NATÁLIA NORIKA YASSUNAKA HATA

PRODUÇÃO DE LEVANA POR BACTÉRIAS DO ÁCIDO ACÉTICO E AVALIAÇÃO DO SEU POTENCIAL PARA APLICAÇÃO BIOMÉDICA E EM ALIMENTOS

Londrina
2022

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Tese apresentada ao Programa de Pós-Graduação em Ciência de Alimentos da Universidade Estadual de Londrina, como requisito parcial para obtenção do título de Doutora em Ciência de Alimentos.

Orientador: Profª. Drª Wilma Aparecida Spinosa

Londrina

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BANCA EXAMINADORA

_____

Orientadora: Profa. Dra. Wilma Aparecida Spinosa
Universidade Estadual de Londrina – UEL

_____

Profa. Dra. Daniele Sartori
Universidade Estadual de Londrina - UEL

_____

Profa. Dra. Adriana Aparecida Bosso Tomal
Universidade do Estado de Minas Gerais - UEMG

_____

Prof. Dr. Pedro Oliva Neto
Universidade Estadual Paulista Júlio de Mesquita Filho - UNESP

_____

Prof. Dr. Edson Marcelino Alves
Universidade Federal do Triângulo Mineiro - UFTM

Londrina, 05 de agosto de 2022

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RESUMO GERAL

Bactérias do ácido acético (BAA) atuam como agentes de deterioração em vários nichos ecológicos, porém, devido à sua habilidade de oxidar diferentes substratos por meio da chamada "fermentação oxidativa", elas exercem papel fundamental na produção de vários alimentos e bebidas, bem como na produção de exopolissacarídeos (EPS) como a levana. Levana é um polímero bastante promissor para aplicação nas áreas médicas e em alimentos. Na indústria de alimentos, por exemplo, levana pode ser utilizado como um potencial prebiótico, emulsificante, agente de textura, estabilizante e como um substituto de gordura. Já na área médica, levana tem demonstrado várias atividades biológicas, tais como anti-inflamatória, antioxidante, antitumoral, antibiofilme, entre outras. Neste contexto, e sabendo que a produção de levana por BAA é ainda relativamente recente, o objetivo deste trabalho foi avaliar o potencial de síntese de levana por BAA isoladas de uvas, caracterizar o EPS, e testar suas atividades antioxidantes, antitumorais, antimicrobianas e antibiofilme. E devido às suas propriedades bioativas, produzí-la *in situ* em bebida de uva durante a fermentação ácido glucônica. Nos ensaios iniciais, foram identificados cinco isolados capazes de produzir EPS, dentre as quais *Gluconobacter cerinus* UELBM11 destacou-se por produzir ~14,0 g/L de levana em condições não otimizadas. Levana a partir de UELBM11 consistia de monômeros de frutose ligadas por β-(2→6) com algumas ramificações do tipo β-(2→1). Levana mostrou uma massa molecular de $8{,}78 \times 10^5$ Da, uma alta estabilidade térmica (227,44 °C), e uma morfologia microporosa. Análises de atividade antioxidante demonstraram que o EPS teve uma alta atividade de captura de radicais ABTS e OH. Em seguida, por meio da metodologia de superfície de resposta, a máxima produção de levana (48,06 g/L) foi obtida em condições otimizadas com sacarose a 24,84 % (m/v) e pH inicial de 6,95. O tratamento com levana a 1000 μg/mL por 24 h, 48 h e 72 h mostrou efeito citotóxico sob células neuroblastoma SH-SY5Y. Apesar de não mostrar atividade antimicrobiana, levana inibiu a formação de biofilme de patógenos de alimentos como *Salmonella* e *Shiguella*. Por fim, *G. cerinus* UELBM11 foi ábil a consumir sacarose e produzir levana *in situ* em bebida de uva suplementada com sacarose. A fermentação glucônica aumentou a biodisponibilidade de compostos fenólicos e preservou o conteúdo de antocianinas. Resultados de FRAP mostraram que a atividade antioxidante da bebida aumentou de 388,03 (T0 h) para 460.21 (T24 h) (mg vitamina C equivalente/L) ($p<0{,}05$). As análises de compostos voláteis mostraram que a fermentação glucônica podem reduzir o conteúdo de compostos voláteis da bebida de uva, mas ao mesmo tempo podem formar novos compostos e preservar compostos como 2,4-di-tert-butilfenol e a maioria dos monoterpenos, que tem impacto direto no aroma da bebida e na saúde humana.

Palavras-chave: Levana, Bioatividade, Fermentação, Bebida, Bactéria do ácido acético

YASSUNAKA-HATA, Natália Norika. **Production of levan by acetic acid bacteria and evaluation of its potential for biomedical and food applications**. 2022. 206 f. Thesis (Doctoral degree in Food Science) — Universidade Estadual de Londrina, Londrina, 2022.

ABSTRACT

Acetic acid bacteria (AAB) act as spoilage agents in various ecological niches. However, due to their ability to oxidize various substrates through the so-called "oxidative fermentation", they play a fundamental role in the production of various foods and beverages, as well as in the production of exopolysaccharides (EPS) such as levan. Levan is a very promising polymer for medical and food applications. For example, in the food industry, Levan can be used as a potential prebiotic, emulsifier, texturizing agent, stabilizer, and fat substitute. In the medical field, levan has shown several biological activities, such as anti-inflammatory, antioxidant, antitumor, antibiofilm, among others. In this context, and knowing that the production of levans by AAB is still relatively new, the aim of this work was to evaluate the potential of levans synthesis by AAB isolated from grapes, to characterize the EPS and to test their antioxidant, antitumor, antimicrobial and antibiofilm activities. After, to produce it *in situ* in grape beverages during gluconic acid fermentation due to its bioactive properties. In the initial experiments, five isolates capable of producing EPS were identified, among which *Gluconobacter cerinus* UELBM11 stood out with a production of ~14.0 g/L of levan under non-optimized conditions. Levan from UELBM11 consisted of fructose monomers linked by β-(2→6) with some β-(2→1)-like branches. Levan exhibited a molecular mass of 8.78×10^5 Da., high thermal stability (227.44 °C), and microporous morphology. Antioxidant activity analysis showed that EPS exhibited high activity in scavenging ABTS and OH radicals. Using the response surface method, maximum production of levan (48.06 g/L) was obtained under optimized conditions with sucrose content of 24.84 % (m/v) and initial pH of 6.95. Treatment with 1000 μg/mL levans for 24, 48, and 72 hours showed a cytotoxic effect on SH-SY5Y neuroblastoma cells. Although it did not show antimicrobial activity, it inhibited biofilm formation of food pathogens such as *Salmonella* and *Shiguella*. Finally, *G. cerinus* UELBM11 was able to consume sucrose and produce levan in situ in a sucrose-enriched grape beverage. Gluconic fermentation increased the bioavailability of phenolic compounds and preserved anthocyanin content. FRAP assays showed that the antioxidant activity of the beverage increased from 388.03 (T0 h) to 460.21 (T24 h) (mg vitamin C equivalent/L) ($p < 0.05$). Volatile compound analysis showed that gluconic fermentation can reduce the content of volatile compounds in grape beverage, but at the same time form new compounds and preserve compounds such as 2,4-di-tert-butylphenol and most monoterpenes, which directly affects the aroma of the beverage and human health.

Keywords: Levan, Bioactivity, Fermentation, Beverage, Acetic acid bacteria

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1. INTRODUÇÃO

Normalmente, quando se fala em bactérias do ácido acético (BAA), logo as associamos com a deterioração de certos tipos de alimentos e com a produção de um produto tradionalmente utilizado como condimento, o vinagre, cujo principal componente é o ácido acético.

De certo modo, as BAA atuam como agentes de deterioração em vários nichos ecológicos, porém, elas também são de grande interesse industrial devido às suas habilidades de oxidar o etanol, açúcares e polióis em seus respectivos ácidos orgânicos, aldeídos e cetonas (SAICHANA *et al.*, 2015), em geral, e em especial, à ácido acético. Por meio deste processo de oxidação incompleta dos substratos, assim chamada de "fermentação oxidativa", as BAA exercem papel fundamental na produção de vários alimentos e bebidas, tais como o vinagre, kombucha, kefir, cerveja lambic e cacau (DE ROOS; DE VUYST, 2018). Além disso, destaca-se a sua importância na produção de produtos específicos como o ácido glucônico, miglitol e ácido ascórbico (LYNCH *et al.*, 2019), bem como na produção de exopolissacarídeos bioativos como a levana.

A levana é um polímero composto por resíduos de D-frutofuranosil unidos por ligações β-(2,6) na cadeia principal e ligações β-(2,1) na cadeia lateral (MANTOVAN *et al.*, 2018), podendo ser produzida por vários microrganismos por meio da ação da enzima levanasacarase (EC 2.4.1.10) a partir de substrato a base de sacarose (ÖNER; HERNÁNDEZ; COMBIE, 2016). Suas propriedades únicas, tais como alta solubilidade em água e óleo, forte adesividade, biocompatibilidade e habilidade de formação de filme, fazem dela um biopolímero bastante promissor nas indústrias de alimentos e farmacêutica. Na indústria de alimentos, por exemplo, levana pode ser utilizado como um potencial prebiótico, emulsificante, estabilizante, agente de cor, sabor e textura, substituto de gordura, material de cobertura, filmes para embalagem e agente encapsulante (DAĞBAĞLI; GÖKSUNGUR, 2017; ÖNER; HERNÁNDEZ; COMBIE, 2016). Já na área médica/farmacêutica, é uma das macromoléculas que tem demonstrado uma ampla gama de atividades biológicas, dentre as quais podemos citar: anti-inflamatória, antioxidante (SRIKANTH *et al.*, 2015b), antidiabética (DAHECH *et al.*, 2011), hipocolesterolêmica (BELGHITH *et al.*, 2012), imunoestimuladora (TAYLAN; YILMAZ; DERTLI, 2019), além de atividade antitumoral (ESAWY *et al.*, 2013).

Na literatura, a produção de levana por BAA ainda é relativamente recente e sua aplicação na área de alimentos e biomédica, pouco explorada. Baseado no exposto, e tendo em vista as diversas propriedades deste polissacarídeo, o objetivo do presente estudo foi avaliar o

potencial de síntese de levana por linhagens de BAA isoladas de uvas, caracterizar o polissacarídeo, otimizar sua produção, e avaliar suas propriedades antioxidantes, seu efeito citotóxico em células de neuroblastoma de linhagem humana (SH-SY5Y), e suas atividades antimicrobiana e antibiofilme contra patógenos de origem alimentar. Para aplicação na área de alimentos, objetivou-se introduzir a bactéria produtora de levana (*Gluconobacter cerinus* UELBM11) em bebida de uva suplementada com sacarose e avaliar a sua capacidade de produção de levana *in situ.* Além disso, investigar os efeitos da fermentação glucônica nos parâmetros cinéticos, no conteúdo de compostos fenólicos, na atividade antioxidante e na composição de compostos voláteis da bebida.

2. OBJETIVOS

2.1. OBJETIVO GERAL

Avaliar o potencial de síntese de levana por linhagens de BAA isoladas de uvas, caracterizar o polissacarídeo, e testar suas atividades antioxidantes, antitumorais, antimicrobianas e antibiofilme. E devido às suas diversas propriedades, produzí-la *in situ* em bebida de uva durante a fermentação ácido glucônica.

Realizar uma seleção de linhagens de BAA isoladas de uvas para a biossíntese de levana, e caracterização química e física do polissacarídeo da linhagem mais promissora. Numa segunda etapa foram contempladas a otimização do bioprocesso de produção da levana, a avaliação do potencial antioxidante, antitumoral, antimicrobiano e antibiofilme desse polissacarídeo produzido, e aplicação da linhagem selecionada em bebida de uva com fermentação ácido glucônica.

2.2. OBJETIVOS ESPECÍFICOS

- Isolar linhagens de BAA produtoras de frutana a partir da uva. Avaliar as suas capacidades de produção de EPS a partir da sacarose e identificar os isolados utilizando-se de métodos bioquímicos e moleculares. A partir dos isolados identificados, comparar a produção de EPS e selecionar a maior produtora para confirmação e caracterização da levana. Caracterizar a levana quanto à sua estrutura química, massa molecular, propriedades térmicas, morfologia de superfície e capacidade antioxidante;

- Otimizar a produção de levana a partir da bactéria *G. cerinus* UELBM11 por metodologia de superfície de resposta. Avaliar o efeito citotóxico da levana contra células de neuroblastoma de linhagem humana (SH-SY5Y) e suas atividades antimicrobiana e antibiofilme contra patógenos de origem alimentar.

- Utilizar o isolado bacteriano *Gluconobacter cerinus* UELBM11 para elaboração de uma bebida de uva sintetizando-se a levana *in situ* por meio da fermentação ácido glucônica. Avaliar a cinética de crescimento bacteriano, de formação de levana e consumo de carboidratos, assim como mudanças na viscosidade, % acidez titulável total, conteúdo de compostos fenólicos, atividade antioxidante e a caracterização dos compostos voláteis presentes.

3. REVISÃO BIBLIOGRÁFICA

3.1. UVA

Uvas (*Vitis* sp.) são frutas amplamente consumidas e representam uma das principais cultivares produzidas em todo o mundo (SAMOTICHA *et al.*, 2017). Neste universo, a China figura-se como a maior produtora de uva, seguida pela Itália, Espanha, França e Estados Unidos. O Brasil ocupa a 15 ª posição com aproximamente 1,4 milhões de toneladas produzidas em 2020 (FAO, 2020a).

A região sul brasileira foi a maior produtora de uva em 2020, contabilizando cerca de 60,24 % da produção total e tendo o Rio grande do Sul como a sua principal representante, com 745.356 toneladas produzidas (MELLO; MACHADO, 2021). A produção nacional de uvas destinadas ao processamento (vinho, suco e derivados) foi estimada em 661.820 toneladas em 2020, representando 46,72 % da produção total. O restante, e a maior parte (53,28 %), destinou-se ao consumo *in natura* (MELLO; MACHADO, 2021). Neste mesmo ano, o consumo per capita de vinhos (população acima de 18 anos), incluindo os espumantes (nacionais mais importados), foi de 2,66 L. Enquanto o consumo médio por habitante de uva de mesa, uvas passas e de suco de uva foi de 3,49 kg, 0,14 kg e de 1,36 L, respectivamente (MELLO; MACHADO, 2021).

Uvas e seus derivados não são consumidos somente pelo seu flavor único, mas também porque os consumidores estão mais conscientes de suas propriedades funcionais (GRANATO *et al.*, 2015). Estes produtos, além de serem ricos em carboidratos, ácidos orgânicos, aminoácidos, vitaminas e melatoninas, representam fontes baratas de compostos fenólicos (WU *et al.*, 2021).

Em geral, os compostos fenólicos da uva variam conforme estrutura e atividade e podem ser essencialmente divididos em duas principais famílias: flavonoides e não-flavonoides (GARRIDO-BAÑUELOS *et al.*, 2019). Os flavonoides mais importantes presentes em uvas incluem as antocianinas, flavan-3-óis, proantocianidinas (procianidinas e prodelfinidinas), e os flavonóis, enquanto na família dos não-flavonoides, têm-se os ácidos fenólicos (ácido hidroxicinâmico e seus derivados e hidroxibenzóico) e os estilbenos (HORNEDO-ORTEGA *et al.*, 2021).

Compostos fenólicos, quando consumidos regularmente têm sido associado à vários benefícios à saúde (NATIVIDADE *et al.*, 2013; GRANATO *et al.*, 2015). As antocianinas e antocianidinas, por exemplo, além de serem responsáveis pela coloração vermelha a azul

arroxeado de frutas e verduras, apresentam atividades antioxidante e antimicrobiana, atuam na prevenção de doenças cardiovasculares, diabetes e obesidade, e melhoram a saúde visual e neurológica (KHOO *et al.*, 2017). Proantocianidinas a partir de uvas, especialmente das sementes, têm demonstrado efeitos contra doenças cardiovasculares, diabetes, obesidade ou câncer relacionados ao estresse oxidativo e processos inflamatórios (RODRÍGUEZ-PÉREZ *et al.*, 2019). Propriedades antioxidantes e cardioprotetivas do resveratrol (grupo estilbeno) e quercetina (flavonoide) também têm sido relatadas por vários estudos (SABRA; NETTICADAN; WIJEKOON, 2021). Já os taninos condensados, nomeados como proantocianadinas, também foram associados à redução de colesterol e consequentemente à diminuição da pressão arterial (KUMAR *et al.*, 2018). As uvas também são ricas em fitoesteróis e ácidos graxos. Estes compostos podem inibir parcialmente a absorção de colesterol produzido endogenamente ou ingerido na dieta, diminuindo seus níveis circulantes e exercendo ação antiaterogênica e efeitos cardioprotetores (SABRA; NETTICADAN; WIJEKOON, 2021).

Vale ressaltar que a composição polifenólica das uvas é altamente dependente de práticas agrícolas, condições ambientais (solo, clima) e ataques de patógenos. No entanto, as diferenças varietais ou genéticas são os fatores que mais contribuem para tais diferenças (HORNEDO-ORTEGA *et al.*, 2021).

Além da sua composição fenólica, o aroma é um dos atributos de qualidade decisivos para a seleção e aceitação de uvas e seus derivados pelo consumidor (WU *et al.*, 2016). O aroma das uvas compreende um vasto número de compostos voláteis de baixa massa molecular e baixa polaridade, sendo derivados de ácidos graxos, aminoácidos e carboidratos (SCHWAB; DAVIDOVICH-RIKANATI; LEWINSOHN, 2008). Este grupo heterogêneo inclui vários grupos funcionais como álcoois, aldeídos, cetonas, esteres, lactonas, terpenoides, ácidos carboxílicos, éteres, norisioprenoides, fenóis, compostos aromáticos, bem como compostos nitrogenados e sulfurosos (SCHWAB; DAVIDOVICH-RIKANATI; LEWINSOHN, 2008; ILC; WERCK-REICHHART; NAVROT, 2016; LIN; MASSONNET; CANTU, 2019). Na uva, estes compostos são predominantemente encontrados no epicarpo (casca) (MATIJAŠEVIĆ *et al.*, 2019) e tipicamente presentes na forma livre, ou como precursores, na forma de glicosídeos e aminoácidos conjugados, voláteis inodoros, ácidos hidroxicinâmicos, entre outros (CANUTI *et al.*, 2009; PARKER FERREIRA *et al.*, 2018; LOPEZ, 2019). Como compostos livres, estes contribuem diretamente para o perfil aromático das uvas e mostos

Enquanto os compostos na forma livre contribuem diretamente para o perfil aromático das uvas e mostos (FERREIRA; LOPEZ, 2019), na forma de precursores, a liberação de aromas ativos se dá durante a maceração física e subsequente hidrólise não-enzimática/enzimática dos conjugados via glicosidases microbianas, esterases, C−S liases, e descarboxilases, bem como pela hidrólise ácida e rearranjos químicos (PARKER *et al.*, 2018). Ademais, moléculas de aromas livres podem também ser liberados a partir de glicosídeos e aminoácidos conjugados por enzimas salivares ou bacterianas presentes na cavidade bucal (PARKER *et al.*, 2018; FERREIRA; LOPEZ, 2019). E semelhante aos compostos fenólicos, a variedade, grau de maturação da uva, bem como fatores ambientais como o clima, exposição ao sol, disponibilidade de água e infecções fúngicas podem influenciar diretamente na formação de seus compostos aromáticos (ROBINSON *et al.*, 2014).

3.2. CARACTERÍSTICAS GERAIS DAS BACTÉRIAS DO ÁCIDO ACÉTICO

As BAA são gram-negativas, mesófilas e classificadas na família *Acetobacteraceae*. Podem ser móveis, com forma elipsoidal a alongada (bastonetes), ocorrendo individualmente, em pares ou em cadeias. Sua largura varia entre 0,4 a 1,0 µm e o comprimento entre 0,8 e 4,5 µm, e não esporulam (SENGUN; KARABIYIKLI, 2011;LAUREYS; BRITTON; DE CLIPPELEER, 2020).

Apresentam reação de catalase positiva e oxidase negativa, e o metabolismo estritamente aeróbio, tendo o oxigênio como aceptor de elétron terminal (SENGUN; KARABIYIKLI, 2011). De acordo com Laureys, Britton e De Clippeleer (2020), BAA crescem bem entre pH 5,0 - 6,5, mas são também capazes de crescer em pH 3,0 - 4,0 e até mesmo em valores menores. A temperatura ótima de crescimento encontra-se entre 25 ºC e 30 ºC, sendo que nenhum crescimento é observado acima de 34 ºC (LYNCH *et al.*, 2019; LAUREYS; BRITTON; DE CLIPPELEER, 2020).

Até o presente momento, dezessete gêneros são descritos na família *Acetobacteraceae*, dentre os quais, os que apresentam maior número de espécies são *Acetobacter, Gluconobacter, Asaia, Komagataeibacter* e *Gluconacetobacter* (MALIMAS *et al.* 2016).

O grupo possui a habilidade em oxidar vários tipos de açúcares, álcoois de açúcar e álcoois em seus respectivos aldeídos, cetonas e ácidos orgânicos correspondentes por meio de um processo incompleto de oxidação denominado “fermentação oxidativa”, da qual obtém-se

sua energia (LYNCH *et al.*, 2019). *Acetobacter* e *Komagataeibacter* spp., por exemplo, são especializados na conversão de etanol a ácido acético via duas etapas oxidativas sucessivas, e por isso, são comumente encontrados em ambientes alcoólicos e ácidos, tal como indústrias de vinagre (GOMES *et al.*, 2018). Apresentam também um conjunto completo de enzimas do ciclo do ácido cítrico (CAC), necessário para a posterior oxidação de ácidos orgânicos em CO_2 e H_2O (LYNCH *et al.*, 2019). Por outro lado, *Gluconobacter* spp. ocorrem preferencialmente em nichos açucarados e são particularmente proficientes na oxidação de açúcares e álcoois de açúcares (MATSUSHITA; MATSUTANI, 2016; JAKOB *et al.*, 2020). São incapazes de superoxidar o acetato a CO_2 e H_2O devido à carência de enzimas do CAC, mas são valiosos para inúmeras aplicações biotecnológicas na síntese de compostos precursores da vitamina C (L-sorbose), ácido glucônico, dihidroxicetona e o miglitol (LA CHINA *et al.*, 2018; LYNCH *et al.*, 2019).

Já as espécies do gênero *Gluconacetobacter* desempenham importante função como bactérias fixadoras de nitrogênio em plantas, tendo como principal representante a bactéria *Gluconacetobacter diazotropicus* (MAMLOUK; GULLO, 2013; SIEVERS; SWINGS, 2015a). O gênero *Asaia*, por sua vez, tem sido associado à deterioração de bebidas e recentemente encontrado como microrganismo simbionte em mosquitos transmissores da malária (BASSENE *et al.*, 2020). Destaca-se também a característica notável das BAA da produção de exopolissacarídeos, das quais os mais valiosos são a celulose e a levana, sintetizadas principalmente por espécies de *Komagataebacter, Kozakia, Gluconacetobacter, Neoasaia* e *Gluconobacter* (GOMES *et al.*, 2018; LYNCH *et al.*, 2019).

As BAA são amplamente distribuídas em ambientes alcoólicos, açucarados e ácidos (MAMLOUK; GULLO, 2013). Muitas vezes são tidas apenas como agentes deteriorantes de cerveja e vinho, onde a acidez é indesejável, mas o entendimento sobre a sua importância na fabricação de alimentos fermentados como o vinagre, kombucha, kefir de água, cacau, assim como a aplicabilidade e funcionalidade de exopolissacarídeos tais como a levana e a celulose, é ainda bastante limitado. Sendo assim, a difusão do conhecimento sobre o vasto número de benefícios das BAA, por meio da pesquisa, possibilita a sua exploração em diversos campos de aplicação.

A Tabela 1 mostra algumas das características diferenciais dos gêneros *Acetobacter, Gluconacetobacter, Gluconobacter, Komagataeibacter* e *Asaia*. Informações adicionais em

relação às características gerais dos gêneros pertencentes à família *Acetobacteraceae* podem ser obtidas em Komagata, Iino e Yamada (2014) e Sievers e Swings (2015b).

Tabela 1. Características diferenciais dos gêneros *Acetobacter, Gluconacetobacter, Gluconobacter, Komagataeibacter* e *Asaia.*

Característica	***A.***	***G.***	***Gl.***	***K.***	***As.***
Flagelo e motilidade	Peritríquio ou não-móveis	Peritríquio ou não-móveis	Polar ou não-móveis	Não-móveis	Peritríquio ou não-móveis
Catalase	+	+	+	+	+
Oxidase	-	-	-	-	-
Oxidação de etanol à ácido acético	+	+	+	+	- ou f
Oxidação do ácido acético à CO_2 e H_2O	+	+ (a)	-	+	+
Oxidação do lactato à CO_2 e H_2O	+	+ ou -	-	+	+
Crescimento em meio com 0,35 % de ácido acético	+	+	+	+	+
Crescimento em D- manitol	+ ou -	+ ou -	+	+	+ ou -
Crescimento na presença de 30 % de D-glicose	-	+ ou -	- ou f	+	+
Produção de celulose	-	+ ou -	-	+ ou -	-
Produção de levana a partir da sacarose	+ ou -	+ ou -	-	-	-
Fixação de nitrogênio molecular	-	+ ou -	-	-	-
Cetogênese (Dihiddroxiacetona) a partir de glicerol	+ ou -	+ ou -	+	+	- ou f
Produção de ácido a partir de D-manitol	+ ou -	+ ou -	+	-	+ ou -
Produção de ácido a partir de D-glicose	+	+	+	+	+
Tipo de Ubiquinona	Q-9	Q-10	Q-10	Q-10	Q-10

Fonte: (KOMAGATA; IINO; YAMADA, 2014; SIEVERS; SWINGS, 2015b)

Legenda: *A*, *Acetobacter; G., Gluconacetobacter; Gl., Gluconobacter; K., Komagataeibacter; A., Asaia;* +, 90 % ou mais cepas são positivas; -, 90 % ou mais das cepas são negativas; f, fracamente positiva; a, a superoxidação do acetato à CO_2 e H_2O depende da concentração do acetato no meio.

3.3. METABOLISMO DAS BACTÉRIAS DO ÁCIDO ACÉTICO

Microrganismos aeróbios normalmente oxidam fontes de carbono completamente a CO_2 e H_2O no CAC. No entanto, o processo de oxidação se torna incompleto quando se tem a presença de substâncias inibitórias, excesso de substratos de carbono e condições fisiológicas e

de crescimento anormais. No caso das BAA, a oxidação incompleta de álcoois e açúcares, por exemplo, ocorre mesmo em condições normais (ADACHI; YAKUSHI, 2016; TABAN; SAICHANA, 2016). Esta habilidade única das BAA é dada pela sua cadeia respiratória, que consiste de ubiquinonas (UQ), ubiquinol oxidases terminais (citocromo *bo3* ubiquinol oxidase e quinol oxidase insensível ao cianeto (CIO)) e de várias desidrogenases localizadas na superfície periplasmática da membrana citoplasmática (ADACHI; YAKUSHI, 2016). Essas enzimas são divididas em quinoproteínas e flavoproteínas, que possuem a pirroloquinolina quinona (PQQ) e a flavina adenina dinucleotídeo covalentemente ligada (FAD) como grupo prostético, respectivamente (LYNCH *et al.*, 2019).

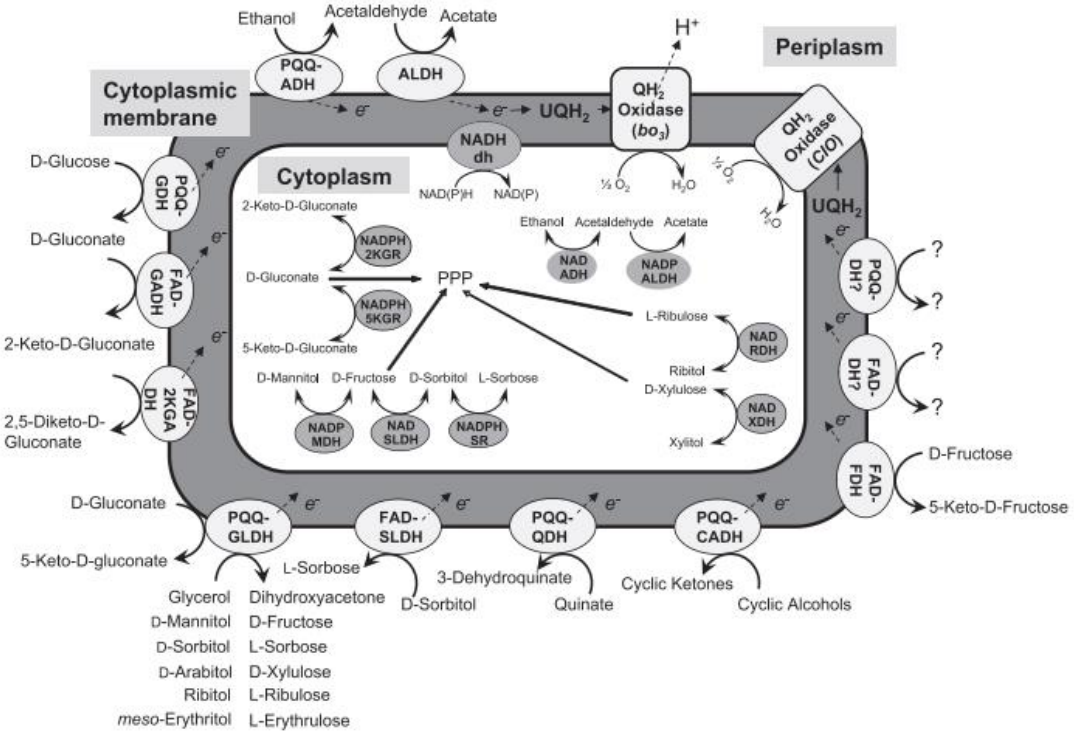
Na cadeia respiratória das BAA, as UQ aceitam elétrons das desidrogenases para liberar a sua forma reduzida (UQH2), que é então oxidada pelas ubiquinol oxidases terminais. Deste modo, as diferentes desidrogenases ligadas à membrana citoplasmática das BAA agem como desidrogenases primárias na cadeia respiratória oxidando substratos, e liberam seus produtos oxidados no meio de cultivo. Os elétrons abstraídos do substrato são então transferidos à oxidase terminal via ubiquinona respiratória para a geração de energia (Figura 1) (SAICHANA *et al.*, 2015; ADACHI; YAKUSHI, 2016).

Já o segundo sistema de enzimas desidrogenases localizadas no citoplasma são dependentes de $NAD(P)^+$ e não têm papel na fermentação oxidativa. Além disso, a cadeia respiratória citosólica parece competir com a cadeias respiratórias da fermentação oxidativa (periplasmática) em relação transferência de elétrons e energia, e por isso ambas as formas de respiração ocorrem em diferentes fases de crescimento (MATSUSHITA; MATSUTANI, 2016; LYNCH *et al.*, 2019). Enquanto as desidrogenases ligadas à membrana são responsáveis pela formação do produto, as enzimas presentes no citoplasma desempenham o papel na assimilação do produto (MATSUSHITA; TOYAMA; ADACHI, 1994; SAICHANA *et al.*, 2015).

A habilidade em oxidar etanol a ácido acético é a característica mais conhecida das BAA (GOMES *et al.*, 2018) (Figura 2). A oxidação parcial do etanol se dá por meio de dois estágios consecutivos: 1) o etanol é oxidado a acetaldeído pela enzima álcool desidrogenase dependente de pirroloquinolina quinona (PQQ-ADH) e em seguida, 2) acetaldeído é oxidado à ácido acético pelo aldeído desidrogenase (ALDH). Ambas as enzimas estão ligadas ao lado periplasmático da membrana citoplasmática e são independentes de $NAD(P)^+$ (MATSUSHITA, TOYAMA; ADACHI, 1994). Por outro lado, a álcool desidrogenase NAD-dependente (NAD-ADH) e aldeído desidrogenase NAD-dependente (NAD-ALDH) podem também realizar a

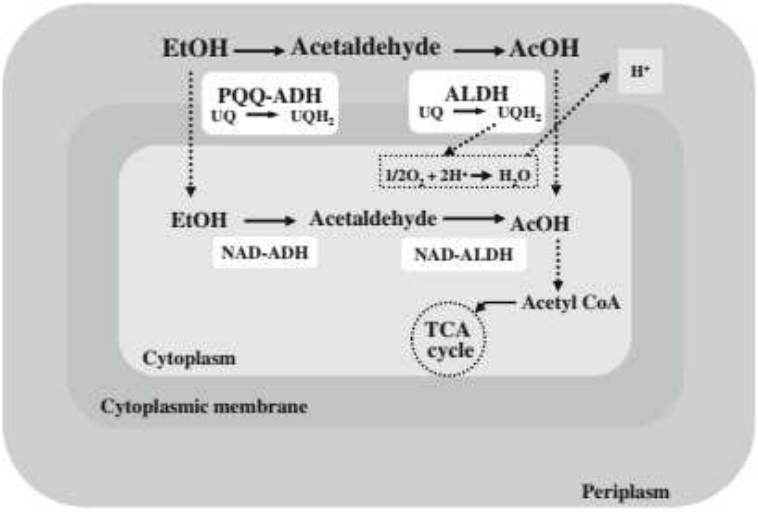
oxidação completa do etanol à nível citoplasmático, sendo o ácido acético resultante utilizado posteriormente pela acetil-CoA sintase e via CAC (MATSUSHITA; TOYAMA; ADACHI, 1994).

Figura 1 - Fermentação oxidativa dos diferentes substratos utilizados pelas BAA.



Legenda: A D-glucono-δ-lactona pode ser convertida em D-gluconato espontaneamente ou por uma gluconolactonase ligada à membrana (não mostrado esta figura). PQQ-ADH: álcool desidrogenase dependente de PQQ; ALDH: aldeído desidrogenase; PQQ-GDH: D-glicose desidrogenase dependente de PQQ; PQQ-GLDH: glicerol desidrogenase dependente de PQQ; FAD-GADH: D-gluconato desidrogenase dependente de FAD; FAD-SLDH: D-sorbitol desidrogenase dependente de FAD; FAD-FDH: dependente de FAD D-frutose desidrogenase; FAD-2KGA-DH: 2-ceto-D-gluconato desidrogenase dependente de FAD; NADH-dh: NADH desidrogenase; QH2 Oxidase (bo3): citocromo bo3 quinol oxidase; QH2 Oxidase (CIO): quinol oxidase insensível ao cianeto; 2KGR: 2-ceto-D-gluconato redutase; 5KGR: 5-ceto-D-gluconato redutase; ADH: álcool desidrogenase; RDH: ribitol desidrogenase; XDH: xilitol desidrogenase; SLDH: sorbitol desidrogenase; MDH: manitol desidrogenase; QDH: quinato desidrogenase; CADH: álcool cíclico desidrogenase; SR: sorbose redutase; UQH2: ubiquinol, forma reduzida de ubiquinona; e PPP: via das pentoses fosfato. **Fonte:** Saichana *et al.* (2015)

Figura 2 - Oxidação do etanol por PQQ-ADH e ALDH na superfície da membrana citoplasmática e por NAD-ADH e NAD-ALDH no citoplasma.



Fonte: Mamlouk e Gullo (2013)

Como observado na Figura 1, BAA oxidam diferentes substratos além do etanol. Como exemplo temos outros álcoois como o glicerol, oxidado à dihidroxiacetona, e polióis como o manitol e sorbitol, oxidados em frutose e sorbose, respectivamente (SAICHANA *et al.*, 2015). Além disso, ácidos orgânicos como o acético, lático, pirúvico, málico, succínico e fumárico podem ser completamente oxidados à CO_2 e H_2O por gêneros de BAA que apresentam a rota do CAC funcional, como é o caso de *Acetobacter, Gluconacetobacter e Komagataeibacter* (MAMLOUK; GULLO, 2013). Já para *Gluconobacter*, a oxidação de ácidos orgânicos não é possível devido à ausência de enzimas como o succinato desidrogenase e α-cetoglutarato desidrogenase, presentes no CAC (MATSUSHITA, KAZUNOBU TOYAMA; ADACHI, 1994; LYNCH *et al.*, 2019). Quanto aos açúcares, BAA têm alta habilidade em oxidar arabinose, frutose, galactose, ribose, sorbose, manose e xilose, mas oxidam preferencialmente a glicose (MAMLOUK; GULLO, 2013).

3.3.1. Oxidação da Glicose por BAA

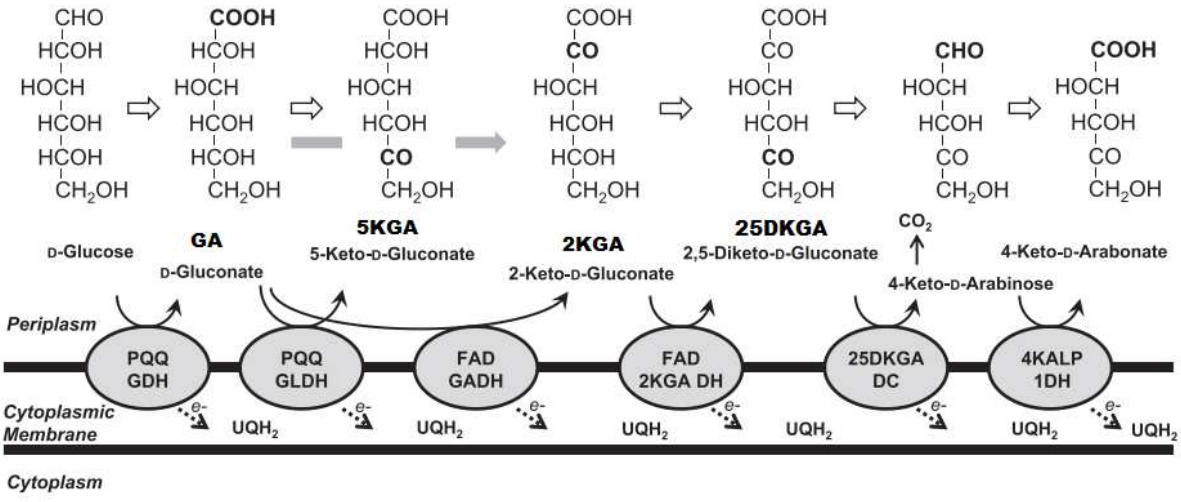
Similarmente à oxidação do etanol, no metabolismo da glicose os dois sistemas de enzimas também estão envolvidos (Figura 3). No entanto, de acordo com Matsushita, Toyama e Adachi (1994), as enzimas citoplasmáticas estão envolvidas principalmente na biossíntese de

precursores e na manutenção celular durante a fase estacionária, sem muita contribuição na fermentação oxidativa.

Já no espaço periplasmático, a glicose é primeiramente oxidada a glicono-δ-lactona por uma glicose desidrogenase dependente de PQQ (PQQ-GDH) ligada à membrana, que é então convertido em ácido D-glucônico (GA) espontaneamente ou por uma gluconolactonase presente na membrana (MATSUSHITA, TOYAMA; ADACHI, 1994). GA é então convertido para 2-ceto-D-gluconato (2KGA) ou 5-ceto-D-gluconato (5KGA) por duas diferentes enzimas oxidantes ligadas à membrana, que realizam a reação competitiva *in vivo*. Enquanto gluconato desidrogenase dependente de FAD (FAD-GADH) é responsável pela formação de 2KGA (SHINAGAWA *et al.*, 1981; TOYAMA *et al.*, 2007), 5KGA é formada pela PQQ-GLDH. Em algumas cepas de BAA, 2KGA é ainda convertido em Ácido 2,5-diceto-D-glucônico (25DKGA) pela 2-ceto-D-gluconato desidrogenase dependente de FAD (SHINAGAWA *et al.*, 1981; MATSUSHITA; TOYAMA; ADACHI, 1994). Por fim, os produtos resultantes da oxidação são parcialmente submetidos à via pentose-fosfato como a principal rota para produção de massa celular durante a fase estacionária de crescimento (SAICHANA *et al.*, 2015).

Sob o ponto de vista industrial, a produção massiva de ácido glucônico por *G. oxydans* é realizada com altas concentrações de glicose, baixo pH e alta taxa de aeração, assim suprimindo a formação de ácidos cetogluconatos (MAMLOUK; GULLO, 2013).

Figura 3 - Metabolismo da glicose em *Gluconobacter.*



Legenda: PQQ-GDH: D-glicose desidrogenase dependente de PQQ; PQQ-GLDH: glicerol desidrogenase dependente de PQQ; FAD-GADH: D-gluconato dependente de FAD desidrogenase;

FAD-2KGA DH: 2-ceto-D-gluconato desidrogenase dependente de FAD; 25DKGA DC: 2,5-diceto-D-gluconato descarboxilase; 4KALP1DH: 4-ceto-D-aldopentose-1-desidrogenase; **Fonte:** Adaptado de Saichana *et al.* (2015).

3.4. OCORRÊNCIA E ISOLAMENTO DE BACTÉRIAS DO ÁCIDO ACÉTICO

Bactérias do ácido acético estão amplamente distribuídas na natureza, e por isso, seu isolamento é proveniente de diversas fontes (Tabela 2). Historicamente, são reconhecidas como "bactérias do vinagre", uma vez que têm sido encontradas primeiramente em vinagre, e mais tarde no vinho e cerveja em decomposição. Sabe-se, no entanto, que este grupo de bactérias também pode ocorrer em nichos açucarados, tais como flores e frutas, e em bebidas fermentadas agindo como deteriorantes. Além disso, têm sido isoladas de rizosfera de plantas e até mesmo de seres humanos, agindo como patógenos oportunistas (CLEENWERCK; DE VOS, 2008; SOLIERI; GIUDICI, 2009; CHASE *et al.*, 2012; MAMLOUK; GULLO, 2013).

Tabela 2. Gêneros e espécies de BAA e fontes de isolamento.

