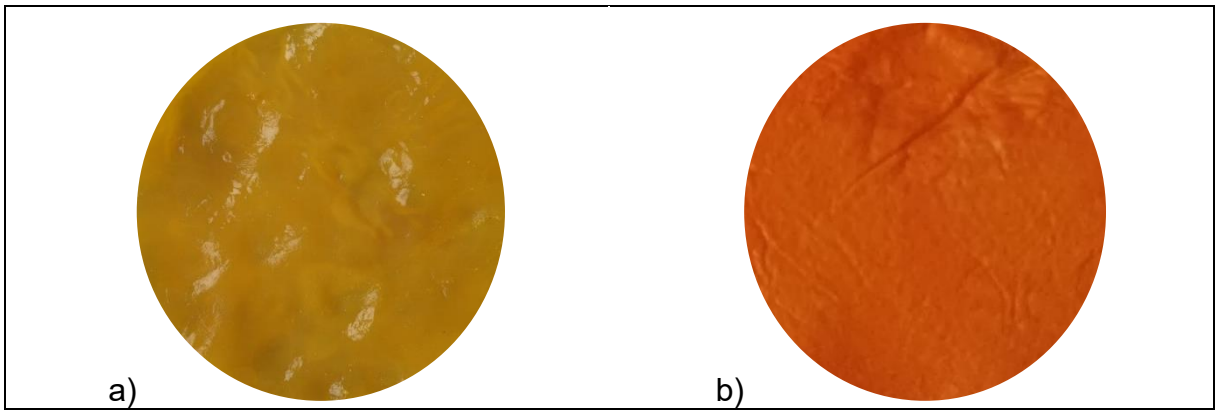
Sample	Color				Curcumin
	a	b	L	hue	
BC/Curc	17.41±1.79	46.30±0.31	67.18±1.03	69.41±1.92	1.09±0.01
	DPPH %Inb				
	0 min	10 min	20 min	30 min	
	79.86±3.27	86.54±1.54	87.69±0.64	89.46±1.02	

Color parameters L*, a*, b* of the BC/CURC is shown in Table 3. Figure 6 shows their visual appearance. BC/curc showed a bright yellow-orange color, and this color indicates the effective incorporation of curcumin into the BC.

The content of curcumin from the curcumin-incorporated BC/Curc is shown in Table 3. The release profile of curcumin is caused by a series of processes such as solubilization and diffusion of curcumin in the film and dissolution of curcumin in solution. The values for BC/CURC is 1.09 μg CC/g dry. The effect of turmeric ethanolic extract incorporated in BC amount on the DPPH free radicals exhibits a remarkable DPPH radical scavenging activity with a scavenging effectiveness value of 89.46 %

after 30 min. BC is free of bioactive compounds responsible for antioxidant properties; thus, by enriching the medium with turmeric ethanolic extract, an extract rich in bioactive compounds, it is possible to demonstrate antioxidant activities.

Figure 5 - Membrane loaded with turmeric ethanolic extract (BC/Curc) (a) before and after drying.



4. Conclusion

In this study we implemented PBD followed by response surface methodology for optimizing BC production. Our results indicate that BC can be produced with a yield of 1.09 $g.L^{-1}$ using TS as alternative carbon source, with culture conditions of 10 days of incubation time, 5% (v:v) inoculum, 0.5 $g.L^{-1}$ yeast extract, 0.5 $g.L^{-1}$ peptone, 0.115 $g.L^{-1}$ citric acid, 0.27 $g.L^{-1}$ Na_2HPO_4, 0.85% ethanol (v:v) and 1.15% of glucose from TS ($g.L^{-1}$) at 30 °C incubation temperature. This represents an effective use of an alternative resource such as syrup to produce a nanobiomaterial suitable for a broad range of biotechnology, pharmaceutical, and cosmetics. The bacterial cellulose membranes produced with TS also presented high crystalline and pure form; thus is possible to say that BC production with TS, like the carbon source used in this work, does not change the structural properties of BC, FT-IR, and XRD results support this statement.

Furthermore, the produced BC revealed antioxidant activities when loaded with turmeric ethanolic extract. The study paves the way for the cost-effective and eco-friendly application of BC for safe food packaging based on natural plant extracts. This was the first demonstration of the application of sugars provided by enzymatic hydrolysis of turmeric in BC production and the application of turmeric as a source of curcumin to provide modifications after BC biosynthesis and promote antioxidant activities on BC properties.