Gênero	**Espécie**	**Fonte**	**Referência**
ACETOBACTER			
Acetobacter	*A. conturbans* / *A. fallax*	Fermentação de cidra	Sombolestani *et al.* (2020)
Acetobacter	*A. oryzoeni*	Vinagre de vinho de arroz	Baek *et al.* (2020)
Acetobacter	*A. lambici*	Cerveja lambic	Spitaels *et al.* (2014)
Acetobacter	*A. orientalis*	Iogurte Caucasiano	Nakashima e Tamura (2018)
ACIDOMONAS			
Acidomonas	*Ac. methanolica*	Paciente com doença crônica granulomatosa	Chase *et al.* (2012)
ASAIA			
Asaia	*As. bogorensis*	Água flavorizada de frutas	Kregiel, Otlewska e Antolak (2014)
Asaia	*As. siamensis*	Flor tropical	Katsura *et al.* (2001)
GLUCONACETOBACTER			

Gluconacetobacter	*Gl. diazotrophicus*	Cenoura/ beterraba/café/ rabanete	Madhaiyan *et al.* (2004)
Gluconacetobacter	*Gl. tumulicola / Gl. asukensis*	Substância viscosa em túmulo de pedra	Tazato *et al.*, (2012)
Gluconacetobacter	*Gl. johannae / Gl. azotocaptans*	Plantas de café	Fuentes-Ramírez *et al.* (2001)
GLUCONOBACTER			
Gluconobacter	*G. cerevisiae*	Ambiente de cervejaria	Spitaels *et al.* (2014b)
Gluconobacter	*G. aidae*	Frutas tropicais	Yukphan *et al.* (2020)
Gluconobacter	*G. oxydans*	Kombucha	Neffe-skocińska *et al.* (2022)
KOMAGATAEIBACTER			
Komagataeibacter	*Kg. cocois*	Leite de coco	Liu *et al.* (2018)
Komagataeibacter	*Kg. diopyri*	Caqui e fruto de sapoti	Naloka *et al.* (2020)
Komagataeibacter	*Kg. xylinus*	Vinagre tradicional iraniano	Lavasani *et al.* (2017)
KOZAKIA			
Kozakia	*K. baliensis*	Açúcar mascavo	Lisdiyanti *et al.* (2002)
NEOASAIA			
Neoasaia	*N. chiangmaiensis*	Flor de gengibre vermelho	Yukphan *et al.* (2005)

Um dos maiores desafios com relação às BAA encontra-se no seu isolamento e cultivo em meio laboratorial. Por serem bastante fastidiosas, mostram uma baixa porcentagem de recuperação, resultando muitas vezes na subestimação de suas contagens microbianas (BARTOWSKY; HENSCHKE, 2008; SOLIERI; GIUDICI, 2009). Por isso, várias formulações de meios contendo principalmente glicose, manitol e etanol foram desenvolvidas visando resolver estes problemas (MAMLOUK; GULLO, 2013). Meios como GYC (m/v; 5% Glicose; 1 % Extrato de levedura; 0,5 % Carbonato de cálcio; 2 % Ágar) (CARR; PASSMORE, 1979) e MYP (m/v; 2,5 % Manitol; 0,5 % Extrato de levedura; 0,5 % Peptona; 2,0 % Ágar) (SOKOLLEK; HERTEL; HAMMES, 1998) têm sido frequentemente utilizados para o isolamento de BAA a partir de amostras de vinhos e frutas (BARTOWSKY *et al.*, 2003; DE

OLIVEIRA *et al.*, 2010; MATEO *et al.*, 2014) e diversos vinagres (KIM *et al.*, 2019; YETIMAN; KESMEN, 2015).

Alguns estudos, no entanto, ainda relatavam dificuldades no isolamento e cultivo de BAA oriundos de indústrias de vinagre com altas concentraçõese ácido acético (10 – 15 %) (ENTANI *et al.*, 1985). Este problema foi parcialmente reduzido pela introdução da técnica de ágar de dupla-camada, cujo meio AE (m/v; 0,2 % Extrato de levedura; 0,3 % Peptona; 1,5 % Glicose; 6,5 % Ácido acético; 2,0 % Etanol (v/v) foi empregado por adição de 0,5 % de ágar na camada inferior e 1 % de ágar na camada superior (ENTANI *et al.*, 1985). O cultivo no ágar de dupla camada sob alta umidade simula a atmosfera dos tanques de acetificação e permite o crescimento de bactérias resistentes a alta acidez (GUILLAMÓN; 2017).

3.5. ANÁLISE POLIFÁSICA PARA CLASSIFICAÇÃO DAS BAA

Após o isolamento e purificação dos microrganismos, BAA podem ser caracterizados por meio de análises polifásicas que combinam métodos fenotípicos, quimiotaxonômicos e moleculares (CLEEWERCK; DE VOS, 2008).

3.5.1. Métodos Fenotípicos

A análise fenotípica clássica, baseada em critérios morfológicos, bioquímicos e fisiológicos, é a ferramenta mais comum para descrição formal das espécies em nível de família (SOLIERI; GIUDICI, 2009; GUILLAMÓN; MAS, 2017). As características gerais mais utilizadas incluem a coloração de Gram, a morfologia celular e aspecto da colônia e a atividade das enzimas oxidase e catalase (KOMAGATA; IINO; YAMADA, 2014). Porém, outros testes têm sido utilizados para a identificação e diferenciação dos diversos gêneros de BAA. Dentre elas podemos citar: a capacidade de oxidação do etanol a ácido acético, a capacidade de oxidação de ácido acético a CO_2 e H_2O, a capacidade de oxidação de lactato a CO_2 e H_2O, produção de celulose, produção de substância mucosa tipo levana a partir de sacarose, crescimento na presença de 30 % de glicose, entre outros (SIEVERS; SWINGS, 2015b).

Nos gêneros que não são monotípicos, e que incluem a maioria das espécies dos gêneros *Acetobacter, Gluconobacter, Gluconacetobacter, Asaia e Komagataeibacter,* a identificação em nível de gênero pode ser realizada pela combinação de apenas dois testes convencionais, que são baseados na oxidação do acetato e lactato e na produção de ácido acético a partir do etanol (YAMADA; YUKPHAN, 2008).

Em testes de oxidação de lactato e acetato, utilizando-se o meio DSM (CIRIGLIANO, 1982), o gênero *Gluconobacter* se mostra incapaz de oxidar lactato, mas oxidam preferencialmente os carboidratos constituintes, produzindo ácido acético e mantendo a coloração amarelada do meio. Por outro lado, para os gêneros *Gluconacetobacter* e *Komagataeibacter,* a mudança de cor para púrpura não é tão pronunciada quanto àquela observada para o gênero *Acetobacter* (CIRIGLIANO, 1982; YAMADA, 2016). Este último também produz um precipitado branco de carbonato de cálcio juntamente ao crescimento celular e forma um halo translúcido ao redor das colônias (CIRIGLIANO, 1982).

Já a diferença na habilidade relativa de oxidação do etanol pelos gêneros *Acetobacter* e *Gluconobacter* pode ser observada com o uso do meio Carr (FUGELSANG; EDWARDS, 2007). A presença do indicador verde de bromocresol como um indicador de pH facilita a identificação de BAA, uma vez que em condições neutras/alcalinas a coloração do meio apresenta-se esverdeada, e em presença de ácidos, amarelada. Ambos os gêneros possuem a capacidade de oxidar etanol à ácido acético, o que leva à uma mudança na cor do meio para o amarelo. No entanto, diferentemente dos gêneros *Acetobacter, Komagataeibacter,* e *Gluconacetobacter*, cepas do gênero *Gluconobacter,* não super-oxidam o ácido acético a CO_2 e H_2O, permanecendo assim, com o meio na cor amarela sob incubação adicional. Para os gêneros que oxidam o acetato, a metabolização leva ao retorno da cor verde do meio (FUGELSANG; EDWARDS, 2007; SIEVERS; SWINGS, 2015b). BAA pertencentes ao gênero *Asaia* diferem dos gêneros já mencionados devido a pouca ou nenhuma oxidação do etanol a ácido acético, e a oxidação de acetato e lactato, visualizada pela mudança de coloração do meio, é bastante lenta. Estes dois testes são bastante úteis quando o número de isolados é elevado (YAMADA, 2016).

3.5.2. Métodos Quimiotaxonômicos

Métodos quimiotaxonômicos são métodos taxonômicos recentemente abordados que tem como base a análise de constituintes celulares como ácidos graxos e quinonas isoprenoides. A extração e purificação de ambas são realizadas por métodos padronizados e a determinação quantitativa de suas composições geralmente se dá por análises cromatográficas (CLEENWERCK; DE VOS, 2008).

A composição de ácidos graxos em BAA é caracterizada pela presença de cadeias C18:1ω7 de ácido graxos insaturados, em sua maioria, e por ácidos 2-hidróxi. E em relação às

quinonas, cepas do gênero *Acetobacter* são as únicas a possuírem Q9 como principal ubiquinona, enquanto as outras restantes, Q10 (CLEENWERCK; DE VOS, 2008; KOMAGATA; IINO; YAMADA, 2014).

3.5.3. Métodos Moleculares

A taxonomia de BAA, inicialmente baseada em métodos fenotípicos, tem sofrido uma contínua reorientação, que é devido, sobretudo pela introdução de métodos moleculares. O principal objetivo da aplicação dos métodos moleculares é permitir a identificação de espécies dos microrganismos, assim como a discriminação ou tipificação de estirpes ou de genótipos dentro de uma mesma espécie. Essa discriminação desempenha um importante papel em aplicações industriais, uma vez que BAA estão envolvidas em diversos processos biotecnológicos, seja como um agente contaminante ou como agentes de transformação, e nem todas as estirpes da mesma espécie geram mudanças similares no produto (KREGIEL, 2013; GUILLAMÓN; MAS, 2017; DE ROOS; DE VUYST, 2018;; IZQUIERDO-CAÑAS *et al.*, 2018). Dentre as técnicas mais comuns utilizadas pode-se citar:

- ✓ Hibridização DNA-DNA: Do ponto de vista taxonômico, é o método mais amplamente utilizado para descrever novas espécies de um determinado grupo. A técnica mede o grau de similaridade entre os genomas de diferentes espécies (GUILLAMÓN; MAS, 2017);
- ✓ Determinação da porcentagem de base: Este é um dos primeiros métodos utilizados na taxonomia bacteriana. É baseado no cálculo de composição de bases do DNA (mol% guanina-G + citosina-C). Diferentes conteúdos de G+C indicam organismos diferentes, no entanto, valores idênticos, por si só, não necessariamente caracterizam uma taxonomia particular (LUDWIG, 2007);
- ✓ Análise do gene 16S rDNA: O gene 16S rDNA é uma região altamente conservada com pequenas regiões que caracterizam diferentes espécies. No entanto, as diferenças nas sequências do gene 16S rDNA são bastante limitadas e algumas espécies possuem poucos pares de nucleotídeos de diferença (GUILLAMÓN; MAS, 2017).

Além desses, vários outros métodos moleculares têm sido reportados (TRČEK; BARJA, 2015), que em combinação com os métodos fenotípicos e quimiotaxonômicos fornecem informações mais precisas para a identificação e classificação das BAA (CLEENWERCK; DE VOS, 2008; YAMADA, 2016).

3.6. PARTICIPAÇÃO DAS BAA NA FABRICAÇÃO DE PRODUTOS FERMENTADOS

3.6.1. Vinagre

A produção do vinagre, que remonta há mais de 10000 anos, é o processo de biotransformação mais conhecido das BAA (SOLIERI; GIUDICI, 2009; LYNCH *et al.*, 2019). Apesar de não ser considerado um "alimento" e não possuir um alto valor nutricional, o vinagre é consumido mundialmente por todas as classes sociais, e difere pelas matérias-primas utilizadas, pelas tecnologias de fabricação e por seu vasto campo de aplicação (SOLIERI; GIUDICI, 2009; SPINOSA *et al.*, 2015; LYNCH *et al.*, 2019).

A definição e os padrões de identidade e qualidade do vinagre possuem algumas diferenças locais, mas em geral, os órgãos regulamentadores de alimentos consideram o vinagre como o resultado de uma dupla fermentação (primeira a alcoólica e em seguida, acética) de substratos açucarados (HO *et al.*, 2017). No Brasil, por exemplo, o MAPA (Ministério da Agricultura, Pecuária e Abastecimento) por meio da Instrução Normativa Nº 6 de 03 de Abril de 2012 e Decreto nº 6.871, de 04 de junho de 2009, define como Fermentado acético, o produto com acidez volátil mínima de 4 % (g/100 mL, expresso em ácido acético), obtido da fermentação acética do fermentado alcoólico de mostos de frutas, de cereais, de outros vegetais, de mel, da mistura de vegetais ou da mistura hidroalcoólica. O fermentado acético poderá ser denominado "vinagre de...", acrescido do nome do substrato utilizado. A legislação também acrescenta que, apesar das denominações fermentado acético e vinagre serem equivalentes, elas são mutuamente excludentes (BRASIL, 2009; BRASIL, 2012).

O substrato utilizado no processamento do vinagre, em sua maioria, é de origem vegetal (ex: vinagres de cereais, cebola, cidra, maçã e manga), com exceção somente dos vinagres de mel e soro de leite (LI *et al.*, 2015; GIUDICI; DE VERO; GULLO, 2017). A composição química da matéria-prima tem forte influência na seleção de microrganismos e determina as espécies dominantes envolvidas no processo de acetificação (MUROOKA, 2016). Em vinagres de frutas, a fermentação alcoólica, conduzida principalmente pela levedura *Saccharomyces cerevisiae,* pode ser realizada por fermentações induzidas espontaneamente (em produções artesanais) ou pela inoculação de culturas puras (starter) (em produções em escala industrial) (LI *et al.*, 2015). Na segunda etapa de oxidação, a fermentação acética, que tem como principais representantes as BAA dos gêneros *Acetobacter, Gluconobacter, Gluconacetobacter* e

Komagataeibacter, a velocidade de acetificação pode ser aprimorada pela inoculação da "mãe do vinagre" ou de culturas starter de cepas benéficas ao processo. Somente na produção de vinagre de vinho tradicional, a fermentação acética acontece espontaneamente (SOLIERI; GIUDICI, 2009; LI *et al.*, 2015).

Os diferentes tipos de vinagres também apresentam variadas aplicações. Vinagres são comumente utilizados como conservantes, agentes flavorizantes e no preparo de maioneses, molhos de saladas, mostarda e outros condimentos (BUDAK *et al.*, 2014; LI *et al.*, 2015; SPINOSA *et al.*, 2015). Seu uso como medicamento rotineiro para pessoas e animais já data desde a antiguidade remota, ademais, pode ser empregado como um agente de limpeza e em alguns países até como uma bebida saudável (SPINOSA *et al.*, 2015; LYNCH *et al.*, 2019).

Apesar de o vinagre ser tradicionalmente utilizado como um tempero e conservante de alimentos, estudos científicos recentes têm reportado que o consumo regular de vinagre pode promover efeitos fisiológicos benéficos à saúde (LYNCH *et al.*, 2019; XIA *et al.*, 2020; OUSAAID *et al.*, 2022). Entre as propriedades terapêuticas apresentadas pelo vinagre podemos incluir: atividade antibacteriana, regulação da pressão arterial e glicemia, atividade antioxidante, prevenção das doenças cardiovasculares e prevenção da obesidade (SAMAD; AZLAN; ISMAIL, 2016; GOMES *et al.*, 2018;). Vinagres contêm, além do ácido acético, vários compostos bioativos como polifenóis e flavonoides que contribuem para o seu gosto, cheiro e funções específicas. Tendo em vista que diferentes vinagres podem ser produzidos a partir de diferentes matérias-primas, processos e espécies de BAA, o entendimento sobre a relação entre os compostos presentes e a funcionalidade do vinagre é de grande importância (LI *et al.*, 2015; SAMAD; AZLAN; ISMAIL, 2016).

3.6.2. Kombucha

Kombucha é uma bebida popular usualmente consumida na Ásia (DE FILIPPIS *et al.*, 2018). É caracterizada por ser uma bebida não alcoólica, refrescante, de acidez acentuada e sabor específico (DE ROOS; DE VUYST, 2018). Tradicionalmente, sua obtenção se dá pela fermentação do chá adocicado, porém, outras matérias-primas de origem vegetal (por exemplo, cereais ou folhas de plantas) ou animal (por exemplo, leite) e até mesmo cogumelos podem ser utilizados (MORALES, 2020). A fermentação, que dura em torno de 7 a 10 dias, ocorre rapidamente após a adição da camada celulósica denominado tea fungus ou SCOBY (Symbiotic Colony of Bacteria and Yeast) ao chá adocicado (LAAVANYA; SHIRKOLE;

BALASUBRAMANIAN, 2021). Esta cultura simbiótica é composta prevalentemente por BAA (espécies dos gêneros *Komagataeibacter*, *Gluconobacter* e *Acetobacter)* (DE ROOS; DE VUYST, 2018), além de BAL (*Lactobacillus*, *Lactococcus*) (VILLARREAL-SOTO *et al.*, 2018) e leveduras *(Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Torulaspora delbrueckii*, *Brettanomyces bruxellensis*) (COTON *et al.*, 2017). Sob condições aeróbias, este consórcio converte o açúcar e os os compostos do chá, produzindo componentes majoritários como ácido acético, ácido glucônico, ácido glucurônico e etanol. Sua composição também inclui aminoácidos, compostos fenólicos, vitaminas e algumas enzimas hidrolíticas (DE FILIPPIS *et al.*, 2018; VILLARREAL-SOTO *et al.*, 2018). O perfil químico do Kombucha pode ser responsável pelos seus múltiplos benefícios à saúde quando associado ao consumo regular da bebida (DE FILIPPIS *et al.*, 2018). Recentemente, o chá tem chamado a atenção de pesquisadores e consumidores devido às suas atividades biológicas *in vitro*, tais como atividade antimicrobiana, atividade antioxidante e anti-inflamatória e potencial anticarcinogênico. No entanto, investigações clínicas adicionais e avaliações *in vivo* devem ser realizadas para a confirmação dos benefícios da bebida à saúde (VILLARREAL-SOTO *et al.*, 2018; KAPP; SUMNER, 2019).

3.6.3. Kefir de Água

O kefir de água é uma bebida espumante, refrescante, de baixo teor alcoólico, com sabores ácidos e frutados (DE ROOS; DE VUYST, 2018). Sua obtenção é dada pela fermentação espontânea de solução de açúcar, frutas secas e grãos de kefir de água (dextrana insolúvel). Estes grãos, translúcidos e em forma semelhante à couve-flor, incorporam microrganismos que servem como inóculo para o processo fermentativo (LYNCH *et al.*, 2021).

A composição microbiana dos grãos, que diferem do kefir de leite, apresenta especialmente leveduras (Ex: *Pichia*, *Lachancea*, *Saccharomyces, Dekkera* e *Kluyveromyces*) e BAL (Ex: *Lactobacillus*, *Lactococcus e Leuconostoc)* (PENDÓN *et al.*, 2021). No entanto, o metabolismo da *Saccharomyces* e outras leveduras estimulam a produção de altas concentrações de álcool, permitindo assim, o crescimento de BAA (Ex: *Gluconobacter*, *Komagataeibacter* e *Acetobacter*) e a oxidação do etanol a ácido acético (LAUREYS *et al.*, 2018; BUENO *et al.*, 2021). Ao final da fermentação, tem-se como produtos principais o etanol, ácido lático, ácido acético e outros metabólitos como manitol, glicerol, ésteres, aldeídos, e

outros ácidos orgânicos (FIORDA *et al.*, 2017; GUZEL-SEYDIM; GÖKIRMAKLI; GREENE, 2021).

Assim como o Kombucha, bebidas de kefir têm sido relacionadas a variados efeitos benéficos à saúde humana. A bebida é amplamente conhecida por seu potencial probiótico e por sua atividade antimicrobiana contra várias espécies de microrganismos patogênicos (WALDHERR *et al.*, 2010; FIORDA *et al.*, 2017). Adicionalmente, estudos têm demonstrado que o kefir de água apresenta atividade antihiperglicêmica (ROCHA-GOMES *et al.*, 2018), atividade antioxidante (BUENO *et al.*, 2021; OZCELIK; AKAN; KINIK, 2021), atividade anticarcinogênica, efeito hepatoprotetivo, efeito anti-inflamatório, efeito gastroprotetivo, entre outros (GUZEL-SEYDIM; GÖKIRMAKLI; GREENE, 2021).

Devido aos numerosos efeitos positivos do kefir, vários substratos têm sido investigados para adaptação dos seus grãos (DESTRO *et al.*, 2019; BUENO *et al.*, 2021; DARVISHZADEH; ORSAT; MARTINEZ, 2021; OZCELIK; AKAN; KINIK, 2021). Isto possibilitou o surgimento de novos produtos funcionais e bebidas probióticas com características similares ao kefir tradicional de açúcar mascavo (FIORDA et al., 2017).

3.6.4. Cerveja Lambic

A cerveja lambic, originária da Bélgica, é provavelmente um dos estilos de cerveja mais antigos fabricados até hoje (SPITAELS *et al.*, 2015; THOMPSON *et al.*, 2017). É caracterizada por ser uma cerveja refrescante, alcoólica, ácida com notas frutadas e poucos carboidratos residuais. O processo fermentativo espontâneo acontece na presença de água, malte de cevada, trigo não maltado e lúpulo seco envelhecido, e a maturação, que ocorre em barris de madeira, pode chegar até três anos (DE ROOS; DE VUYST, 2018).

Uma análise profunda durante os três anos de fermentação da cerveja lambic revelou um padrão de sucessão na dominância microbiana, na qual, quatro fases distintas são identificadas (POTHAKOS *et al.*, 2016).

A primeira fase (do início até o primeiro mês de fermentação) inicia-se com os membros da família *Enterobacteriaceae,* que são posteriormente inibidos pela acumulação de etanol produzida por leveduras selvagens (oxidativa), acidificação por enterobactérias e AAB (*A. orientalis*), e pela redução da glicose (utilizada para o crescimento microbiano em geral) (DE ROOS; DE VUYST, 2018; DE ROOS; VANDAMME; DE VUYST, 2018). Espécies de *Gluconobacter*, tais como *G. cerevisiae*, também tem sido isolada durante esta fase,

provavelmente devido à combinação de ambientes ricos em monossacarídeos e pela baixa concentração de etanol (DE ROOS *et al.*, 2018).

A segunda fase ou fase da fermentação do etanol, referida como a mais importante, se estende até o quarto mês, tendo como principais representantes, as leveduras *Saccharomyces cerevisiae*, *S. bayanus/pastorianus* e *S. uvarum*, que são responsáveis pela conversão de carboidratos à etanol (SPITAELS *et al.*, 2014c). Após 4 a 10 meses de fermentação, a fase da acidificação se inicia com a predominância de BAL (*Pediococcus damnosus* e/ou *Lactobacillus brevis*), que juntas produzem altas quantidades de ácido lático (DE ROOS; VANDAMME; DE VUYST, 2018).

Finalmente, após 10 meses, a fase da maturação inicia-se com a presença de leveduras (*Dekkera bruxellensis*) e BAL (*P. damnosus* e/ou *L. brevis*), caracterizando-se pela produção de etil-acetato e etil-lactato (SPITAELS *et al.*, 2015; POTHAKOS *et al.*, 2016; DE ROOS; VANDAMME; DE VUYST, 2018). Durante a terceira fase até os 24 meses de maturação, o crescimento de *A. pasteurianus* (BAA) foi também observado por De Roos *et al.* (2018).

BAA são abundantes durante os principais períodos de fermentação da produção cerveja lambic tradicional, produzindo concentrações muito maiores de ácido acético e acetoína (da oxidação do ácido lático via piruvato) na interfase líquido/ar dos barris. Embora a formação de ácido acético por BAA e a subsequente formação de acetato de etila sejam compostos desejáveis para a cervejas lambic, o desenvolvimento excessivo de BAA deve ser controlado para evitar um perfil de flavor desfavorável (DE ROOS *et al.*, 2018; DE ROOS; VANDAMME; DE VUYST, 2018).

3.6.5. Cacau

A semente de cacau (*Theobroma cacao* L) é a matéria-prima principal para a fabricação do chocolate (HO; FLEET; ZHAO, 2018). Devido ao seu sabor adstringente e amargo, a fermentação espontânea, que envolve mudanças bioquímicas e uma complexa atividade microbiana, desempenha uma função essencial na composição e sabor do chocolate e produtos à base de cacau (DE VUYST; WECKX, 2016; VISINTIN *et al.*, 2016). A fermentação do cacau consiste de uma sucessão microbiana de várias espécies de leveduras, BAL, BAA, além de espécies de *Bacillus* sp., outras bactérias e fungos filamentosos (DE MELO PEREIRA *et al.*, 2013; HO; FLEET; ZHAO, 2018).

As leveduras são os microrganismos dominantes durante os dois primeiros dias, que por meio da fermentação alcoólica (anaerobiose) convertem os açúcares da polpa em etanol e dióxido de carbono. A produção de enzimas pectinolíticas pelas leveduras também permite a quebra da pectina e a entrada de oxigênio na polpa do cacau, criando condições microaeróbias para o desenvolvimento de BAL. Por meio da via homofermentativa, BAL fermentam os carboidratos à ácido lático, enquanto que pela via heterofermentativa, ácido lático, ácido acético ou etanol e dióxido de carbono são produzidos (POTHAKOS *et al.*, 2016). No último estágio da fermentação, o aumento da temperatura, aeração e a disponibilidade de substratos (etanol, ácido acético e lático, manitol) torna favorável o crescimento de BAA, que tem como principal função a oxidação desses substratos em ácido acético e acetoína (DE VUYST; LEROY, 2020). O ácido acético produzido difunde-se no interior das sementes, mata o embrião e a conversão enzimática dos substratos fornece cor e o flavor característicos dos grãos de cacau fermentados (SANDHYA *et al.*, 2016).

Recentemente, vários trabalhos que anseiam a padronização e aumento da produtividade do processo fermentativo fazem uso de culturas starter, entre as quais as BAA, pela sua importância, também estão presentes (DE VUYST; WECKX, 2016; SANDHYA *et al.*, 2016; DE ROOS *et al.*, 2018).

3.7. OUTROS PRODUTOS PRODUZIDOS POR BAA

As BAA, além de serem utilizadas comercialmente na produção de vinagres e outros alimentos fermentados, são também capazes de atuar como biocatalisadores para a produção industrial de uma grande variedade de compostos (MAMLOUK; GULLO, 2013; GOMES *et al.*, 2018). Cepas do gênero *Gluconobacter*, em especial, possuem a habilidade de realizar a fermentação oxidativa de açúcares e álcoois de açúcares levando a formação de compostos como L-sorbose, ácidos cetoglucônicos, di-hidroxiacetona (DHA), cetonas cíclicas, além de outras substâncias (SAICHANA *et al.*, 2015; SHINJOH; TOYAMA, 2016).

A fermentação oxidativa de L-sorbose a partir de D-sorbitol, é o exemplo mais clássico observado durante a produção de vitamina C por *Gluconobacter*. Outros intermediários precursores, como 2-ceto-D-ácido glucônico (2KGA) a partir de D-ácido glucônico, 2,5-diceto-D-ácido glucônico (25DKGA), e 5-ceto-D-ácido glucônico (5KGA) também estão presentes na rota de síntese (MAMLOUK; GULLO, 2013; SHINJOH; TOYAMA, 2016). O 5KGA tem aplicações potenciais para a síntese de ácido tartárico e ácido xilárico, além de ser precursor

para a fabricação de compostos aromáticos como 4-hidroxi-5-metil-2,3-di-hidrofuranona-3, um valioso produto utilizado na indústria de alimentos (SALUSJÄRVI *et al.*, 2004).

A produção microbiana de DHA a partir do glicerol tem sido bastante explorada na indústria farmacêutica, podendo ser utilizado como agente de bronzeamento e como um intermediário para a síntese de vários produtos químicos e surfactantes (SENGUN; KARABIYIKLI, 2011; MAMLOUK; GULLO, 2013). *Gluconobacter* spp. pode também ser aplicado na biotransformação de precursores de miglitol, uma droga utilizada para o tratamento de diabetes tipo II; na produção de ácido glucônico, considerado um ácido multifuncional nas indústrias de alimentos, rações, bebidas, têxteis, farmacêuticas e de construção; e na fabricação do ácido xiquímico, um intermediário chave para inúmeros antibióticos (MAMLOUK; GULLO, 2013; SAICHANA *et al.*, 2015).

3.8. DESENVOLVIMENTO DE NOVOS PRODUTOS A PARTIR DE BAA

3.8.1. Bebidas De Frutas Fermentadas Por BAA

Atualmente, os consumidores têm demonstrado um interesse crescente em alimentos que, além de saciar a sua fome, possam também prevenir doenças relacionadas à nutrição e melhorar a saúde mental (DONNO; TURRINI, 2020). De acordo com a Organização das Nações Unidas para a Alimentação e a Agricultura (FAO), frutas constituem uma importante parte de uma dieta saudável. Além de serem fontes de fibras dietéticas, vitaminas, minerais e fitoquímicos benéficos, frutas podem ajudar na diminuição dos fatores de risco para doenças como o sobrepeso/obesidade, inflamação crônica, pressão arterial elevada, e alto colesterol (FAO, 2020b).

A fermentação baseada em frutas tem melhorado a qualidade nutricional e funcional de bebidas. Aliado a isso, o aumento da demanda do consumidor por produtos livres de lactose, com baixo teor de gordura e contendo poucos aditivos, faz deste tipo de fermentação uma ferramenta bastante promissora para atender os requisitos de pessoas obesas e com doenças cardiovasculares, alérgicos, intolerantes, veganos e vegetarianos (MARRERO *et al.*, 2019; MIN *et al.*, 2019; AYED; M'HIR; HAMDI, 2020).

Dentre as pesquisas atuais, destaca-se o desenvolvimento de bebidas de frutas incorporadas com bactérias probióticas (KAPRASOB *et al.*, 2018; MANTZOURANI *et al.*, 2018; YANG *et al.*, 2018). Estes microrganismos, além de fornecerem uma bebida funcional, melhoram a biodisponibilidade de minerais, a digestibilidade e as propriedades organolépticas

como cor, sabor e aroma (MARRERO *et al.*, 2019). No entanto, o ambiente ácido e a presença de fatores antinutricionais e inibitórios das frutas acaba se tornando um grande desafio para a manutenção da viabilidade e estabilidade das bactérias durante o processamento e armazenamento (MIN *et al.*, 2019).

Tendo isso em vista, a fermentação de bebidas de frutas a partir de BAA torna-se uma alternativa viável, uma vez que estas são capazes de oxidar diferentes substratos e são comumente encontradas em ambientes açucarados e de alta acidez.

Bebidas de vinagre de frutas, por exemplo, estão se tornando cada vez mais populares no mercado norte americano. Por definição, o produto deve ser elaborado por ao menos um tipo de fruta e conter no mínimo, 300 g do suco da fruta a cada litro de fermentado. As bebidas têm sido categorizadas de acordo com a concentração de ácido acético como: baixa acidez (< 3% v/v) e alta acidez (5 - 7% v/v) (NANDASIRI; RUPASINGHE, 2013). E o conteúdo de açúcares totais e sólidos solúveis, acidez titulável e a densidade podem depender do método de fermentação utilizado e da concentração de ácido acético (NANDASIRI; RUPASINGHE, 2013). Quanto aos seus benefícios, estudos *in vivo* realizados em animais indicaram que bebidas de vinagre de tomate podem ser consumidas como uma bebida funcional que previne a obesidade visceral e a resistência à insulina (SEO *et al.*, 2014), enquanto que, bebidas de vinagres de romã demonstraram reduzir o tecido adiposo visceral em humanos (PARK *et al.*, 2014). Outros autores também sugerem que bebidas de vinagres de frutas como cranberry, blueberry e tomate poderiam servir como alternativas no controle da hipertensão e hipecolesterolemia (NANDASIRI; RUPASINGHE, 2013). No entanto, Chang *et al.* (2020) adverte que o consumo contínuo de bebidas de vinagre deve ser controlado de modo a evitar injúrias no trato gastrointestinal.

Outra abordagem bastante recente tem sido focada na fermentação ácido glucônica. Apesar de trabalhos relacionados a este tipo de fermentação serem ainda escassos, seus resultados mostram-se bastante promissores. A fermentação glucônica de bebida de morango por *G. japonicus* transforma a glicose em ácido glucônico e mantém a frutose naturalmente presente no fruto como um adoçante. Isto permite que diabéticos consumam uma bebida que mantém os compostos fenólicos (não antocininas) e a atividade antioxidante praticamente inalteradas (ÁLVAREZ-FERNÁNDEZ *et al.*, 2014). Além disso, sua composição permanece estável durante 15 dias a temperatura ambiente (27−30 °C) e até 30 dias sob refrigeração (4 ºC) (ÁLVAREZ-FERNÁNDEZ *et al.*, 2016). Em outro estudo, Hornedo-Ortega *et al.* (2016)

compararam a atividade antioxidante e a composição de antocianinas de fermentado alcoólico e glucônico de bebida de morango. Os autores concluíram que a fermentação glucônica de bebidas de morango por *G. japonicus* é um processo inovador que preserva a composição de antocininas e mostra valores de atividade antioxidante maiores que do fermentado alcoólico (HORNEDO-ORTEGA *et al.*, 2016). Ainda, Ordóñez *et al.* (2015) comprovaram a segurança do fermentado glucônico de morango, mostrando que nenhuma das 8 aminas biogênicas pesquisadas foram detectadas.

Além dos compostos bioativos proveniente das frutas, há evidências de que o ácido glucônico e seus derivados possuem propriedades prebióticas. No cólon humano, este ácido favorece o crescimento de *Lactobacillus* sp. and *Bifidobacterium adolescentis* e altera o perfil metabólico no intestino (CAÑETE-RODRÍGUEZ *et al.*, 2016). GA e seus derivados são reconhecidos como seguros para uso em alimentos, e são comumente adicionados à produtos lácteos e refrigerantes para preservar e/ou melhorar suas propriedades sensoriais (CAÑETE-RODRÍGUEZ *et al.*, 2016). Em estudo conduzido por Li *et al.* (2022), por exemplo, a presença de maiores proporções de GA em Kombucha proporcionou uma melhoria no sabor da bebida. GA contribui para o sabor ácido agradável, enquanto o ácido acético formado produz um off-flavor ácido e adstringente.

3.9. EXOPOLISSACARÍDEOS PRODUZIDOS POR BAA

Os polissacarídeos microbianos são produzidos por uma ampla gama de bactérias, apresentando uma extrema diversidade em termos de estrutura química e composição (ROCA *et al.*, 2015). BAA, por exemplo, são capazes de produzir grandes quantidades de exopolissacarídeos, incluindo homopolissacarídeos como a levana e a celulose bacteriana (CB), assim como heteropolissacarídeos como a acetana ou xilanana e gluconacetana (BRANDT *et al.*, 2017; DANKE, 1993; KORNMANN *et al.*, 2004).

A CB é um EPS que tem chamado atenção devido às suas propriedades únicas derivadas de suas características físico-químicas, o que faz dela um polímero promissor em diversas áreas de aplicação. Na indústria de alimentos, a utilização mais popular da celulose é na fabricação da Nata de coco, uma sobremesa tradicional bastante consumida no sudeste asiático, produto da fermentação realizada pela bactéria *Komagataeibacter xylinus* (SHI *et al.*, 2014; ULLAH; SANTOS; KHAN, 2016). Além da área de alimentos, a celulose tem sido utilizada na área

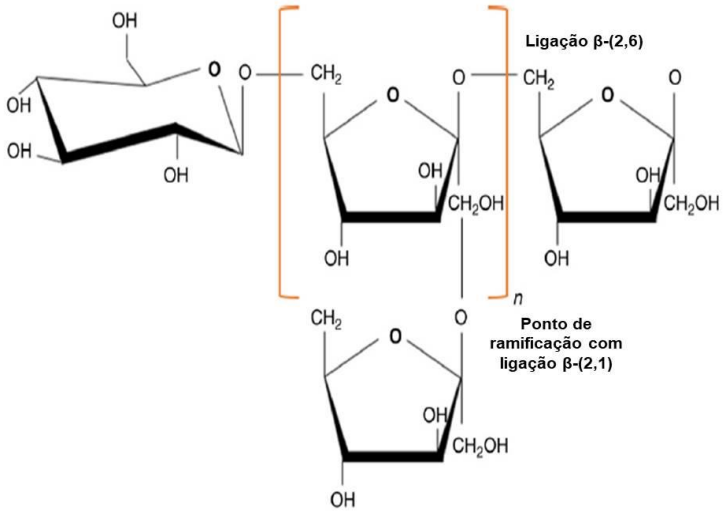
médica, farmacêutica, de cosméticos, em materiais de embalagens e fabricação de papéis (SHI *et al.*, 2014).

Por outro lado, pesquisas relacionadas à levana produzida por BAA são ainda bastante recentes, e, além disso, é sabido que este EPS também possui propriedades ímpares e que pode lançar uma série de oportunidades promissoras para vários setores industriais (ARAMSANGTIENCHAI *et al.*, 2020; JAKOB; STEGER; VOGEL, 2012; SEMJONOVS *et al.*, 2016; SRIKANTH *et al.*, 2015b; UA-ARAK; JAKOB; VOGEL, 2017a).

3.9.1. Levana

Levana é um polímero composto por resíduos de D-frutofuranosil unidos por ligações β-(2,6) na cadeia principal e ligações β-(2,1) na cadeia lateral, além de possuir um resíduo de D-glucopiranosil na extremidade de sua cadeia (MANTOVAN *et al.*, 2018) (Figura 4).

Figura 4 - Estrutura química da levana



Fonte: Modificado de Srikanth *et al.* (2015a)

Este EPS é comumente biossintetizado por um número restrito de plantas, mas pode ser produzido por vários microrganismos, que incluem Archaea, fungos e uma gama de bactérias (ÖNER; HERNÁNDEZ; COMBIE, 2016; SRIKANTH *et al.*, 2015a). Entre as BAA, espécies dos gêneros *Acetobacter*, *Gluconobacter*, *Gluconacetobacter*, *Kozakia* e *Neoasaia* também são

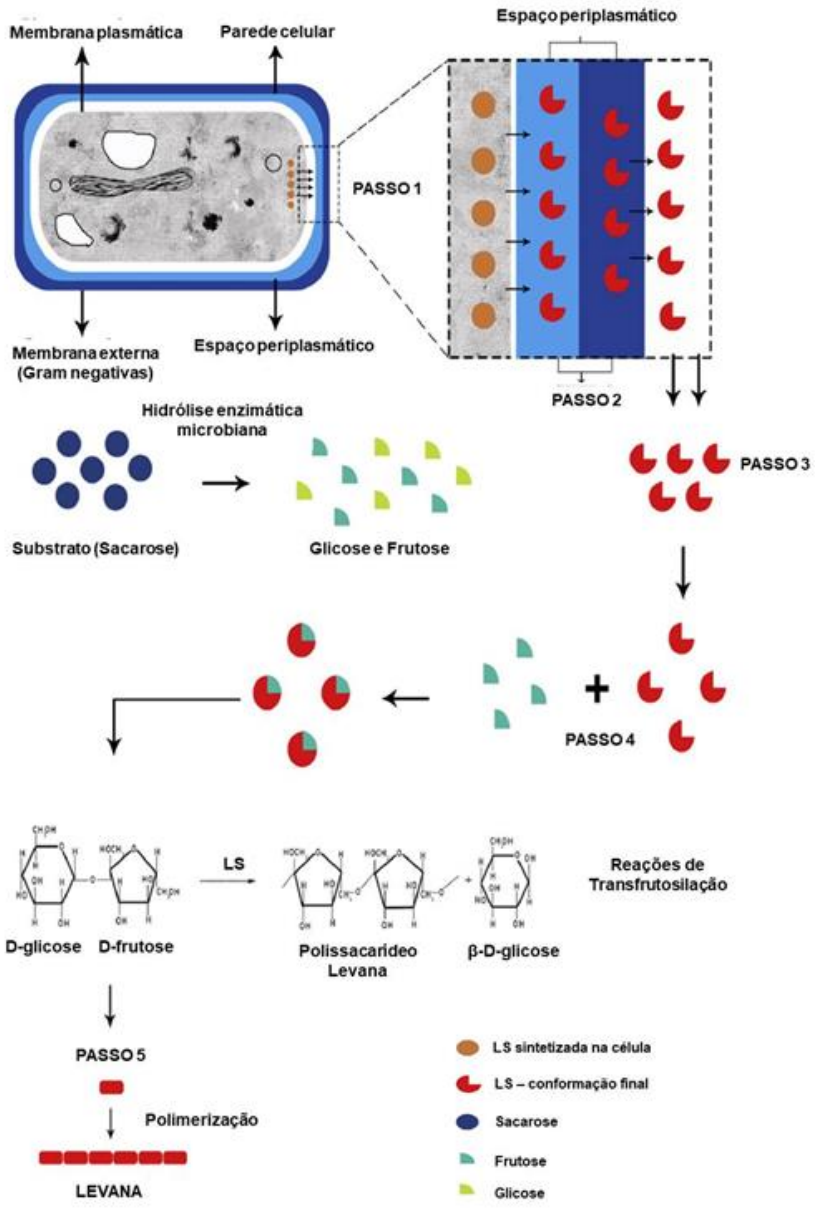
capazes de produzí-la (IDOGAWA *et al.*, 2014; JAKOB; STEGER; VOGEL, 2012; SEMJONOVS *et al.*, 2016; SRIKANTH *et al.*, 2015b; UA-ARAK; JAKOB; VOGEL, 2017a).

A síntese e polimerização da levana ocorre na matriz extracelular pela ação da enzima levanasacarase (LS), cuja principal função é transferir os resíduos de frutose da sacarose por meio de reações de transfrutosilação (SRIKANTH *et al.*, 2015a). A Figura 5 mostra o processo de biossíntese da levana a partir da sacarose.

Primeiramente, a LS é biossintetizada no citoplasma da célula (Passo 1). Em seguida, a enzima é acumulada no espaço periplasmático onde ganha sua conformação final (Passo 2) e é excretada no meio circundante (Passo 3) (GONZÁLEZ-GARCINUÑO *et al.*, 2018; SRIKANTH *et al.*, 2015a). Durante o 4º passo, a LS age sobre o substrato e a síntese de levana inicia-se por reações de transfrutosilação. Por último (Passo 5), a contínua adição de subunidades de frutose pela LS permite o aumento da cadeia polissacarídica de levana, assim finalizando a sua síntese (SRIKANTH *et al.*, 2015a).

A enzima tem alta especificidade sobre a sacarose e menor atividade sobre a frutose, manose, rafinose, manitol, etc. Por outro lado, a inibição é observada na presença de glicose e outros açúcares que possuem configuração semelhante à glicose, como lactose, galactose e maltose, além de outros álcoois de açúcar (HAN, 1990; SRIKANTH *et al.*, 2015a). Outra característica importante da LS é a sua atuação na clivagem de ligações β-(2,6) da própria cadeia de levana quando o substrato sacarose é totalmente metabolizado. Neste caso, a liberação consecutiva de frutose terminal da levana é somente cessada quando pontos de ramificação β-(2,1) de ligações frutose-frutosil presentes em sua estrutura parece impedir a extensa hidrólise da cadeia pela própria enzima sintetizadora (MÉNDEZ-LORENZO *et al.*, 2015; ÖNER; HERNÁNDEZ; COMBIE, 2016).

Figura 5 - Representação esquemática da biossíntese da levana



Fonte: Adaptado de Srikanth et al. (2015a).

3.9.1.1. Estrutura e propriedades

Em geral, levanas sintetizadas por diferentes organismos apresentam similaridade na estrutura, porém diferem em relação à massa molecular e em graus de polimerização (GP) e ramificação (HAN, 1990; MUTANDA *et al.*, 2014; PARK; KHAN, 2009). Levanas bacterianas possuem massa molecular e GP maiores que a de plantas. Isto é atribuído às múltiplas ramificações presentes em sua estrutura (2 % a 12 %), o que faz com que a sua massa molecular

varie de 2 a 100 milhões de Dalton (Da) com GP >100, enquanto que em levanas de plantas, a faixa se apresenta entre 2000 a 33000 Da e o GP < 100 (MUTANDA *et al.*, 2014; PARK; KHAN, 2009; STOJKOVIĆ *et al.*, 2015).

A característica estrutural da levana, em forma de anéis de furanose, também desempenha um importante papel na conformação das moléculas em solução, na qual lhe proporciona uma adicional flexibilidade quando comparada a polissacarídeos formados por anéis de piranose. Além disso, a cadeia semi-flexível dos anéis interage intra- e intermolecularmente, resultando em uma estrutura esférica densamente empacotada e fornece soluções aquosas de baixa viscosidade (temperatura ambiente) em concentrações em que para outros polissacarídeos seriam formados pastas ou géis (BENIGAR *et al.*, 2015; COMBIE, 2006; HAN W. YOUN, 1990; MANANDHAR; VIDHATE; D'SOUZA, 2009; SRIKANTH *et al.*, 2015a; STOJKOVIĆ *et al.*, 2015).

Xu *et al.* (2018) ao estudarem as propriedades reológicas da levana, verificaram que soluções aquosas de levana (*Brenneria* sp. EniD312) apresentam comportamento de fluido Newtoniano em baixas concentrações (3 %; m/V) e não-Newtoniano (fluido pseudoplástico) em altas concentrações (6, 9 e 12 %; m/V). Resultados semelhantes foram reportados para soluções de levana derivadas de *Zymomonas mobilis* e *Erwinia herbicola* (BENIGAR *et al.*, 2014). No entanto, para soluções de levanas de *Bacillus subtilis,* o comportamento mostrou-se totalmente Newtoniano em concentrações entre 1 – 8 % (BENIGAR *et al.*, 2014)(BENIGAR et al., 2014). De acordo com Xu *et al.* (2018), a levana poderia ser um bom aditivo na indústria de alimentos, uma vez que seu comportamento não-Newtoniano é bastante interessante para a fabricação de produtos lácteos, xaropes e molhos de saladas.

Soluções de levana também são caracterizadas por exibir um comportamento bastante atípico quando comparados a outros polissacarídeos, na qual a formação de gel não é observada (MAJEE; AVLANI; BISWAS, 2017). Porém, Jakob *et al.* (2013) ao estabelecerem a relação estrutura/função de levanas isoladas de BAA, sugeriram que em solução, o aumento do seu peso molecular reforça as interações intramoleculares de modo a alcançar uma estrutura mais compacta característica de um "microgel" com propriedades hidrocoloides. Os autores ainda reforçam que levanas produzidas por BAA podem, assim, oferecer novas possibilidades para aplicações em alimentos.

Ao contrário de muitos outros polímeros, levana não é entumescida em água, porém apresenta alta solubilidade em água quente, variada solubilidade em água fria e é insolúvel na

maioria dos solventes orgânicos, sendo o dimetil sulfóxido (DMSO) uma exceção. A alta solubilidade da levana em água é atribuída principalmente à sua ligação β-(2,6) do que para a ligação β-(2,1), e as ramificaçãoes poderiam ser somente um fator suporte (HAN W. YOUN, 1990; MANANDHAR; VIDHATE; D'SOUZA, 2009; SRIKANTH *et al.*, 2015a). Levanas são agentes não-redutores, não são hidrolisadas por ação de invertases e amilases de leveduras, mas são bastante suscetíveis à hidrólise ácida (HAN, 1990). Sua decomposição acontece em torno de 225 °C e a temperatura de transição vítrea encontra-se a 141 °C (MANANDHAR; VIDHATE; D'SOUZA, 2009).

Outra propriedade importante da levana é a sua força adesiva. Embora os açúcares sejam caracterizados pela pegajosidade, a força adesiva de levana é significativamente maior que a de outros polímeros naturais. As ramificações colaboram para a sua força coesiva e a capacidade de formar ligações adesivas com uma ampla variedade de substratos é dada ao seu grande número de hidroxilas. Comumente, levana é referida como um adesivo "verde", uma vez que é facilmente removida com água e está associada às aplicações de alto valor em indústrias médicas, de alimentos, farmacêuticas e químicas (COMBIE, 2006; GOMES *et al.*, 2018; SRIKANTH *et al.*, 2015a).

3.9.1.2. Ferramentas para a produção de levana

Devido à múltipla aplicabilidade da levana, este polímero tem despertado a atenção de pesquisadores para a sua produção em grande escala. Diferentes estratégias visando o aumento do seu rendimento por variados microrganismos e em condições específicas tem sido proposto, inclusive pelo uso de ferramentas estatísticas.

Métodos estatísticos, além de utilizarem eficientemente os dados experimentais, reduzem significativamente o número e o tempo necessário para os experimentos, o que consequentemente diminui também os custos de desenvolvimento e produção (KEKEZ *et al.*, 2015). Atualmente, a técnica estatística multivariada e sua modelagem têm sido aplicada com sucesso em estudos científicos, uma vez que é possível estudar o efeito de vários fatores e as interações que levam à otimização do processo (LIU *et al.*, 2010; MELO *et al.*, 2007).

A Metodologia de Superfície de Resposta (MSR), por exemplo, é uma coleção de técnicas estatísticas e matemáticas para modelagem e análise de problemas em que a variável resposta é influenciada por vários fatores. Dessa forma, os fatores que influenciam no processo

podem ser otimizados de acordo com as especificações do produto final. A superfície de resposta pode ser expressa como:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=1}^{k} \beta_{ij} x_i x_j$$

Onde *Y* é a resposta (variável dependente), x_i e x_j são as variáveis independentes, e β_0, β_i, β_{ii} e β_{ij} são os coeficientes de regressão das variáveis para os termos do intercepto, linear, quadrático e de interação, respectivamente (MYERS; MONTGOMERY; ANDERSON-COOK, 2016).

Na biotecnologia, por exemplo, a MSR tem sido extensivamente utilizada para a otimização da composição de meios de cultivo e parâmetros de processo essenciais para a produção de vários metabólitos, inclusive para a levana (BOUALLEGUE *et al.*, 2020; SILBIR *et al.*, 2014).

Jathore *et al.*(2012), com o objetivo de otimizar a produção de levana a partir de *Pseudomonas fluorescens* utilizaram primeiramente o delineamento de Plackett-Burman para a determinação dos fatores mais relevantes e em seguida, o delineamento central composto rotacional (DCCR). Por meio da otimização das variáveis mais importantes (sacarose, NH_4Cl, $NaNO_3$ e peptona de caseína), os autores obtiveram um aumento significativo de 5,27 para 15,42 g/L na produção de levana. Similarmente, Silbir *et al.* (2014) otimizaram a produção da levana por *Zymomonas mobilis* B-14023 em sistemas de batelada e alcançaram uma produção máxima de 40,2 g/L, na qual os níveis ótimos das variáveis foram: 299,1 g/L de concentração inicial de sacarose, 42,3 h de tempo de incubação e pH inicial 6,0.

Para a otimização da produção de levana pela bactéria do ácido acético *Acetobacter xylinum* NCIM 2526, cinco variáveis foram consideradas para o método DCCR (pH inicial, porcentagem de inóculo, concentração de sacarose, concentração de peptona bacteriológica e concentração inicial de levana como indutor). Neste trabalho, após otimização, Srikanth *et al.* (2015b) obtiveram um aumento significativo de 0,54 para 13,25 g/L na produção de levana.

Semjonovs *et al.* (2017) aplicaram a MSR como ferramenta para a otimização das condições de produção simultânea de FOS e levana por outra bactéria do ácido acético, *Gluconobacter nephelii* P1464. Empregando-se os delineamentos fatoriais Plackett-Burman e Box-Behnken, o modelo de regressão para a síntese de frutanas mostrou uma produção global

de 31,66 g/L com 208,63 g/L de sacarose, 11,13 g/L de extrato de levedura e 298 rpm de velocidade de agitação.

Após uma pré-seleção dos fatores que influenciavam a produção de levana por *Bacillus licheniformis* NS032 pelo método de um fator por vez, Kekez *et al.* (2015) consideraram três fatores (sacarose, cloreto de amônio e pH inicial do meio) a serem utilizados no delineamento Box-Behnken. O estudo comparou a produção de levana em dois sistemas com níveis diferentes de sacarose, designados como baixa sacarose (60–200 g/L) e alta sacarose (300–400 g/L). De acordo com o modelo, em sistemas de baixa sacarose, a máxima produção predita de levana foi de 47,8 g/L (sacarose 196,8 g/L, cloreto de amônio 2,4 g/L, pH 7,0), enquanto em sistemas de alta sacarose, a produção alcançada foi de 99,2 g/L (sacarose 397,6 g/L, cloreto de amônio 4,6 g/L, pH 7,4). Em ambos os sistemas, os autores afirmaram que o pH inicial do meio exerceu um papel decisivo na produção do EPS.

Além dos trabalhos acima citados, vários outros trabalhos reportam um aumento significativo na produção da levana utilizando diferentes microrganismos e parâmetros de processo (Tabela 3). Deste modo, a MSR demonstra ser uma ferramenta estatística valiosa para a produção da levana em grande escala e possibilita o seu emprego em diversas áreas de aplicação.

Tabela 3. Estratégias para otimização da produção de levana por Metodologia de Superfície de Resposta (MSR).

Microrganismo	**Delineamento experimental**	**Condições ótimas**	**Produção/Produtividade de levana**	**Referência**
Zymomonas mobilis	Fatorial completo	Conc. Sacarose: 150 g/L Fermentação em batelada	40,14 g/L (m/v)	Borsari *et al.* (2006)
Paenibacillus polymyxa EJS-3	Fatorial Fracionado e CCR	Conc. Sacarose: 188,2 g/L Extrato de levedura: 25,8 g/L $CaCl_2$: 0,34 g/L	35,26 g/L (m/v)	Liu *et al.* (2010)
Bacillus subtilis Natto	Fatorial completo	Conc. Sacarose: 400 g/L Tempo de cultivo: 16 h	111,6 g/L (m/v)	Dos Santos *et al.*(2013)
Bacillus subtilis Natto	Box-Behnken	Temperatura: 39.5°C Tempo de cultivo: 21 h pH: 8,0	5.82 g/L.h	De Melo *et al.* (2015)
Bacillus licheniformis ANT 179	CCR	Caldo de cana: 20 % (v/v) Peptona de caseína: 2 % (m/v)	50,25 g/L (m/v)	Xavier; Ramana (2017)
Bacillus subtilis B58	Fatorial Fracionado e Box-Behnken	Conc. Sacarose: 188,2 g/L Extrato de levedura: 25,8 g/L K_2HPO_4: 5,0 g/L	26,65 g/L (m/v)	Hamid *et al.* (2018)
Bacillus subtilis AF17	Plackett-Burman e Box-Behnken	Conc. Sacarose: 162,5 g/L Triptona: 10 g/L pH: 7,0	7,9 g/L (m/v)	Bouallegue *et al.* (2020)
Leuconostoc citreum BD1707	Box-Behnken	Conc. Sacarose: 172 g/L Temperatura: 26 ºC Tempo de cultivo: 112 h pH: 6,12	34,86 g/L (m/v)	Han *et al.* (2021)

3.9.1.3. Aplicações da levana

3.9.1.3.1. *Aplicações da levana na área médica/farmacêutica*

Exopolissacarídeos são polímeros conhecidos por possuir uma ampla gama de atividades biológicas. Tais atividades estão diretamente relacionadas à composição de monômeros, peso molecular, grau de ramificação, assim como da presença de grupos funcionais em sua estrutura (SARILMISER; ONER, 2014).

A levana é uma das macromoléculas que tem demonstrado uma ampla gama de atividades biológicas, dentre as quais podemos citar: anti-inflamatória (SRIKANTH *et al.*, 2015b), antidiabética (DAHECH *et al.*, 2011), hipocolesterolêmica (BELGHITH *et al.*, 2012), imunoestimuladora (TAYLAN; YILMAZ; DERTLI, 2019), atividade antitumoral (ESAWY *et al.*, 2013), atividade antimicrobiana (AĞÇELI; CIHANGIR, 2020; MUMMALETI *et al.*, 2022), antioxidante (MUMMALETI *et al.*, 2022; VU *et al.*, 2021) e antibiofilme (ABID *et al.*, 2018; TILWANI *et al.*, 2021).

A atividade antitumoral da levana, por exemplo, tem sido avaliada em diversas linhagens celulares (ABDEL-FATTAH *et al.*, 2012; KAZAK SARILMISER; TOKSOY ONER, 2014), que variam desde aquelas que mais causam mortes no mundo, como as do pulmão (OPAS, 2020), até aquelas que mais afetam as crianças, como as de neuroblastoma humano (ESCOBAR *et al.*, 2019). Alguns estudos reportam que a efetividade da levana neste tipo de tratamento depende de uma massa molecular específica, que na maioria das vezes parece envolver aquelas de maior MM (CALAZANS *et al.*, 2000; YOO *et al.*, 2004) ou pode ser influenciada pelo seu grau de ramificação (YOON *et al.*, 2004). Além disso, a dose aplicada, o tempo de ação e o tipo de células tumorais-alvo podem influenciar em sua toxicicidade (DAHECH *et al.*, 2012; ESAWY *et al.*, 2013; KAZAK SARILMISER; TOKSOY ONER, 2014; VIEIRA *et al.*, 2021).

A síntese de derivados da levana também vem trazendo oportunidades para o desenvolvimento de novos agentes terapêuticos Esses derivados exibem uma potente atividade inibitória contra diferentes células tumorais quando comparados à sua forma nativa (não modificada) (ABDEL-FATTAH *et al.*, 2012; SARILMISER; ONER, 2014; LIU *et al.*, 2012). Este aumento na bioatividade das moléculas é principalmente atribuído à introdução dos grupos na levana, que aumentam a habilidade de doação de elétrons e a afinidade com receptores nas células do sistema imunológico (ABDEL-FATTAH *et al.*, 2012; LIU *et al.*, 2012).

Outra característica importante da levana é a sua habilidade de captura de radicais livres, demonstrando assim, uma alta atividade antioxidante. A levana a partir da bactéria do ácido acético *A. xylinum* NCIM2526, por exemplo, exibiu 100 % de atividade de captura sob radicais DPPH na concentração de 1,25 mg/mL (SRIKANTH *et al.*, 2015b). Resultados similares foram reportados por levana a partir de *Bacillus subtilis,* na qual, doses de 1,2 mg/mL já foram suficientes para inibir totalmente os radicais (MUMMALETI *et al.*, 2022). Em contrapartida, para levanas de *Bacillus velezensis* VTX20 (VU *et al.*, 2021) e *Bacillus megaterium* PFY-147 (PEI *et al.*, 2020) foram necessárias concentrações superiores de 4 mg/mL e 5 mg/mL para que 64.0 ± 4.0% e 94.78 ± 1.57% do radical DPPH fosse capturado, respectivamente.

Além do radical DPPH, estudos com outros radicais livres como os radicais OH (•OH), ânion superóxido (O^{2-}) e o cátion 2,20-azino-bis (ácido 3-etilbenzotiazolina-6-sulfônico) ($ABTS^{+}$) foram avaliados por Pei *et al.* (2020), Huang *et al.* (2020), Liu *et al.*(2012) e Liu *et al.* (2009). Apesar de todos os trabalhos terem demonstrado a efetividade da levana em inibir esses radicais, destaca-se aqui os estudos de Pei *et al.* (2020) e (LIU *et al.*, 2009), na qual inibições de 79.29 ± 1.04 % (PEI *et al.*, 2020) e 87.58 % (Levana bruta), 76.73 % (Fração 1-EPS-1) e 68.55% (Fração 2 – EPS-2) (LIU *et al.*, 2009) do •OH foram observadas com doses de 5 mg/mL e 1 mg/mL, respectivamente. Estes resultados demonstram que a levana é um EPS promissor no combate aos radicais livres, principalmente pelo fato de exibir alta atividade contra este radical (•OH), que é um dos radicais reativos mais conhecidos e que podem atacar e causar danos em quase todas as biomacromoléculas de células vivas (LIU *et al.*, 2012). De acordo com Kim e Chung (2016) e Hertadi, Amari e Ratnaningsih (2020), a atividade antioxidante da levana pode ser atribuída à presença dos vários grupos funcionais hidroxila, que podem reagir com os radicais livres e cessar as reações em cadeia dos radicais.

O efeito antimicrobiano da levana contra vários patógenos de origem alimentar (*Listeria monocytogenes, Salmonella abony, Staphylococcus aureus* e *Escherichia coli*) e deteriorantes (*Pseudomonas aeruginosa*) também tem sido reportado recentemente por Ağçeli e Cihangir (2020) e Mummaleti *et al.* (2022). Utilizando o método de disco-difusão em ágar, para ambos os estudos, a máxima zona de inibição foi mais pronunciada para *Escherichia coli* utilizando-se doses de 1000 µg/mL (16 mm) (AĞÇELI; CIHANGIR, 2020) e 200 µg/mL (16,17 mm) (MUMMALETI *et al.*, 2022). Este efeito inibitório da levana foi

atribuído ao estresse osmótico e redução da atividade da água, assim como pela obstrução da entrada de nutrientes pelo EPS (LI *et al.*, 2018; MUMMALETI *et al.*, 2022).

Ademais, a atividade antibiofime da levana na formação de biofilme de vários patógenos como *Salmonella*, *Vibrio*, *Escherichia coli* e *Enterococcu*s também tem mostrado resultados promissores, abrindo novas possibilidades para sua aplicação em ambientes de processamento de alimentos (ABID *et al.*, 2018; TILWANI *et al.*, 2021).

3.9.1.3.2.*Aplicações da levana em alimentos*

Levana tem grande potencial para aplicações na indústria de alimentos, podendo ser utilizado como prebiótico, emulsificante, estabilizante, agente de cor, sabor e textura, substituto de gordura, como material de cobertura, fonte de frutose, filmes para embalagem e agente encapsulante (DAĞBAĞLI; GÖKSUNGUR, 2017; ÖNER; HERNÁNDEZ; COMBIE, 2016).

Os variados aspectos do papel dos FOS-L e levana como prebióticos sobre bactérias probióticas e na complexa microbiota intestinal têm sido analisados em diversos estudos, apesar de não haver evidências conclusivas de testes em humanos (ADAMBERG *et al.*, 2015, 2014; LI; KIM, 2013; PORRAS-DOMÍNGUEZ *et al.*, 2014; ZHAO; WANG; KIM, 2013a, 2013b).

Em modelos animais, por exemplo, dietas suplementadas com levana mostraram um aumento na viabilidade de *Lactobacillus* e *Bifidobacteria* e inibição de *Escherichia coli* e *Clostridium perfringers* (ZHAO; WANG; KIM, 2013a, 2013b). Em outra abordagem, Adamberg *et al.* (2015) utilizaram a microbiota fecal humana e por meio da microcalorimetria isotérmica verificaram que a levana altera a composição da microbiota fecal e o perfil de metabólitos, podendo ser considerada uma potencial candidata à prebiótico. Utilizando o sequenciamento metagenômico para avaliação da atividade prebiótica da levana, Cheng *et al.* (2021) também verificaram alterações na microbiota intestinal de camundongos e a estimulação da produção de ácidos graxos de cadeia curta.

Na panificação, os EPS são conhecidos por melhorar as propriedades reológicas da massa e a textura, o valor nutricional, a vida de prateleira e maquinabilidade do trigo, centeio e de pães sem glúten (HERMANN; PETERMEIER; VOGEL, 2015). Jakob, Steger e Vogel (2012) avaliaram os efeitos funcionais de diferentes levanas produzidas por BAA (*G. frateurii* TMW 2.767, *G. cerinus* DSM 9533, *N. chiangmaiensis* NBRC 101099, e *K. baliensis* DSM

14400) em pães a base de trigo. Os autores verificaram que a adição de duas dosagens do EPS (1 e 2 % p/p de farinha) proporcionou um aumento do volume, um nítido amolecimento dos pães frescos, e além disso, durante o armazenamento de uma semana observou-se um retardamento do endurecimento dos pães.

Apesar de BAL e leveduras serem microrganismos típicos em massa lêveda ("sourdoughs") (UA-ARAK; JAKOB; VOGEL, 2017a), estudos realizados por Hermann, Petermeier e Vogel (2015) verificaram que *N. chiangmaiensis* NBRC 101099 e *K. baliensis* DSM 14400 possuem a habilidade de crescer em diferentes farinhas (trigo, trigo integral, espelta e centeio) e produzir altas quantidades de levana *in situ* a partir da sacarose. Mais tarde, a massa lêveda de trigo sarraceno e melaço, fermentadas pelas BAA *G. albidus* TMW 2.1191e *K. baliensis* NBRC 16680, foi empregada no preparo de pães sem glúten, e estes avaliados em relação ao volume, dureza do miolo e às características sensoriais (UA-ARAK; JAKOB; VOGEL, 2017b). Os pães obtidos a partir da massa apresentaram melhoria nos aspectos sensoriais e na qualidade, incluindo maior volume específico e menor dureza do miolo. No entanto, os autores salientaram que a forte acidificação durante a fermentação poderia se tornar um desafio em produções de grande escala (UA-ARAK; JAKOB; VOGEL, 2017b)

Outras vantagens da aplicação de levana em alimentos incluem seu uso como substituto de gordura. Frutanas possuem propriedades suaves semelhantes à gordura que melhoram o paladar, o sabor e espalhabilidade dos produtos lácteos. Além disso, levanas de alto peso molecular são dificilmente detectadas pelos sensores gustativos e a detecção de odores é quase imperceptível devido à sua baixa volatilidade (ÖNER; HERNÁNDEZ; COMBIE, 2016). Sua baixa viscosidade e alta solubilidade fazem dela um ótimo substituto da goma arábica para uma grande variedade de alimentos, visto que esta última também apresenta excelentes propriedades estabilizantes e emulsificantes (HAN, 1990). No setor de embalagens, a adição de levana em filmes a base de amido aumentou suas propriedades mecânicas e de barreira, além de se uma alternativa econômica (MANTOVAN *et al.*, 2018). Gan *et al.* (2022) também desenvolveram filmes comestíveis a base de levan/pullulan/quitosana enriquecidos com ε-polilisina e aplicaram em morangos. Como resultado, eles demonstraram que os filmes podem ajudar a preservar a qualidade pós-colheita do morango, minimizando a perda de água, inibindo o desenvolvimento microbiano e diminuindo a taxa de respiração durante o armazenamento.

REFERÊNCIAS

ABDEL-FATTAH, A. M.; GAMAL-ELDEEN, A. M.; HELMY, W. A.; ESAWY, M. A. Antitumor and antioxidant activities of levan and its derivative from the isolate *Bacillus subtilis* NRC1aza. **Carbohydrate Polymers**, v. 89, n. 2, p. 314–322, 2012.

ABID, Y.; CASILLO, A.; GHARSALLAH, H.; JOULAK, I.; LANZETTA, R.; CORSARO, M. M.; ATTIA, H.; AZABOU, S. Production and structural characterization of exopolysaccharides from newly isolated probiotic lactic acid bacteria. **International Journal of Biological Macromolecules**, v. 108, p. 719–728, 2018.

ADACHI, O.; YAKUSHI, T. Membrane-Bound Dehydrogenases of Acetic Acid Bacteria. *In*: **Acetic Acid Bacteria Ecology and Physiology**. [s.l.] Springer Nature, 2016. p. 273–298.

ADAMBERG, K.; TOMSON, K.; TALVE, T.; PUDOVA, K.; PUURAND, M.; VISNAPUU, T.; ALAMÄE, T.; ADAMBERG, S. Levan enhances associated growth of *Bacteroides, Escherichia, Streptococcus* and *Faecalibacterium* in fecal microbiota. **PLoS ONE**, v. 10, n. 12, p. 1–18, 2015.

ADAMBERG, S.; TOMSON, K.; VIJA, H.; PUURAND, M.; KABANOVA, N.; VISNAPUU, T.; JÕGI, E.; ALAMÄE, T.; ADAMBERG, K. Degradation of fructans and production of propionic acid by bacteroides thetaiotaomicron are enhanced by the shortage of amino acids. **Frontiers in Nutrition**, v. 1, n. December, p. 1–10, 2014.

ÁLVAREZ-FERNÁNDEZ, M. A.; HORNEDO-ORTEGA, R.; CEREZO, A. B.; TRONCOSO, A. M.; GARCÍA-PARRILLA, M. C. Effects of the strawberry (*Fragaria ananassa*) purée elaboration process on non-anthocyanin phenolic composition and antioxidant activity. **Food Chemistry**, v. 164, p. 104–112, 2014.

ÁLVAREZ-FERNÁNDEZ, M. A.; HORNEDO-ORTEGA, R.; CEREZO, A. B.; TRONCOSO, A. M.; GARCÍA-PARRILLA, M. C. Determination of nonanthocyanin phenolic compounds using high-resolution mass spectrometry (UHPLC-Orbitrap-MS/MS) and Impact of storage conditions in a beverage made from strawberry by fermentation. **Journal of Agricultural and Food Chemistry**, v. 64, n. 6, p. 1367–1376, 2016.

ARAMSANGTIENCHAI, P.; KONGMON, T.; PECHROJ, S.; SRISOOK, K. Enhanced production and immunomodulatory activity of levan from the acetic acid bacterium, *Tanticharoenia sakaeratensis.* **International Journal of Biological Macromolecules**, v. 163, p. 574–581, 2020.

AYED, L.; M'HIR, S.; HAMDI, M. Microbiological, biochemical, and functional aspects of fermented vegetable and fruit beverages. **Journal of Chemistry**, v. 2020, n. Table 1, 2020.

BAEK, J. H.; KIM, K. H.; MOON, J. Y.; YEO, S. H.; JEON, C. O. *Acetobacter oryzoeni* sp. Nov., isolated from korean rice wine vinegar. **International Journal of Systematic and Evolutionary Microbiology**, v. 70, n. 3, p. 2026–2033, 2020.

BARTOWSKY, E. J.; HENSCHKE, P. A. Acetic acid bacteria spoilage of bottled red wine-A review. **International Journal of Food Microbiology**, v. 125, n. 1, p. 60–70, 2008.

BARTOWSKY, E. J.; XIA, D.; GIBSON, R. L.; FLEET, G. H.; HENSCHKE, P. A. Spoilage of bottled red wine by acetic acid bacteria. **Letters in Applied Microbiology**, v. 36, n. 5, p. 307–314, 2003.

BASSENE, H.; NIANG, E. H. A.; FENOLLAR, F.; DOUCOURE, S.; FAYE, O.; RAOULT, D.; SOKHNA, C.; MEDIANNIKOV, O. Role of plants in the transmission of *Asaia* sp., which potentially inhibit the *Plasmodium* sporogenic cycle in *Anopheles* mosquitoes. **Scientific Reports**, v.

10, n. 1, p. 1–10, 2020.

BELGHITH, K. S.; DAHECH, I.; HAMDEN, K.; FEKI, A.; MEJDOUB, H.; BELGHITH, H. Hypolipidemic effect of diet supplementation with bacterial levan in cholesterol-fed rats. **International Journal of Biological Macromolecules**, v. 50, n. 4, p. 1070–1074, 2012.

BENIGAR, E.; DOGSA, I.; STOPAR, D.; JAMNIK, A.; CIGIĆ, I. K.; TOMŠIČ, M. Structure and dynamics of a polysaccharide matrix: Aqueous solutions of bacterial levan. **Langmuir**, v. 30, n. 14, p. 4172–4182, 2014.

BENIGAR, E.; TOMŠIČ, M.; SRETENOVIC, S.; STOPAR, D.; JAMNIK, A.; DOGSA, I. Evaluating SAXS results on aqueous solutions of various bacterial Levan utilizing the string-of-beads model. **Acta Chimica Slovenica**, v. 62, n. 3, p. 509–517, 2015.

BORSARI, R. R. J.; CELLIGOI, M. A. P. C.; BUZATO, J. B.; DA SILVA, R. S. D. S. F. Influence of carbon source and the fermentation process on levan production by *Zymomonas mobilis* analyzed by the surface response method. **Ciencia e Tecnologia de Alimentos**, v. 26, n. 3, p. 604–609, 2006.

BOUALLEGUE, A.; CASILLO, A.; CHAARI, F.; CIMINI, D.; CORSARO, M. M.; BACHOUAL, R.; ELLOUZ-CHAABOUNI, S. Statistical optimization of levan: Influence of the parameter on levan structure and angiotensin I-converting enzyme inhibitory. **International Journal of Biological Macromolecules**, v. 158, p. 945–952, 2020.

BRANDT, J. U.; BORN, F. L.; JAKOB, F.; VOGEL, R. F. Environmentally triggered genomic plasticity and capsular polysaccharide formation are involved in increased ethanol and acetic acid tolerance in *Kozakia baliensis* NBRC 16680. **BMC Microbiology**, v. 17, n. 1, p. 1–12, 2017.

BRASIL, MINISTÉRIO DA AGRICULTURA, P. e A. Instrução normativa nº 6 de abril de 2012. Brasília, DF, BRASIL. **Diário Oficial da República Federativa do Brasil**, 2012.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Decreto nº 6.871, de 04 de junho de 2009. Regulamenta a Lei nº 8.918, de 14 de julho de 1994, dispõe sobre a padronização, a classificação, o registro, a inspeção, a produção e a fiscalização de bebidas. Brasília, DF, BRASIL. **Diário Oficial da República Federativa do Brasil**, 2012.

BUDAK, N. H.; AYKIN, E.; SEYDIM, A. C.; GREENE, A. K.; GUZEL-SEYDIM, Z. B. Functional Properties of Vinegar. **Journal of Food Science**, v. 79, n. 5, 2014.

BUENO, R. S.; RESSUTTE, J. B.; HATA, N. N. Y.; HENRIQUE-BANA, F. C.; GUERGOLETTO, K. B.; DE OLIVEIRA, A. G.; SPINOSA, W. A. Quality and shelf life assessment of a new beverage produced from water kefir grains and red pitaya. **Lwt**, v. 140, n. December 2020, 2021.

CALAZANS, G. M. T.; LIMA, R. C.; DE FRANÇA, F. P.; LOPES, C. E. Molecular weight and antitumour activity of *Zymomonas mobilis* levans. **International Journal of Biological Macromolecules**, v. 27, n. 4, p. 245–247, 2000.

CAÑETE-RODRÍGUEZ, A. M.; SANTOS-DUEÑAS, I. M.; JIMÉNEZ-HORNERO, J. E.; EHRENREICH, A.; LIEBL, W.; GARCÍA-GARCÍA, I. Gluconic acid: Properties, production methods and applications—An excellent opportunity for agro-industrial by-products and waste bio-valorization. **Process Biochemistry**, v. 51, n. 12, p. 1891–1903, 2016.

CANUTI, V.; CONVERSANO, M.; CALZI, M. L.; HEYMANN, H.; MATTHEWS, M. A.; EBELER, S. E. Headspace solid-phase microextraction-gas chromatography-mass spectrometry for profiling free volatile compounds in *Cabernet Sauvignon* grapes and wines. **Journal of Chromatography A**, v. 1216, n. 15, p. 3012–3022, 2009.

CARR, J. G.; PASSMORE, S. M. Methods for identifying acetic acid bacteria. *In*: SKINNER, F. A.; LOVELOCK, D. W. **Identification Methods for Microbiologists**. [s.l: s.n.]p. 33–47.

CHANG, J.; HAN, S. E.; PAIK, S. S.; KIM, Y. J. Corrosive esophageal injury due to a commercial vinegar beverage in an adolescent. **Clinical Endoscopy**, v. 53, n. 3, p. 366–369, 2020.

CHASE, J. M.; HOLLAND, S. M.; GREENBERG, D. E.; MARSHALL-BATTY, K.; ZELAZNY, A. M.; CHURCH, J. A. *Acidomonas methanolica*-associated necrotizing lymphadenitis in a patient with chronic granulomatous disease. **Journal of Clinical Immunology**, v. 32, n. 6, p. 1193–1196, 2012.

CHENG, R.; CHENG, L.; ZHAO, Y.; WANG, L.; WANG, S.; ZHANG, J. Biosynthesis and prebiotic activity of a linear levan from a new *Paenibacillus* isolate. **Applied Microbiology and Biotechnology**, v. 105, n. 2, p. 769–787, 2021.

CIRIGLIANO, M. C. A Selective Medium for the Isolation and Differentiation of *Gluconobacter* and *Acetobacter*. **Journal of Food Science**, v. 47, n. 3, p. 1038–1039, 1982.

CLEENWERCK, I.; DE VOS, P. Polyphasic taxonomy of acetic acid bacteria: An overview of the currently applied methodology. **International Journal of Food Microbiology**, v. 125, n. 1, p. 2–14, 2008.

COMBIE, J. Properties of levan and potential medical uses. **ACS Symposium Series**, v. 934, p. 263–269, 2006.

COTON, M.; PAWTOWSKI, A.; TAMINIAU, B.; BURGAUD, G.; DENIEL, F.; COULLOUMME-LABARTHE, L.; FALL, A.; DAUBE, G.; COTON, E. Unraveling microbial ecology of industrial-scale Kombucha fermentations by metabarcoding and culture-based methods. **FEMS Microbiology Ecology**, v. 93, n. 5, p. 1–16, 2017.

DAĞBAĞLI, S.; GÖKSUNGUR, Y. Exopolysaccharide production of acetic acid bacteria. *In*: SENGUN, I. Y. **Acetic Acid Bacteria: Fundamentals and Food Applications**. [s.l.] CRC Press. Taylor & Francis Group, 2017. p. 120–141.

DAHECH, I.; BELGHITH, K. S.; BELGHITH, H.; MEJDOUB, H. Partial purification of a *Bacillus licheniformis* levansucrase producing levan with antitumor activity. **International Journal of Biological Macromolecules**, v. 51, n. 3, p. 329–335, 2012. Disponível em:

DAHECH, I.; BELGHITH, K. S.; HAMDEN, K.; FEKI, A.; BELGHITH, H.; MEJDOUB, H. Antidiabetic activity of levan polysaccharide in alloxan-induced diabetic rats. **International Journal of Biological Macromolecules**, v. 49, n. 4, p. 742–746, 2011. Disponível em:

DANKE, C. E. S. M. A. In vitro biosynthesis of acetan using electroporated *Acetobactev xylinum* cells as enzyme preparations. **Journal of General Microbiology**, v. 193, n. 1 993, p. 2745–2756, 1993.

DARVISHZADEH, P.; ORSAT, V.; MARTINEZ, J. L. Correction to: process optimization for development of a novel water kefir drink with high antioxidant activity and potential probiotic properties from russian olive fruit (*Elaeagnus angustifolia*) (Food and Bioprocess Technology, (2021), 14, 2, (248-260),. **Food and Bioprocess Technology**, v. 14, n. 2, p. 261, 2021.

DE FILIPPIS, F.; TROISE, A. D.; VITAGLIONE, P.; ERCOLINI, D. Different temperatures select distinctive acetic acid bacteria species and promotes organic acids production during Kombucha tea fermentation. **Food Microbiology**, v. 73, p. 11–16, 2018. Disponível em:

DE MELO, F. C. B. C.; BORSATO, D.; DE MACEDO JÚNIOR, F. C.; MANTOVANI, M. S.; LUIZ, R. C.; CELLIGOI, M. A. P. C. Study of levan productivity from *Bacillus subtilis* Natto by surface response methodology and its antitumor activity against HepG2 cells using metabolomic approach. **Pakistan Journal of Pharmaceutical Sciences**, v. 28, n. 6, p. 1917–1926, 2015.

DE MELO PEREIRA, G. V.; MAGALHÃES, K. T.; DE ALMEIDA, E. G.; DA SILVA COELHO, I.; SCHWAN, R. F. Spontaneous cocoa bean fermentation carried out in a novel-design stainless steel tank: Influence on the dynamics of microbial populations and physical-chemical properties. **International Journal of Food Microbiology**, v. 161, n. 2, p. 121–133, 2013.

ESCOBAR, S. J.; SIMONE, M.; MARTIN, N.; DE OLIVEIRA RIBEIRO, C. A.; MARTINEZ, G. R.; WINNISCHOFER, S. M. B.; WITTING, P. K.; ROCHA, M. E. M. Cytotoxic effects of 4′-hydroxychalcone on human neuroblastoma cells (SH-SY5Y). **Toxicology in Vitro**, v. 61, n. May 2018, p. 104640, 2019.

OLIVEIRA, A. L. D.; SANTOS, V.; LIOTTI, R. G.; ZILIOLI, E.; SPINOSA, W. A.; RIBEIRO-PAES, J. T. Estudo de bactérias do gênero *Gluconobacter*: Isolamento, purificação, identificação fenotípica e molecular. **Ciencia e Tecnologia de Alimentos**, v. 30, n. 1, p. 106–112, 2010.

DE ROOS, J.; DE VUYST, L. Acetic acid bacteria in fermented foods and beverages. **Current Opinion in Biotechnology**, v. 49, p. 115–119, 2018.

DE ROOS, J.; VANDAMME, P.; DE VUYST, L. Wort substrate consumption and metabolite production during lambic beer fermentation and maturation explain the successive growth of specific bacterial and yeast species. **Frontiers in Microbiology**, v. 9, n. NOV, p. 1–20, 2018.

DE ROOS, J.; VERCE, M.; AERTS, M.; VANDAMME, P.; DE VUYST, L. Temporal and spatial distribution of the acetic acid bacterium communities throughout the wooden casks used for the fermentation and maturation of lambic beer underlines their functional role. **Applied and Environmental Microbiology**, v. 84, n. 7, p. 1–15, 2018.

DE VUYST, L.; LEROY, F. Functional role of yeasts, lactic acid bacteria and acetic acid bacteria in cocoa fermentation processes. **FEMS Microbiology Reviews**, v. 44, n. 4, p. 432–453, 2020.

DE VUYST, L.; WECKX, S. The cocoa bean fermentation process: from ecosystem analysis to starter culture development. **Journal of Applied Microbiology**, v. 121, n. 1, p. 5–17, 2016.

DESTRO, T. M.; PRATES, D. da F.; WATANABE, L. S.; GARCIA, S.; BIZ, G.; SPINOSA, W. A. Organic brown sugar and jaboticaba pulp influence on water kefir fermentation. **Ciencia e Agrotecnologia**, v. 43, 2019.

DONNO, D.; TURRINI, F. Plant foods and underutilized fruits as source of functional food ingredients: Chemical composition, quality traits, and biological properties. **Foods**, v. 9, n. 10, p. 7–10, 2020.

DOS SANTOS, L. F.; BAZANI CABRAL DE MELO, F. C.; MARTINS PAIVA, W. J.; BORSATO, D.; CORRADI CUSTÓDIO DA SILVA, M. D. L.; COLABONE CELLIGOI, M. A. P. Characterization and optimization of levan production by *Bacillus subtilis* NATTO. **Romanian Biotechnological Letters**, v. 18, n. 4, p. 8413–8422, 2013.

ENTANI, E.; OHMORI, S.; MASAI, H.; SUZUKI, K. I. *Acetobacter polyoxogenes* sp. nov., a new species of an acetic acid bacterium useful for producing vinegar with high acidity. **The Journal of General and Applied Microbiology**, v. 31, n. 5, p. 475–490, 1985.

ESAWY, M. A.; AMER, H.; GAMAL-ELDEEN, A. M.; EL ENSHASY, H. A.; HELMY, W. A.;

ABO-ZEID, M. A. M.; MALEK, R.; AHMED, E. F.; AWAD, G. E. A. Scaling up, characterization of levan and its inhibitory role in carcinogenesis initiation stage'. **Carbohydrate Polymers**, v. 95, n. 1, p. 578–587, 2013.

FAO, Food and Agriculture Organization of the United Nations. **FAOSTAT.** Disponível em: https://www.fao.org/faostat/en/#rankings/countries_by_commodity.Acesso em: jul. 2022.

FAO, Food and Agriculture Organization of the United Nations. 2020. **Fruit and vegetables – your dietary essentials.** The International Year of Fruits and Vegetables. Disponível em: https://www.fao.org/3/cb2395en/online/cb2395en.html. Acesso em: jul. 2022.

FERREIRA, V.; LOPEZ, R. The actual and potential aroma of winemaking grapes. **Biomolecules**, v. 9, n. 12, 2019.

FIORDA, F. A.; DE MELO PEREIRA, G. V.; THOMAZ-SOCCOL, V.; RAKSHIT, S. K.; PAGNONCELLI, M. G. B.; VANDENBERGHE, L. P. de S.; SOCCOL, C. R. Microbiological, biochemical, and functional aspects of sugary kefir fermentation - A review. **Food Microbiology**, v. 66, p. 86–95, 2017.

FUENTES-RAMÍREZ, L. E.; BUSTILLOS-CRISTALES, R.; TAPIA-HERNÁNDEZ, A.; JIMÉNEZ-SALGADO, T.; WANG, E. T.; MARTÍNEZ-ROMERO, E.; CABALLERO-MELLADO, J. Novel nitrogen-fixing acetic acid bacteria, *Gluconacetobacter johannae* sp. nov. and *Gluconacetobacter azotocaptans* sp. nov., associated with coffee plants. **International Journal of Systematic and Evolutionary Microbiology**, v. 51, n. 4, p. 1305–1314, 2001.

FUGELSANG, KENNETH C. EDWARDS, C. G. **IDENTIFICATION OF WINE MICROORGANISMS**. Boston, MA.: Springer, 2007. 241–272 p.