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CAPÍTULO IV

SCIENTIFIC ARTICLE 3

Enhancement of curcumin content in water by enzyme-assisted extraction: a study of interactions of curcumin and starch from turmeric

Abstract

Curcuma longa L. belongs to the family Zingiberaceae, commonly known as turmeric. The turmeric rhizomes usually contain more than 40% of starch. Curcumin is a phenolic compound, a secondary metabolite in *C. longa* L., investigated intensively because of its potential positive effects on human health, such as anticancer and antioxidant. The interactions of polyphenols with compounds present in food, such as starch, influence the content and activity of phenolic compounds. In this context, this paper reviews the interactions between curcumin and starch, and their impact on polyphenol activity. A turmeric syrup (TS) by amylolytic enzymes (α-amylase and amyloglucosidase) and a turmeric (*C. longa* L.) were evaluated for the polyphenolic compounds content (TPC) by Folin-Ciocalteu, contend of curcumin (CC) in UPLC and the tautomeric conformation of curcumin was determined by FT-IR. The TPC results were 16.00±0.12 mg GAE/g for TF and 12.28 ± 0.22 mg GAE/g for TH, extract in ethanol 80:20 (v:v). The CCs of TF and TH were comparable to each other ($p < 0.05$), 1.74±0.0 for TF and 1.88±0.00 g curcumin.100 g^{-1}. In TS, starch was completely converted into sugars, which contributed to the release of curcumin from the starch matrix, and this explains the same CC in TS in aqueous solution and TPC results. FT-IR spectra of TS and TH exhibited similar peaks for curcumin structural conformation in the keto-enol form. This study offers insights into the interaction mechanism between curcumin and starch, suggesting that starch can serve as a source of curcumin in the food industry, ensuring curcumin's aqueous dispersibility and stability.

Keywords: Interaction mechanism, *Curcuma longa* L., Hydrolysate, Phenolic Compound.

1. Introduction

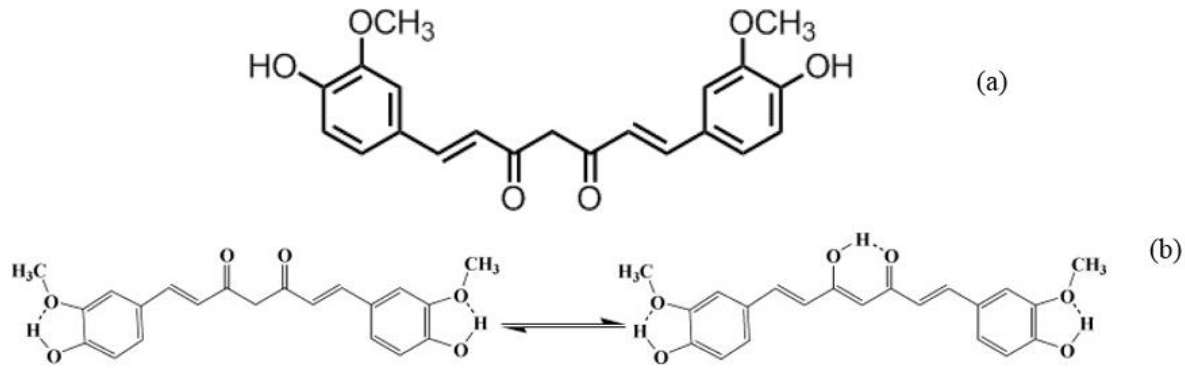
The ingestion of plant-based foods abundant in phenolic compounds has been associated with a lowered probability of various chronic diseases, including cardiovascular and neurodegenerative diseases, cancers, and type II diabetes. Turmeric (*Curcuma longa* L.) is a significant crop that has historically been used worldwide as a flavoring agent, natural colorant, stabilizer, and, especially, spice in food preparations. Turmeric is also valued for its therapeutic properties and is a significant ingredient in cosmetic industries (VISAKH *et al.*, 2023).

The chemical composition of turmeric has starch as one the major components, more than 40%, and contains the curcuminoids present in 1–5%, which confer its orange-yellowish appearance: curcumin, demethoxycurcumin, and bisdemethoxycurcumin. The health benefits of turmeric have been attributed mainly to curcumin, which may contribute to its biological activities, such as anti-inflammatory, anticancer, neuroprotective, antioxidant, and antimicrobial (KOUR *et al.*, 2022; LAN, XIANG *et al.*, 2023; PRIYADARSINI, 2009; WANG, SHUMIN *et al.*, 2023). Curcumin is a phenolic compound, a secondary metabolite chemically known as [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], molecular structure of curcumin was illustrated in Figure 1.