GAN, L.; JIANG, G.; YANG, Y.; ZHENG, B.; ZHANG, S.; LI, X.; TIAN, Y.; PENG, B. Development and characterization of levan/pullulan/chitosan edible films enriched with ε-polylysine for active food packaging. **Food Chemistry**, v. 388, n. April, p. 132989, 2022.

GARRIDO-BAÑUELOS, G.; BUICA, A.; SCHÜCKEL, J.; ZIETSMAN, A. J. J.; WILLATS, W. G. T.; MOORE, J. P.; DU TOIT, W. J. Investigating the relationship between cell wall polysaccharide composition and the extractability of grape phenolic compounds into Shiraz wines. Part II: Extractability during fermentation into wines made from grapes of different ripeness levels. **Food Chemistry**, v. 278, n. March 2018, p. 26–35, 2019.

GIUDICI, P.; DE VERO, L.; GULLO, M. Vinegars. *In*: **Acetic Acid Bacteria: Fundamentals and Food Applications**. [s.l.] CRC Press. Taylor & Francis Group, 2017. p. 261–87.

GOMES, R. J.; BORGES, M. de F.; ROSA, M. de F.; CASTRO-GÓMEZ, R. J. H.; SPINOSA, W. A. Acetic acid bacteria in the food industry: Systematics, characteristics and applications. **Food Technology and Biotechnology**, v. 56, n. 2, p. 139–151, 2018.

GONZÁLEZ-GARCINUÑO, Á.; TABERNERO, A.; DOMÍNGUEZ, Á.; GALÁN, M. A.; MARTIN DEL VALLE, E. M. Levan and levansucrases: Polymer, enzyme, micro-organisms and biomedical applications. **Biocatalysis and Biotransformation**, v. 36, n. 3, p. 233–244, 2018.

GRANATO, D.; KOOT, A.; SCHNITZLER, E.; VAN RUTH, S. M. Authentication of geographical origin and crop system of grape juices by phenolic compounds and antioxidant activity using chemometrics. **Journal of Food Science**, v. 80, n. 3, p. C584–C593, 2015.

GUILLAMÓN, J. M.; MAS, A. Acetic Acid Bacteria. *In*: **Biology of Microorganisms on Grapes, in Must and in Wine**. [s.l: s.n.]p. 48–64.

GUZEL-SEYDIM, Z. B.; GÖKIRMAKLI, Ç.; GREENE, A. K. A comparison of milk kefir and water kefir: Physical, chemical, microbiological and functional properties. **Trends in Food Science and Technology**, v. 113, n. April, p. 42–53, 2021.

HAMID, K. R. A.; ELSAYED, E. A.; ENSHASY, H. A. E.; ESAWY, M.; MALEK, R. A. Bioprocess optimization for levan production by *Bacillus subtilis* B58. **Journal of Scientific and Industrial Research**, v. 77, n. 7, p. 386–393, 2018.

HAN, J.; FENG, H.; WANG, X.; LIU, Z.; WU, Z. Levan from *Leuconostoc citreum* BD1707: production optimization and changes in molecular weight distribution during cultivation. **BMC biotechnology**, v. 21, n. 1, p. 14, 2021.

HAN W. YOUN. **Microbial LevanAdvances in Applied Microbiology**1990.

HERMANN, M.; PETERMEIER, H.; VOGEL, R. F. Development of novel sourdoughs with in situ formed exopolysaccharides from acetic acid bacteria. **European Food Research and Technology**, v. 241, n. 2, p. 185–197, 2015.

HERTADI, R.; AMARI, M. M. S.; RATNANINGSIH, E. Enhancement of antioxidant activity of levan through the formation of nanoparticle systems with metal ions. **Heliyon**, v. 6, n. 6, p. e04111, 2020.

HO, C. W.; LAZIM, A. M.; FAZRY, S.; ZAKI, U. K. H. H.; LIM, S. J. Varieties, production, composition and health benefits of vinegars: A review. Food chemistry. v. 221, p.1621–1630, 2017.

HO, V. T. T.; FLEET, G. H.; ZHAO, J. Unravelling the contribution of lactic acid bacteria and acetic acid bacteria to cocoa fermentation using inoculated organisms. **International Journal of Food Microbiology**, v. 279, p. 43–56, 2018.

HORNEDO-ORTEGA, R.; GONZÁLEZ-CENTENO, M. R.; CHIRA, K.; JOURDES, M.; TEISSEDRE, P.-L. Phenolic compounds of grapes and wines: key compounds and implications in sensory perception. **IntechOpen**, p. 13, 2021.

HORNEDO-ORTEGA, R.; KRISA, S.; CARMEN GARCÍA-PARRILLA, M.; RICHARD, T. Effects of gluconic and alcoholic fermentation on anthocyanin composition and antioxidant activity of beverages made from strawberry. **LWT - Food Science and Technology**, v. 69, p. 382–389, 2016.

HUANG, T. Y.; HUANG, M. Y.; TSAI, C. K.; SU, W. T. Phosphorylation of levan by microwave-assisted synthesis enhanced anticancer ability. **Journal of Bioscience and Bioengineering**, v. xxx, n. xxx, 2020.

IDOGAWA, N.; AMAMOTO, R.; MURATA, K.; KAWAI, S. Phosphate enhances levan production in the endophytic bacterium *Gluconacetobacter diazotrophicus* Pal5. **Bioengineered**, v. 5, n. 3, p. 173–179, 2014.

ILC, T.; WERCK-REICHHART, D.; NAVROT, N. Meta-analysis of the core aroma components of grape and wine aroma. **Frontiers in Plant Science**, v. 7, n. September2016, 2016.

IZQUIERDO-CAÑAS, P. M.; LÓPEZ-MARTÍN, R.; GARCÍA-ROMERO, E.; GONZÁLEZ-ARENZANA, L.; MÍNGUEZ-SANZ, S.; CHATONNET, P.; PALACIOS-GARCÍA, A.; PUIG-PUJOL, A. Effect of kaolin silver complex on the control of populations of *Brettanomyces* and acetic acid bacteria in wine. **Journal of Food Science and Technology**, v. 55, n. 5, p. 1823–1831, 2018.

JAKOB, F.; GEBRANDE, C.; BICHLER, R. M.; VOGEL, R. F. Insights into the pH-dependent,

extracellular sucrose utilization and concomitant levan formation by *Gluconobacter albidus* TMW 2.1191. **Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology**, v. 113, n. 7, p. 863–873, 2020.

JAKOB, F.; PFAFF, A.; NOVOA-CARBALLAL, R.; RÜBSAM, H.; BECKER, T.; VOGEL, R. F. Structural analysis of fructans produced by acetic acid bacteria reveals a relation to hydrocolloid function. **Carbohydrate Polymers**, v. 92, n. 2, p. 1234–1242, 2013.

JAKOB, F.; STEGER, S.; VOGEL, R. F. Influence of novel fructans produced by selected acetic acid bacteria on the volume and texture of wheat breads. **European Food Research and Technology**, v. 234, n. 3, p. 493–499, 2012.

JATHORE, N. R.; BULE, M. V.; TILAY, A. V.; ANNAPURE, U. S. Microbial levan from *Pseudomonas fluorescens*: Characterization and medium optimization for enhanced production. **Food Science and Biotechnology**, v. 21, n. 4, p. 1045–1053, 2012.

KAPP, J. M.; SUMNER, W. Kombucha: a systematic review of the empirical evidence of human health benefit. **Annals of Epidemiology**, v. 30, p. 66–70, 2019.

KAPRASOB, R.; KERDCHOECHUEN, O.; LAOHAKUNJIT, N.; SOMBOONPANYAKUL, P. B vitamins and prebiotic fructooligosaccharides of cashew apple fermented with probiotic strains *Lactobacillus* spp., *Leuconostoc mesenteroides* and *Bifidobacterium longum*. **Process Biochemistry**, v. 70, n. February, p. 9–19, 2018.

KATSURA, K.; KAWASAKI, H.; POTACHAROEN, W.; SAONO, S.; SEKI, T.; YAMADA, Y.; UCHIMURA, T.; KOMAGATA, K. *Asaia siamensis* sp. nov., an acetic acid bacterium in the α-Proteobacteria. **International Journal of Systematic and Evolutionary Microbiology**, v. 51, n. 2, p. 559–563, 2001.

KAZAK SARILMISER, H.; TOKSOY ONER, E. Investigation of anti-cancer activity of linear and aldehyde-activated levan from *Halomonas smyrnensis* AAD6T. **Biochemical Engineering Journal**, v. 92, p. 28–34, 2014.

KEKEZ, B. D.; GOJGIC-CVIJOVIC, G. D.; JAKOVLJEVIC, D. M.; STEFANOVIC KOJIC, J. R.; MARKOVIC, M. D.; BESKOSKI, V. P.; VRVIC, M. M. High levan production by *Bacillus licheniformis* ns032 using ammonium chloride as the sole nitrogen source. **Applied Biochemistry and Biotechnology**, v. 175, n. 6, p. 3068–3083, 2015.

KHOO, H. E.; AZLAN, A.; TANG, S. T.; LIM, S. M. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. **Food and Nutrition Research**, v. 61, n. 1, 2017.

KIM, D. H.; CHON, J. W.; KIM, H.; SEO, K. H. Development of a novel selective medium for the isolation and enumeration of acetic acid bacteria from various foods. **Food Control**, v. 106, n. June, p. 106717, 2019.

KIM, S. J.; CHUNG, B. H. Antioxidant activity of levan coated cerium oxide nanoparticles. **Carbohydrate Polymers**, v. 150, p. 400–407, 2016.

KOMAGATA, K. .; IINO, T. .; YAMADA, Y. The family *Acetobacteraceae*. *In*: **The Prokaryotes**. [s.l.] Springer Berlin Heidelberg, 2014. p. 3–78.

KORNMANN, H.; VALENTINOTTI, S.; DUBOC, P.; MARISON, I.; VON STOCKAR, U. Monitoring and control of *Gluconacetobacter xylinus* fed-batch cultures using in situ mid-IR spectroscopy. **Journal of Biotechnology**, v. 113, n. 1–3, p. 231–245, 2004.

KOŞARSOY AĞÇELI, G.; CIHANGIR, N. Nano-sized biopolymer levan: Its antimicrobial, anti-biofilm and anti-cancer effects. **Carbohydrate Research**, v. 494, n. June, p. 108068, 2020.

KREGIEL, D. Attachment of *Asaia lannensis* to materials commonly used in beverage industry. **Food Control**, v. 32, n. 2, p. 537–542, 2013.

KREGIEL, D.; OTLEWSKA, A.; ANTOLAK, H. Attachment of *Asaia bogorensis* originating in fruit-flavored water to packaging materials. **BioMed Research International**, v. 2014, 2014.

KUMAR, A.; MOSA, K. A.; JI, L.; KAGE, U.; DHOKANE, D.; KARRE, S.; MADALAGERI, D.; PATHANIA, N. Metabolomics-assisted biotechnological interventions for developing plant-based functional foods and nutraceuticals. **Critical Reviews in Food Science and Nutrition**, v. 58, n. 11, p. 1791–1807, 2018.

LA CHINA, S.; ZANICHELLI, G.; DE VERO, L.; GULLO, M. Oxidative fermentations and exopolysaccharides production by acetic acid bacteria: a mini review. **Biotechnology Letters**, v. 40, n. 9–10, p. 1289–1302, 2018.

LAAVANYA, D.; SHIRKOLE, S.; BALASUBRAMANIAN, P. Current challenges, applications and future perspectives of SCOBY cellulose of Kombucha fermentation. **Journal of Cleaner Production**, v. 295, p. 126454, 2021.

LAUREYS, D.; AERTS, M.; VANDAMME, P.; DE VUYST, L. Oxygen and diverse nutrients influence the water kefir fermentation process. **Food Microbiology**, v. 73, p. 351–361, 2018.

LAUREYS, D.; BRITTON, S. J.; DE CLIPPELEER, J. Kombucha Tea Fermentation: A Review. **Journal of the American Society of Brewing Chemists**, v. 78, n. 3, p. 165–174, 2020.

LAVASANI, P. S.; MOTEVASELI, E.; SHIRZAD, M.; MODARRESSI, M. H. Isolation and identification of *Komagataeibacter xylinus* from Iranian traditional vinegars and molecular analyses. **Iranian Journal of Microbiology**, v. 9, n. 6, p. 338–347, 2017.

LI, J.; KIM, I. H. Effects of levan-type fructan supplementation on growth performance, digestibility, blood profile, fecal microbiota, and immune responses after lipopolysaccharide challenge in growing pigs. **Journal of Animal Science**, v. 91, n. 11, p. 5336–5343, 2013.

LI, R.; XU, Y.; CHEN, J.; WANG, F.; ZOU, C.; YIN, J. Enhancing the proportion of gluconic acid with a microbial community reconstruction method to improve the taste quality of Kombucha. **Lwt**, v. 155, p. 112937, 2022.

LI, S.; LI, P.; FENG, F.; LUO, L. X. Microbial diversity and their roles in the vinegar fermentation process. **Applied Microbiology and Biotechnology**, v. 99, n. 12, p. 4997–5024, 2015.

LI, X. L.; THAKUR, K.; ZHANG, Y. Y.; TU, X. F.; ZHANG, Y. S.; ZHU, D. Y.; ZHANG, J. G.; WEI, Z. J. Effects of different chemical modifications on the antibacterial activities of polysaccharides sequentially extracted from peony seed dreg. **International Journal of Biological Macromolecules**, v. 116, p. 664–675, 2018.

LIN, J.; MASSONNET, M.; CANTU, D. The genetic basis of grape and wine aroma. **Horticulture Research**, v. 6, n. 1, 2019.

LISDIYANTI, P.; KAWASAKI, H.; WIDYASTUTI, Y.; SAONO, S.; SEKI, T.; YAMADA, Y.; UCHIMURA, T.; KOMAGATA, K. *Kozakia baliensis* gen. nov., sp. nov., a novel acetic acid bacterium in the α-Proteobacteria. **International Journal of Systematic and Evolutionary Microbiology**, v. 52, n. 3, p. 813–818, 2002.

LIU, J.; LUO, J.; YE, H.; SUN, Y.; LU, Z.; ZENG, X. Production, characterization and antioxidant activities in vitro of exopolysaccharides from endophytic bacterium *Paenibacillus polymyxa* EJS-3. **Carbohydrate Polymers**, v. 78, n. 2, p. 275–281, 2009.

LIU, J.; LUO, J.; YE, H.; SUN, Y.; LU, Z.; ZENG, X. Medium optimization and structural characterization of exopolysaccharides from endophytic bacterium *Paenibacillus polymyxa* EJS-3. **Carbohydrate Polymers**, v. 79, n. 1, p. 206–213, 2010.

LIU, J.; LUO, J.; YE, H.; ZENG, X. Preparation, antioxidant and antitumor activities in vitro of different derivatives of levan from endophytic bacterium *Paenibacillus polymyxa* EJS-3. **Food and Chemical Toxicology**, v. 50, n. 3–4, p. 767–772, 2012.

LIU, L. X.; LIU, S. X.; WANG, Y. M.; BI, J. C.; CHEN, H. M.; DENG, J.; ZHANG, C.; HU, Q. S.; LI, C. F. *Komagataeibacter cocois* sp. Nov., a novel cellulose-producing strain isolated from coconut milk. **International Journal of Systematic and Evolutionary Microbiology**, v. 68, n. 10, p. 3125–3131, 2018.

LUDWIG, W. Nucleic acid techniques in bacterial systematics and identification. **International Journal of Food Microbiology**, v. 120, n. 3, p. 225–236, 2007.

LYNCH, K. M.; WILKINSON, S.; DAENEN, L.; ARENDT, E. K. An update on water kefir: Microbiology, composition and production. **International Journal of Food Microbiology**, v. 345, n. January 2020, p. 109128, 2021.

LYNCH, K. M.; ZANNINI, E.; WILKINSON, S.; DAENEN, L.; ARENDT, E. K. Physiology of acetic acid bacteria and their role in vinegar and fermented beverages. **Comprehensive Reviews in Food Science and Food Safety**, v. 18, n. 3, p. 587–625, 2019.

MADHAIYAN, M.; SARAVANAN, V. S.; JOVI, D. B. S. S.; LEE, H.; THENMOZHI, R.; HARI, K.; SA, T. Occurrence of *Gluconacetobacter diazotrophicus* in tropical and subtropical plants of Western Ghats, India. **Microbiological Research**, v. 159, n. 3, p. 233–243, 2004.

MAJEE, S. B.; AVLANI, D.; BISWAS, G. R. Rheological behavior and pharmaceutical applications of bacterial exopolysaccharides. **Journal of Applied Pharmaceutical Science**, v. 7, n. 9, p. 224–232, 2017.

MAMLOUK, D.; GULLO, M. Acetic acid bacteria: physiology and carbon sources oxidation. **Indian Journal of Microbiology**, v. 53, n. 4, p. 377–384, 2013.

MANANDHAR, S.; VIDHATE, S.; D'SOUZA, N. Water soluble levan polysaccharide biopolymer electrospun fibers. **Carbohydrate Polymers**, v. 78, n. 4, p. 794–798, 2009.

MANTOVAN, J.; BERSANETI, G. T.; FARIA-TISCHER, P. C. S.; CELLIGOI, M. A. P. C.; MALI, S. Use of microbial levan in edible films based on cassava starch. **Food Packaging and Shelf Life**, 2018.

MANTZOURANI, I.; KAZAKOS, S.; TERPOU, A.; MALLOUCHOS, A.; KIMBARIS, A.; ALEXOPOULOS, A.; BEZIRTZOGLOU, E.; PLESSAS, S. Assessment of volatile compounds evolution, antioxidant activity, and total phenolics content during cold storage of pomegranate beverage fermented by *Lactobacillus paracasei* K5. **Fermentation**, v. 4, n. 4, 2018.

MARRERO, S. C.; MARTÍNEZ-RODRÍGUEZ, A.; PÉREZ, S. E. M.; MOYA, S. P. **New trends and applications in fermented beverages**. [s.l.] Elsevier Inc., 2019. 31–66 p.

MATEO, E.; TORIJA, M. J.; MAS, A.; BARTOWSKY, E. J. Acetic acid bacteria isolated from

grapes of South Australian vineyards. **International Journal of Food Microbiology**, v. 178, p. 98–106, 2014.

MATIJAŠEVIĆ, S.; POPOVIĆ-DJORDJEVIĆ, J.; RISTIĆ, R.; ĆIRKOVIĆ, D.; ĆIRKOVIĆ, B.; POPOVIĆ, T. Volatile aroma compounds of brandy ‘lozovača0 produced from Muscat table grapevine cultivars (*Vitis vinifera* L.). **Molecules**, v. 24, n. 13, p. 1–15, 2019.

MATSUSHITA, KAZUNOBU TOYAMA, H.; ADACHI, O. Respiratory chains and bioenergetics of acetic acid bacteria. *In*: ROSE, A. H.; TEMPEST, D. W. **Advances in MICROBIAL PHYSIOLOGY**. [s.l.] ACADEMIC PRESS LIMITED, 1994. p. 247–297.

MATSUSHITA, K.; MATSUTANI, M. Distribution, Evolution, and Physiology of Oxidative Fermentation. *In*: **Acetic Acid Bacteria: Ecology and Physiology**. [s.l.] Springer Japan, 2016. p. 159–178.

MELLO, L. M. R. de; MACHADO, C. A. E. Vitivinicultura brasileira: panorama 2020. **Panorama**, v. Embrapa Uv, p. 1–18, 2021. Disponível em: <http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1135990>. Acesso em: jul. 2022.

MELO, I. R.; PIMENTEL, M. F.; LOPES, C. E.; CALAZANS, G. M. T. Application of fractional factorial design to levan production by *Zymomonas mobilis*. **Brazilian Journal of Microbiology**, v. 38, n. 1, p. 45–51, 2007.

MÉNDEZ-LORENZO, L.; PORRAS-DOMÍNGUEZ, J. R.; RAGA-CARBAJAL, E.; OLVERA, C.; RODRÍGUEZ-ALEGRÍA, M. E.; CARRILLO-NAVA, E.; COSTAS, M.; MUNGUÍA, A. L. Intrinsic levanase activity of *Bacillus subtilis* 168 levansucrase (SacB). **PLoS ONE**, v. 10, n. 11, p. 1–15, 2015.

MIN, M.; BUNT, C. R.; MASON, S. L.; HUSSAIN, M. A. Non-dairy probiotic food products: An emerging group of functional foods. **Critical Reviews in Food Science and Nutrition**, v. 59, n. 16, p. 2626–2641, 2019.

MORALES, D. Biological activities of kombucha beverages: The need of clinical evidence. **Trends in Food Science and Technology**, v. 105, n. September, p. 323–333, 2020.

MUMMALETI, G.; SARMA, C.; KALAKANDAN, S. K.; GAZULA, H.; SIVANANDHAM, V.; ANANDHARAJ, A. Characterization of levan produced from coconut inflorescence sap using *Bacillus subtilis* and its application as a sweetener. **Lwt**, v. 154, n. July 2021, p. 112697, 2022.

MUROOKA, Y. Acetic acid bacteria in production of vinegars and traditional fermented foods. *In*: **Acetic Acid Bacteria: Ecology and Physiology**. [s.l.] Springer Japan, 2016. p. 51–72.

MUTANDA, T.; MOKOENA, M. P.; OLANIRAN, A. O.; WILHELMI, B. S.; WHITELEY, C. G. Microbial enzymatic production and applications of short-chain fructooligosaccharides and inulooligosaccharides: Recent advances and current perspectives. **Journal of Industrial Microbiology and Biotechnology**, v. 41, n. 6, p. 893–906, 2014.

MYERS, R. H.; MONTGOMERY, D. C.; ANDERSON-COOK, C. M. A.-C. **RESPONSE SURFACE METHODOLOGY: Process and Product Optimization Using Designed Experiments**. [s.l.] WILEY SERIES IN PROBABILITY AND STATISTICS Established, 2016. 1–821 p.

NAKASHIMA, N.; TAMURA, T. Whole-genome sequence of *Acetobacter orientalis* Strain FAN1, isolated from caucasian yogurt nobutaka. **Genome announcements**, v. 6, n. 13, p. 1–2, 2018.

NALOKA, K.; YUKPHAN, P.; MATSUTANI, M.; MATSUSHITA, K.; THEERAGOOL, G. *Komagataeibacter diospyri* sp. Nov., a novel species of thermotolerant bacterial nanocellulose-producing bacterium. **International Journal of Systematic and Evolutionary Microbiology**, v. 70, n. 1, p. 251–258, 2020.

NANDASIRI, R.; RUPASINGHE, V. Inhibition of Low Density Lipoprotein Oxidation and Angiotensin Converting Enzyme in vitro by Functional Fruit Vinegar Beverages. **Journal of Food Processing & Beverages**, v. 1, n. 2, p. 2–6, 2013.

NATIVIDADE, M. M. P.; CORRÊA, L. C.; SOUZA, S. V. C. de; PEREIRA, G. E.; LIMA, L. C. de O. Simultaneous analysis of 25 phenolic compounds in grape juice for HPLC: Method validation and characterization of São Francisco Valley samples. **Microchemical Journal**, v. 110, p. 665–674, 2013.

NEFFE-SKOCIŃSKA, K.; KRUK, M.; ŚCIBISZ, I.; ZIELIŃSKA, D. The Novel Strain of Gluconobacter oxydans H32 Isolated from kombucha as a proposition of a starter culture for sour ale craft beer production. **Applied Sciences**, v. 12, n. 6, p. 3047, 2022.

NGUYEN VU, T. H.; QUACH, N. T.; NGUYEN, N. A.; NGUYEN, H. T.; NGO, C. C.; NGUYEN, T. D.; HO, P. H.; HOANG, H.; CHU, H. H.; PHI, Q. T. Genome mining associated with analysis of structure, antioxidant activity reveals the potential production of levan-rich exopolysaccharides by food-derived bacillus velezensis vtx20. **Applied Sciences (Switzerland)**, v. 11, n. 15, 2021.

ÖNER, E. T.; HERNÁNDEZ, L.; COMBIE, J. Review of Levan polysaccharide: From a century of past experiences to future prospects. **Biotechnology Advances**, v. 34, n. 5, p. 827–844, 2016.

OPAS, Organização Panamericana da Saúde. Câncer. Disponível em: https://www.paho.org/pt/topicos/cancer#:~:text=Folha%20informativa%20atualizada%20em%20outubro%20de%202020&text=A%20met%C3%A1stase%20%C3%A9%20a%20principal,Am%C3%A9rica%20Latina%20e%20no%20Caribe. Acesso em: jul. 2022. 2020.

ORDÓÑEZ, J. L.; SAINZ, F.; CALLEJÓN, R. M.; TRONCOSO, A. M.; TORIJA, M. J.; GARCÍA-PARRILLA, M. C. Impact of gluconic fermentation of strawberry using acetic acid bacteria on amino acids and biogenic amines profile. **Food Chemistry**, v. 178, p. 221–228, 2015.

OUSAAID, D.; MECHCHATE, H.; LAAROUSSI, H.; HANO, C.; BAKOUR, M.; GHOUIZI, A. El; CONTE, R.; LYOUSSI, B.; ARABI, I. El. Fruits vinegar: quality characteristics, phytochemistry, and functionality. **Molecules**, v. 27, n. 222, p. 1–16, 2022.

OZCELIK, F.; AKAN, E.; KINIK, O. Use of Cornelian cherry, hawthorn, red plum, roseship and pomegranate juices in the production of water kefir beverages. **Food Bioscience**, v. 42, n. June, p. 101219, 2021.

PARK, J. E.; KIM, J. Y.; KIM, J.; KIM, Y. J.; KIM, M. J.; KWON, S. W.; KWON, O. Pomegranate vinegar beverage reduces visceral fat accumulation in association with AMPK activation in overweight women: A double-blind, randomized, and placebo-controlled trial. **Journal of Functional Foods**, v. 8, n. 1, p. 274–281, 2014.

PARK, J. K.; KHAN, T. **Other microbial polysaccharides: Pullulan, scleroglucan, elsinan, levan, alternant, dextran**. [s.l.] Woodhead Publishing Limited, 2009. 592–614 p.

PARKER, M.; CAPONE, D. L.; FRANCIS, I. L.; HERDERICH, M. J. Aroma Precursors in Grapes and Wine: Flavor Release during Wine Production and Consumption. **Journal of Agricultural and Food Chemistry**, v. 66, n. 10, p. 2281–2286, 2018.

PEI, F.; MA, Y.; CHEN, X.; LIU, H. Purification and structural characterization and antioxidant activity of levan from Bacillus megaterium PFY-147. **International Journal of Biological Macromolecules**, v. 161, p. 1181–1188, 2020.

PENDÓN, M. D.; BENGOA, A. A.; IRAPORDA, C.; MEDRANO, M.; GARROTE, G. L.; ABRAHAM, A. G. Water kefir: Factors affecting grain growth and health-promoting properties of the fermented beverage. **Journal of Applied Microbiology**, n. November, p. 1–19, 2021.

PORRAS-DOMÍNGUEZ, J. R.; ÁVILA-FERNÁNDEZ, Á.; RODRÍGUEZ-ALEGRÍA, M. E.; MIRANDA-MOLINA, A.; ESCALANTE, A.; GONZÁLEZ-CERVANTES, R.; OLVERA, C.; LÓPEZ MUNGUÍA, A. Levan-type FOS production using a *Bacillus licheniformis* endolevanase. **Process Biochemistry**, v. 49, n. 5, p. 783–790, 2014.

POTHAKOS, V.; ILLEGHEMS, K.; LAUREYS, D.; SPITAELS, F.; VANDAMME, P.; DE VUYST, L. Acetic acid bacteria in fermented food and beverage ecosystems. *In*: **Acetic Acid Bacteria Ecology and Physiology**. [s.l.] Springer Japan, 2016. p. 73–99.

ROBINSON, A. L.; BOSS, P. K.; SOLOMON, P. S.; TRENGOVE, R. D.; HEYMANN, H.; EBELER, S. E. Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. **American Journal of Enology and Viticulture**, v. 65, n. 1, p. 1–24, 2014.

ROCA, C.; ALVES, V. D.; FREITAS, F.; REIS, M. A. M. Exopolysaccharides enriched in rare sugars: Bacterial sources, production, and applications. **Frontiers in Microbiology**, v. 6, n. APR, p. 1–7, 2015.

ROCHA-GOMES, A.; ESCOBAR, A.; SOARES, J. S.; ALVES, A.; SILVA, D.; RIUL, T. R. Chemical composition and hypocholesterolemic effect of milk kefir and water kefir in Wistar rats Composição química e efeito hipocolesterolêmico do kefir de leite e do kefir de água em ratos Wistar. **Revista de Nutrição**, v. 31, n. 2, p. 137–145, 2018.

RODRÍGUEZ-PÉREZ, C.; GARCÍA-VILLANOVA, B.; GUERRA-HERNÁNDEZ, E.; VERARDO, V. Grape seeds proanthocyanidins: An overview of in vivo bioactivity in animal models. **Nutrients**, v. 11, n. 10, p. 1–18, 2019.

SABRA, A.; NETTICADAN, T.; WIJEKOON, C. Grape bioactive molecules, and the potential health benefits in reducing the risk of heart diseases. **Food Chemistry: X**, v. 12, p. 100149, 2021.

SAICHANA, N.; MATSUSHITA, K.; ADACHI, O.; FRÉBORT, I.; FREBORTOVA, J. Acetic acid bacteria: A group of bacteria with versatile biotechnological applications. **Biotechnology Advances**, v. 33, n. 6, p. 1260–1271, 2015.

SALUSJÄRVI, T.; POVELAINEN, M.; HVORSLEV, N.; ENEYSKAYA, E. V.; KULMINSKAYA, A. A.; SHABALIN, K. A.; NEUSTROEV, K. N.; KALKKINEN, N.; MIASNIKOV, A. N. Cloning of a gluconate/polyol dehydrogenase gene from *Gluconobacter suboxydans* IFO 12528, characterisation of the enzyme and its use for the production of 5-ketogluconate in a recombinant Escherichia coli strain. **Applied Microbiology and Biotechnology**, v. 65, n. 3, p. 306–314, 2004.

SAMAD, A.; AZLAN, A.; ISMAIL, A. Therapeutic effects of vinegar: A review. **Current Opinion in Food Science**, v. 8, p. 56–61, 2016.

SAMOTICHA, J.; WOJDYŁO, A.; CHMIELEWSKA, J.; OSZMIAŃSKI, J. The effects of flash release conditions on the phenolic compounds and antioxidant activity of Pinot noir red wine. **European Food Research and Technology**, v. 243, n. 6, p. 999–1007, 2017.

SANDHYA, M. V. S.; YALLAPPA, B. S.; VARADARAJ, M. C.; PURANAIK, J.; RAO, L. J.; JANARDHAN, P.; MURTHY, P. S. Inoculum of the starter consortia and interactive metabolic process in enhancing quality of cocoa bean (*Theobroma cacao*) fermentation. **LWT - Food Science and Technology**, v. 65, p. 731–738, 2016.

SCHWAB, W.; DAVIDOVICH-RIKANATI, R.; LEWINSOHN, E. Biosynthesis of plant-derived flavor compounds. **Plant Journal**, v. 54, n. 4, p. 712–732, 2008.

SEMJONOVS, P.; SHAKIROVA, L.; BROKS, R.; KISTKINS, S.; ZIKMANIS, P. Influence of environmental factors on extracellular fructan and oligosaccharide production by *Gluconobacter nephelii.* **Research Journal of Microbiology**, v. 12, n. 1, p. 33–41, 2017.

SEMJONOVS, P.; SHAKIROVA, L.; TREIMANE, R.; SHVIRKSTS, K.; AUZINA, L.; CLEENWERCK, I.; ZIKMANIS, P. Production of extracellular fructans by *Gluconobacter nephelii* P1464. **Letters in Applied Microbiology**, v. 62, n. 2, p. 145–152, 2016.

SENGUN, I. Y.; KARABIYIKLI, S. Importance of acetic acid bacteria in food industry. **Food Control**, v. 22, n. 5, p. 647–656, 2011.

SEO, K. Il; LEE, J.; CHOI, R. Y.; LEE, H. I.; LEE, J. H.; JEONG, Y. K.; KIM, M. J.; LEE, M. K. Anti-obesity and anti-insulin resistance effects of tomato vinegar beverage in diet-induced obese mice. **Food and Function**, v. 5, n. 7, p. 1579–1586, 2014.

SHI, Z.; ZHANG, Y.; PHILLIPS, G. O.; YANG, G. Utilization of bacterial cellulose in food. **Food Hydrocolloids**, v. 35, p. 539–545, 2014.

SHINAGAWA, E.; MATSUSHITA, K.; ADACHI, O.; AMEYAMA, M. Purification and characterization of 2-keto-d-gluconate dehydrogenase from *Gluconobacter melanogenus.* **Agricultural and Biological Chemistry**, v. 45, n. 5, p. 1079–1085, 1981.

SHINJOH, M.; TOYAMA, H. Industrial Application of Acetic Acid Bacteria (Vitamin C and Others). *In*: **Acetic Acid Bacteria Ecology and Physiology**. [s.l.] Springer Japan, 2016. p. 321–337.

SIEVERS, M.; SWINGS, J. *Gluconacetobacter*. **Bergey's Manual of Systematics of Archaea and Bacteria**, p. 1–11, 2015a.

SIEVERS, M.; SWINGS, J. *Acetobacteraceae*. **Bergey's Manual of Systematics of Archaea and Bacteria**, n. C, p. 1–20, 2015b.

SILBIR, S.; DAGBAGLI, S.; YEGIN, S.; BAYSAL, T.; GOKSUNGUR, Y. Levan production by *Zymomonas mobilis* in batch and continuous fermentation systems. **Carbohydrate Polymers**, v. 99, p. 454–461, 2014.

SOKOLLEK, S. J.; HERTEL, C.; HAMMES, W. P. Cultivation and preservation of vinegar bacteria. **Journal of Biotechnology**, v. 60, n. 3, p. 195–206, 1998.

SOLIERI, L.; GIUDICI, P. Vinegars of the world. **Vinegars of the World**, p. 1–297, 2009.

SOMBOLESTANI, A. S.; CLEENWERCK, I.; CNOCKAERT, M.; BORREMANS, W.; WIEME, A. D.; DE VUYST, L.; VANDAMME, P. Novel acetic acid bacteria from cider fermentations: Acetobacter conturbans sp. nov. and acetobacter fallax sp. nov. **International Journal of Systematic and Evolutionary Microbiology**, v. 70, n. 12, p. 6163–6171, 2020.

SPINOSA, W. A.; DOS SANTOS JÚNIOR, V.; GALVAN, D.; FIORIO, J. L.; GOMEZ, R. J. H. C. Vinegar rice (*Oryza sativa* L.) produced by a submerged fermentation process from alcoholic fermented rice. **Food Science and Technology (Brazil)**, v. 35, n. 1, p. 196–201, 2015.

SPITAELS, F.; LI, L.; WIEME, A.; BALZARINI, T.; CLEENWERCK, I.; VAN LANDSCHOOT, A.; DE VUYST, L.; VANDAMME, P. *Acetobacter lambici* sp. nov., isolated from fermenting lambic beer. **International Journal of Systematic and Evolutionary Microbiology**, v. 64, n. PART 4, p. 1083–1089, 2014a.

SPITAELS, F.; WIEME, A.; BALZARINI, T.; CLEENWERCK, I.; VAN LANDSCHOOT, A.; DE VUYST, L.; VANDAMME, P. *Gluconobacter cerevisiae* sp. nov., isolated from the brewery environment. **International Journal of Systematic and Evolutionary Microbiology**, v. 64, n. PART 4, p. 1134–1141, 2014b.

SPITAELS, F.; WIEME, A. D.; JANSSENS, M.; AERTS, M.; DANIEL, H. M.; VAN LANDSCHOOT, A.; DE VUYST, L.; VANDAMME, P. The microbial diversity of traditional spontaneously fermented lambic beer. **PLoS ONE**, v. 9, n. 4, 2014c.

SPITAELS, F.; WIEME, A. D.; JANSSENS, M.; AERTS, M.; VAN LANDSCHOOT, A.; DE VUYST, L.; VANDAMME, P. The microbial diversity of an industrially produced lambic beer shares members of a traditionally produced one and reveals a core microbiota for lambic beer fermentation. **Food Microbiology**, v. 49, p. 23–32, 2015.

SRIKANTH, R.; REDDY, C. H. S. S. S.; SIDDARTHA, G.; RAMAIAH, M. J.; UPPULURI, K. B. Review on production, characterization and applications of microbial levan. **Carbohydrate Polymers**, v. 120, p. 102–114, 2015a.

SRIKANTH, R.; SIDDARTHA, G.; SUNDHAR REDDY, C. H. S. S.; HARISH, B. S.; JANAKI RAMAIAH, M.; UPPULURI, K. B. Antioxidant and anti-inflammatory levan produced from *Acetobacter xylinum* NCIM2526 and its statistical optimization. **Carbohydrate Polymers**, v. 123, p. 8–16, 2015b.

STOJKOVIĆ, B.; SRETENOVIC, S.; DOGSA, I.; POBERAJ, I.; STOPAR, D. Viscoelastic properties of levan-DNA mixtures important in microbial biofilm formation as determined by micro- and macrorheology. **Biophysical Journal**, v. 108, n. 3, p. 758–765, 2015.

TABAN, B. M.; SAICHANA, N. Physiology and biochemistry of acetic acid bacteria. *In*: **Acetic Acid Bacteria: Fundamentals and Food Applications**. [s.l.] Taylor & Francis Group, 2016. p. 71–91.

TAWEESAK MALIMAS, HUONG THI LAN VU, YUKI MURAMATSU, PATTARAPORN YUKPHAN, S. T. and Y. Y. Systematics of Acetic Acid Bacteria. *In*: **Acetic Acid Bacteria: Fundamentals and Food Applications**, 2016.

TAYLAN, O.; YILMAZ, M. T.; DERTLI, E. Partial characterization of a levan type exopolysaccharide (EPS) produced by *Leuconostoc mesenteroides* showing immunostimulatory and antioxidant activities. **International Journal of Biological Macromolecules**, v. 136, p. 436–444, 2019.

TAZATO, N.; NISHIJIMA, M.; HANDA, Y.; KIGAWA, R.; SANO, C.; SUGIYAMA, J. Gluconacetobacter tumulicola sp. nov. and *Gluconacetobacter asukensis* sp. nov., isolated from the stone chamber interior of the Kitora Tumulus. **International Journal of Systematic and Evolutionary Microbiology**, v. 62, n. 8, p. 2032–2038, 2012.

THOMPSON WITRICK, K.; DUNCAN, S. E.; HURLEY, K. E.; O'KEEFE, S. F. Acid and volatiles of commercially-available lambic beers. **Beverages**, v. 3, n. 4, 2017.

TILWANI, Y. M.; LAKRA, A. K.; DOMDI, L.; YADAV, S.; JHA, N.; ARUL, V. Optimization and physicochemical characterization of low molecular levan from *Enterococcus faecium* MC-5 having

potential biological activities. **Process Biochemistry**, v. 110, n. August, p. 282–291, 2021.

TOYAMA, H.; FURUYA, N.; SAICHANA, I.; ANO, Y.; ADACHI, O.; MATSUSHITA, K. Membrane-bound, 2-keto-D-gluconate-yielding D-gluconate dehydrogenase from "*Gluconobacter dioxyacetonicus*" IFO 3271: Molecular properties and gene disruption. **Applied and Environmental Microbiology**, v. 73, n. 20, p. 6551–6556, 2007.

TRČEK, J.; BARJA, F. Updates on quick identification of acetic acid bacteria with a focus on the 16S-23S rRNA gene internal transcribed spacer and the analysis of cell proteins by MALDI-TOF mass spectrometry. **International Journal of Food Microbiology**, v. 196, p. 137–144, 2015.

UA-ARAK, T.; JAKOB, F.; VOGEL, R. F. Influence of levan-producing acetic acid bacteria on buckwheat-sourdough breads. **Food Microbiology**, v. 65, p. 95–104, 2017a.

UA-ARAK, T.; JAKOB, F.; VOGEL, R. F. Fermentation pH modulates the size distributions and functional properties of *Gluconobacter albidus* TMW 2.1191 levan. **Frontiers in Microbiology**, v. 8, n. MAY, p. 1–11, 2017b.

ULLAH, H.; SANTOS, H. A.; KHAN, T. Applications of bacterial cellulose in food, cosmetics and drug delivery. **Cellulose**, v. 23, n. 4, p. 2291–2314, 2016.

VIEIRA, A. M.; ZAHED, F.; CRISPIM, A. C.; DE SOUZA BENTO, E.; FRANÇA, R. de F. O.; PINHEIRO, I. O.; PARDO, L. A.; CARVALHO, B. M. Production of levan from *Bacillus subtilis* var. natto and apoptotic effect on SH-SY5Y neuroblastoma cells. **Carbohydrate Polymers**, v. 273, n. June, p. 118613, 2021.

VILLARREAL-SOTO, S. A.; BEAUFORT, S.; BOUAJILA, J.; SOUCHARD, J. P.; TAILLANDIER, P. Understanding kombucha tea fermentation: A Review. **Journal of Food Science**, v. 83, n. 3, p. 580–588, 2018.

VISINTIN, S.; ALESSANDRIA, V.; VALENTE, A.; DOLCI, P.; COCOLIN, L. Molecular identification and physiological characterization of yeasts, lactic acid bacteria and acetic acid bacteria isolated from heap and box cocoa bean fermentations in West Africa. **International Journal of Food Microbiology**, v. 216, p. 69–78, 2016.

WALDHERR, F. W.; DOLL, V. M.; MEISSNER, D.; VOGEL, R. F. Identification and characterization of a glucan-producing enzyme from *Lactobacillus hilgardii* TMW 1.828 involved in granule formation of water kefir. **Food Microbiology**, v. 27, n. 5, p. 672–678, 2010.

WU, B.; LIU, J.; YANG, W.; ZHANG, Q.; YANG, Z.; LIU, H.; LV, Z.; ZHANG, C.; JIAO, Z. Nutritional and flavor properties of grape juice as affected by fermentation with lactic acid bacteria. **International Journal of Food Properties**, v. 24, n. 1, p. 906–922, 2021.

WU, Y.; DUAN, S.; ZHAO, L.; GAO, Z.; LUO, M.; SONG, S.; XU, W.; ZHANG, C.; MA, C.; WANG, S. Aroma characterization based on aromatic series analysis in table grapes. **Scientific Reports**, v. 6, n. July, p. 1–16, 2016.

XAVIER, J. R.; RAMANA, K. V. Optimization of levan production by cold-active *Bacillus licheniformis* ANT 179 and Fructooligosaccharide synthesis by its levansucrase. **Applied Biochemistry and Biotechnology**, v. 181, n. 3, p. 986–1006, 2017.

XIA, T.; ZHANG, B.; DUAN, W.; ZHANG, J.; WANG, M. Nutrients and bioactive components from vinegar: A fermented and functional food. **Journal of Functional Foods**, v. 64, n. August 2019, p. 103681, 2020.

XU, W.; LIU, Q.; BAI, Y.; YU, S.; ZHANG, T.; JIANG, B.; MU, W. Physicochemical properties of a high molecular weight levan from *Brenneria* sp. EniD312. **International Journal of Biological Macromolecules**, v. 109, p. 810–818, 2018.

YAMADA, Y. Systematics of acetic acid bacteria. *In*: **Acetic Acid Bacteria: Ecology and PhysiologyEcology and Physiology**. [s.l.] Springer Japan, 2016. p. 1–50.

YAMADA, Y.; YUKPHAN, P. Genera and species in acetic acid bacteria. **International Journal of Food Microbiology**, v. 125, n. 1, p. 15–24, 2008.

YANG, X.; ZHOU, J.; FAN, L.; QIN, Z.; CHEN, Q.; ZHAO, L. Antioxidant properties of a vegetable–fruit beverage fermented with two *Lactobacillus plantarum* strains. **Food Science and Biotechnology**, v. 27, n. 6, p. 1719–1726, 2018.

YETIMAN, A. E.; KESMEN, Z. Identification of acetic acid bacteria in traditionally produced vinegar and mother of vinegar by using different molecular techniques. **International Journal of Food Microbiology**, v. 204, p. 9–16, 2015.

YOO, S. H.; YOON, E. J.; CHA, J.; LEE, H. G. Antitumor activity of levan polysaccharides from selected microorganisms. **International Journal of Biological Macromolecules**, v. 34, n. 1–2, p. 37–41, 2004.

YOON, E. J.; YOO, S. H.; CHA, J.; LEE, H. G. Effect of levan's branching structure on antitumor activity. **International Journal of Biological Macromolecules**, v. 34, n. 3, p. 191–194, 2004.

YUKPHAN, P.; CHAROENYINGCHAROEN, P.; MALIMAS, S.; MURAMATSU, Y.; NAKAGAWA, Y.; TANASUPAWAT, S.; YAMADA, Y. *Gluconobacter aidae* sp. Nov., an acetic acid bacteria isolated from tropical fruits in Thailand. **International Journal of Systematic and Evolutionary Microbiology**, v. 70, n. 7, p. 4351–4357, 2020.

YUKPHAN, P.; MALIMAS, T.; POTACHAROEN, W.; TANASUPWAT, S.; TANTICHAROEN, M.; YAMADA, Y. *Neoasaia chiangmaiensis* gen. nov., sp. nov., a novel osmotolerant acetic acid bacterium in the α-Proteobacteria. **Journal of General and Applied Microbiology**, v. 51, n. 5, p. 301–311, 2005.

ZHAO, P. Y.; WANG, J. P.; KIM, I. H. Effect of dietary levan fructan supplementation on growth performance, meat quality, relative organ weight, cecal microflora, and excreta noxious gas emission in broilers. **Journal of animal science**, v. 91, n. 11, p. 5287–5293, 2013a.

ZHAO, P. Y.; WANG, J. P.; KIM, I. H. Evaluation of dietary fructan supplementation on growth performance, nutrient digestibility, meat quality, fecal microbial flora, and fecal noxious gas emission in finishing pigs. **Journal of Animal Science**, v. 91, n. 11, p. 5280–5286, 2013b.

4. MATERIAIS E MÉTODOS

O material e métodos estão descritos nos quatro artigos científicos apresentados no próximo item, **5. RESULTADOS E DISCUSSÃO**.

5. RESULTADOS E DISCUSSÃO

Os resultados e discussão foram redigidos na forma de quatro artigos científicos e estão apresentados nos subitens de 5.1 a 5.4

5.1. ARTIGO CIENTÍFICO1[1]

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[1] Artigo submetido na Revista **Food Technology and Biotechnology**

Natália Norika Yassunaka Hata[1*], Monica Surek[2], Daniele Sartori[3], Rodrigo Vassoler Serrato[4] and Wilma Aparecida Spinosa[1]

[1]Departamento de Ciência e Tecnologia de Alimentos / Centro de Ciências Agrárias / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil; [2]Departamento de Análises Clínicas / Universidade Federal do Paraná – UFPR, Campus III – Sede Botânico, 80210-170, Curitiba, PR, Brazil; [3]Departamento de Bioquímica e Biotecnologia / Centro de Ciências Exatas / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil; [4]Departamento de Bioquímica e Biologia Molecular / Universidade Federal do Paraná – UFPR, Centro Politécnico, 81531-990, Curitiba, PR, Brazil
*Corresponding author. E-mail address: naty_ea@hotmail.com

Functional Aspects of Acetic Acid Bacteria in Food and Beverages

Functionality of acetic acid bacteria in food

SUMMARY

Acetic acid bacteria (AAB) are microorganisms widely distributed in nature. Although this group is involved in the spoilage of some foods, AAB are of great industrial interest and their functionality is still poorly understood. Through oxidative fermentation, AAB oxidize ethanol, sugars and polyols into different organic acids, aldehydes and ketones. These metabolites are produced during a succession of biochemical reactions of various fermented foods and beverages, such as vinegar, kombucha, water kefir, lambic beer and cocoa. Furthermore, important products like gluconic acid and ascorbic acid precursors can be extracted industrially from their metabolism. The development of new AAB-fermented fruit drinks with healthy and functional appeal is an interesting niche for research and the food industry to explore, as it can meet the needs of a wide range of consumers. Exopolysaccharides like levan and bacterial cellulose (BC) have unique properties, but they need to be produced on a larger scale to expand their applications in this area. This work emphasizes the importance and functionality of AAB during the fermentation process of various foods, as well as the role of AAB in the development of new beverages and the numerous applications of levan and BC.

Keywords: Acetic; Food; Beverage; Oxidative fermentation

INTRODUCTION

AAB are mesophilic, gram-negative bacteria that belong to the *Acetobacteraceae* family. They can be single, in pairs, or in chains, and have an ellipsoidal to elongated shape (rods). Their width varies from 0.4 to 1.0 µm, and their length ranges from 0.8 to 4.5 µm. AAB do not sporulate (*1,2*).

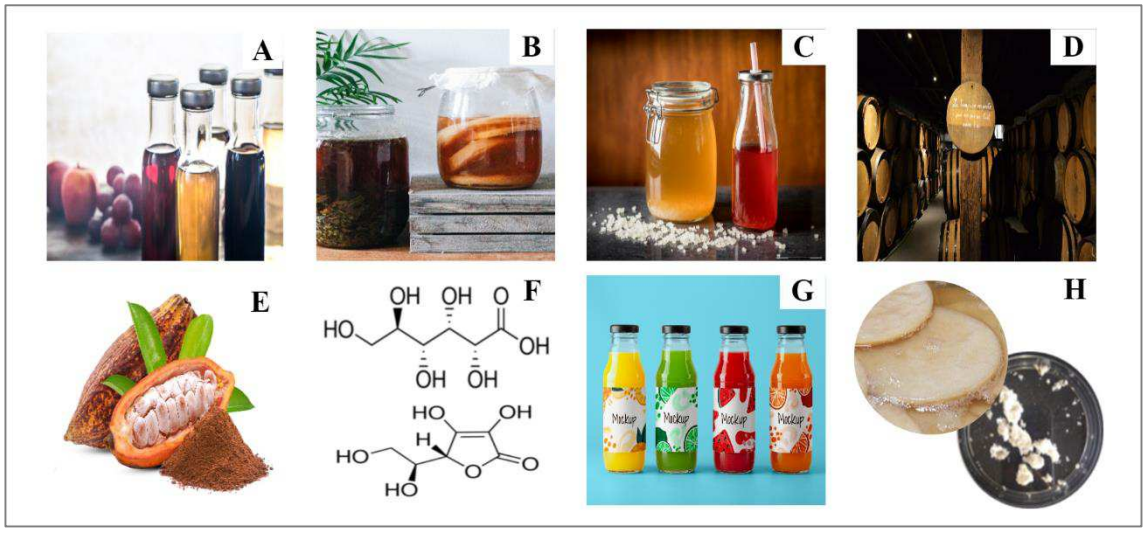
They show positive catalase and negative oxidase reactions, as well as strictly aerobic metabolism with oxygen as the terminal electron acceptor (*1*). According to Laureys *et al.* (*2*), AAB grow well between pH 5.0 and 6.5, but can also grow at pH 3.0 - 4.0 and even lower. The optimum temperature for growth is between 25 and 30 ºC, with no growth occurring above 34 ºC (*2,3*).

So far, seventeen genera are described in the family *Acetobacteraceae*, among which the ones with the highest number of species are *Acetobacter, Gluconobacter, Asaia, Komagataeibacter and Gluconacetobacter* (*4*). The group has the ability to oxidize various types of sugars, sugar alcohols and alcohols to their respective aldehydes, ketones and corresponding organic acids through an incomplete oxidation process called “oxidative fermentation”, from which they obtain their energy (*3*). *Acetobacter* and *Komagataeibacter* spp., for example, are specialized in converting ethanol to acetic acid via two successive oxidative steps, and are thus common in alcoholic and acidic environments, such as vinegar industry (*5*). They also present a complete set of citric acid cycle (CAC) enzymes, which are required for the further oxidation of organic acids to CO_2 and H_2O (*3*). On the other hand, *Gluconobacter* spp. occur preferentially in sugary niches and are particularly proficient in the oxidation of sugars and sugar alcohols (*6,7*). Due to a lack of CAC enzymes, they are unable to superoxidize acetate to CO_2 and H_2O, but they are valuable in the biotechnological synthesis of precursor compounds of vitamin C (L-sorbose), gluconic acid, dihydroxyketone, and miglitol (*3,8*).

The bacterium *Gluconacetobacter diazotropicus* is the most well-known member of the genus *Gluconacetobacter*, which plays an important role as a nitrogen-fixing bacteria in plants (*9,10*). The genus *Asaia*, on the other hand, has been linked to beverage spoilage and has recently been found as a symbiotic microorganism in malaria-carrying mosquitos (*11*). Also noteworthy is AAB's role in the production of exopolysaccharides, the most valuable of which are bacterial cellulose (BC) and levan, which are produced mainly by *Komagataebacter, Kozakia, Gluconacetobacter, Neoasaia*, and *Gluconobacter* species (*3,5*). AAB are widely distributed in alcoholic, sugary and acidic environments (*10*). They are often seen only as spoiling agents in wine, where acidity is undesirable, but the understanding of their role in the production of fermented foods like vinegar, kombucha, water kefir, lambic

beer and cocoa, as well as the applicability and functionality of EPS as the levan and cellulose, is still quite limited (Fig. 1). Thus, the dissemination of knowledge about the vast number of benefits of AAB, through research, makes it possible to explore them in different fields of application.

Fig. 1. Various foods, beverages, chemicals, and exopolysaccharides in which acetic acid bacteria are involved. A) Vinegars from different raw materials; B) Kombucha; C) Water kefir; D) Lambic beer; E) Cocoa; F) Organic acids (gluconic and ascorbic); G) New fruit drinks; and Exopolysaccharides (bacterial cellulose and levan). Parts of the figure designed by iStock.



PARTICIPATION OF AAB IN FOOD AND BEVERAGE

Vinegar

Vinegar production has been around for over 10,000 years (*3,12*). Despite not being considered a “food” and not having a high nutritional value, vinegar is consumed by people of all social classes all over the world, and it differs in terms of the raw materials used, manufacturing technologies, and its wide range of applications (Fig.1A) (*3,12,13*).

The definition and standards of vinegar identity and quality have some local differences, but in general, food regulatory agency consider vinegar to be the result of a double fermentation (first alcoholic, then acetic) of sugary substrates (*14*). In Brazil, the MAPA (Ministry of Agriculture, Livestock and Supply) defines acetic fermented as a product with a minimum volatile acidity of 4 % (g/100 mL, expressed in acetic acid) obtained from acetic fermentation of alcoholic fermented of fruit, cereal, other vegetable, honey, vegetable

mixture, or hydroalcoholic mixture. The acetic fermented product can be called “vinegar of...”, plus the name of the substrate used (*15*).

The substrate used in the processing of vinegar is mostly of vegetable origin (e.g. cereal, onion, cider, apple and mango vinegars), with the exception of honey and whey vinegars (*12,16*). The chemical composition of the raw material has a strong influence on the selection of microorganisms and determines the dominant species involved in the acetification process (*7*). In fruit vinegars, the alcoholic fermentation, carried out mainly by the yeast *Saccharomyces cerevisiae*, can be conducted by spontaneously induced fermentations (in artisanal productions) or by inoculation of pure cultures (starter) (in industrial scale productions) (*16*). In the second oxidation stage, the acetic fermentation, whose main representatives are the AAB of the *Acetobacter, Gluconobacter, Gluconacetobacter* and *Komagataeibacter* genera, the acetification rate can be improved by inoculating the "mother of vinegar" or starter cultures of beneficial strains to the process. Only in traditional wine vinegar production does acetic fermentation happen spontaneously (*12,16*).

Vinegars have a wide range of applications. Vinegars are frequently used as preservatives, flavoring agents, and as ingredients in mayonnaise, salad dressings, mustard, and other condiments (*13,16,17*). Its use as a routine medicine for humans and animals dates back to remote antiquity, in addition, it can be used as a cleaning agent and in some countries even as a healthy drink (*3,13*).

Although vinegar is traditionally used as a flavoring and food preservative, recent scientific studies have reported that regular consumption of vinegar can promote beneficial physiological health effects (*3,18,19*). Among the therapeutic properties presented by vinegar can be included: antibacterial activity, regulation of blood pressure and glycemia, antioxidant activity, prevention of cardiovascular diseases and prevention of obesity (*5,20*). Vinegars contain, in addition to acetic acid, several bioactive compounds such as polyphenols that contribute to their taste, smell and specific functions. Considering that different vinegars can be produced from different raw materials, processes and species of AAB, understanding the relationship between the compounds present and the functionality of vinegar is of great importance (*16,20*).

Kombucha

Kombucha is a popular drink usually consumed in Asia (*21*) (Fig.1B). It is characterized by being a non-alcoholic beverage, refreshing, with accentuated acidity and specific flavor (*22*). It is traditionally made by fermenting sweetened tea, but other plant-based (for example, cereals or plant leaves) or animal-based (for example, milk) raw materials, as

well as mushrooms, can be used (*23*). Fermentation, which lasts around 7 to 10 days, occurs quickly after adding the cellulosic layer called tea fungus or SCOBY (Symbiotic Colony of Bacteria and Yeast) to the sweetened tea (*24*). This symbiotic culture is predominantly composed of AAB (species of the genera *Komagataeibacter, Gluconobacter* and *Acetobacter*) (*22*), in addition to lactic acid bacteria (LAB) (*Lactobacillus, Lactococcus*) (*25*) and yeasts *(Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Torulaspora delbrueckii*, *Brettanomyces bruxellensis*) (*26*).

Under aerobic conditions, this consortium converts sugar and tea, producing major components such as acetic acid, gluconic acid, glucuronic acid and ethanol. The composition of kombucha also includes vitamins, phenolic compounds, amino acids, and some hydrolytic enzymes (*21,25*). The chemical profile of Kombucha may be responsible for its multiple health benefits when associated with regular consumption of the drink (*21*). Recently, tea has attracted the attention of researchers and consumers due to its in vitro biological activities, such as antimicrobial activity, antioxidant and anti-inflammatory activity and anticarcinogenic potential. However, more clinical investigations and in vivo evaluations should be carried out to confirm the health benefits of the beverage (*25,27*).

Water kefir

Water kefir is a sparkling, refreshing, low-alcohol drink with acidic and fruity flavors (*22*) (Fig.1C). Its obtainment is given by the spontaneous fermentation of sugar solution, dried fruits and water kefir grains (insoluble dextran). These translucent grains, which resemble cauliflower in shape, contain microorganisms that act as an inoculum for the fermentation process (*28*).

The microbial composition of the grains, which differ from milk kefir, features especially yeasts (e.g. *Pichia, Lachancea, Saccharomyces, Dekkera* and *Kluyveromyces*) and LAB (e.g. *Lactobacillus, Lactococcus* and *Leuconostoc*) (*29*). However, the metabolism of *Saccharomyces* and other yeasts stimulates the production of high concentrations of alcohol, thus allowing the growth of AAB (e.g. *Gluconobacter, Komagataeibacter* and *Acetobacter*) and the oxidation of ethanol to acetic acid (*30,31*). At the end of fermentation, the main products are ethanol, lactic acid, acetic acid and other metabolites such as mannitol, glycerol, esters, aldehydes, and other organic acids (*32,33*).

Kefir drinks, like Kombucha, have been linked to numerous health benefits. The beverage is well-known for having potentially ‘probiotic’ and antimicrobial properties against a wide range of pathogenic bacteria (*32,34*). Furthermore, studies have shown that water

kefir has antihyperlipidemic property (*35*), antioxidant activity (*31,36*), anticarcinogenic activity, hepatoprotective effect, anti-inflammatory effect, gastroprotective effect, among others (*33*). Due to the numerous positive effects of kefir, several substrates have been investigated for the adaptation of its grains (*31,36–38*). This enabled the emergence of new functional drinks with characteristics similar to traditional brown sugar kefir (*32*).

Lambic beer

Lambic beer, originally from Belgium, is probably one of the oldest beer styles brewed to date (*39,40*) (Fig.1D). It's a refreshing, alcoholic, acidic beer with fruity notes and only a few residual carbohydrates. The spontaneous fermentation process occurs in the presence of water, barley malt, unmalted wheat, and aged dry hops, and maturation takes up to three years in wooden barrels (*22*).

An in-depth examination of the microbial dominance pattern over the course of three years of lambic beer fermentation revealed four distinct phases (*7*). The first fase (from the start up to 1 month of fermentation) begins with members of the *Enterobacteriaceae* family, which are inhibited by ethanol accumulation produced by wild (oxidative) yeast, acidification by enterobacterial and AAB (*A. orientalis)*, and glucose reduction by microbial growth in general (*22,41*). *Gluconobacter* species such as *G. cerevisiae* also have been isolated during this phase, probably as a result of the combination of a monosaccharide-rich environment and low ethanol concentrations (*42*).

The second or phase of ethanol fermentation, referred to as the most important, extends until the fourth month, having as main representatives, yeasts (*Saccharomyces cerevisiae*, *S. bayanus/pastorianus and S. uvarum*), responsible for the conversion of carbohydrates to ethanol and carbon dioxide (*43*). After 4 to 10 months of fermentation, the acidification phase takes place. This phase is characterised by the predominance of the LAB species *Pediococcus damnosus* and/or *Lactobacillus brevis*, which together produce high amount of lactic acid (*41*). Finally, after 10 months, the maturation phase begins with yeast species *Dekkera bruxellensis* and LAB species *P. damnosus* and/or *L. brevis*, which is characterized by the production of ethyl acetate and ethyl lactate (*7,41,43*) During the third phase until 24 months of maturation, the growth of AAB *A. pasteurianus* was also observed by De Roos *et al.* (*42*). AAB are abundant during major periods of traditional lambic beer production's first fermentation year producing much larger concentrations of acetic acid and acetoin (from lactic acid oxidation via pyruvate) at the liquid/air interphase of the casks. Although the formation of acetic acid by AAB and the subsequent formation of ethyl acetate

are desired compounds for complex lambic beers, excessive AAB development must be controled to avoid an unfavorable flavor profile (*41,42*).

Cocoa

Cocoa bean (*Theobroma cacao* L) is the main raw material for the manufacture of chocolate (*44*) (Fig.1E). Due to its astringent and bitter taste, spontaneous cocoa fermentation plays an essential role for the composition and flavor of chocolate and cocoa-based products (*45,46*). This process involves biochemical changes and is characterized by a complex microbial succession of several species of yeasts, LAB, AAB, in addition to species of *Bacillus* sp., other bacteria and filamentous fungi (*44,47*).

During the first two days, yeasts are the most prevalent microorganisms, converting the pulp sugars to ethanol and carbon dioxide through alcoholic fermentation (anaerobiosis). The production of pectinolytic enzymes by yeasts also allows for the breakdown of pectin and the entry of oxygen into the cocoa pulp, resulting in microaerobic conditions contributing to the growth of LAB. LAB convert carbohydrates to lactic acid via the homofermentation pathway, while lactic acid, acetic acid, or ethanol, as well as carbon dioxide, are produced via the heterofermentation pathway (*7*). Increased temperature, aeration, and the availability of substrates (ethanol, acetic and lactic acid, mannitol) favor the growth of AAB, whose main function is to oxidize these substrates into acetic acid and acetoin in the final stages of fermentation. Several recent studies have used starter cultures to standardize and increase the productivity of the fermentation process, with AAB being one of the most important (*22,45,48*).

OTHER PRODUCTS PRODUCTED BY AAB

AAB can participate as biocatalysts for the industrial manufacturing a wide range of compounds, in addition to being employed commercially in the manufacturing of vinegars and other fermented foods (*5,10*) (Fig. 1F). *Gluconobacter* strains, in particular, have the ability to carry out oxidative fermentation of sugars and sugar alcohols, resulting in the formation of L-sorbose, ketogluconic acids, dihydroxyacetone (DHA), and cyclic ketones, among other compounds (*7,49*). The oxidative fermentation of L-sorbose from D-sorbitol is the most classic example observed during the production of vitamin C by *Gluconobacter*. Other precursor intermediates such as 2-keto-D-gluconic acid (2KGA) from D-Gluconic acid (GA), 2,5-diketo-D-gluconic acid (25DKGA), and 5-keto-D-gluconic acid (5KGA) are also present in the synthesis route (*7,10*). 5KGA has potential applications for the synthesis of

tartaric acid and xylaric acid, in addition to being a precursor for the manufacture of aromatic compounds such as 4-hydroxy-5-methyl-2,3-dihydrofuranone-3, a valuable product used in the food industry (*50*). The microbial production of DHA from glycerol has been explored in the pharmaceutical industry, and it can be used as a tanning agent and as an intermediate for the synthesis of various chemicals and surfactants (*1,10*). *Gluconobacter* species can also be applied in the biotransformation of miglitol precursors, a drug used for the treatment of type II diabetes; in the production of gluconic acid, considered a multifunctional acid in the food, feed, beverage, textile, pharmaceutical and construction industries; and in the manufacture of shikimic acid, a key intermediate for numerous antibiotics (*10,49*).

DEVELOPMENT OF NEW PRODUCTS FROM AAB

Currently, consumers have shown a growing interest in foods that, in addition to satisfying their hunger, can also prevent nutrition-related diseases and improve mental health (*51*). According to the Food and Agriculture Organization of the United Nations (FAO), fruits constitute an important part of a healthy diet. In addition to being a source of dietary fiber, vitamins, minerals, and beneficial phytochemicals, fruits may help lower risk factors for diseases such as overweight/obesity, chronic inflammation, high blood pressure, and high cholesterol (*52*).

Fruit-based fermentation has improved the nutritional and functional quality of beverages. In addition, rising consumer demand for lactose-free products with low fat content and few additives makes this type of fermentation a promising tool for meeting the needs of obese people with cardiovascular diseases, allergies, and intolerances, vegans, and vegetarians (*53*–*55*).

The development of fruit drinks containing probiotic bacteria stands out among current research (*56*–*59*). These microorganisms improve mineral bioavailability, digestibility, and organoleptic properties such as color, flavor, and aroma, in addition to providing a functional beverage (*55*). However, the acidic environment, as well as the presence of anti-nutritional and inhibitory factors in fruits, make maintaining bacteria viability and stability during processing and storage a major challenge (*54*). In view of this, AAB fermentation of fruit drinks becomes a viable alternative, since they can oxidize a wide range of substrates and are typically found in sugary and high-acid environments.

Fruit vinegar drinks, for instance, are gaining popularity in North America (Fig. 1G). By definition, the product must be made from at least one type of fruit and contain at least 300 g of fruit juice for each liter of fermented product. These beverages have been categorized according to the concentration of acetic acid as: low acidity (< 3% v/v) and high

acidity (5 - 7% v/v) (*60*). Furthermore, the fermentation method used and the concentration of acetic acid may affect the content of total sugars and soluble solids, titratable acidity, and density (60). As for its benefits, in vivo animal studies have shown that tomato vinegar drinks can prevent visceral obesity and insulin resistance (*61*), while pomegranate vinegar drinks have been shown to reduce visceral adipose tissue in humans (*62*). Other research suggests that fruit vinegar drinks like cranberry, blueberry, and tomato could be used to treat hypertension and hypercholesterolemia (*60*). However, according to Chang *et al.* (*63*), continuous consumption of vinegar drinks should be avoided to prevent gastrointestinal injuries.

Another recent approach has been focused on GA fermentation. Although works related to this type of fermentation are still scarce, their results are very promising. The gluconic fermentation of strawberry drink by *G. japonicus* converts glucose into GA while keeping the fructose naturally present in the fruit as a sweetener. This allows diabetics to consume a drink that keeps phenolic compounds (non-anthocianins) and antioxidant activity practically unchanged (*64*). Furthermore, its composition remains stable for 15 days at room temperature (27−30 °C) and up to 30 days when refrigerated (4 °C) (*65*). In another study, Hornedo-Ortega *et al*. (*66*) compared the antioxidant activity and anthocyanin composition of alcoholic and gluconic fermented strawberry drinks. The authors concluded that the gluconic fermentation of strawberry beverages by *G. japonicus* is a novel process that preserves the anthocyanin composition and shows higher antioxidant activity values than the alcoholic fermented (*66*). Ordóez *et al.* (*67*) also demonstrated the safety of strawberry gluconic fermented by demonstrating that none of the eight biogenic amines studied were detected. In addition to the bioactive compounds found in fruits, GA and its derivatives have been shown to have prebiotic properties. This acid promotes the growth of *Lactobacillus* sp. and *Bifidobacterium adolescentis* in the human colon and alters the intestine's metabolic profile (*68*). GA and its derivatives are approved for use in food and are commonly used to preserve and/or improve the sensory properties of dairy products and soft drinks (*68*). The presence of higher proportions of GA in Kombucha, for example, improved the taste of the drink in a study conducted by Li *et al.* (*69*). GA contributes to the pleasant sour taste, while the acetic acid formed produces an acidic and astringent off-flavor (*69*).

EXOPOLYSACCHARIDES PRODUCED BY AAB

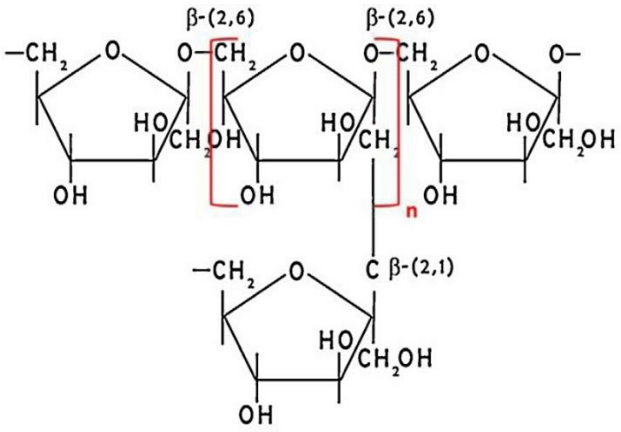
Microbial polysaccharides are produced by a wide range of bacteria, presenting an extreme diversity in terms of chemical structure and composition (*70*). AAB, for example, can produce large amounts of EPS, including both homopolysaccharides like levan and BC and

heteropolysaccharides like acetan or xylinan, and gluconacetan (*71–73*). Due the importance of levan and BC (Fig. 1H). in research and industrial applications, some of their characteristics and main applications in the food industry are discussed in this paper.

Levan

Levan is a polymer composed of D-fructofuranosyl residues joined by β-(2,6) bonds in the main chain and β-(2,1) bonds in the side chain, in addition to having a D-glucopyranosyl residue at the end of its chain (*74*). Fig. 2 shows the structure of the levan. Levan is commonly biosynthesized by a restricted number of plants in nature, but it can also be produced by several microorganisms, including Archaea, fungi and a wide range of bacteria (*75,76*). Among the AAB, species of the genera *Acetobacter, Gluconobacter, Gluconacetobacter, Kozakia* and *Neoasaia* are also capable of producing it (*76–80*).

Fig. 2. Chemical structure of levan (81).



The synthesis and polymerization of levan occurs in the extracellular matrix by the action of the enzyme levanasucrase, whose main function is to transfer fructose residues from sucrose by transfructosylation reactions (*81*). The enzyme has high specificity on sucrose and lower activity on fructose, mannose, raffinose, maltose, etc. On the other hand, inhibition is observed in the presence of glucose and other sugars that have a configuration similar to glucose, such as lactose, galactose and maltose, as well as other sugar alcohols (*81,82*).

In general, the structure of levans produced by different organisms is similar, but differ in terms of molecular mass, degrees of polymerization (DP) and branching (*82,83*). Plant levans have a lower molecular mass and DP than bacteria. Plant levans have a molecular mass of 2000 to 33000 Da and a DP < 100, whereas bacterial levans have multiple

ramifications (2 % to 12 %) and a molecular mass of 2 to 100 million Daltons (Da) with DP >100 (*81,83,84*).

In comparison to polysaccharides formed by pyranose rings, the structural characteristic of levan, in the form of furanose rings, plays an important role in the conformation of molecules in solution, providing additional flexibility. Furthermore, the semi-flexible chain of the rings interacts intramolecularly and intermolecularly, resulting in a densely packed spherical structure and low viscosity aqueous solutions (room temperature) at concentrations where other polysaccharides would form pastes or gels (*81,82,84–87*).

Xu *et al.* (*88*) observed that aqueous solutions of levan (*Brenneria* sp. EniD312) exhibit Newtonian fluid behavior at low concentrations (3 %; m/V) and non-Newtonian fluid (pseudoplastic fluid) behavior at high concentrations (6, 9 and 12%; m/V) when studying the rheological properties of levan. Levan solutions derived from *Zymomonas mobilis* and Erwinia herbicola showed similar results (*89*). At concentrations between 1 and 8%, however, the behavior of Bacillus subtilis levan solutions was completely Newtonian (89). According to Xu *et al.* (*88*), levan could be a good additive in the food industry, since its non-Newtonian behavior is interesting for the manufacture of dairy products, syrups and salad dressings.

Levan solutions are also characterized by exhibiting an atypical behavior when compared to other polysaccharides, in which gel formation is not observed (*85,90*). However, Jakob *et al.* (*91*), when establishing the structure/function relationship of isolated AAB levans, suggested that in solution, increasing their molecular weight reinforces intramolecular interactions in order to achieve a more compact structure characteristic of a “microgel” with hydrocolloid properties. The authors also reinforce that levans produced by AAB may thus offer new possibilities for applications in food.

Unlike many other polymers, levan does not swell in water, but it has a high solubility in hot water, a variable solubility in cold water, and is insoluble in most organic solvents, with the exception of dimethyl sulfoxide (DMSO). The high solubility of levan in water is mainly attributed to its β-(2,6) bond than to the β-(2,1) bond, and the ramifications could only be a supporting factor (*81,82,87*). Levans are non-reducing agents that aren't hydrolyzed by yeast invertases or amylases, but they are susceptible to acid hydrolysis (*82*). It decomposes at around 225 ºC, and the glass transition temperature is 141 ºC (*87*). Another important property of levan is its adhesive strength. Although sugars are characterized by stickiness, the adhesive strength of levan is significantly higher than that of other natural polymers. The branches contribute to its cohesive strength and the ability to form adhesive bonds with a wide range of substrates is given to its large number of hydroxyls. Levan is commonly

referred to as a "green" adhesive because it is water-removable and has high-value applications in several areas (*5,81,86*).

In food area, several studies have explored the effects of FOS-L and levan as prebiotics on probiotic bacteria and the complex gut microbiota, however there is no conclusive evidence from human trials (*92–97*) (Fig. 3). In animal models, for instance, levan supplementation increased *Lactobacillus* and *Bifidobacteria* viability, while inhibiting *Escherichia coli* and *Clostridium perfringers* (*96,97*). Adamberg *et al.* (*92*) using isothermal microcalorimetry and the human fecal microbiota reported that levan alters the composition of the fecal microbiota and the profile of metabolites, making it a potential candidate for prebiotics. Using metagenomic sequencing to assess the prebiotic activity of levan, Cheng (*98*) also verified alterations in the intestinal microbiota of mice and the stimulation of the production of short-chain fatty acids.

Fig. 3. Levan applications in food area. Parts of the figure designed by Freepik.

In bakery, EPS are known to improve the rheological properties of dough, and the texture, nutritional value, shelf life and machinability of wheat, rye and gluten-free breads (*99*). Jakob *et al.* (*77*) evaluated the functional effects of different AAB levans (*G. frateurii* TMW 2767, *G. cerinus* DSM 9533, *N. chiangmaiensis* NBRC 101099, and *K. baliensis* DSM 14400) added to wheat-based breads. The addition of two doses of EPS (1 and 2 % w/w flour) resulted in an increase in volume, a noticeable softening of the fresh breads, and a delay in hardening of the breads after a week of storage. Despite the fact that LAB and yeasts are common microorganisms in sourdoughs (*79*), Hermann *et al.* (*99*) reported that AAB like *N. chiangmaiensis* NBRC 101099 and *K. baliensis* DSM 14400 can grow on a variety of flours (wheat, whole wheat, spelled, and rye) and produce large amounts of levan. Later, gluten-free breads were made with buckwheat and molasses dough fermented by *G. albidus* TMW 2.1191 and *K. baliensis* NBRC 16680, and their volume, crumb hardness, and sensory characteristics were evaluated (*79*). Breads made from the dough had better sensory and

quality characteristics, such as higher specific volume and lower crumb hardness. However, the authors pointed out that strong acidification during fermentation could become a challenge in large-scale productions (*79*).

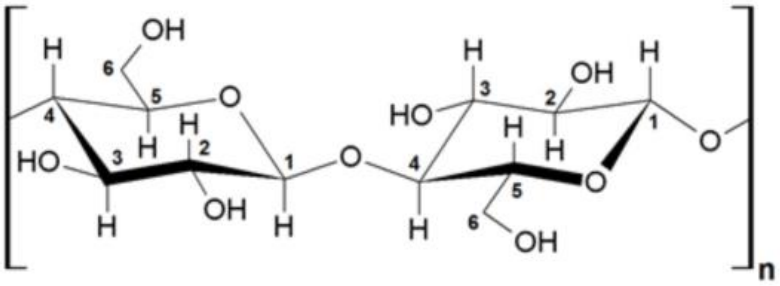
Other advantages of applying levan in foods include its use as a fat substitute. Fructans have fat-like properties that improve the flavor and spreadability of dairy products. Furthermore, high molecular weight levans are hardly detected by taste sensors and odor detection is almost imperceptible due to their low volatility (*75*). Its low viscosity and high solubility make it an excellent substitute for gum arabic, as the latter also has excellent stabilizing and emulsifying properties for food application (*82*). In the food packaging industry, levan as a component in edible starch films increased its barrier and mechanical properties besides being a cost-effective alternative (*74*). Gan *et al.* (*100*) also developed levan/pullulan/chitosan edible films enriched with ε-polylysine and applied on strawberry. As a result, they demonstrated that films could help preserve strawberry post-harvest quality by minimizing water loss, inhibiting microbial development, and decreasing respiration rate during storage.

Although this study is not focused on biomedical or cosmetic applications of levan, this EPS has shown several bioactive properties, including antitumor, antimicrobial, anti-inflammatory, hypocholesterolemic, antidiabetic, and immunostimulating activity (*75,81*). As a result, in addition to being a useful EPS for food production, its consumption alone or in food can provide several health benefits to the host.

Bacterial cellulose

BC is a linear glucan composed of several glucose monomers linked by β-(1–4) bonds (*5*) (Fig. 4). This biopolymer can be synthesized by various microorganisms, such as algae and fungi, as well as by various bacteria of the *Achromobacter, Alcaligenes*, *Aerobacter, Agrobacterium, Azotobacter, Komagataeibacter Pseudomonas, Rhizobium, Sarcina, Dickeya,* and *Rhodobacter* (*102,103*). Among these bacteria, *Komagataeibacter* species (AAB) are frequently utilized in research and commercial production, and are employed as a model strain because of their high productivity and ability to metabolize a wide range of carbon/nitrogen sources (*104*).

Fig. 4. Chemical structure of bacterial cellulose (101).



When cultivated under controlled conditions, *Komagataeibacter* produces highly porous BC structures in the form of pellicules (static culture) or as fibrous suspension, pellets, spheres or irregular masses (agitated culture) (*102,105*). The synthesis of BC from glucose involves several individual enzymes, catalytic complexes, and regulatory proteins. Briefly, β-glucan chains are formed first, followed by the assembly and crystallization of cellulose chains. In this final stage, the cellulose chains are released from the cell and self-assemble into fibrils (*103*). When compared to plant cellulose, BC has a great number of unique physicochemical and mechanical properties, including higher crystallinity, degree of polymerization, water absorbing and holding capacity, tensile strength, and biological adaptability (*104*). Moreover, plant-derived cellulose is usually incorporated with hemicellulose and lignin, necessitating harsh chemical treatments to remove these impurities (*106*). BC generated by microbial fermentation, on the other hand, has a greater purity and requires less energy and chemical processing to purify (*106*). As a result, BC has been used in a variety of food-related applications (Fig.5), since it is a dietary fiber that has been approved as "generally recognized as safe" (GRAS) food by the US Food and Drug Administration (FDA) (*107*).

The "nata de coco," a traditional food consumed in the Philippines and other Southeast Asian countries, is one of BC's most well-known industrial applications. In its manufacturing process, BC is synthetized by fermenting coconut water, then cleaning, washing, chopping, and immersing it in sugar syrup to be served as a dessert (*102,106,108*). Actually, a variety of nata-like products have been developed to meet consumer demands. To change the color and flavor of the dessert, different fruit juices, syrups, and other ingredients have been employed. Other items containing nata de coco, such as fruit-flavored pudding, drinks, and jellys, have been marketed all over the world.

In other food systems, BC has also shown promise as a stabilizer, noncaloric bulking agent, and texture modifier. After heat sterilization, the use of an aqueous suspension of BC

in liquids such as chocolate drinks prevent cocoa precipitation and stabilizes the dispersion (*109*). In pasty condiments BC reduces the stickiness and controlls the syneresis during storage (*109*). Furthermore, BC promotes firmness in solid foods like tofu, while it changes the texture of kamaboko by increasing hardness and fraturability (*109*). The addition of BC to meat products such as hamburguer and sausage can also reduce fat content without compromising tenderness and juiciness, as well as produce stable emulsions, respectively (*109*).

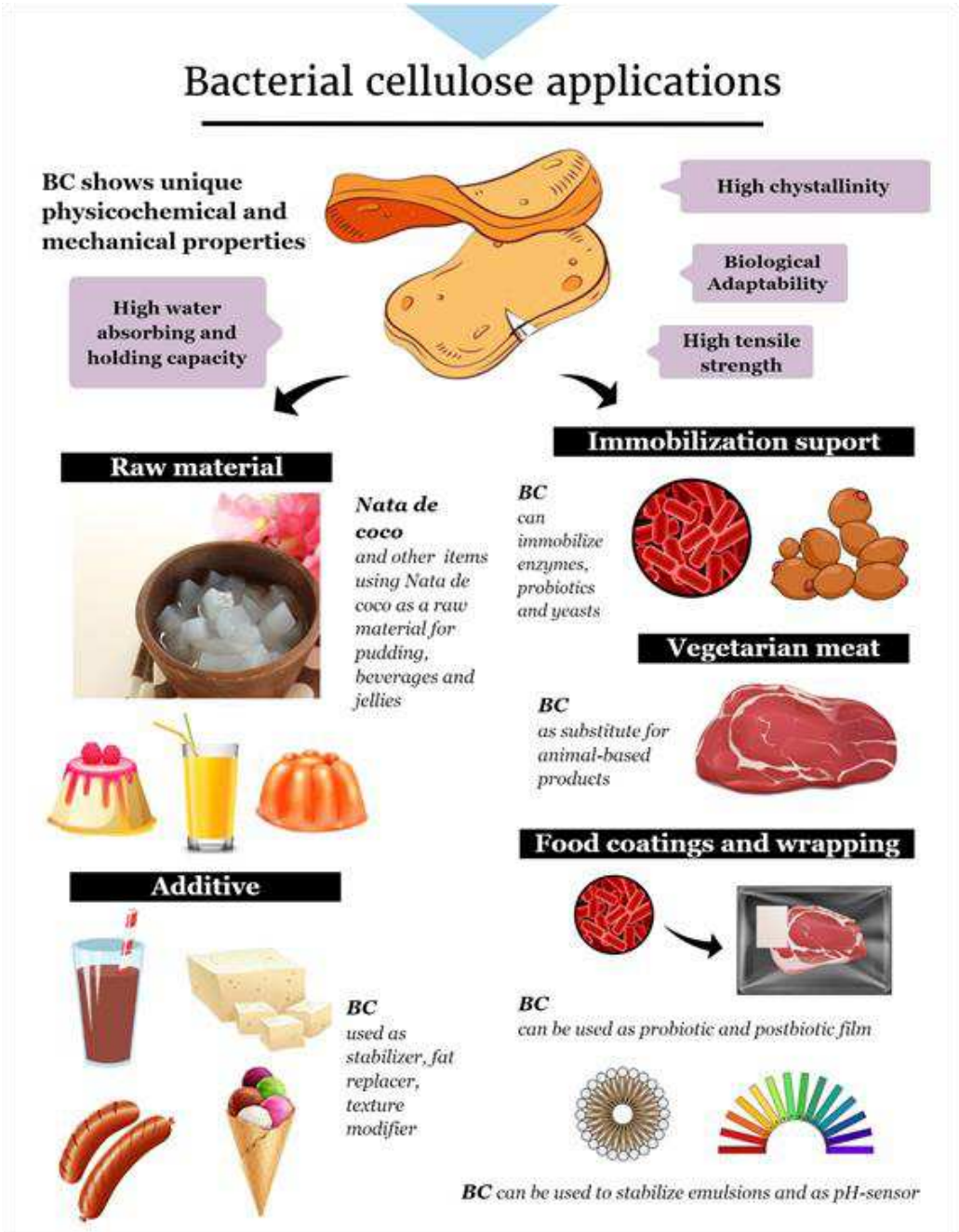
Following the example of earlier products that were lower in calories, Guo *et al.* (*110*) demonstrated that adding BC/soy protein isolate blends to ice cream as a cream substitute can result in ice creams with low calories, melt resistance, and good texture properties. Surimi products (*111*), cheese (*112*), meatballs (*113*), pork Frankfurters (*114*), and mayonnaise (*115*) are among the other applications of BC as a fat replacer. These findings suggest that BC could be widely used as a food additive in processed foods to improve their quality and shelf life while also lowering the calories in the final products.