The non-covalent interactions between starch and phenolic compounds may impact on the physicochemical and nutritional properties of food (JAKOBEK, 2015; ZHU, 2015). In a previous study carried out in 2017, our research group developed a turmeric-and-black-pepper-based hydrolysate (HTBP). The production of HTBP via enzymatic conversion reached 100% conversion of starch into sugars (glucose - 97.43% and maltose - 2.31%). The hydrolyzed displayed *in vitro* antioxidant activity.

The mechanisms of antioxidant action involve the scavenging and stabilization of free cationic radicals by reactions involving the transfer of hydrogen atoms and electrons (QUEIROZ-CANCIAN *et al.*, 2018).

Figure 1 Chemical structures of curcumin (a) and curcumin keto-enol tautomerism. (b).



Fonte: PRIYADARSINI, 2009

The interactions of polyphenols with compounds present in food, such as starch, influence the content and activity of phenolic compounds. In this context, this paper study the interactions between curcumin and starch, and their impact on polyphenol activity.

2. Material and methods

TS, a turmeric hydrolysate was prepared according to Queiroz-Cancian et al. (2018) with modifications. Briefing, the turmeric flour was suspended in distilled water (150 g/L) for the enzymatic hydrolysis. For liquefaction, 0.23 g.100g^{-1} for α-amylase (204 KNU.g^{-1}) was added at pH 6.03, 91.5 °C at 10 minutes in a constant agitation 70 rpm. For saccharification, 1.60 mL.100g^{-1} for amyloglucosidase was added at pH 4.5, 60 °C at 8 hours in a constant agitation of 150 rpm.

2.8 Extraction procedure

The curcumin extracts from turmeric and TS were prepared according to Roy & Rhim (2020), with modifications, to determine curcumin content (CC) by ultra-performance liquid chromatography (UPLC). The samples were weight, then put in a constant agitation overnigth at 120 rpm with acetonitrile (ACN). After, the samples were treated with 60 min ultrasonic and then centrifuged at 11 000 rpm for 50 min. The supernatant was diluted with ACN 50:50 (v:v) and then filtered through a 0.22 µm PVDF membrane filter before the injection (1 µL) into the ultra-performance liquid chromatography (UPLC®) Waters (Acquity UPLC System, Waters, USA).

2.9 Total phenolic content (TPC)

The total phenolic content (TPC) in the extracts' samples was estimated using the Folin-Ciocalteu reagent with a microplate adaptation (BOBO-GARCÍA *et al.*, 2015). Briefly, for the reaction, 20 µL of each extract, 100 µL of Folin-Ciocalteau reagent (diluted 1:4 in H_2O), and 75 µL of 10% sodium carbonate solution were mixed. After thorough mixing, the samples were kept at room temperature with light protection for 2 hours. Absorbance was measured at a wavelength of 750 nm using a UV-VIS spectrophotometer. A standard curve [TPC = slope coefficient × absorbance + y-intercept coefficient; R^2 = 0.999] was plotted using different concentrations of gallic acid (4 - 24 µg mL^{-1}). The results were expressed in milligrams of gallic acid equivalent per gram of sample (mg GAE g^{-1}).

2.10 Curcumin contend (CC)

The UPLC was equipped with a UV-Vis detector and a C18 column (180×4.6 mm, 5 µm; Shiseido, Japan). The curcumin was quantified according to Cheng et al.

(2010) with modifications. The mobile phase consisting of a mixture of 0.05% aqueous phosphoric acid and acetonitrile in the ratio of 50:50 (v:v), isocratic with the flow rate of 0.3 $mL.min^{-1}$ was employed at 30 °C. The detection wavelength was set at 420 nm. The calibration curve was prepared using curcumin standard in the concentration range 0.10 – 2.5 µg/mL, obtaining a correlation coefficient (R^2) of 0.999. The injections were made in triplicate, and the results were expressed as mg CC/g sample dry weight.

2.11 Fourier-transform infrared (FT-IR) spectroscopy

Infrared spectra were recorded to determine the main bands that characterize the curcumin in the turmeric sample and TS. Dried TS and and turmeric samples were incorporated into potassium bromide and pressed into tablets under high pressure. The spectra were recorded on a Shimadzu IR-Prestige-21 spectrometer (Brazil) in the region of 4000 to 400 cm^{-1} at a resolution of 1 cm^{-1}.

2.12 Statistical analysis

All analyses were undertaken in triplicate, except when mentioned, and the experimental results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) and Tukey's test were carried out at $p < 0.05$. Statistical analysis was performed using Statistica 14.0 (TIBCO Software).

3. Results and discussion

3.1. Curcumin contend and TPC

The non-covalent interactions between starch and phenolic compounds in food systems involve hydrogen bonds, hydrophobic interaction, and electrostatic and ionic interactions (ZHU, 2021). This interaction can interfere with the potential antioxidant effects of turmeric.