BC has also been used as a vegetarian meat preparation when combined with Monascus extract obtained from a naturally red pigmented mould (*116*). The product has a natural meat flavor and is resistant to color and morphology changes. Furthermore, because of its non-animal origin, this ingredient may be a suitable substitute for animal-based products for some dietary restrictions (*102,104*).

BC has gained notoriety in research related to the immobilization of enzymes, cells, and probiotics for use in food. Because of its superior characteristics in relation to plant cellulose, BC has provided stability to enzymes against temperature and pH variations (*117*). When compared to free laccase, laccase immobilized on magnetically modified BC showed superior thermal stability at 70 °C and maintained 65 % of initial activity after 8 cycles of use (*118*). Similarly, Chen *et al.* (*119*) obtained a retention of 69 % of the original activity after seven recyclings when immobilizing fungal laccase on natural BC.

BC has also been explored as a carrier for cell immobilization, primarily for yeasts in the winemaking process. This approach reduces inoculum preparation costs, as the yeast can be recovered and separated at the end of the fermentation process (*106*). BC has been shown to protect wine yeast from adverse conditions such as high osmotic pressure and low pH (*120*). As a result, the growth of immobilized yeast was better than that of free yeast (*120*). Furthermore, metabolic activities of immobilized yeast in BC were reported to be higher than those of free yeast (*121*). Immobilized yeast in BC was also shown to have no negative impact on the sensory quality of the final product during repeated batch fermentation (*122*), and can increase the amount of alcohol produced as compared to free cells (*123*)

Fig. 5. Different applications of bacterial cellulose (BC) in food area. Parts of the figure designed by Freepik and iStock.



BC has been proved in studies to be an effective matrix for immobilization of the probiotic bacteria (*124*). In this context, Fijałkowski *et al.* (*125*) showed that BC as an immobilization support improves the probiotic viability, providing protection against adverse conditions of gastrointestinal tract (*125*). Furthermore, the authors established that the

immobilization effciency depends on the cellulose form, its synthesis method, and immobilization method (*125*). Similarly, Phromthep and Leenanon (*126*) have demonstrated that the BC produced from fruit juice residues and coconut milk resulted in improved survival of the immobilized *Lactobacillus plantarum* than free cells. Under prolonged incubation, Zywicka *et al.* (*127*) used BC pellicle as a support for immobilization during prolonged incubation and reported that the cell viability of *L. delbruecki* was affected after 72 hours. On the other hand, the viability of *L. acidophilus* 016 immobilized in BC Nano Fiber, was found to be 71 % for up to 24 days when stored at ambient temperature (35 ºC) (*124*). This finding shows potential because one of the requirements for a microorganism to be administered for therapeutic purposes is that it remain viable in the food that will be consumed (*125*).

More recently, BC has been reported as a matrix for probiotic films (*128,129*). The films can be used as coatings or wrapping over a variety of foods, providing consumer health benefits, as well as potentially inhibiting the growth of spoilage bacteria and fungus on food surfaces, thus extending the shelf life of the product (*128,129*). Similarly, the development of BC-based films and probiotics-derived bioactive metabolites (so-called postbiotics) have also gained attention for antimicrobial food packaging (*130,131*). For meat applications, the rapid release of postbiotics from BC-based films into food is ideal for food with a finite shelf life, as it can effectively control foodborne pathogens like *Listeria monocytogenes* while also extending the shelf life without affecting the meat's sensorial attributes (*130,131*). In addition, several studies have performed ex-situ and in-situ modifications of BC to improve its properties for use in food packaging. However, to assess potential usefulness as active packaging, more research is needed to investigate mechanical, permeability, interactions, and release rates in semisolid and solid food model media (*132*).

Other recent BC applications include its use as a food-grade emulsion stabilizer (*133–135*), whose function can also be extended to cosmetics and medical emulsions. In addition to developing pH-sensing indicators based on BC nanofibers incorporated with anthocyanins from various fruits, vegetables, and flowers for freshness monitoring and shelf life extension of fish (*136*), beverages (*137*), fruits (*138*), and shrimp (*139,140*).

CONCLUSIONS

AAB are well-known for causing wine spoilage. However, their importance and functionality for food applications were demonstrated in this study. Through oxidative fermentation, AAB can produce a variety of metabolites. Its role in various biochemical processes during food fermentation, such as vinegar, kombucha, water kefir, cocoa, and lambic beer, results in unique sensory and biochemical characteristics, as well as health

benefits. Although commercial production of vinegar-based drinks is well established, research on fruit drinks fermented by AAB is still relatively new and scarce. Beverage production of fruits through gluconic fermentation is very promising, since several fruits can be used (including non-standard fruits, for example) and can be more adaptable to AAB metabolism than to the growth of LAB. Furthermore, due to the formation of GA and the presence of phenolic compounds in the fruits, the functional drink could meet the demand of lactose intolerant, allergic to milk proteins, and those seeking a vegetarian, vegan and healthy diet. Due to bioactive properties, levan could also be explored in beverage development. Levan could be produced *in situ* during the gluconic fermentation of fruit juices, since several species of *Gluconobacter* are able to produce levan from sucrose and oxidize glucose to gluconic acid. BC has numerous applications in food, with several products already marketed worldwide. However, similar to levan, its main challenge lies in large-scale production and in reducing production costs to expand its applications in this area.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTION

Natália Norika Yassunaka Hata – Drafting the article, and conception or design of the work.
Mônica Surek - critical revision
Daniele Sartori - critical revision
Rodrigo Vassoler Serrato - critical revision
Wilma Aparecida Spinosa - critical revision and final approval of the version to be published.

ORCID ID

Natália Norika Yassunaka Hata – https://orcid.org/0000-0003-2319-1156
Monica Surek - https://orcid.org/0000-0003-4048-3046
Daniele Sartori - https://orcid.org/0000-0002-0465-9932
Rodrigo Vassoler Serrato - https://orcid.org/0000-0001-8868-6825
Wilma Aparecida Spinosa - https://orcid.org/0000-0001-9532-0135

REFERENCES

1. Sengun IY, Karabiyikli S. Importance of acetic acid bacteria in food industry. Food Control. 2011;22(5):647–56. http://dx.doi.org/10.1016/j.foodcont.2010.11.008
2. Laureys D, Britton SJ, De Clippeleer J. Kombucha tea fermentation: a review. J Am Soc Brew Chem. 2020;78(3):165–74. https://doi.org/10.1080/03610470.2020.1734150
3. Lynch KM, Zannini E, Wilkinson S, Daenen L, Arendt EK. Physiology of acetic acid bacteria and their role in vinegar and fermented beverages. Compr Rev Food Sci Food Saf. 2019;18(3):587–625. https://doi.org/10.1111/1541-4337.12440
4. Taweesak Malimas, Huong Thi Lan Vu, Yuki Muramatsu, Pattaraporn Yukphan ST and YY. Systematics of acetic acid bacteria. In: Acetic acid bacteria: fundamentals and food applications. 2014.
5. Gomes RJ, Borges M de F, Rosa M de F, Castro-Gómez RJH, Spinosa WA. Acetic acid bacteria in the food industry: Systematics, characteristics and applications. Food Technol Biotechnol. 2018;56(2):139–51. https://doi.org/10.17113/ftb.56.02.18.5593
6. Jakob F, Gebrande C, Bichler RM, Vogel RF. Insights into the pH-dependent, extracellular sucrose utilization and concomitant levan formation by *Gluconobacter albidus* TMW 2.1191. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol. 2020;113(7):863–73. https://doi.org/10.1007/s10482-020-01397-3
7. Matsushita K, Toyama H, Tonouchi N, Kainuma AO-. Acetic Acid Bacteria. Ecology and physiology, Springer, Japan, 2016. https://doi.org/10.1007/978-4-431-55933-7
8. La China S, Zanichelli G, De Vero L, Gullo M. Oxidative fermentations and exopolysaccharides production by acetic acid bacteria: a mini review. Biotechnol Lett 2018;40(9–10):1289–302. https://doi.org/10.1007/s10529-018-2591-7
9. Sievers M, Swings J. *Gluconacetobacter*. Bergey's Man Syst Archaea Bact. 2015;1–11. https://doi.org/10.1002/9781118960608.gbm00883
10. Mamlouk D, Gullo M. Acetic acid bacteria: physiology and carbon sources oxidation. Indian J Microbiol. 2013;53(4):377–84. https://doi.org/10.1007/s12088-013-0414-z
11. Bassene H, Niang EHA, Fenollar F, Doucoure S, Faye O, Raoult D, et al. Role of plants in the transmission of *Asaia* sp., which potentially inhibit the *Plasmodium* sporogenic cycle in *Anopheles* mosquitoes. Sci Rep. 2020;10(1):1–10. https://doi.org/10.1038/s41598-020-64163-5
12. Solieri L, Giudici P. Vinegars of the world. Vinegars of the World. 2009;1–297. https://doi.org/10.1007/978-88-470-0866-3
13. Spinosa WA, dos Santos Júnior V, Galvan D, Fiorio JL, Gomez RJHC. Vinegar rice (*Oryza sativa* L.) produced by a submerged fermentation process from alcoholic fermented rice. Food Sci Technol. 2015;35(1):196–201. https://doi.org/10.1590/1678-457X.6605
14. Ho CW, Lazim AM, Fazry S, Zaki UKHH, Lim SJ. Varieties, production, composition and health benefits of vinegars: A review. Vol. 221, Food Chemistry. 2017. 1621–1630 https://doi.org/10.1016/j.foodchem.2016.10.128
15. BRASIL. Ministério da Agricultura Pecuária e Abastecimento (MAPA). Instrução Normativa n.º 6 de abril de 2012. Estabelece os padrões de identidade e qualidade e

a classificação dos fermentados acéticos. Diário Oficial da União República Federativa do Brasil, Brasília, DF, 4 de abril de 2012.
16. Li S, Li P, Feng F, Luo LX. Microbial diversity and their roles in the vinegar fermentation process. Appl Microbiol Biotechnol. 2015;99(12):4997–5024. https://doi.org/10.1007/s00253-015-6659-1
17. Budak NH, Aykin E, Seydim AC, Greene AK, Guzel-Seydim ZB. Functional properties of vinegar. J Food Sci. 2014;79(5). https://doi.org/10.1111/1750-3841.12434
18. Xia T, Zhang B, Duan W, Zhang J, Wang M. Nutrients and bioactive components from vinegar: A fermented and functional food. J Funct Foods. 2020;64(August 2019):103681. https://doi.org/10.1016/j.jff.2019.103681
19. Ousaaid D, Mechchate H, Laaroussi H, Hano C, Bakour M, Ghouizi A El, et al. and Fruits vinegar: quality characteristics, phytochemistry, and functionality. 2022;1–16. https://doi.org/10.3390/molecules27010222
20. Samad A, Azlan A, Ismail A. Therapeutic effects of vinegar: A review. Curr Opin Food Sci. 2016;8:56–61. http://dx.doi.org/10.1016/j.cofs.2016.03.001
21. De Filippis F, Troise AD, Vitaglione P, Ercolini D. Different temperatures select distinctive acetic acid bacteria species and promotes organic acids production during Kombucha tea fermentation. Food Microbiol. 2018;73:11–6. https://doi.org/10.1016/j.fm.2018.01.008
22. De Roos J, De Vuyst L. Acetic acid bacteria in fermented foods and beverages. Curr Opin Biotechnol. 2018;49:115–9. http://dx.doi.org/10.1016/j.copbio.2017.08.007
23. Morales D. Biological activities of kombucha beverages: The need of clinical evidence. Trends Food Sci Technol. 2020;105(September):323–33. https://doi.org/10.1016/j.tifs.2020.09.025
24. Laavanya D, Shirkole S, Balasubramanian P. Current challenges, applications and future perspectives of SCOBY cellulose of Kombucha fermentation. J Clean Prod. 2021;295:126454. https://doi.org/10.1016/j.jclepro.2021.126454
25. Villarreal-Soto SA, Beaufort S, Bouajila J, Souchard JP, Taillandier P. Understanding kombucha tea fermentation: a review. J Food Sci. 2018;83(3):580–8. https://doi.org/10.1111/1750-3841.14068
26. Coton M, Pawtowski A, Taminiau B, Burgaud G, Deniel F, Coulloumme-Labarthe L, et al. Unraveling microbial ecology of industrial-scale Kombucha fermentations by metabarcoding and culture-based methods. FEMS Microbiol Ecol. 2017;93(5):1–16. https://doi.org/10.1093/femsec/fix048
27. Kapp JM, Sumner W. Kombucha: a systematic review of the empirical evidence of human health benefit. Ann Epidemiol. 2019;30:66–70. https://doi.org/10.1016/j.annepidem.2018.11.001
28. Lynch KM, Wilkinson S, Daenen L, Arendt EK. An update on water kefir: Microbiology, composition and production. Int J Food Microbiol. 2021;345(January 2020):109128. https://doi.org/10.1016/j.ijfoodmicro.2021.109128
29. Pendón MD, Bengoa AA, Iraporda C, Medrano M, Garrote GL, Abraham AG. Water kefir: Factors affecting grain growth and health-promoting properties of the fermented beverage. J Appl Microbiol. 2021;(November):1–19. https://doi.org/10.1111/jam.15385
30. Laureys D, Aerts M, Vandamme P, De Vuyst L. Oxygen and diverse nutrients influence the water kefir fermentation process. Food Microbiol. 2018;73:351–61.

https://doi.org/10.1016/j.fm.2018.02.007
31. Bueno RS, Ressutte JB, Hata NNY, Henrique-Bana FC, Guergoletto KB, de Oliveira AG, et al. Quality and shelf life assessment of a new beverage produced from water kefir grains and red pitaya. Lwt. 2021;140. https://doi.org/10.1016/j.lwt.2020.110770
32. Fiorda FA, de Melo Pereira GV, Thomaz-Soccol V, Rakshit SK, Pagnoncelli MGB, Vandenberghe LP de S, et al. Microbiological, biochemical, and functional aspects of sugary kefir fermentation - a review. Food Microbiol. 2017;66:86–95. https://doi.org/10.1016/j.fm.2017.04.004
33. Guzel-Seydim ZB, Gökırmaklı Ç, Greene AK. A comparison of milk kefir and water kefir: Physical, chemical, microbiological and functional properties. Trends Food Sci Technol. 2021;113(April):42–53. https://doi.org/10.1016/j.tifs.2021.04.041
34. Waldherr FW, Doll VM, Meißner D, Vogel RF. Identification and characterization of a glucan-producing enzyme from *Lactobacillus hilgardii* TMW 1.828 involved in granule formation of water kefir. Food Microbiol. 2010;27(5):672–8. http://dx.doi.org/10.1016/j.fm.2010.03.013
35. Rocha-Gomes A, Escobar A, Soares JS, Alves A, Silva D, Riul TR. Chemical composition and hypocholesterolemic effect of milk kefir and water kefir in Wistar rats Composição química e efeito hipocolesterolêmico do kefir de leite e do kefir de água em ratos Wistar. Rev Nutr. 2018;31(2):137–45. http://dx.doi.org/10.1590/1678-9865201800020001
36. Ozcelik F, Akan E, Kinik O. Use of Cornelian cherry, hawthorn, red plum, roseship and pomegranate juices in the production of water kefir beverages. Food Biosci. 2021;42(June):101219. https://doi.org/10.1016/j.fbio.2021.101219
37. Darvishzadeh P, Orsat V, Martinez JL. Correction to: Process optimization for development of a novel water kefir drink with high antioxidant activity and potential probiotic properties from russian olive fruit (*Elaeagnus angustifolia*). Food Bioprocess Technol. 2021;14(2):261. https://doi.org/10.1007/s11947-020-02563-1
38. Destro TM, Prates D da F, Watanabe LS, Garcia S, Biz G, Spinosa WA. Organic brown sugar and jaboticaba pulp influence on water kefir fermentation. Cienc e Agrotecnologia. 2019;43. https://doi.org/10.1590/1413-7054201943005619
39. Spitaels F, Wieme AD, Janssens M, Aerts M, Van Landschoot A, De Vuyst L, et al. The microbial diversity of an industrially produced lambic beer shares members of a traditionally produced one and reveals a core microbiota for lambic beer fermentation. Food Microbiol. 2015;49:23–32. http://dx.doi.org/10.1016/j.fm.2015.01.008
40. Thompson Witrick K, Duncan SE, Hurley KE, O'keefe SF. Acid and volatiles of commercially-available lambic beers. Beverages. 2017;3(4). https://doi.org/10.3390/beverages3040051
41. De Roos J, Vandamme P, De Vuyst L. Wort substrate consumption and metabolite production during lambic beer fermentation and maturation explain the successive growth of specific bacterial and yeast species. Front Microbiol. 2018;9(NOV):1–20. https://doi.org/10.3389/fmicb.2018.02763
42. De Roos J, Verce M, Aerts M, Vandamme P, De Vuyst L. Temporal and spatial distribution of the acetic acid bacterium communities throughout the wooden casks used for the fermentation and maturation of lambic beer underlines their functional role. Appl Environ Microbiol. 2018;84(7):1–15. https://doi.org/10.1128/AEM.02846-17

43. Spitaels F, Wieme AD, Janssens M, Aerts M, Daniel HM, Van Landschoot A, et al. The microbial diversity of traditional spontaneously fermented lambic beer. PLoS One. 2014;9(4). https://doi.org/10.1371/journal.pone.0095384
44. Ho VTT, Fleet GH, Zhao J. Unravelling the contribution of lactic acid bacteria and acetic acid bacteria to cocoa fermentation using inoculated organisms. Int J Food Microbiol. 2018;279:43–56. https://doi.org/10.1016/j.ijfoodmicro.2018.04.040
45. De Vuyst L, Weckx S. The cocoa bean fermentation process: from ecosystem analysis to starter culture development. J Appl Microbiol. 2016;121(1):5–17. https://doi.org/10.1111/jam.13045
46. Visintin S, Alessandria V, Valente A, Dolci P, Cocolin L. Molecular identification and physiological characterization of yeasts, lactic acid bacteria and acetic acid bacteria isolated from heap and box cocoa bean fermentations in West Africa. Int J Food Microbiol 2016;216:69–78. http://dx.doi.org/10.1016/j.ijfoodmicro.2015.09.004
47. de Melo Pereira GV, Magalhães KT, de Almeida EG, da Silva Coelho I, Schwan RF. Spontaneous cocoa bean fermentation carried out in a novel-design stainless steel tank: Influence on the dynamics of microbial populations and physical-chemical properties. Int J Food Microbiol. 2013;161(2):121–33. http://dx.doi.org/10.1016/j.ijfoodmicro.2012.11.018
48. Sandhya MVS, Yallappa BS, Varadaraj MC, Puranaik J, Rao LJ, Janardhan P, et al. Inoculum of the starter consortia and interactive metabolic process in enhancing quality of cocoa bean (*Theobroma cacao*) fermentation. LWT - Food Sci Technol. 2016;65:731–8. http://dx.doi.org/10.1016/j.lwt.2015.09.002
49. Saichana N, Matsushita K, Adachi O, Frébort I, Frebortova J. Acetic acid bacteria: A group of bacteria with versatile biotechnological applications. Biotechnol Adv. 2015;33(6):1260–71. http://dx.doi.org/10.1016/j.biotechadv.2014.12.001
50. Salusjärvi T, Povelainen M, Hvorslev N, Eneyskaya E V., Kulminskaya AA, Shabalin KA, et al. Cloning of a gluconate/polyol dehydrogenase gene from *Gluconobacter* suboxydans IFO 12528, characterisation of the enzyme and its use for the production of 5-ketogluconate in a recombinant *Escherichia coli* strain. Appl Microbiol Biotechnol. 2004;65(3):306–14. https://doi.org/10.1007/s00253-004-1594-6
51. Donno D, Turrini F. Plant foods and underutilized fruits as source of functional food ingredients: Chemical composition, quality traits, and biological properties. Foods. 2020;9(10):7–10. https://doi.org/10.3390/foods9101474
52. FAO. Fruit and vegetables – your dietary essentials. Fruit and vegetables – your dietary essentials. The International Year of Fruits and Vegetables. 2020.
53. Ayed L, M'Hir S, Hamdi M. Microbiological, Biochemical, and functional aspects of fermented vegetable and fruit beverages. J Chem. 2020;2020. https://doi.org/10.1155/2020/5790432
54. Min M, Bunt CR, Mason SL, Hussain MA. Non-dairy probiotic food products: An emerging group of functional foods. Crit Rev Food Sci Nutr. 2019;59(16):2626–41. https://doi.org/10.1080/10408398.2018.1462760
55. Marrero SC, Martínez-Rodríguez A, Pérez SEM, Moya SP. New trends and applications in fermented beverages. Fermented Beverages: Volume 5. The Science of Beverages. Elsevier Inc.; 2019. 31–66 p. https://doi.org/10.1016/B978-0-12-815271-3.00002-6

56. Kaprasob R, Kerdchoechuen O, Laohakunjit N, Somboonpanyakul P. B vitamins and prebiotic fructooligosaccharides of cashew apple fermented with probiotic strains *Lactobacillus* spp., *Leuconostoc mesenteroides* and *Bifidobacterium longum*. Process Biochem. 2018;70(February):9–19. https://doi.org/10.1016/j.procbio.2018.04.009
57. Yang X, Zhou J, Fan L, Qin Z, Chen Q, Zhao L. Antioxidant properties of a vegetable–fruit beverage fermented with two *Lactobacillus plantarum* strains. Food Sci Biotechnol. 2018;27(6):1719–26. https://doi.org/10.1007/s10068-018-0411-4
58. Saska S, Teixeira LN, de Castro Raucci LMS, Scarel-Caminaga RM, Franchi LP, dos Santos RA, et al. Nanocellulose-collagen-apatite composite associated with osteogenic growth peptide bone regeneration. Int J Biol Macromol. 2017;103:467–76. http://dx.doi.org/10.1016/j.ijbiomac.2017.05.086
59. Mantzourani I, Kazakos S, Terpou A, Mallouchos A, Kimbaris A, Alexopoulos A, et al. Assessment of volatile compounds evolution, antioxidant activity, and total phenolics content during cold storage of pomegranate beverage fermented by *Lactobacillus paracasei* K5. Fermentation. 2018;4(4). https://doi.org/10.3390/fermentation4040095
60. Ruchira Nandasiri HPVR. Inhibition of low density lipoprotein oxidation and angiotensin converting enzyme in vitro by functional fruit vinegar beverages. J Food Process Beverages. 2013;1(2):2–6.
61. Seo K Il, Lee J, Choi RY, Lee HI, Lee JH, Jeong YK, et al. Anti-obesity and anti-insulin resistance effects of tomato vinegar beverage in diet-induced obese mice. Food Funct. 2014;5(7):1579–86. http://dx.doi.org/10.1039/C4FO00135D
62. Park JE, Kim JY, Kim J, Kim YJ, Kim MJ, Kwon SW, et al. Pomegranate vinegar beverage reduces visceral fat accumulation in association with AMPK activation in overweight women: A double-blind, randomized, and placebo-controlled trial. J Funct Foods. 2014;8(1):274–81. http://dx.doi.org/10.1016/j.jff.2014.03.028
63. Chang J, Han SE, Paik SS, Kim YJ. Corrosive esophageal injury due to a commercial vinegar beverage in an adolescent. Clin Endosc. 2020;53(3):366–9. http://dx.doi.org/10.5946/ce.2019.066
64. Álvarez-Fernández MA, Hornedo-Ortega R, Cerezo AB, Troncoso AM, García-Parrilla MC. Effects of the strawberry (*Fragaria ananassa*) purée elaboration process on non-anthocyanin phenolic composition and antioxidant activity. Food Chem. 2014;164:104–12. https://doi.org/10.1016/j.foodchem.2014.04.116
65. Álvarez-Fernández MA, Hornedo-Ortega R, Cerezo AB, Troncoso AM, García-Parrilla MC. Determination of nonanthocyanin phenolic compounds using high-resolution mass spectrometry (UHPLC-Orbitrap-MS/MS) and impact of storage conditions in a beverage made from strawberry by fermentation. J Agric Food Chem. 2016;64(6):1367–76. https://doi.org/10.1021/acs.jafc.5b05617
66. Hornedo-Ortega R, Álvarez-Fernández MA, Cerezo AB, Troncoso AM, García-Parrilla MC. Influence of storage conditions on the anthocyanin profile and colour of an innovative beverage elaborated by gluconic fermentation of strawberry. J Funct Foods. 2016;23:198–209. http://dx.doi.org/10.1016/j.jff.2016.02.014
67. Ordóñez JL, Sainz F, Callejón RM, Troncoso AM, Torija MJ, García-Parrilla MC. Impact of gluconic fermentation of strawberry using acetic acid bacteria on amino acids and biogenic amines profile. Food Chem. 2015;178:221–8.

https://doi.org/10.1016/j.foodchem.2015.01.085
68. Cañete-Rodríguez AM, Santos-Dueñas IM, Jiménez-Hornero JE, Ehrenreich A, Liebl W, García-García I. Gluconic acid: Properties, production methods and applications—An excellent opportunity for agro-industrial by-products and waste bio-valorization. Process Biochem. 2016;51(12):1891–903. https://doi.org/10.1016/j.procbio.2016.08.028
69. Li R, Xu Y, Chen J, Wang F, Zou C, Yin J. Enhancing the proportion of gluconic acid with a microbial community reconstruction method to improve the taste quality of Kombucha. Lwt. 2022;155:112937. https://doi.org/10.1016/j.lwt.2021.112937
70. Roca C, Alves VD, Freitas F, Reis MAM. Exopolysaccharides enriched in rare sugars: Bacterial sources, production, and applications. Front Microbiol. 2015;6(APR):1–7. https://doi.org/10.3389/fmicb.2015.00288
71. Brandt JU, Born FL, Jakob F, Vogel RF. Environmentally triggered genomic plasticity and capsular polysaccharide formation are involved in increased ethanol and acetic acid tolerance in *Kozakia baliensis* NBRC 16680. BMC Microbiol. 2017;17(1):1–12. https://doi.org/10.1186/s12866-017-1070-y
72. Kornmann H, Valentinotti S, Duboc P, Marison I, Von Stockar U. Monitoring and control of *Gluconacetobacter xylinus* fed-batch cultures using in situ mid-IR spectroscopy. J Biotechnol. 2004;113(1–3):231–45. https://doi.org/10.1016/j.jbiotec.2004.03.029
73. Semino CE, Dankert, MA. In vitro biosynthesis of acetan using electroporated *Acetobacter xylinum* cells as enzyme preparations. J Gen Microbiol. 1993;193(1 993):2745–56. https://doi.org/10.1099/00221287-139-11-2745
74. Mantovan J, Bersaneti GT, Faria-Tischer PCS, Celligoi MAPC, Mali S. Use of microbial levan in edible films based on cassava starch. Food Packag Shelf Life. 2018; https://doi.org/10.1016/j.fpsl.2018.08.003
75. Öner ET, Hernández L, Combie J. Review of Levan polysaccharide: From a century of past experiences to future prospects. Biotechnol Adv. 2016;34(5):827–44. http://dx.doi.org/10.1016/j.biotechadv.2016.05.002
76. Srikanth R, Siddartha G, Sundhar Reddy CHSS, Harish BS, Janaki Ramaiah M, Uppuluri KB. Antioxidant and anti-inflammatory levan produced from *Acetobacter xylinum* NCIM2526 and its statistical optimization. Carbohydr Polym. 2015;123:8–16. http://dx.doi.org/10.1016/j.carbpol.2014.12.079
77. Jakob F, Steger S, Vogel RF. Influence of novel fructans produced by selected acetic acid bacteria on the volume and texture of wheat breads. Eur Food Res Technol. 2012;234(3):493–9. https://doi.org/10.1007/s00217-011-1658-7
78. Semjonovs P, Shakirova L, Treimane R, Shvirksts K, Auzina L, Cleenwerck I, et al. Production of extracellular fructans by *Gluconobacter nephelii* P1464. Lett Appl Microbiol. 2016;62(2):145–52. https://doi.org/10.1111/lam.12521
79. Ua-Arak T, Jakob F, Vogel RF. Influence of levan-producing acetic acid bacteria on buckwheat-sourdough breads. Food Microbiol. 2017;65:95–104. http://dx.doi.org/10.1016/j.fm.2017.02.002
80. Idogawa N, Amamoto R, Murata K, Kawai S. Phosphate enhances levan production in the endophytic bacterium *Gluconacetobacter diazotrophicus* Pal5. Bioengineered. 2014;5(3):173–9. https://doi.org/10.4161/bioe.28792

81. Srikanth R, Reddy CHSSS, Siddartha G, Ramaiah MJ, Uppuluri KB. Review on production, characterization and applications of microbial levan. Carbohydr Polym. 2015;120:102–14. https://doi.org/10.1016/j.carbpol.2014.12.003
82. Han W. Youn. Microbial Levan. Advances in Applied Microbiology. 1990. https://doi.org/10.1016/S0065-2164(08)70244-2
83. Mutanda T, Mokoena MP, Olaniran AO, Wilhelmi BS, Whiteley CG. Microbial enzymatic production and applications of short-chain fructooligosaccharides and inulooligosaccharides: Recent advances and current perspectives. J Ind Microbiol Biotechnol. 2014;41(6):893–906. https://doi.org/10.1007/s10295-014-1452-1
84. Stojković B, Sretenovic S, Dogsa I, Poberaj I, Stopar D. Viscoelastic properties of levan-DNA mixtures important in microbial biofilm formation as determined by micro- and macrorheology. Biophys J. 2015;108(3):758–65. https://doi.org/10.1016/j.bpj.2014.10.072
85. Benigar E, Tomšič M, Sretenovic S, Stopar D, Jamnik A, Dogsa I. Evaluating SAXS results on aqueous solutions of various bacterial Levan utilizing the string-of-beads model. Acta Chim Slov. 2015;62(3):509–17. http://dx.doi.org/10.17344/acsi.2015.1437
86. Combie J. Properties of levan and potential medical uses. ACS Symp Ser. 2006;934:263–9. http://dx.doi.org/10.1021/bk-2006-0934.ch013
87. Manandhar S, Vidhate S, D'Souza N. Water soluble levan polysaccharide biopolymer electrospun fibers. Carbohydr Polym. 2009;78(4):794–8. http://dx.doi.org/10.1016/j.carbpol.2009.06.023
88. Xu W, Liu Q, Bai Y, Yu S, Zhang T, Jiang B, et al. Physicochemical properties of a high molecular weight levan from *Brenneria* sp. EniD312. Int J Biol Macromol. 2018;109:810–8. http://dx.doi.org/10.1016/j.ijbiomac.2017.11.056
89. Benigar E, Dogsa I, Stopar D, Jamnik A, Cigić IK, Tomšič M. Structure and dynamics of a polysaccharide matrix: Aqueous solutions of bacterial levan. Langmuir. 2014;30(14):4172–82. https://doi.org/10.1021/la500830j
90. Majee SB, Avlani D, Biswas GR. Rheological behavior and pharmaceutical applications of bacterial exopolysaccharides. J Appl Pharm Sci. 2017;7(9):224–32. http://dx.doi.org/10.7324/JAPS.2017.70931
91. Jakob F, Pfaff A, Novoa-carballal R, Rübsam H, Becker T, Vogel RF. Structural analysis of fructans produced by acetic acid bacteria reveals a relation to hydrocolloid function. Carbohydr Polym. 2013;92(2):1234–42. http://dx.doi.org/10.1016/j.carbpol.2012.10.054
92. Adamberg K, Tomson K, Talve T, Pudova K, Puurand M, Visnapuu T, et al. Levan enhances associated growth of *Bacteroides*, *Escherichia*, *Streptococcus* and *Faecalibacterium* in fecal microbiota. PLoS One. 2015;10(12):1–18. https://doi.org/10.1371/journal.pone.0144042
93. Adamberg S, Tomson K, Vija H, Puurand M, Kabanova N, Visnapuu T, et al. Degradation of fructans and production of propionic acid by bacteroides thetaiotaomicron are enhanced by the shortage of amino acids. Front Nutr. 2014;1(December):1–10. https://doi.org/10.3389/fnut.2014.00021
94. Porras-Domínguez JR, Ávila-Fernández Á, Rodríguez-Alegría ME, Miranda-Molina A, Escalante A, González-Cervantes R, et al. Levan-type FOS production using a *Bacillus licheniformis* endolevanase. Process Biochem. 2014;49(5):783–90.

http://dx.doi.org/10.1016/j.procbio.2014.02.005
95. Li J, Kim IH. Effects of levan-type fructan supplementation on growth performance, digestibility, blood profile, fecal microbiota, and immune responses after lipopolysaccharide challenge in growing pigs. J Anim Sci. 2013;91(11):5336–43. https://doi.org/10.2527/jas.2013-6665
96. Zhao PY, Wang JP, Kim IH. Effect of dietary levan fructan supplementation on growth performance, meat quality, relative organ weight, cecal microflora, and excreta noxious gas emission in broilers. J Anim Sci. 2013;91(11):5287–93. https://academic.oup.com/jas/article-abstract/91/11/5287/4731425
97. Zhao PY, Wang JP, Kim IH. Evaluation of dietary fructan supplementation on growth performance, nutrient digestibility, meat quality, fecal microbial flora, and fecal noxious gas emission in finishing pigs. J Anim Sci. 2013;91(11):5280–6. https://doi.org/10.2527/jas.2012-5393
98. Cheng R, Cheng L, Zhao Y, Wang L, Wang S, Zhang J. Biosynthesis and prebiotic activity of a linear levan from a new *Paenibacillus* isolate. Appl Microbiol Biotechnol. 2021;105(2):769–87. https://doi.org/10.1007/s00253-020-11088-8
99. Hermann M, Petermeier H, Vogel RF. Development of novel sourdoughs with in situ formed exopolysaccharides from acetic acid bacteria. Eur Food Res Technol. 2015;241(2):185–97. https://doi.org/10.1007/s00217-015-2444-8
100. Gan L, Jiang G, Yang Y, Zheng B, Zhang S, Li X, et al. Development and characterization of levan/pullulan/chitosan edible films enriched with ε-polylysine for active food packaging. Food Chem. 2022;388(April):132989. https://doi.org/10.1016/j.foodchem.2022.132989
101. Perotti, G. F., Barud, H. S., Ribeiro, S. J., & Constantino, V. R. Bacterial cellulose as a template for preparation of hydrotalcite-like compounds. Journal of the Brazilian Chemical Society. 2014;25:1647-1655. https://doi.org/10.5935/0103-5053.20140153
102. Ullah H, Santos HA, Khan T. Applications of bacterial cellulose in food, cosmetics and drug delivery. Cellulose. 2016;23(4):2291–314. https://doi.org/10.1007/s10570-016-0986-y
103. Azeredo HMC, Barud H, Farinas CS, Vasconcellos VM, Claro AM. Bacterial cellulose as a raw material for food and food packaging applications. Front Sustain Food Syst. 2019;3(February). https://doi.org/10.3389/fsufs.2019.00007
104. Lin D, Liu Z, Shen R, Chen S, Yang X. Bacterial cellulose in food industry: Current research and future prospects. Int J Biol Macromol. 2020;158:1007–19. https://doi.org/10.1016/j.ijbiomac.2020.04.230
105. Andriani D, Apriyana AY, Karina M. The optimization of bacterial cellulose production and its applications: a review. Cellulose. 2020;27(12):6747–66. https://doi.org/10.1007/s10570-020-03273-9
106. Marestoni LD, Barud H da S, Gomes RJ, Catarino RPF, Hata NNY, Ressutte JB, et al. Commercial and potential applications of bacterial cellulose in Brazil: Ten years review. Polimeros. 2021;30(4). https://doi.org/10.1590/0104-1428.09420
107. Shi Z, Zhang Y, Phillips GO, Yang G. Utilization of bacterial cellulose in food. Food Hydrocoll. 2014;35:539–45. http://dx.doi.org/10.1016/j.foodhyd.2013.07.012
108. Iguchi M, Yamanaka S, Budhiono A. Bacterial cellulose - a masterpiece of nature's arts. J Mater Sci. 2000;35(2):261–70. https://doi.org/10.1023/A:1004775229149

109. Okiyama A, Motoki M, Yamanaka S. Bacterial cellulose IV. Application to processed foods. Top Catal. 1993;6(6):503–11. http://dx.doi.org/10.1016/S0268-005X(09)80074-X
110. Guo Y, Zhang X, Hao W, Xie Y, Chen L, Li Z, et al. Nano-bacterial cellulose/soy protein isolate complex gel as fat substitutes in ice cream model. Carbohydr Polym 2018;198(March):620–30. https://doi.org/10.1016/j.carbpol.2018.06.078
111. Lin S, Chen L, Chen H. Physical characteristics of surimi and bacterial cellulose composite gel. 2009;34(2011):1363–79. https://doi.org/10.1111/j.1745-4530.2009.00533.x
112. Karahan AG, Kart A, Akoğlu A, Çakmakçi ML. Physicochemical properties of low-fat soft cheese Turkish Beyaz made with bacterial cellulose as fat mimetic. Int J Dairy Technol. 2011;64(4):502–8. https://doi.org/10.1111/j.1471-0307.2011.00718.x
113. Lin KW, Lin HY. Quality characteristics of chinese-style meatball containing bacterial cellulose (nata). J Food Sci. 2004;69(3). https://doi.org/10.1111/j.1365-2621.2004.tb13378.x
114. Yu SY, Lin KW. Influence of bacterial cellulose (nata) on the physicochemical and sensory properties of Frankfurter. J Food Sci. 2014;79(6). https://doi.org/10.1111/1750-3841.12494
115. Akoğlu A, Cakir I, Karahan AG, Cakmakci ML. Effects of bacterial cellulose as a fat replacer on some properties of fat-reduced mayonnaise. Rom Biotechnol Lett. 2017;23(3):13674–80.
116. Purwadaria T, Gunawan L, Gunawan AW. The Production of Nata Colored by Monascus purpureus J1 Pigments as Functional Food. Microbiol Indones. 4(1), 2. https://doi.org/10.5454/mi.4.1.2
117. Wu SC, Lia YK. Application of bacterial cellulose pellets in enzyme immobilization. J Mol Catal B Enzym. 2008;54(3–4):103–8. https://doi.org/10.1016/j.molcatb.2007.12.021
118. Drozd R, Rakoczy R, Wasak A, Junka A, Fijałkowski K. The application of magnetically modified bacterial cellulose for immobilization of laccase. Int J Biol Macromol 2018;108:462–70. http://dx.doi.org/10.1016/j.ijbiomac.2017.12.031
119. Chen L, Zou M, Hong FF. Evaluation of fungal laccase immobilized on natural nanostructured bacterial cellulose. Front Microbiol. 2015;6(NOV):1–10. https://doi.org/10.3389/fmicb.2015.01245
120. Ton NMN, Nguyen MD, Pham TTH, Le VVM. Influence of initial pH and sulfur dioxide content in must on wine fermentation by immobilized yeast in bacterial cellulose. Int Food Res J. 2010;17(3):743–9.
121. Nguyen DN, Ton NMN, Le VVM. Optimization of *Saccharomyces cerevisiae* immobilization in bacterial cellulose by ‘adsorption- incubation‘ method. Int Food Res J. 2009;16(1):59–64.
122. Ton NMN, Le VVM. Application of immobilized yeast in bacterial cellulose to the repeated batch fermentation in wine-making. Int Food Res J. 2011;18(3):983–7.
123. Żywicka A, Peitler D, Rakoczy R, Junka AF, Fijałkowski K. Wet and dry forms of bacterial cellulose synthetized by different strains of *Gluconacetobacter xylinus* as carriers for yeast immobilization. Appl Biochem Biotechnol. 2016;180(4):805–16. https://doi.org/10.1007/s12010-016-2134-4
124. Jayani T, Sanjeev B, Marimuthu S, Uthandi S. Bacterial Cellulose Nano Fiber (BCNF) as carrier support for the immobilization of probiotic, *Lactobacillus acidophilus* 016. Carbohydr Polym. 2020;250(March):116965. https://doi.org/10.1016/j.carbpol.2020.116965

125. Fijałkowski K, Peitler D, Rakoczy R, Zywicka A. Survival of probiotic lactic acid bacteria immobilized in different forms of bacterial cellulose in simulated gastric juices and bile salt solution. Lwt. 2016;68:322–8. https://doi.org/10.1016/j.lwt.2015.12.038
126. Phromthep K, Leenanon B. Survivability of immobilized *Lactobacillus plantarum* cells within bacterial cellulose in mamao juice. Int Food Res J. 2017;24(3):939–49.
127. Żywicka A, Wenelska K, Junka A, Chodaczek G, Szymczyk P, Fijałkowski K. Immobilization pattern of morphologically different microorganisms on bacterial cellulose membranes. World J Microbiol Biotechnol. 2019;35(1):1–11. http://dx.doi.org/10.1007/s11274-018-2584-7
128. Oliveira-Alcântara AV, Abreu AAS, Gonçalves C, Fuciños P, Cerqueira MA, Gama FMP, et al. Bacterial cellulose/cashew gum films as probiotic carriers. Lwt. 2020;130(March):109699. https://doi.org/10.1016/j.lwt.2020.109699
129. Motalebi Moghanjougi Z, Rezazadeh Bari M, Alizadeh Khaledabad M, Almasi H, Amiri S. Bio-preservation of white brined cheese (Feta) by using probiotic bacteria immobilized in bacterial cellulose: Optimization by response surface method and characterization. Lwt 2020;117(September 2019):108603. https://doi.org/10.1016/j.lwt.2019.108603
130. Rasouli Y, Moradi M, Tajik H, Molaei R. Fabrication of anti-Listeria film based on bacterial cellulose and *Lactobacillus sakei*-derived bioactive metabolites; application in meat packaging. Food Biosci. 2021;42(June):101218. https://doi.org/10.1016/j.fbio.2021.101218
131. Shafipour Yordshahi A, Moradi M, Tajik H, Molaei R. Design and preparation of antimicrobial meat wrapping nanopaper with bacterial cellulose and postbiotics of lactic acid bacteria. Int J Food Microbiol. 2020;321(December 2019):108561. https://doi.org/10.1016/j.ijfoodmicro.2020.108561
132. Cazón P, Vázquez M. Bacterial cellulose as a biodegradable food packaging material: A review. Food Hydrocoll. 2021;113. https://doi.org/10.1016/j.foodhyd.2020.106530
133. Yan H, Chen X, Song H, Li J, Feng Y, Shi Z, et al. Synthesis of bacterial cellulose and bacterial cellulose nanocrystals for their applications in the stabilization of olive oil pickering emulsion. Food Hydrocoll. 2017;72:127–35. http://dx.doi.org/10.1016/j.foodhyd.2017.05.044
134. Paximada P, Koutinas AA, Scholten E, Mandala IG. Effect of bacterial cellulose addition on physical properties of WPI emulsions. Comparison with common thickeners. Food Hydrocoll. 2016;54:245–54. http://dx.doi.org/10.1016/j.foodhyd.2015.10.014
135. Zhai X, Lin D, Liu D, Yang X. Emulsions stabilized by nanofibers from bacterial cellulose: New potential food-grade Pickering emulsions. Food Res Int. 2018;103(July 2017):12–20. https://doi.org/10.1016/j.foodres.2017.10.030
136. Moradi M, Tajik H, Almasi H, Forough M, Ezati P. A novel pH-sensing indicator based on bacterial cellulose nanofibers and black carrot anthocyanins for monitoring fish freshness. Carbohydr Polym. 2019;222(March):115030. https://doi.org/10.1016/j.carbpol.2019.115030
137. Kuswandi B, Asih NPN, Pratoko DK, Kristiningrum N, Moradi M. Edible pH sensor based on immobilized red cabbage anthocyanins into bacterial cellulose membrane for intelligent food packaging. Packag Technol Sci. 2020;33(8):321–32. https://doi.org/10.1002/pts.2507
138. Kuswandi B, Maryska C, Jayus, Abdullah A, Heng LY. Real time on-package

freshness indicator for guavas packaging. J Food Meas Charact. 2013;7(1):29–39. https://doi.org/10.1007/s11694-013-9136-5

139. Mohammadalinejhad S, Almasi H, Moradi M. Immobilization of *Echium amoenum* anthocyanins into bacterial cellulose film: A novel colorimetric pH indicator for freshness/spoilage monitoring of shrimp. Food Control. 2020;113(August 2019):107169. https://doi.org/10.1016/j.foodcont.2020.107169
140. Wen Y, Liu J, Jiang L, Zhu Z, He S, He S, et al. Development of intelligent/active food packaging film based on TEMPO-oxidized bacterial cellulose containing thymol and anthocyanin-rich purple potato extract for shelf life extension of shrimp. Food Packag Shelf Life. 2021;29(June):100709. https://doi.org/10.1016/j.fpsl.2021.100709

5.2. ARTIGO CIENTÍFICO2[1]

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Natália Norika Yassunaka Hata[a*], Daniele Sartori[b], Matheus Mertz Ribeiro[b], Rodrigo Vassoler Serrato[e], Adriana Aparecida Bosso Tomal[c], Leonel Vinícius Constantino[d], Mariana Assis de Queiroz Cancian[a], Francisco de Assis Marques[f], Fernando Teruhiko Hata[g], Fernanda Carla Henrique Bana[a], Fernando César de Macedo Júnior[d], Wilma Aparecida Spinosa[a]

[a]Departamento de Ciência e Tecnologia de Alimentos / Centro de Ciências Agrárias / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil; [b]Departamento de Bioquímica e Biotecnologia / Centro de Ciências Exatas / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil; [c]Departamento de Ciências Naturais / Universidade do Estado de Minas Gerais / Av. Paraná, 3001, Jardim Belvedere, 35501–170, Divinópolis, MG, Brazil; [d]Departamento de Química / Centro de Ciências Exatas / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil; [e]Departamento de Bioquímica e Biologia Molecular / Universidade Federal do Paraná – UFPR, Centro Politécnico, 81531-990, Curitiba, PR, Brazil; [f]Departamento de Química / Centro de Ciências Exatas / Universidade Federal do Paraná – UFPR, Centro Politécnico, 81531-990, Curitiba, PR, Brazil; [g]Departamento de Agronomia / Centro de Ciências Agrárias / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil
*Corresponding author. E-mail address: naty_ea@hotmail.com

Structural and biochemical characterization of levan produced by *Gluconobacter cerinus* UELBM11 from Brazilian grapes

Abstract

Acetic acid bacteria (AAB) are versatile organisms which catalyze a wide range of carbon sources and alcohols into biomolecules of great industrial interest. In this work, we exploited the ability of *Gluconobacter* spp. to synthesize levan. Among the isolates found, *Gluconobacter cerinus* UELBM11 produced about 14.0 g/L of levan under non-optimized conditions. Gas chromatography–mass spectrometry (GC-MS), Fourier transform infrared (FT-IR) spectroscopy, and Nuclear Magnetic Resonance (NMR) analysis confirmed that levan from *G. cerinus* UELBM11 consisted of D-fructose bound by β-(2→6) with some β-(2→1) linked branch chains. The average molecular weight (Mw) of purified levan was 8.78×10^5 Da. Thermogravimetric/Differential Thermogravimetric (TGA)/(DTG) analysis indicated high thermal stability with the maximum decomposition rate observed at 227.44 °C. Scanning Electron Microscopy (SEM) revealed a microporous morphology and the assay of the antioxidant activity demonstrated that levan had high scavenging of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and hydroxyl radicals. Thus, we demonstrated that levan produced by *G. cerinus* UELBM11 is a promising natural antioxidant and, due its excellent properties, may be used as a useful additive for applications in cosmetics, pharmaceutics, and food products.