In the enzymatic hydrolysis process, turmeric starch is heated in the presence of water, the granules start to absorb water and swell, and some components leach out and solubilize. With the increasing temperature and water absorption, the granules rupture with the disordering of chain organization (gelatinization). When the gelatinized starch is cooling, the disordered chains undergo re-association through hydrophobic interactions and hydrogen bonding (retrogradation) (QUEIROZ-CANCIAN *et al.*, 2018; ZHU, 2015). These steps and organization within the turmeric matrix can affect the availability of curcumin and phenolic compounds. Curcumin content and TPC of turmeric and TS were comparable to each other ($p < 0.05$) (Table 2).

Table 1 - Total polyphenolic content (TPC) and curcumin content obtained by extraction from turmeric and turmeric syrup.

Matriz	Curcumin Content g curcumin.100 g^{-1}	Total polyphenolic content mg EAG.g for total polyphenolic content^{-1}
Turmeric	2.58^{a}±0.05	16.00^{a}±0.12
Turmeric Syrup	2.55^{a}±0.30	12.28^{b} ±0.22

Data expressed as mean ± SD of three data points (n=3).
Different letters within the same column mean significant differences at P < 0.05.

TPC results were 16.00±0.12 mg GAE/g for turmeric and 12.28 ± 0.22 mg GAE/g for TS. A high number of compounds belonging to the group of phenolics (phenolic acids, acetophenones, phenylacetic acid, hydroxycinnamic acids, coumarins, naphthoquinones, xanthones, stilbenes, flavonoids) and tannins (JAKOBEK, 2015), Salem et al. (2022) profile the phytochemical composition for the rhizomes of *Curcuma longa* L by LC- and GC–MS analysis resulted in the identification of a total of 161 metabolites belonged to various phytochemical classes, like

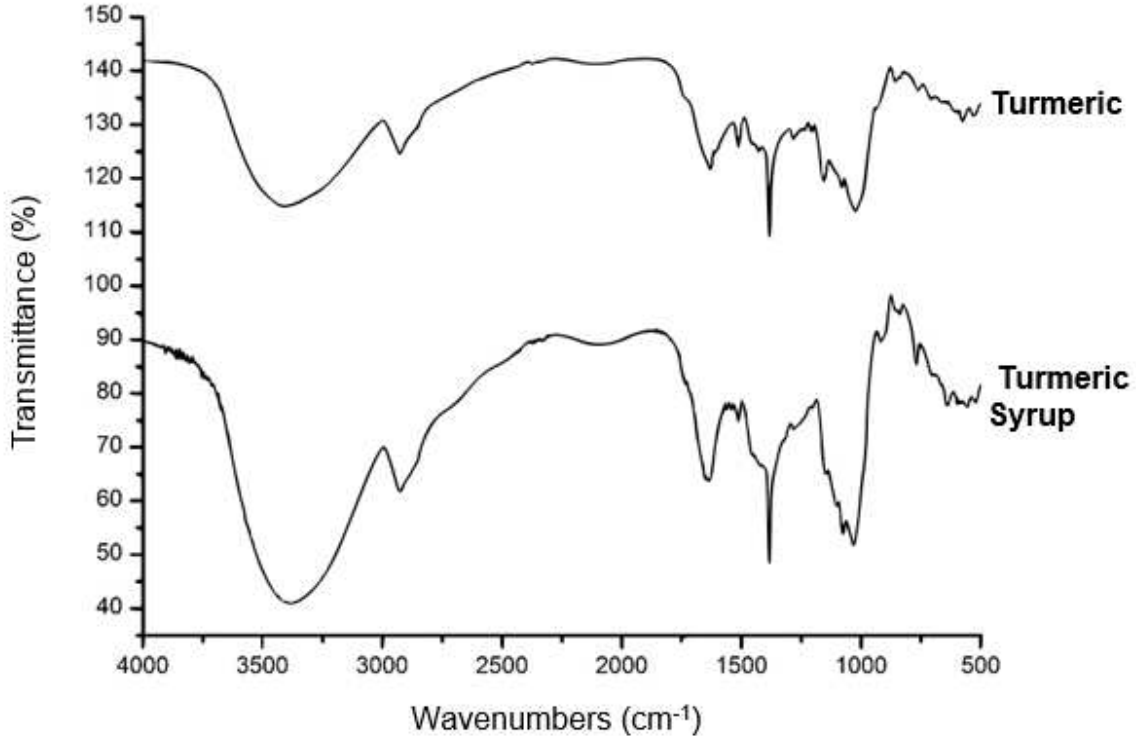
curcuminoids, sesquiterpenoids and terpecurcumins. The difference in results for TPC (Table 1) may have been significant considering that other phytochemical compounds, like tannins and flavonoids, may not have been recovered in water or were lost in the hydrolysis process, thus, the values obtained for TS are lower than those found for turmeric ($p < 0.05$).