Keywords: Acetic Acid Bacteria, *Gluconobacter*, Biotechnology, Fructan

1. Introduction

Acetic acid bacteria (AAB) are well-known spoilage microorganisms that can easily be isolated from foods or beverages such as wine, beer, sweet drinks, and fruits [1,2]. Naturally spoiled grapes are an excellent medium for their proliferation since sugars and alcohols present can be quickly oxidized to their corresponding organic acids. Furthermore, in this substrate, the tolerance of AAB to ethanol and their ability to convert it to acetic acid, restrict the multiplication of several microorganisms [3]. On the other hand, AAB are versatile organisms of great industrial interest. They play a key role in the production of ascorbic acid (vitamin C) and cellulose, and in the food industry, the group is involved in the production of several foods and beverages, which include vinegar, cocoa, kombucha, and other similar fermented beverages [1].

Recently, AAB species of *Asaia, Gluconobacter*, *Gluconacetobacter*, *Komagataeibacter*, *Kozakia*, *Neoasaia,* and *Tanticharoenia* genera have been exploited for their ability to produce fructan of levan type with multiple applications [4]. Levan is a homopolysaccharide constituted by fructose units linked by β-(2→6)-glycosidic bonds, with some β-(2→1) linked branch chains, and a terminal D-glucosyl residue [5]. The synthesis reaction of levan by bacteria is catalyzed by levansucrase (EC 2.4.1.10); an enzyme belonging to the family of glycoside hydrolases (GH68) that catalyzes the hydrolysis of sucrose and the transfer of fructosyl units to various acceptors [6,7].

The unique properties presented by levan, such as its high solubility in water and oil, strong adhesiveness, biocompatibility, and film-forming ability, enable levan as a potential functional biopolymer mainly in food and pharmaceutical industries [8]. Levan may be considered a potential candidate to the prebiotic, since it modulates the composition and function of the human colon microbiota, increasing the growth of probiotic strains [9]. In

bread making, Jakob et al. [10] used levan from AAB and reported a significant increase in volume, reduced crumb hardness, and retarded staling of wheat bread. Other applications of levan in food involve its use as an emulsifying, fat substitute, and encapsulating and stabilizing agent [8,11].

In the medical area, levan is one of the macromolecules that have demonstrated a wide range of biological activities, namely: anti-oxidant [12], anti-inflammatory [12], hypocholesterolemic [11], immunomodulatory [4], anti-cancer activities [13,14], and curative effect on peptic ulcers [15].

Taking into consideration the wide application field of levan and that its production from AAB is still relatively recent, looking for new and potent producing members is extremely relevant to reduce its production costs. Moreover, levan produced from different microbial sources may differ significantly regarding to physicochemical and bioactive properties. Therefore, in this study, we isolated exopolysaccharide (EPS) producing *Gluconobacter* strains from Brazilian grapes, tested their ability to synthesize homopolysaccharides from sucrose and characterized the levan produced from *G. cerinus* UELBM11. Futhermore, *in vitro* antioxidant activity of levan was determined by ABTS and hydroxyl radical scavenging.

2. Materials and Methods

2.1. Microorganism isolation

Over ripen grapes (*Vitis vinifera* L.) samples were collected from two areas of Brazil: Barreiras, Bahia (11°57’14.0”S, 45°31’51.7”W) and Sarandi, Paraná (23º25’00.4”S, 51º48’58.3”W). Posteriorly, the samples were cut into small pieces and inoculated in two enrichment mediums denominated MYP (Manitol, Yeast extract, Peptone) [16] and GEPYA

(Glucose, Ethanol, Peptone, Yeast extract, Acetic acid, Cycloheximide) [17] and incubated under aerobic conditions at 30 °C for 120 h. After incubation, serial dilutions were prepared from both enrichment mediuns and spread on MYP agar plates. The plates were incubated at 30 °C for 96 h and the bacterial colonies that grew were purified by repeating streaking. All isolates were stored in 20 % malt extract (w/v) at -20 °C.

2.2. Biochemical and morphological identification of bacterial strains

Morphological and biochemical analyses were carried out according to Bergey's Manual of Systematic Bacteriology [18,19] and others report [10,20–22]. Firstly, gram staining and a test of oxidation of ethanol to acetic acid were carried out in order to identify AAB. Only strains that presented as gram-negative and produced acetic acid were subjected to test of production of catalase, production of cellulose, production of dihydroxyacetone from glycerol (ketogenesis), production of brown pigment, acid production from glucose, overoxidation of acetic acid to carbon dioxide and water, oxidation of lactate to carbon dioxide and water and finally, production of mucous EPS on sucrose agar plates. The EPS-producing activity was observed macroscopically and classified according to Jakob et al. [10] as: (+) weak, (+++) strong, and (-) no production of mucous EPS from sucrose.

2.3. Molecular identification

The molecular technique for the species identification was performed only for isolates that demonstrated (+++) strong EPS-producing activity.

2.3.1. DNA extraction

One mL cell suspension in sterile ultrapure water was prepared from a fresh culture on MYP agar for each strain studied. The DNA extraction from the bacteria was performed as described by Cheng and Jiang [23]. The quantification of extracted DNA was estimated by electrophoresis using 1% agarose gel (w/v).

2.3.2. Polymerase Chain Reaction (PCR) amplification

PCR primers employed to amplify the 16S rDNA were 16Sd 5'-GCTGGCGGCATGCTTAACACAT-3' and 16Sr 5'-GGAGGTGATCCAGCCGCAGGT-3' [24]. The 25 µL reaction mixture contained 10 ng bacterial DNA, 0.4 µmol/L of each primer, 0.25 mmol/L of the dNTPs mix, 2.0 mmol/L $MgCl_2$, 1U Taq DNA polymerase, amplification buffer and ultrapure water. The PCR was performed in a thermal cycler (Eppendorf, Germany) and the amplification was performed according to Ruiz et al. [24]. Amplified PCR products were checked by 1% (w/v) agarose gel electrophoresis and a 1 kb DNA Ladder marker. Purified PCR products were sequenced by BPI – Biotecnologia Pesquisa e Inovação, Brazil. The obtained sequences were aligned with known sequences deposited in the National Center for Biotechnology Information (NCBI) database [25] using the software MEGA 7 (https://www.megasoftware.net/). Phylogenetic tree reconstruction was carried out using Neighbor Joining distance method. All identified bacteria were registered in Genbank.

2.4. Cultivation of selected strains and production of levans

All identified strains were evaluated for their ability to produce fructose EPS from medium containing sucrose. Firstly, one loopful of preinocula cultivated for 72 h on MYP agar (1%, w/v) was transferred to 250 mL flasks containing 100 mL of HS medium (%, w/v - glucose 2.0; peptone 0.5; yeast extract 0.5; anhydrous disodium phosphate 0.27; monohydrate

citric acid 0.115; pH 6.0) [26]. After cultivation at 30 °C on a rotary shaker (120 rpm) (TECNAL-4200) until 0.6 of absorbance (600 nm) (Thermo Electron Corporation®, Spectronic Genesys 6®, EUA), the cells were washed and used as inoculum. The levan fermentative production was conducted in Erlenmeyer flasks with a working volume of 100 mL. Modified HS medium (50 g/L sucrose) was inoculated with standardized inoculum (1%, v/v) and kept at 30 °C for 48 h under shaking conditions (150 rpm).

2.5. Isolation, purification and quantification of levans

The fermentation process was interrupted by centrifugation at 5000 rpm for 20 min to remove the cells. The supernatant obtained was precipitated with cold ethanol 99% (3:1, v/v) and left overnight at -20 °C [27]. The precipitate was recovered by centrifugation and dried at 50 °C (partially purified levan). Prior to identification and structural characterization, partially purified levan was redissolved in ultrapure water, followed by deproteinization with 1% Alcalase® 2.4L FG (v/v) (Novozymes Latin American Limited; Brazil) at pH 8.0 and incubation at 55 °C for 1.5 h. After the procedure, the solution was heated at 85 ºC for 10 min to inactivate the enzyme, centrifuged and the supernatant reprecipitated with ethanol, dialyzed (MWCO 14 kDa) (Sigma-Aldrich, USA) for at least 48 h at 4 °C and lyophilized [10].

The lyophilized sample was dissolved in deionized water, then loaded on a Sepharose CL-2B column (20 mm × 500 mm, Sigma), and eluted with 50 mmol/L phosphate buffer (pH 7.0) at a flow rate of 0.5 mL/min. The main fractions were detected by phenol-sulfuric acid method [28] pooled, dialyzed, and lyophilized (purified levan). The protein content was estimated by the Bradford method using bovine serum albumin as standard [29].

For quantification, levan was analyzed by HPAEC-PAD (ICS 5000, Dionex Canada Ltd., Oakville, Canada), equipped with a CarboPac PA-10 column (250 mm x 4 mm, i.d.,

Dionex, CA). Briefly, levan was resuspended and hydrolyzed in 0.05 mol/L oxalic acid for 1 h at 100 °C [30] neutralized with 0.1 mol/L NaOH, and filtered through a 0.22 µm membrane (Millipore, MA, USA). Aliquots of 10 µL were analyzed at 1 mL/min at 25 °C with isocratic elution of 20 mmol/L NaOH through the use of ultrapure water (resistivity of 18.2 MΩ, 90% solvent A) and 200 mmol/L NaOH (10% solvent B) for 20 min. For each chromatographic run a regeneration step was performed with 200 mmol/L NaOH (10 min), followed by re-equilibration with 20 mmol/L NaOH for 15 minutes. Fructose and glucose (Sigma-Aldrich, USA) standard treated under the same conditions were used for quantification.

2.6. Structural characterization of levan from Gluconobacter cerinus UELBM11

2.6.1. Molecular weight determination

The molecular mass determination was performed in high-pressure steric exclusion chromatograph (HPSEC) (DSP-F, Wyatt Technology, USA) with four gel permeation columns Ultrahydrogel (Waters) (2000, 500, 250 and 120). This system is equipped with a differential refractive index (IR) detector and a multi angle laser light scattering detector (MALLS). The levan samples were prepared and analyzed according to the procedures described by Serrato et al. [27].

2.6.2. Fourier-Transform Infrared (FT-IR) Spectroscopy

The determination of functional groups and chemical bonds of levan was performed by FT-IR spectroscopy. The sample was prepared as a KBr pellet and the spectroscopic measurements were carried out within the wavelength range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1} using a FT-IR spectrometer (Shimadzu, Kyoto, Japan).

2.6.3. Monosaccharide composition analysis

The monosaccharide composition of purified levan was determined as its alditol acetate (AA) derivatives. Levan was hydrolyzed (2 mol/L Trifluoroacetic acid, 30 min, 50 °C) [31], followed by reduction with sodium borohydride ($NaBH_4$) at room temperature and then acetylated with a mixture of pyridine and acetic anhydride (1:1 v/v) for 24 h at 25 ºC. The alditol acetates obtained were extracted with chloroform, washed successively with 5% $CuSO_4$ solution (m/v) for removal of residual pyridine, and then washed with water twice. The resulting organic phase was then dried and submitted to GC-MS (Marca, País) analysis according to Serrato et al. [27].

2.6.4. Methylation analysis

The linkage analysis of monosaccharide units of levan was performed according to the method described by Gojgic-Cvijovic et al. [32]. Briefly, levan (60 mg) was dissolved in dimethyl sulfoxide (DMSO) with an excess of NaOH and fully methylated with iodomethane (CH_3I). Permethylated levan was dialyzed (MWCO 14 kDa) against destilled water and submitted to hydrolysis with 1% acetic acid at 100 °C for 4 h [33], reduction and acetylation as described above (item 2.6.3). The partially metilated alditol acetate (PMAA) monosaccharide derivatives were determined after GC-MS analysis as described by Sassaki et al. [34].

2.6.5. Nuclear Magnetic Ressonance (NMR)

The type of linkage in the biosynthesized levan was identified by ^{1}H and ^{13}C NMR analysis. 0.5 mL of purified levan solution (30 mg/mL) was homogenized with 0.1 mL of deuterium oxide (D_2O) and transferred to a 5 mm broad-band probehead. All NMR spectra

were recorded at 25 ºC on a NMR spectrometer (Bruker Avance III, Germany) at 400 MHz (^{1}H and ^{13}C). Chemical shifts (δ) were expressed in ppm.

2.7. Thermogravimetric analysis (TGA)/ Differential Thermogravimetric Analysis (DTG)

The TGA was performed with a TGA-50 analyzer (Shimadzu, Japan) under N_2 atmosphere at a flow rate of 20 mL/min. The sample was placed in a crucible and heated at a linear heating rate of 10 °C/min over a temperature of 30–800 ºC. The DTG was applied with the derivatization of the TGA data of levan.

2.8. Scanning Electron Microscopy (SEM)

Scanning electron microscopy was used to investigate the levan morphology. Micrographs of the coated samples with a thin layer of gold nanoparticles were taken with a MEV FEI Quanta 200 scanning electron microscope (Hitachi S-4800, Japan).

2.9. Antioxidant activity

2.9.1. ABTS free radical scavenging activity

The ABTS radical scavenging activity was determined according to Huang et al. [35] with some modifications. Firstly, 7 mM ABTS and 140 mM potassium persulfate solutions were prepared in ultrapure water. Then, 5 mL 7 mM ABTS and 88 µL 140 mM potassium persulfate solutions were reacted in the dark for 16 h to generate the ABTS radical ($ABTS^{\bullet+}$). The $ABTS^{\bullet+}$ solution was diluted with 20 mM monobasic potassium phosphate (pH 7.4) to obtain an absorbance of 0.70 ± 0.02 at 734 nm. The analysis consisted of 100 µL of aqueous levan solution added to 1000 µL of diluted $ABTS^{\bullet+}$ solution. The reaction mixture containing different concentration of levan (0, 0.05, 0.09, 0.18, 0.27, 0.36, 0.45 and 0.91 mg/mL) was

incubated in dark for 6 min at room temperature and the absorbance was measured at 734 nm. The $ABTS^{\bullet+}$ scavenging activities were calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = [(\text{Abs. control} - \text{Abs. sample})]/(\text{Abs. control})]\times 100 \quad \text{Eq (1)}$$

Where Abs. control is the absorbance of $ABTS^{\bullet+}$ without the sample and Abs. sample is the absorbance of sample solution. Vitamin C was used as a positive control.

2.9.2. Assay of Hydroxyl Radical Scavenging Activity

The ability of levan to scavenge hydroxyl radicals generated by Fenton reaction was assayed as described by Gomaa and Yousef [36] with some modifications. Levan's solutions (1 mL) were added to 1 mL of 9 mM ferrous sulfate (m/v), 1 mL of 0.3% H_2O_2 (v/v) and 0.5 mL of 9 mM salicylic acid/ethanol solution (m/v). The mixture containing different concentration of levan (0, 0.14, 0.29, 0.57, 0.86, 1.14, 1.43 and 2.86 mg/mL) was incubated at 37 °C for 30 min and the absorbance of the samples was measured by spectrophotometer at 510 nm. Vitamin C was used for comparison. The hydroxyl radical scavenging activities were calculated according to Eq (1).

2.10. Statistical analysis

The results were analyzed by one-way ANOVA and Tukey test ($p \leq 0.05$) using the Statistica 10, and the graphical analysis was performed GraphPad Prism 5 software and OriginPro 8.

3. Results and Discussion

3.1. Identification and selection of higher levan-producing Gluconobater strains from sucrose

A total of 42 bacterial strains were isolated from rotten grapes from Bahia and Paraná states. All the bacteria exhibited gram-negative staining and capacity to oxidize ethanol to acetic acid (data not shown). Based on morphological, physiological, and biochemical characteristics, and on the analysis of EPS-producing activity, five isolates demonstrated (+++) strong activity on sucrose agar plates were posteriorly identified (Table SM1). The Fig. SM1 shows that all the isolates belong to the genera *Gluconobacter.*

Their cells were rod shape, non-spore-forming, and catalase-positive as described in Bergey's Manual of Systematic Bacteriology [19]. *Gluconobacter* isolates were able to convert ethanol to acetic acid, but not acetate and lactate to CO_2 and H_2O. The conversion of acetate to CO_2 and H_2O, which is known as acetate "overoxidation" is common in some genera of AAB as *Acetobacter*, *Gluconacetobacter* and *Komagataeibacter*, however, is absent in *Gluconobacter* due to lack of key enzymes in the tricarboxylic acid cycle and glyoxylate shunt [3,22]. Likewise, *Acetobacter* readily convert lactate to CO_2 and H_2O, while *Gluconobacter* preferentially oxidize minor carbohydrates to produce acetic acid [21,22]. All of the strains produced acid from glucose, and dihydroxycetone from glycerol, but none of them produced cellulose. In none of the isolates, the formation of water-soluble brown pigment was observed. This characteristic varies by species [20] and is linked to the synthesis of 2,5-diketogluconic acid and γ-pyrones from D-glucose [19]. Similar to our findings, Jakob et al. [10] also demonstrated a strong activity of EPS production (+++) by species of *G. cerinus* DSM 9533 and *G. frateurii* TMW 2.767.

The five isolates were identified by sequencing and phylogenetic analysis of the 16S rRNA gene. The UELBM13, UELMM2, UELBM1, UELBM11 and UELMM4 strains that

showed profiles close to the *Gluconobacter* reference strains (Fig. SM1) and were later named and deposited in Genbank database as *Gluconobacter frateurii* UELBM13 (GenBank accession No. ON149511), *Gluconobacter frateurii* UELMM2 (GenBank accession No. ON149512), *Gluconobacter cerinus* UELBM1 (GenBank accession No. ON149513), *Gluconobacter cerinus* UELBM11 (GenBank accession No. ON149510), and *Gluconobacter kondonii* UELMM4 (GenBank accession No. ON149514).

The production of a levan-like mucous substance from *Gluconobacter* genus is not described in Bergey's Manual, nonetheless, recent reports [30,37] showed that these bacteria have the ability to synthesize high amounts of homopolysaccharides from sucrose. Therefore, the production of the polymer by isolates on HS-medium-containing sucrose was also verified. Sucrose-based substrates are requered in microbial production of levans, since levansucrase shows high specificity for this sugar [6]. In the experimental conditions employed, the Table 1 shows that the concentrations of levan by strains isolated from Bahia and Paraná states ranged from 4.68 g/L to 13.89 g/L.

Table 1. Production of levan on modified HS-medium from *Gluconobacter* strains.

Strain	Levan yield (g/L)	Origin (Brazilian state)
G. cerinus UELBM11	13.89 ± 0.90 [a]	Bahia
G. kondonii UELMM4	12.60 ± 1.39 [a]	Paraná
G. cerinus UELBM1	10.80 ± 0.77 [b]	Bahia
G. frateurii UELMM2	9.52 ± 0.49 [b]	Paraná
G. frateurii UELBM13	4.68 ± 0.84 [c]	Bahia

Data represent mean ± standards deviations. Means with same letters in the same column are not significantly different by Tukey test at 5%.

Despite the fermentation conditions are not optimized, the production of levan from *G. cerinus* UELBM11 was very promising, since it was higher or close to those found by

Brachybacterium phenoliresistens [38] and *Acetobacter xylinum* NCIM 2526 [12]. The purified levan from *G. cerinus* UELBM11 was quantified by total sugars and showed a yield of 11.68 g/L. Preliminary analysis performed by FT-IR and NMR confirmed that all strains were able to synthesize levan (data not shown), however, for the purposes of this study only the levan produced by *G. cerinus* UELBM11 was chosen to be analyzed due to the high yield observed.

3.2. Structural characterization of levan

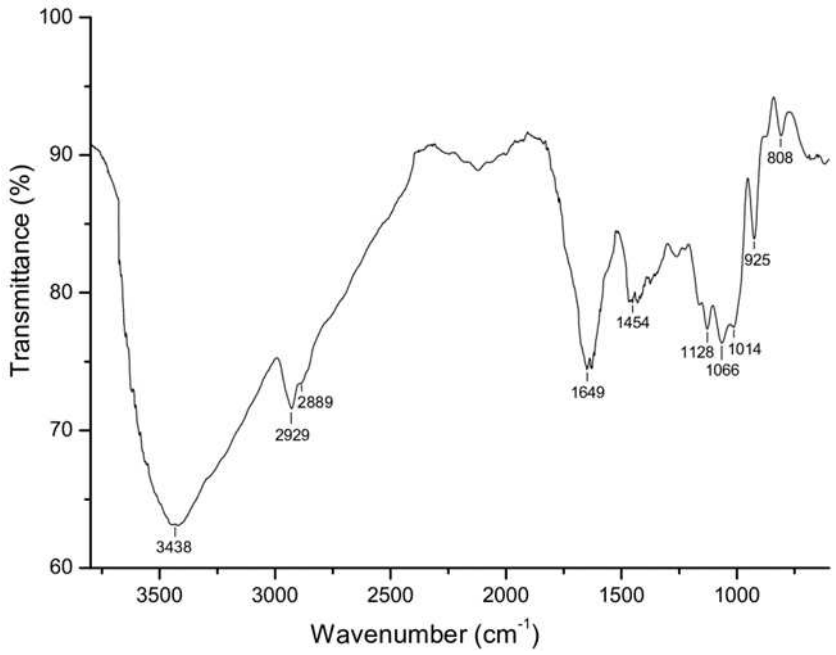
3.2.1. Molecular weight and FT-IR spectra analysis

Levan after purification on Sepharose CL-2B column showed a single major peak by taking the absorbance at 490 nm (Fig. SM2). The obtained fraction was checked for purity and had a negative response to the Bradford test, indicating the absence of protein. Despite levan-producing AAB are known to produce relatively high molecular weight levan (e.g. *N. chiangmaiensis* 100 – 575 MDa; *K. baliensis* 1000–2000 MDa; *G. frateurii* 4–98 MDa; *G. cerinus* 6–98 MDa) [39], HPSEC–MALLS analysis revealed that levan from *G. cerinus* UELBM11 has a lower molecular weight of 8.78×10^5 Da. Levan from an acetic acid bacterium, *Tanticharoenia sakaeratensis,* showed a similar result to our study, in which the majority of molecular weight ranged from approximately $1 \times 10^5 – 6.8 \times 10^5$ Da [4]. The application of levan, whether as a bioactive, functional or natural compound, is directly related to its molecular weight [40,41]. According to Ortiz-Soto et al. [41], the molecular weight of levan can be classified into two categories: low molecular weight (LMW; 8 kDa to 50 kDa) and high molecular weight (HMW; > 50 kDa). In general, LMW levans can be used as potential prebiotic candidates to modulate human intestinal microflora [9], while HMW levans have a variety of applications. HMW levans have been used as a fat replacer in dairy products

due its mouthfeel, taste and spread ability [11] and have a strong influence on bread quality and staling rate [10]. In cosmetical and biomedical area, HMW levans have been shown to have moisturizing properties [42], hypocholesterolemic effect [43], antitumor activity, and have great potential in nanocarrier systems for drug delivery [41,44]. In terms of antioxidant activity, LMW and HMW levans have shown quite varied responses [33,45–47].

The FT-IR spectra of levan samples (Fig. 1) were analyzed in comparison with the FT-IR spectra of the polysaccharides documented in the literature, and absorption bands were assigned indicating the typical polymeric structure of levan.

Fig. 1. FT-IR spectrum of levan biosynthesized from *Gluconobacter cerinus* UELBM11.



The confirmation of the polysaccharide nature of the samples was indicated by a wide absorption range in the region of 3.400 cm^{-1}, resulting from the stretching vibration of hydroxyl groups (ν O-H). The bands in the regions close to 2930 cm^{-1} and 2880 cm^{-1} were

attributed to vibrations of symmetrical and asymmetric stretching of C-H bonds. The absorption band around 1650 cm^{-1} was attributed to the symmetrical angular deformation in the hydroxyl plane, related to fundamental water vibrations [48]. The FT-IR spectra also show weak signals around 1450 cm^{-1} attributed to angular deformation in the plane of methylene C-H bonds. The region of the number of waves from 1200 to 800 cm^{-1} is the region of the fingerprint and can be used to characterize different polysaccharides [49]. The bands close to 1125, 1065, and 1015 cm^{-1}, correspond to the stretches of the carbohydrate glycosidic bonds (ν C - O - C) and (ν C - O - H). The characteristic absorption at 925 and 808 cm^{-1} indicates the presence of the furanoside ring of the sugar units, values corresponding to levan from *Bacillus licheniformis* [50].

3.2.2. Monosaccharide composition and glycosydic linkage analysis

After due derivatization of the levan produced by *G. cerinus* UELBM11 to yield alditol acetates (AA), GC-MS analysis revealed the presence of Man (51.6%) and Glc (48.4%) as the monosaccharide composition. This result was expected since D-fructose units of levan give rise to these epimers after reduction with sodium borohydride, and thus mannitol hexaacetate (R_t 18.83 min) and glucitol hexaacetate (R_t 18.92 min) were found on the chromatogram after hydrolysis, reduction and acetylation of this homopolysaccharide.

The same phenomenon is observed when partially methylated alditol acetates (PMAA) were produced to determine the glycosidic linkage of D-fructose present on the levan structure (Table 2 and Fig. 2). After derivatization, PMAA were analysed by GC-MS, which showed mainly two peaks at 15.32 and 15.40 min corresponding to 1,3,4-Me_3-Glc and 1,3,4-Me_3-Man, respectively. Together, these 1,3,4-Me_3-hexitol derivatives accounted for 86.13% of the PMAA found for the levan produced by *G. cerinus* UELBM11, and both represent the

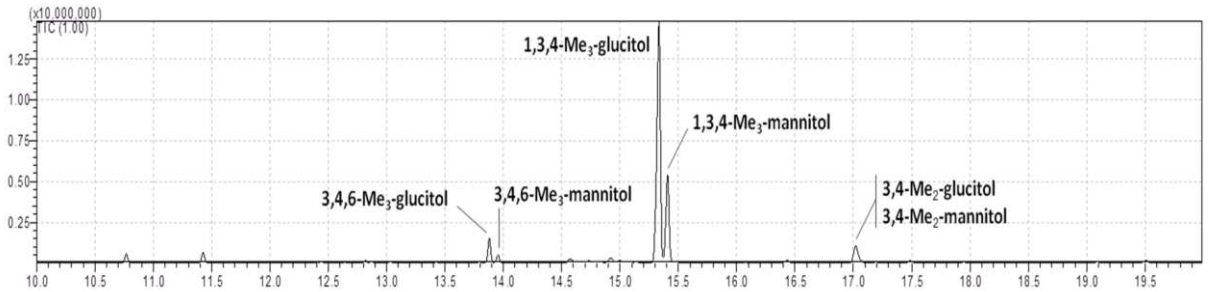
(2→6)-linked Fru*f* residues that form the levan backbone [31]. Branching units of (1,2→6)-linked Fru*f* were also observed since 3,4-Me_2-hexitol (5.87%) derivatives arose at R_t 17.2 min [51]. 1-O-substituted fructose units (7.09%) were determined by the presence of 3,4,6-Me_3-Glc (R_t 13.88 min) and 3,4,6-Me_3-Man (R_t 13.95 min) [51].

Table 2. PMAA derivatives found for the levan produced by *Gluconobacter cerinus* UELBM11.

R*t* (min)	PMAA[ab]	D-fructose linkage on levan	%
12.44	1,3,4,6-Me_4-glucitol	*t*-Fru*f*	0.91
12.53	1,3,4,6-Me_4-mannitol		
13.88	3,4,6-Me_3-glucitol	1-O-Fru*f*	7.09
13.95	3,4,6-Me_3-mannitol		
15.32	1,3,4-Me_3-glucitol	(2→6)-Fru*f*	86.13
15.40	1,3,4-Me_3-mannitol		
17.20	3,4-Me_2-glucitol/mannitol	(1,2→6)-Fru*f*	5.87

[a]Partially methylated alditol acetate derivatives obtained after permethylation with CH_3I, hydrolysis, reduction with $NaBH_4$ and acetylation. [b]The derivative 2,3,4,6-Me_4-glucitol (< 0.5%) was also found at R_t = 12.39 min, representing the terminal t-Glc*p* non-reducing unit of the levan.

Fig. 2. Chromatogram of the partially methylated alditol acetate (PMAA) derivatives found for the levan produced by *Gluconobacter cerinus* UELBM11.



*2,3,4,6-Me4-glucitol, 1,3,4,6-Me4-glucitol and 1,3,4,6-Me4-mannitol derivatives representing non-reducing terminals of t-Glcp and t-Fruf are not indicated in the chromatogram since they represent together <1.5% of the total partially methylated alditol acetate (PMAA) derivatives found.

Both terminal non-reducing units of the levan structure were found on the chromatogram as 2,3,4,6-Me_4-Glc*p*, at R_t 12.39 min (< 0.5%), indicating the D-glucopyranosyl terminal unit [31], and as 1,3,4,6-Me_4-Glc and 1,3,4,6-Me_4-Man, found respectively at R_t 12.44 min and R_t 12.53 min, together accounting for 0,91% of the totality of PMAA in the sample and representing the D-fructofuranosyl terminal units [51].

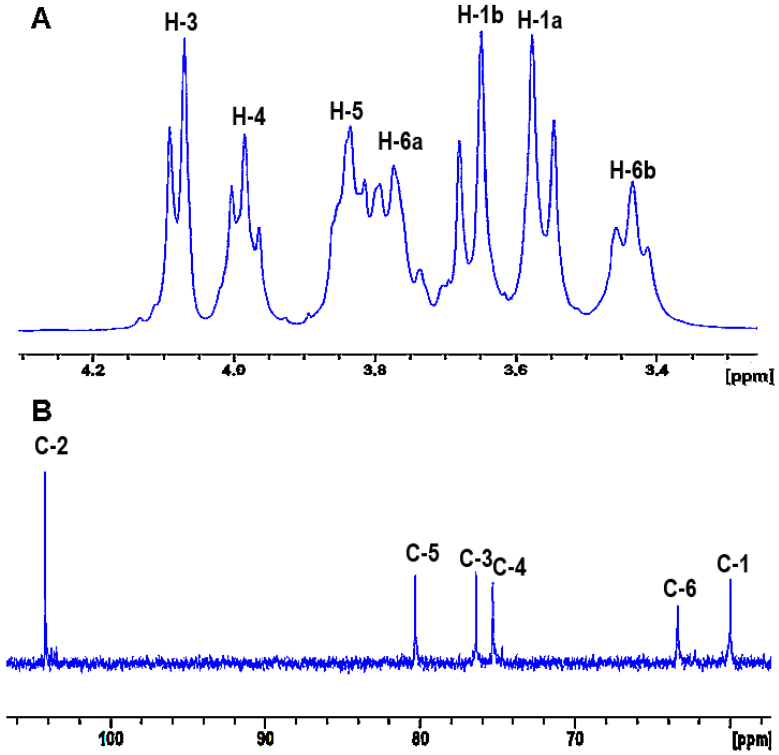
3.2.3. NMR spectroscopy

In order to obtain more structural details, the sample of levan biosynthesized by *G. cerinus* UELBM11 was analyzed by ^{1}H NMR (Fig. 3A), which showed chemical shifts in 3.78 (d, H-6a); 3.45 (t, H-6b); 3.56 (d, H-1a); 3.67 (d, H-1b); 3.84 (m, H-5); 3.97 (t, H-4) and 4.07 ppm (d, H-3), corresponding to fructofuranosyl residues as a levan repetition unit. This result corroborates with the absorption bands in the FT-IR at 925 and 808 cm^{-1} corresponding to the furanoside ring of the sugar units.

^{13}C NMR of levan sample (Fig. 3B) was performed to assign the chemical displacements of anomeric and non-anomeric carbons. Spectral data from ^{13}C NMR showed six signals at δ C 104.2; 80.06, 76.40, 75.52, 63.75 and 60.25, referring to the D-fructofuranosyl residue carbons. The (C-2) showed δC 104.2 ppm corresponding to the presence of glycosidic bonds β-(2→6). The same anomeric pattern was found in studies by Joaquim et al. [52], where they indicate that levans are usually formed by repeated units of D-fructose residues linked by β-(2→6) glycosidic bonds. The δC 60.25 and 63.75 signals (C-1 and C-6) were assigned to the methylene group. The signal at δC 80.06 (C-5) was assigned to

the furanoside ring, and signals at δC 75.52 and 76.40 correspond to oxymetinic groups of the D-fructose residue (C-4 and C-3). These results confirm that levan from *G. cerinus* UELBM11 consists of D-fructose units bound by β-(2→6) bonds with a terminal residue of D-glucopyranosyl and some chains of branches connected β-(2→1) as verified earlier in the GC-MS analysis.

Fig. 3. ^{1}H NMR (A) and ^{13}C NMR (B) spectra (400 MHz, in D_2O) for levan biosynthesized by *Gluconobacter cerinus* UELBM11.

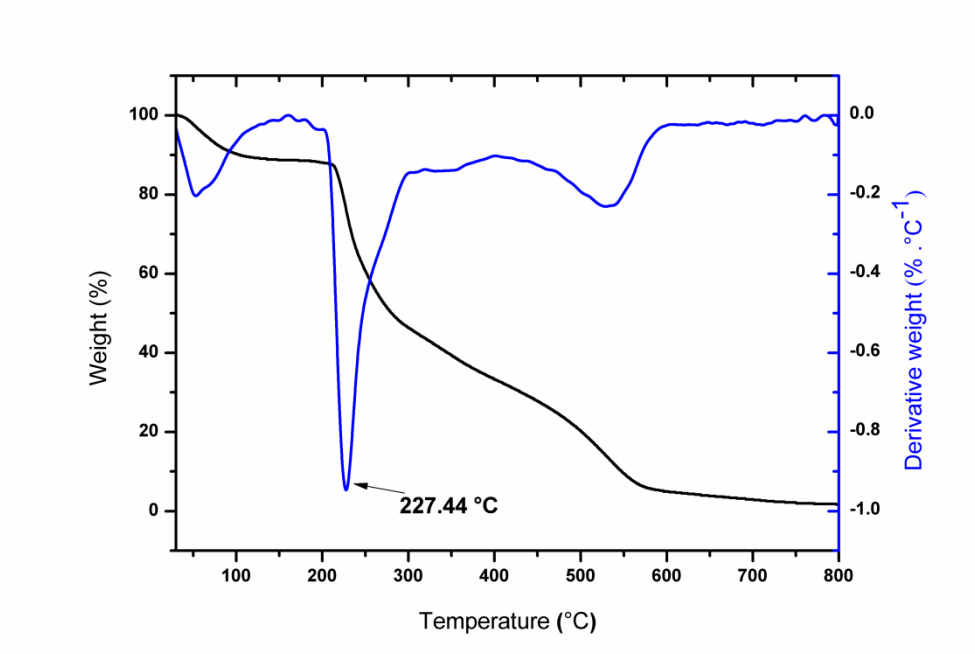


3.3. Thermal stability of levan by TGA/DTG profile

TGA analysis is a method that provides relevant information related to the thermal stability of the samples from the change in weight and/or rate of change in weight (DTG) as a function of temperature. Results from TGA and DTG of levan from *G. cerinus* UELBM11

are shown in Fig. 5. The thermal degradation of the levan occurred in three main stages. At the first stage, between 30 and 200 °C (Fig. 4), approximately 11.94 % of the weight loss was observed, which could be attributed to the residual moisture [12]. The second stage is directly associated with the thermal decomposition of fructans, observed between 200 - 300 °C [12,53,54] and also at 200 – 400 °C [55].

Fig. 4. TGA and DTG as a function of temperature for levan from *Gluconobacter cerinus* UELBM11.



During this stage (200–300 °C), levan from *G. cerinus* UELBM11 showed considerable weight losses (41.46 %) and the greatest rates of change of the derivative weight curve were observed at 227.44 °C. Other authors have also reported the maximum decomposition rate over this temperature range, with derivative peak temperatures at 216.67 °C [54], and 253 °C [53]. With the increment of the temperature from 300 to 400 °C, levan showed lower weight losses, around 13.1 %. According to Stivala et al. [56], the decomposition in this stage is probably caused by the gradual breaking of levan β-(2→1) branch linkages and subsequent breakdown of the branch-chains. After further heating, the

more thermally-resistant β-(2→6) backbone bonds and pyranose rings are broken. Finally, in the third stage between 400 and 650 °C, as just reported by Espinosa-Andrews and Rodríguez-Rodríguez [55] ashes were detected and approximately 30 % of weight losses were observed.