Curcumin is a polyphenolic phytochemical, the major disadvantage associated with the use of curcumin, however, is its low systemic bioavailability due to its poor aqueous solubility. Statistically, the CC did not show a significant difference (Table 1) between TS and turmeric samples ($p < 0.05$). In TS, starch was completely converted into sugars, which contributed to the release of curcumin from the starch matrix and this explains the same CC in TS.

3.2. FT-IR

The tautomerism of curcumin in the turmeric sample and TS was evaluated by FTIR. Both, the FTIR spectra exhibited similar peaks that corresponded to phenolic hydroxyl groups of curcumin spectrum. The curcumin structural conformation was the keto-enol form. FTIR proved that functional groups were not affected by the interactions formed between turmeric starch and curcumin. The peaks were identified as hydroxyl groups (3420 cm^{-1}), hydroxyls from enol groups (2925–2920 cm^{-1}), methyl groups in phenol (1385 cm^{-1}), C=O and C=C (1630–1450 cm^{-1}), and C-O-C (1300–1000 cm^{-1}). The curcumin structural conformation in TS and in turmeric was in the keto-enol form (Figure 2).

Figure 2 *FT-IR spectra of (a) turmeric and (b) turmeric syrup (TS).*



4. Conclusion

Curcumin has poor water-solubility and chemical stability, which limits its utilization as a nutraceutical in many applications. This problem can be partly overcome by starch hydrolysis, in this study we showed enhancement of curcumin content in water by enzyme-assisted extraction.

In TS starch was completely converted into sugars, which contributed to the release of curcumin from the starch matrix and this explains the same CC in TS in aqueous solution and TPC results. Both, the FT-IR spectra of turmeric and TS exhibited similar peaks that corresponded to phenolic hydroxyl groups of curcumin spectrum. The curcumin structural conformation was the keto-enol form. FTIR proved that functional groups were not affected by the interactions formed between turmeric starch and curcumin. This study provides evidence that the interaction breakdown

between curcumin and starch enhances the biodisponibility da curcumin in aqueous media, thus TS can be used as a font of curcumin in the food industry, ensuring curcumin aqueous dispersibility and stability.

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CAPÍTULO V: CONCLUSÕES

Este trabalho integra três estudos de pesquisa distintos centrados na cúrcuma (*Curcuma longa* L.), seu composto bioativo curcumina e suas interações com vários aspectos da ciência alimentar e biotecnologia. As conclusões finais são:

- Aplicação de análise multivariada pode ser utilizada para determinar a similaridade entre as amostras de diferentes origens geográficas de cúrcuma e de diferentes sistemas de cultivo.

- Sistema de cultivo, orgânico ou convencional, não pode ser diferenciado analisando o conjunto de dados com 66 amostras de 4 países de origem diferente.

- A quebra das cadeias de amido, ocasionada pela hidrolise enzimática, liberou curcumina em meio aquoso, gerando um aumento na disponibilidade do composto polifenólico em meio aquoso.

- A quebra da interação entre a curcumina e o amido aumenta a biodisponibilidade da curcumina em meio aquoso.

- A conformação estrutural da curcumina não é afetada pela hidrólise enzimática do amido da cúrcuma.

- A síntese de celulose bacteriana, pela cepa *Komagataeibacter xylinus,* atinge rendimento de 1,09 $g.L^{-1}$ ao utilizar como fonte de carbono açúcares proveniente da hidrolise enzimática da cúrcuma nas condições, otimizadas: 1,15% ($g.L^{-1}$) de xarope de cúrcuma, 10 dias de incubação, 5% (v:v) inóculo, 0,5 $g.L^{-1}$ extrato de levedura, 0,5 $g.L^{-1}$ peptona, 0,115 $g.L^{-1}$ ácido cítrico, 0,27 $g.L^{-1}$ Na_2HPO_4 and 0,85% etanol (v:v) a uma temperatura de incubação de 30 °C.

- A curcumina incorporada na síntese de celulose bacteriana é perdida na etapa de purificação da celulose bacteriana.

- A celulose bacteriana produzida apresentou cristalinidade e pureza característica de celulose tipo I.

- A celulose bacteriana carregada por extrato etanólico de cúrcuma apresenta atividade antioxidante.