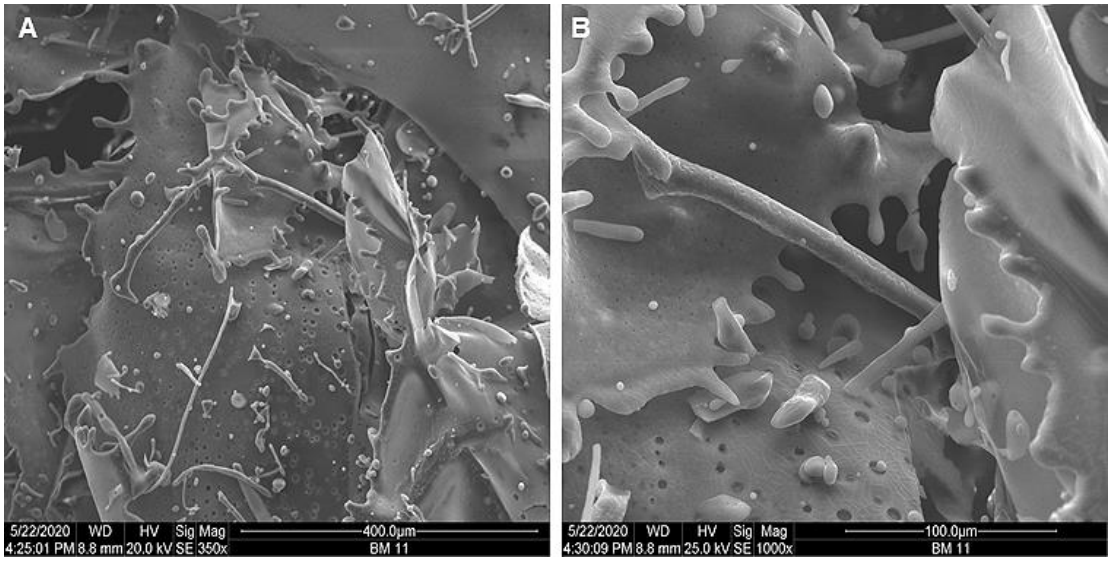
The thermal characteristic of the levan from *G. cerinus* UELBM11 suggests that it could be a good additive for food applications since TGA/DTG displayed that the decomposition of fructans occurred at temperatures close to 200 °C and the numerous food preparations rarely exceed 150 °C [57]. Furthermore, levans could also be used as a stabilizing agent for pharmaceuticals/cosmeceutical formulations [58] and carrying agent of powdered foods [59]. Levan could act by protecting the active ingredients from thermal and environmental decomposition and, consequently, would prolong the shelf life of the product [60].

3.4. Morphology of levan by SEM

SEM is a useful tool to study the surface characteristics of polysaccharides and assists in understanding their physical properties [61]. Fig. 5 (A-B) shows the microstructure of levans from *G. cerinus* UELBM11. From SEM scans, it can be predicted that both levans exhibited some rod structure and raindrops shape granules [62] and a very pronounced microporous matrix. This porous network appears to be linked by many sheets composed of millions of pores with regular round or elliptic shapes. Previous studies have also reported a similar porous morphology observed in *Brenneria* sp. EniD312 levan [54], *Erwinia amylovora* levan [63], *Bacillus subtilis* natto KB1 [45], and *Bacillus mojavensis* levan [64]. According to Haddar et al. [64] and Lobo et al. [61], the microporosity allows greater water holding capacity; which makes levans an interesting component for cosmetics production [38]. Besides that, this property suggests that levan from *G. cerinus* UELBM11 will be a

potential functional agent that enhances thickening, gelling, stabilizing, emulsifying, and the water-binding in food processing [64,65].

Fig. 5. Scanning electron microscopy showing the surface morphology of levan from *Gluconobacter cerinus* UELBM11 (A: 350 ×, B: 1.000 ×).



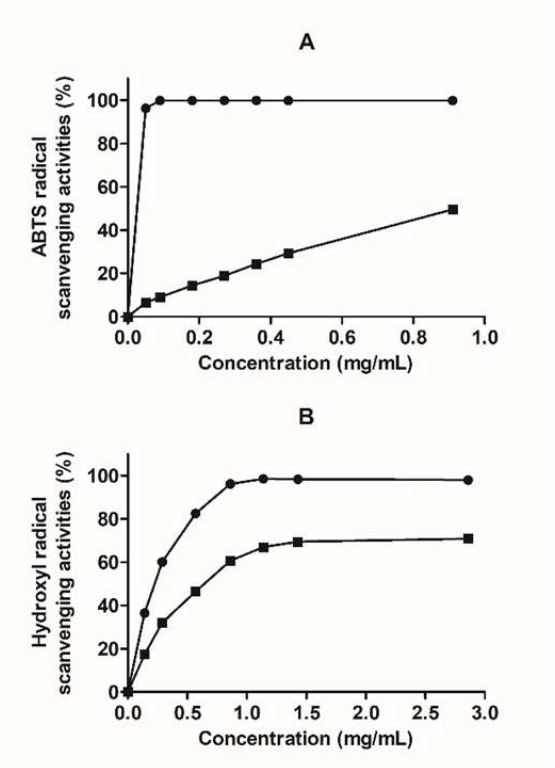
3.5. Antioxidant capacity of levan

3.5.1. ABTS and Hydroxyl radical scavenging ability

The ABTS•+ method is widely used to assess the radical scavenging capacities of hydrophilic and lipophilic antioxidants [61]. The ABTS•+ scavenging ability of levan compared to vitamin C is shown in Fig. 6A. Levan demonstrated potential as antioxidant agent despite having lower inhibitory activity than vitamin C at all concentrations tested. It was observed radical scavenging ability increased in a dose-dependent manner. *G. cerinus* levan reached nearly 50 % of the scavenging rate at higher concentrations (0.9 mg/mL). This scavenging activity was not as high as that of *Lactobacillus plantarum* KX041 (~90 %; 0.6 mg/mL) [66], but it was similar to levan from *Bacillus megaterium* PFY-147 (~50 %; 1

mg/mL) [46] and higher than that of *Bacillus licheniformis* (strain FRI MY-55) (~15-20 %; 2.5 mg/mL) [35]. The ability of levan from *G. cerinus* UELBM11 to act as an antioxidant may be attributed to their large number of hydroxyl groups, which could convert reactive free radicals into a more stable form and stop the free radical chain reaction, most likely due to its ability to donate hydrogens [57].

Fig. 6. Antioxidant activities by scavenging activity of the radicals: ABTS (A) and hydroxyl (B) from (■) levan by *Gluconobacter cerinus* UELBM11 and (●) Vitamin C. Data is presented as the mean ± SD of the triplicates.



Hydroxyl radicals are the most well-known reactive radicals that can attack and destroy almost all bio-macromolecules in cells. This action results in accelerated aging and may facilitate the occurrence and development of related diseases about oxidative stress [46].

Knowing the importance of protecting the organism from free radical damage, this study also evaluated the ability of *G. cerinus* UELBM11 levan to scavenge hydroxyl radicals. Fig. 6B shows that the scavenging rate of levan sample and vitamin C was in a concentration-dependent way. When vitamin C showed nearly 100 % scavenging activity at 1.1 mg/mL, *G. cerinus* BM11's levan exhibited ~67 % activity. The radical scavenging rate gradually stabilized as the levan concentration increased, reaching a maximum of 70.80 % at 2.9 mg/mL. Similar to our findings, Liu et al. [67] found a scavenging activity of 68.55 % at the highest concentration of levan evaluated (1 mg/ml). Pei et al. [46] also reported a scavenging effect close to our work (79.29 %), however at a higher concentration of levan (5 mg/mL). Furthermore, our results were more promising to those of EPS from *Leuconostoc citreum* B-2 (12.92 % at 15 mg/mL) [68] and levan from *Bacillus licheniformis* (strain FRI MY- 55) (15 % to 2.5 mg/mL) [35]. It is possible that the levan from *G. cerinus* UELBM11 reduced the ferrous ion concentration in the Fenton reaction, and that its scavenging effects have been due to its ability to donate active hydrogen through hydroxyl substitutions [57]. These findings showed that levan from *G. cerinus* UELBM11 has a significant effect on radical scavenging and can be used as a natural alternative to commercial antioxidants.

4. Conclusion

In the present study, the isolation and identification of five isolates belonging to the genus *Gluconobacter* was demonstrated. Among them, *G. cerinus* UELBM11 exhibited an expressive production of levan (13.89 g/L) under non-optimized conditions. The structure of levan from *G. cerinus* UELBM11 was mainly composed of D-fructose units linked by β-(2→6) bonds with some β-(2→1) linked branch chains, and a molecular weight of 8.78×10^5 Da. Levan revealed a microporous structure, excellent thermal properties, and has potential as

a free radical scavenger, particularly due to its high hydroxyl radical scavenging ability. Thus, this work showed the versatility of AAB and the potential of *G. cerinus* UELBM11 in producing bioactive metabolites like levan, which may be used as a natural antioxidant or additive in cosmetics, pharmaceutics, and food products.

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6. References

[1] J. De Roos, L. De Vuyst, Acetic acid bacteria in fermented foods and beverages, Curr. Opin. Biotechnol. 49 (2018) 115–119. https://doi.org/10.1016/j.copbio.2017.08.007.

[2] M.J. Valera, F. Laich, S.S. González, M.J. Torija, E. Mateo, A. Mas, Diversity of acetic acid bacteria present in healthy grapes from the Canary Islands, Int. J. Food Microbiol. 151 (2011) 105–112. https://doi.org/10.1016/j.ijfoodmicro.2011.08.007.

[3] K.M. Lynch, E. Zannini, S. Wilkinson, L. Daenen, E.K. Arendt, Physiology of acetic acid bacteria and their role in vinegar and fermented beverages, Compr. Rev. Food Sci. Food Saf. 18 (2019) 587–625. https://doi.org/10.1111/1541-4337.12440.

[4] P. Aramsangtienchai, T. Kongmon, S. Pechroj, K. Srisook, Enhanced production and immunomodulatory activity of levan from the acetic acid bacterium, *Tanticharoenia sakaeratensis*, Int. J. Biol. Macromol. 163 (2020) 574–581. https://doi.org/10.1016/j.ijbiomac.2020.07.001.

[5] J. Mantovan, G.T. Bersaneti, P.C.S. Faria-Tischer, M.A.P.C. Celligoi, S. Mali, Use of microbial levan in edible films based on cassava starch, Food Packag. Shelf Life. (2018). https://doi.org/10.1016/j.fpsl.2018.08.003.

[6] D. Ni, W. Xu, Y. Bai, W. Zhang, T. Zhang, W. Mu, Biosynthesis of levan from sucrose using a thermostable levansucrase from *Lactobacillus reuteri* LTH5448, Int.

J. Biol. Macromol. 113 (2018) 29–37. https://doi.org/10.1016/j.ijbiomac.2018.01.187.

[7] G.T. Bersaneti, N.C. Pan, C. Baldo, M.A.P.C. Celligoi, Co-production of Fructooligosaccharides and Levan by Levansucrase from *Bacillus subtilis* natto with Potential Application in the Food Industry, Appl. Biochem. Biotechnol. 184 (2018) 838–851. https://doi.org/10.1007/s12010-017-2587-0.

[8] F. Küçükaşik, H. Kazak, D. Güney, I. Finore, A. Poli, O. Yenigün, B. Nicolaus, E.T. Öner, Molasses as fermentation substrate for levan production by *Halomonas* sp., Appl. Microbiol. Biotechnol. 89 (2011) 1729–1740. https://doi.org/10.1007/s00253-010-3055-8.

[9] C. Liu, S. Kolida, D. Charalampopoulos, R.A. Rastall, An evaluation of the prebiotic potential of microbial levans from *Erwinia* sp. 10119, J. Funct. Foods. 64 (2020). https://doi.org/10.1016/j.jff.2019.103668.

[10] F. Jakob, S. Steger, R.F. Vogel, Influence of novel fructans produced by selected acetic acid bacteria on the volume and texture of wheat breads, Eur. Food Res. Technol. 234 (2012) 493–499. https://doi.org/10.1007/s00217-011-1658-7.

[11] E.T. Öner, L. Hernández, J. Combie, Review of Levan polysaccharide: From a century of past experiences to future prospects, Biotechnol. Adv. 34 (2016) 827–844. https://doi.org/10.1016/j.biotechadv.2016.05.002.

[12] R. Srikanth, G. Siddartha, C.H.S.S. Sundhar Reddy, B.S. Harish, M. Janaki Ramaiah, K.B. Uppuluri, Antioxidant and anti-inflammatory levan produced from *Acetobacter xylinum* NCIM2526 and its statistical optimization, Carbohydr. Polym. 123 (2015) 8–16. https://doi.org/10.1016/j.carbpol.2014.12.079.

[13] H. Kazak Sarilmiser, E. Toksoy Oner, Investigation of anti-cancer activity of linear and aldehyde-activated levan from *Halomonas smyrnensis* AAD6T, Biochem. Eng. J. 92 (2014) 28–34. https://doi.org/10.1016/j.bej.2014.06.020.

[14] A.A. Gamal, H.Y. Abbas, N.A.M. Abdelwahed, M.T. Kashef, K. Mahmoud, M.A. Esawy, M.A. Ramadan, Optimization strategy of *Bacillus subtilis* MT453867 levansucrase and evaluation of levan role in pancreatic cancer treatment, Int. J. Biol. Macromol. 182 (2021) 1590–1601. https://doi.org/10.1016/j.ijbiomac.2021.05.056.

[15] T.I.M. Ragab, A.S.G. Shalaby, S.A.E. Awdan, G.T. El-Bassyouni, B.M. Salama, W.A. Helmy, M.A. Esawy, Role of levan extracted from bacterial honey isolates in curing peptic ulcer: In vivo, Int. J. Biol. Macromol. 142 (2020) 564–573. https://doi.org/10.1016/j.ijbiomac.2019.09.131.

[16] S.J. Sokollek, C. Hertel, W.P. Hammes, Cultivation and preservation of vinegar bacteria, J. Biotechnol. 60 (1998) 195–206. https://doi.org/10.1016/S0168-1656(98)00014-5.

[17] Y. Yamada, R. Hosono, P. Lisdyanti, Y. Widyastuti, S. Saono, T. Uchimura, K. Komagata, Identification of acetic acid bacteria isolated from Indonesian sources, especially of isolates classified in the genus *Gluconobacter*, J. Gen. Appl. Microbiol. 45 (1999) 23–28. https://doi.org/10.2323/jgam.45.23.

[18] M. Sievers, J. Swings, *Acetobacteraceae*, Bergey's Man. Syst. Archaea Bact. (2015) 1–20. https://doi.org/10.1002/9781118960608.fbm00174.

[19] M. Sievers, J. Swings, *Gluconobacter*, Bergey's Man. Syst. Archaea Bact. (2015) 1–

9. https://doi.org/10.1002/9781118960608.gbm00884.

[20] T. Asai, H. Iizuka, K. Komagata, T. Asap, K. Komagata, The flagellation and taxonomy of genera *Gluconobacter* and *Acetobacter* with reference to the existence of intermediate strains, J. Gen. Appl. Microbiol. 10 (1964) 95–126. https://doi.org/10.2323/jgam.10.95.

[21] M.C. Cirigliano, A selective medium for the isolation and differentiation of *Gluconobacter* and *Acetobacter*, J. Food Sci. 47 (1982) 1038–1039. https://doi.org/10.1111/j.1365-2621.1982.tb12782.x.

[22] C. Fungelsang, K.; Edwards, Fungelsang, K., & Edwards, C. (2007). Wine microbiology, practical applications and procedures. (2nd ed.). New York: Springer Science+Business Media., 2nd ed, Springer Science+Business Media, New York, 2007.

[23] H.R. Cheng, N. Jiang, Extremely rapid extraction of DNA from bacteria and yeasts, Biotechnol. Lett. 28 (2006) 55–59. https://doi.org/10.1007/s10529-005-4688-z.

[24] A. Ruiz, M. Poblet, A. Mas, J.M. Guillamón, Identification of acetic acid bacteria by RFLP of PCR-amplified 16S rDNA and 16S-23S rDNA intergenic spacer, Int. J. Syst. Evol. Microbiol. 50 (2000) 1981–1987. https://doi.org/10.1099/00207713-50-6-1981.

[25] NCBI - National Center for Biotechnology Information, (n.d.). https://www.ncbi.nlm.nih.gov/BLAST.

[26] S. Hestrin, M. Schramm, Synthesis of cellulose by *Acetobacter xylinum.* II. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose., Biochem. J. 58 (1954) 345–352. https://doi.org/10.1042/bj0580345.

[27] R. V. Serrato, C.H.S.G. Meneses, M.S. Vidal, A.P. Santana-Filho, M. Iacomini, G.L. Sassaki, J.I. Baldani, Structural studies of an exopolysaccharide produced by *Gluconacetobacter diazotrophicus* Pal5, Carbohydr. Polym. 98 (2013) 1153–1159. https://doi.org/10.1016/j.carbpol.2013.07.025.

[28] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356. https://doi.org/10.1021/ac60111a017.

[29] M.M. Bradford, A Rapid and sensitive method for the quantitation microgram quantities of protein utilizing the principle of protein-dye binding, 254 (1976) 248–254.

[30] P. Semjonovs, L. Shakirova, R. Treimane, K. Shvirksts, L. Auzina, I. Cleenwerck, P. Zikmanis, Production of extracellular fructans by *Gluconobacter nephelii* P1464, Lett. Appl. Microbiol. 62 (2016) 145–152. https://doi.org/10.1111/lam.12521.

[31] C.X. Dong, L.J. Zhang, R. Xu, G. Zhang, Y.B. Zhou, X.Q. Han, Y. Zhang, Y.X. Sun, Structural characterization and immunostimulating activity of a levan-type fructan from *Curcuma kwangsiensis*, Int. J. Biol. Macromol. 77 (2015) 99–104. https://doi.org/10.1016/j.ijbiomac.2015.03.009.

[32] G.D. Gojgic-Cvijovic, D.M. Jakovljevic, B.D. Loncarevic, N.M. Todorovic, M. V. Pergal, J. Ciric, K. Loos, V.P. Beskoski, M.M. Vrvic, Production of levan by *Bacillus licheniformis* NS032 in sugar beet molasses-based medium, Int. J. Biol. Macromol. 121 (2019) 142–151. https://doi.org/10.1016/j.ijbiomac.2018.10.019.

[33] A. Bouallegue, A. Casillo, F. Chaari, A. La Gatta, R. Lanzetta, M.M. Corsaro, R. Bachoual, S. Ellouz-Chaabouni, Levan from a new isolated *Bacillus subtilis* AF17: Purification, structural analysis and antioxidant activities, Int. J. Biol. Macromol. 144 (2020) 316–324. https://doi.org/10.1016/j.ijbiomac.2019.12.108.

[34] G.L. Sassaki, P.A.J. Gorin, L.M. Souza, P.A. Czelusniak, M. Iacomini, Rapid synthesis of partially O-methylated alditol acetate standards for GC-MS: Some relative activities of hydroxyl groups of methyl glycopyranosides on Purdie methylation, Carbohydr. Res. 340 (2005) 731–739. https://doi.org/10.1016/j.carres.2005.01.020.

[35] T.Y. Huang, M.Y. Huang, C.K. Tsai, W.T. Su, Phosphorylation of levan by microwave-assisted synthesis enhanced anticancer ability, J. Biosci. Bioeng. 131 (2021) 98–106. https://doi.org/10.1016/j.jbiosc.2020.08.007.

[36] M. Gomaa, N. Yousef, Optimization of production and intrinsic viscosity of an exopolysaccharide from a high yielding *Virgibacillus salarius* BM02: Study of its potential antioxidant, emulsifying properties and application in the mixotrophic cultivation of Spirulina platensis, Int. J. Biol. Macromol. 149 (2020) 552–561. https://doi.org/10.1016/j.ijbiomac.2020.01.289.

[37] T. Ua-Arak, F. Jakob, R.F. Vogel, Fermentation pH modulates the size distributions and functional properties of *Gluconobacter albidus* TMW 2.1191 levan, Front. Microbiol. 8 (2017) 1–11. https://doi.org/10.3389/fmicb.2017.00807.

[38] T.A.A. Moussa, S.A.S. Al-Qaysi, Z.A. Thabit, S.B. Kadhem, Microbial levan from Brachybacterium phenoliresistens: Characterization and enhancement of production, Process Biochem. 57 (2017) 9–15. https://doi.org/10.1016/j.procbio.2017.03.008.

[39] F. Jakob, A. Pfaff, R. Novoa-carballal, H. Rübsam, T. Becker, R.F. Vogel, Structural analysis of fructans produced by acetic acid bacteria reveals a relation to hydrocolloid function, Carbohydr. Polym. 92 (2013) 1234–1242. https://doi.org/10.1016/j.carbpol.2012.10.054.

[40] A.K. Lakra, L. Domdi, Y.M. Tilwani, V. Arul, Physicochemical and functional characterization of mannan exopolysaccharide from *Weissella confusa* MD1 with bioactivities, Int. J. Biol. Macromol. 143 (2020) 797–805. https://doi.org/10.1016/j.ijbiomac.2019.09.139.

[41] M.E. Ortiz-Soto, J.R. Porras-Domínguez, J. Seibel, A.L.M. López-Munguía, A close look at the structural features and reaction conditions that modulate the synthesis of low and high molecular weight fructans by levansucrases, Carbohydr. Polym. 219 (2019) 130–142. https://doi.org/10.1016/j.carbpol.2019.05.014.

[42] X. Zhang, Y. Liang, H. Yang, H. Yang, S. Chen, F. Huang, Y. Hou, R. Huang, A novel fusion levansucrase improves thermostability of polymerization and production of high molecular weight levan, Lwt. 150 (2021) 111951. https://doi.org/10.1016/j.lwt.2021.111951.

[43] Y. Yamamoto, Y. Takahashi, M. Kawano, M. Iizuka, T. Matsumoto, S. Saeki, H. Yamaguchi, In vitro digestibility and fermentability of levan and its hypocholesterolemic effects in rats, J. Nutr. Biochem. 10 (1999) 13–18. https://doi.org/10.1016/S0955-2863(98)00077-1.

[44] B.M. Salama, W.A. Helmy, T.I.M. Ragab, M.M. Ali, H.A.A. Taie, M.A. Esawy,

Characterization of a new efficient low molecular weight *Bacillus subtilis* NRC 16 levansucrase and its levan, J. Basic Microbiol. 59 (2019) 1004–1015. https://doi.org/10.1002/jobm.201900170.

[45] M. Domżał-Kędzia, A. Lewińska, A. Jaromin, M. Weselski, R. Pluskota, M. Łukaszewicz, Fermentation parameters and conditions affecting levan production and its potential applications in cosmetics, Bioorg. Chem. 93 (2019) 102787. https://doi.org/10.1016/j.bioorg.2019.02.012.

[46] F. Pei, Y. Ma, X. Chen, H. Liu, Purification and structural characterization and antioxidant activity of levan from *Bacillus megaterium* PFY-147, Int. J. Biol. Macromol. 161 (2020) 1181–1188. https://doi.org/10.1016/j.ijbiomac.2020.06.140.

[47] Y.M. Tilwani, A.K. Lakra, L. Domdi, S. Yadav, N. Jha, V. Arul, Optimization and physicochemical characterization of low molecular levan from *Enterococcus faecium* MC-5 having potential biological activities, Process Biochem. 110 (2021) 282–291. https://doi.org/10.1016/j.procbio.2021.08.021.

[48] J. Han, H. Feng, X. Wang, Z. Liu, Z. Wu, Levan from Leuconostoc citreum BD1707: production optimization and changes in molecular weight distribution during cultivation, BMC Biotechnol. 21 (2021) 14. https://doi.org/10.1186/s12896-021-00673-y.

[49] M.A. Čopíková, J.; Barros, A. S.; Šmídová, I.; Černá, M.; Teixeira, D.; Delgadillo, I.; Synytsya, A.; Coimbra, Influence of hydration of food additive polysaccharides on FT-IR spectra distinction, 63 (2006) 355–359. https://doi.org/10.1016/j.carbpol.2005.08.049.

[50] C. Liu, J. Lu, L. Lu, Y. Liu, F. Wang, M. Xiao, Bioresource Technology Isolation , structural characterization and immunological activity of an exopolysaccharide produced by *Bacillus licheniformis* 8-37-0-1, Bioresour. Technol. 101 (2010) 5528–5533. https://doi.org/10.1016/j.biortech.2010.01.151.

[51] G. Cai, Y. Liu, X. Li, J. Lu, New Levan-Type Exopolysaccharide from Bacillus amyloliquefaciens as an antiadhesive agent against enterotoxigenic *Escherichia coli*, (2019). https://doi.org/10.1021/acs.jafc.9b03234.

[52] E.O. Joaquim, A.H. Hayashi, L.M.B. Torres, R.C.L. Figueiredo-ribeiro, N. Shiomi, F.S. De Sousa, J.H.G. Lago, Chemical structure and localization of levan , the predominant fructan type in underground systems of gomphrena marginata (Amaranthaceae), 9 (2018) 1–10. https://doi.org/10.3389/fpls.2018.01745.

[53] A. Poli, H. Kazak, B. Gürleyendağ, G. Tommonaro, G. Pieretti, E.T. Öner, B. Nicolaus, High level synthesis of levan by a novel *Halomonas* species growing on defined media, Carbohydr. Polym. 78 (2009) 651–657. https://doi.org/10.1016/j.carbpol.2009.05.031.

[54] X. Wei, Q. Liu, Y. Bai, S. Yu, T. Zhang, B. Jiang, W. Mu, Physicochemical properties of a high molecular weight levan from *Brenneria* sp. EniD312, Int. J. Biol. Macromol. 109 (2018) 810–818. https://doi.org/10.1016/j.ijbiomac.2017.11.056.

[55] H. Espinosa-Andrews, R. Rodríguez-Rodríguez, Water state diagram and thermal properties of fructans powders, J. Therm. Anal. Calorim. 132 (2018) 197–204. https://doi.org/10.1007/s10973-017-6868-1.

[56] S.S. Stivala, J. Kimura, L. Reich, Thermal degradation of Levan, Thermochim. Acta.

50 (1981) 111–122. https://doi.org/10.1016/0040-6031(81)85049-6.

[57] F.S. Farinazzo, L.J. Valente, M.B. Almeida, A.S. Simionato, M.T. Carlos Fernandes, C.S. Ishii Mauro, A.A. Bosso Tomal, S. Garcia, Characterization and antioxidant activity of an exopolysaccharide produced by *Leuconostoc pseudomesenteroides* JF17 from juçara fruits (*Euterpe edulis* Martius), Process Biochem. 91 (2020) 141–148. https://doi.org/10.1016/j.procbio.2019.12.005.

[58] S. Sreenivasan, R. Kandasamy, Levan: a biocompatible homopolysaccharide excipient for stabilization of peptide drugs, Int. J. Pept. Res. Ther. 23 (2017) 305–311. https://doi.org/10.1007/s10989-016-9562-4.

[59] Booten K, De Soete J, Booten AF. Fructan - and/or polydextrose - containing dairy process for preparing same and their use. Eur. patent appl. EP 0821885 A1, 1998

[60] C. Leyva-porras, P. Cruz-alcantar, V. Espinosa-sol, M.Z. Saavedra-leos, polymers application of differential scanning calorimetry (dsc) and modulated differential scanning, (n.d.) 1–21.

[61] R.E. Lobo, M.I. Gómez, G. Font de Valdez, M.I. Torino, Physicochemical and antioxidant properties of a gastroprotective exopolysaccharide produced by *Streptococcus thermophilus* CRL1190, Food Hydrocoll. 96 (2019) 625–633. https://doi.org/10.1016/j.foodhyd.2019.05.036.

[62] B. Kekez, G. Gojgić-Cvijović, D. Jakovljević, V. Pavlović, V. Beškoski, A. Popović, M.M. Vrvić, V. Nikolić, Synthesis and characterization of a new type of levan-graft-polystyrene copolymer, Carbohydr. Polym. 154 (2016) 20–29. https://doi.org/10.1016/j.carbpol.2016.08.001.

[63] W. Xu, J. Peng, D. Ni, W. Zhang, H. Wu, W. Mu, Preparation, characterization and application of levan/montmorillonite biocomposite and levan/BSA nanoparticle, Carbohydr. Polym. 234 (2020) 115921. https://doi.org/10.1016/j.carbpol.2020.115921.

[64] A. Haddar, M. Hamed, A. Bouallegue, R. Bastos, E. Coelho, M.A. Coimbra, Structural elucidation and interfacial properties of a levan isolated from *Bacillus mojavensis*, Food Chem. (2020) 128456. https://doi.org/10.1016/j.foodchem.2020.128456.

[65] F. Feng, Q. Zhou, Y. Yang, F. Zhao, R. Du, Y. Han, H. Xiao, Z. Zhou, Characterization of highly branched dextran produced by *Leuconostoc citreum* B-2 from pineapple fermented product, Int. J. Biol. Macromol. 113 (2018) 45–50. https://doi.org/10.1016/j.ijbiomac.2018.02.119.

[66] X. Wang, C. Shao, L. Liu, X. Guo, Y. Xu, X. Lü, Optimization, partial characterization and antioxidant activity of an exopolysaccharide from *Lactobacillus plantarum* KX041, Int. J. Biol. Macromol. 103 (2017) 1173–1184. https://doi.org/10.1016/j.ijbiomac.2017.05.118.

[67] J. Liu, J. Luo, H. Ye, X. Zeng, Preparation, antioxidant and antitumor activities in vitro of different derivatives of levan from endophytic bacterium *Paenibacillus polymyxa* EJS-3, Food Chem. Toxicol. 50 (2012) 767–772. https://doi.org/10.1016/j.fct.2011.11.016.

[68] Y. Wang, R. Du, X. Qiao, B. Zhao, Z. Zhou, Y. Han, Optimization and characterization of exopolysaccharides with a highly branched structure extracted

from *Leuconostoc citreum* B-2, Int. J. Biol. Macromol. 142 (2020) 73–84. https://doi.org/10.1016/j.ijbiomac.2019.09.071.

5.3. ARTIGO CIENTÍFICO3[1]

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[1]Artigo a ser submetido na Revista **Food Research International**

Natália Norika Yassunaka Hata[a]*; Mariana Assis de Queiroz Cancian[a]; Mônica Surek[e]; André Vinícius Rederd de Oliveira dos Santos[d]; Maria Eliane Merlin Rocha[d]; Rodrigo Vassoler Serrato[d]; Daniele Sartori[b]; Leonel Vinícius Constantino[c]; Wilma Aparecida Spinosa[a]

[a]Departamento de Ciência e Tecnologia de Alimentos / Centro de Ciências Agrárias / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil; [b]Departamento de Bioquímica e Biotecnologia / Centro de Ciências Exatas / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil; [c]Departamento de Química / Centro de Ciências Exatas / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil; [d]Departamento de Bioquímica e Biologia Molecular / Universidade Federal do Paraná – UFPR, Centro Politécnico, 81531-990, Curitiba, PR, Brazil; [e]Departamento de Análises Clínicas / Universidade Federal do Paraná – UFPR, Campus III – Sede Botânico, 80210-170, Curitiba, PR, Brazil
*Corresponding author. E-mail address: naty_ea@hotmail.com

Statistical optimization of levan from *Gluconobacter cerinus* UELBM11 and its cytotoxic effect on SH-SY5Y neuroblastoma cells and antimicrobial and antibiofilm activities

ABSTRACT

Gluconobacter cerinus UELBM11 is an acetic acid bacterium isolated from Brazilian grapes that has shown a great ability to produce levan. Levan is a polysaccharide that has highlighted due to a wide range of applications in food, cosmetic and pharmaceutical industry. Therefore, the aim of this study was to optimize the levan production by *G. cerinus* UELBM11 using the Response Surface Methodology (RSM). Furthermore, the cytotoxicity assay against human neuroblastoma cells (SH-SY5Y) as well as its antimicrobial and antibiofilm activity, was evaluated. Results showed that the maximum levan production (48.06 g/L) was obtained in HS medium supplemented with sucrose at 24.84 % (w/v) and initial pH of 6.95. The RSM is a useful tool for large-scale levan production, since it promoted an increase of approximately 3.5 times in levan yield when compared to non-optimized conditions (13.89 g/L). The treatment with 1000 μg/mL of levan for 24 h, 48 h and 72 h triggered a cytotoxic effect in SH-SY5Y cancer cells. Although our results did not show efficacy in inhibiting food pathogens, levan shows promise as a natural and non-toxic alternative for controlling biofilms in food processing environments and can also be used as an additive in new product development.

Keywords: Sucrose, *Gluconobacter cerinus*, response surface methodology, cytotoxicity, antimicrobial, antibiofilm

1. INTRODUCTION

Levan of bacterial origin constitute a significant part among other microbial exopolysaccharides (Donot et al., 2012). This homopolysaccharide of fructose is composed of β-(2,6) fructofuranosyl linkages in the backbone with occasional β-(2, 1) linkages in the branch chains (Han et al., 2021). Several microorganisms of the genera *Halomonas, Lactobacillus, Azotobacter, Bacillus, Erwinia, Pseudomonas, Streptococcus* and *Zymomonas* produce levan through the action of enzyme levansucrase (EC 2.4.1.10) (LS) from sucrose-based substrate (Erkorkmaz et al., 2018; Hövels et al., 2021; Ni et al., 2018; Öner et al., 2016). Recently, studies have shown that levan can also be synthesized by acetic acid bacteria (BAA), particularly by strains of the genus *Komagataeibacter, Gluconobacter, Gluconacetobacter, Kozakia* and *Neoasaia* (Idogawa et al., 2014; Semjonovs et al., 2016; Srikanth et al., 2015; Ua-Arak et al., 2017b).

Levan can be synthesized in two ways: by fermentation with LS-active bacteria, or by enzymatic synthesis employing LS in crude, purified, recombinant, or immobilized form in a medium containing sucrose as a substrate (Li et al., 2015). The microbial production of levan is highly dependent on the strain used as a source of the LS enzyme, as well as on the reaction conditions, such as pH and carbon source concentration (Bouallegue et al., 2020; Han et al., 2021; Li et al., 2015).

Aiming to increase its production, multivariate optimization methods, such as RSM, have been widely applied in several scientific studies (Bouallegue et al., 2020; Han et al., 2021; Semjonovs et al., 2017; Srikanth et al., 2015). The RSM is a collection of statistical and mathematical techniques for modeling and analyzing problems in which the response variable is influenced by several factors (Bouallegue et al., 2020). Through it, it is possible to study

the effect of several factors and the interactions that lead to the optimal conditions of different biotechnological processes (Bouallegue et al., 2020; Esawy et al., 2013).

The interest in optimizing the conditions for the large-scale production of levan is mainly due to its multiple applications. In the food industry, levan can be used as a prebiotic, a stabilizer, fat substitute, and flavoring agent in dairy products (Gamal et al., 2021). In medical and pharmaceutical areas, levan finds many applications as anti-inflammatory-, anti-clotting-, antidiabetic-, and immunostimulant- agent (Bouallegue et al., 2020). Furthermore, levan has demonstrated antibacterial efficacy against pathogens (Mummaleti et al., 2022) and antibiofilm activity (Abid et al., 2018; Tilwani et al., 2021). Levan has been extensively investigated in the treatment against a variety of tumor cells (Abdel-Fattah et al., 2012; Liu et al., 2012); nevertheless, it has been poorly evaluated in human neuroblastoma cells (SH-SY5Y).

Thus, the aim of the present work was to optimize the production of levan by *G. cerinus* UELBM11 isolated from Brazilian grapes using the Response Surface Methodology (RSM). In addition, the cytotoxic activity of levan in human neuroblastoma cells, as well as its antimicrobial and antibiofilm activity, was evaluated.

2. MATERIALS AND METHODS

2.1. Bacterial strain and storage conditions

Gluconobacter cerinus UELBM11 (GenBank accession No. ON149510) was isolated from Brazilian grapes and provided by the Department of Food Science and Technology at the State University of Londrina. The bacterial strain was maintained at 4 °C on MYP agar containing mannitol (25 g/L), yeast extract (5 g/L), peptone (3 g/L) and agar

(10 g/L) (Sokollek et al., 1998) and routinely streaked every month. Stock culture was maintained at −20 °C in malt extract (200 g/L) broth.

2.2. Bacterial cultivation for levan production

G. cerinus UEL BM11 was first grown on MYP agar at 30 °C for 72 h. A loopful of this inoculum was then transferred to a 250 mL flask containing 100 mL of HS medium (20 g/L glucose; 5 g/L peptone; 5 g/L yeast extract; 2.7 g/L disodium phosphate; 1.15 g/L citric acid monohydrate) (pH 6.0) (Hestrin & Schramm, 1954) and maintained at 30 °C on a rotary shaker (120 rpm) until 0.6 of absorbance (600 nm). Afterwards, the cultivation was interrupted by centrifugation (5,000 rpm; 20 min), the cells were washed with distilled water and the absorbance adjusted to 0.7 to provide an inoculum containing 2.5×10^8 CFU/mL. Fermentations were conducted using 250 mL flasks containing 100 mL of modified HS medium (with commercial sucrose) and incubated at 30 °C, 150 rpm for 72 hours. Yeast extract was used as the only source of nitrogen with the content fixed at 10 g/L. The other constituents of the HS medium were remained at the same concentrations. The cellular concentration of inoculum was standardized in 1 % (v/v) for all the conditions.

2.3. Statistical optimization of levan production

RSM based on a Central Composite Rotational Design (CCRD) was used to explain the effects of the sucrose concentration (x_1) and initial pH (x_2) for the maximum production of levan from *G. cerinus* UELBM11. 2^2 CCRD was carried out including 4 axial points ($\alpha = \pm 1.41$) and 3 replications in the central points, totalizing 11 runs. The independent variables and their coded levels were determined through preliminary testing and are shown in Table 1. The levan production was evaluated using the following model equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=1}^{k} \beta_{ij} x_i x_j$$

where Y is the response function (dependent variable), x_i and x_j are the independent variables and the β_0, β_i, β_j, and β_{ij} values are the regression coefficients of the variables for intercept, linear, quadratic, and interaction terms, respectively (Myers et al., 2016). The response function was used to carry out the regression analysis and analyses of variance (ANOVA). Statistica 10.0 software (StatSoft Inc., Tulsa, USA) was used for all running analysis and response surfaces. The proposed model was validated by performing tests in triplicate, and the results were validated in relation to the estimated response by Student's t-test ($p < 0.05$).

2.4. Levan extraction and quantification

Levan was isolated from the supernatant by precipitation with ethanol according to Serrato et al. (2013) with some modifications. In summary, the cells were removed by centrifugation at 5000 rpm for 20 min and the supernatant was precipitated by addition of cold ethanol 99% (3:1, v/v). The mixture was left overnight at - 20 °C, centrifuged, rewashed with cold ethanol and again centrifuged. Further, levan was dried at 50 °C and subjected to acid hydrolysis with 0.5 mol/L oxalic acid for 1 h at 100 °C (Semjonovs et al., 2016). Levan concentration was estimated based on the amount of fructose monomers using Somogyi and Nelson methods (Nelson, 1944; Somogyi, 1952). Alternatively, levan was dialyzed (MWCO 14 kDa) for the cytotoxicity assay (Sigma-Aldrich, USA) for at least 48 h at 4 °C and lyophilized. The analyses were performed in triplicate.

2.5. Cytotoxicity activity

2.5.1. Cell culture

Human neuroblastoma (SH-SY5Y) cells from ATCC (Manassas, VA, USA) were used to evaluate the cytotoxicity activity of the levan. The cells were cultured in medium mixture (1:1) Dulbecco's Modified Eagle's Medium-low glucose /Nutrient Mixture Ham's F12 (DMEM/F12) supplemented with 1% MEM non-essential amino acid solution (100×), 10 % fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin. The cells were maintained in a humidified air containing 5 % CO_2 at 37 °C (Escobar et al., 2019).

2.5.2. Cell viability test

The cellular metabolic activity based on the conversion of (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) MTT into formazan crystals by living cells was determined using MTT assay according to Mosmann (1983) and Escobar et al., (2019). The cells were seeded (1×10^4 cells/well) in 96 well plates and incubated for 24 h at 37 °C. Cells were then exposed to the levan solution (previously diluted in ultrapure water) at different concentrations (50-1000 µg/mL) for 24, 48 and 72 h. The control contained only the culture medium. The cells were incubated with MTT solution (5 mg/mL) at a final concentration of 0.5 mg/mL for 3 h and then formed formazan crystals were solubilized by the addition of dimethyl sulfoxide (200 µL/well). The absorbance of the samples was measured at 550 nm in microplate reader (BioTek Epoch). The results were expressed as cell viability (%) compared with to untread cells (100%).

2.6. Antimicrobial activity

2.6.1. Microorganisms

The antimicrobial activity was assessed against *Shigella sonnei* (ATCC 25931) and *Salmonella enterica* subspecies *enterica* serotype Typhimurium (ATCC 14028). The strains were stored in glycerol at -80°C, reactivated in BHI broth and cultivated in Mueller-Hinton agar.

2.6.2. Antimicrobial assay

The antibacterial activity was evaluated by the broth microdilution technique, according to the Clinical and Laboratory Standards Institute - CLSI (Cockerill et al., 2012). The levan solution was diluted in series (0.078-10 mg/mL) in Mueller Hinton II broth to a final volume of 100 µL in 96-well plate (U-bottom). The bacterial inoculum was prepared in 0.85 % saline solution from bacterial plates cultivated in overnight, with a turbidity equivalent to the Mcfarland 0.5 scale (~1.0×10^8 CFU/mL). This suspension was diluted 1:20 (~5×10^6 UFC/mL) and 10 µL were added to each well. As a negative control, the microorganisms were treated with 1 % ultrapure water. The plates were incubated for 18 h at 35 ± 1 °C.

2.7. Antibiofilm assay

Antibiofilm activity was determined according to the method described by Tilwani et al., (2021). For the assay, 180 µL of BHI containing 2 % glucose was added to the wells of a flat bottom microtiter plate. After, 10 µL of different pathogenic bacteria (*S. enterica* ser. Typhimurium and *S. sonnei*) (final concentration 1.0×10^5 UFC/mL) and 10 µL of levan at different concentrations (0.3125-10 mg/mL) were added to the wells of the microtiter plate and incubated for 18 hours at 37 ºC. The EPS final concentrations were 15.63, 62.50, 125, 250 and 500 µg/mL. Control wells contained broth and bacterial inoculum only, without addition of levan. Unbound cells were carefully discarded, and the plate was gently washed with PBS

buffer (pH 7.2), fixed in metanol, and then 0.2 % crystal violet. The plate was incubated for 20 min and washed three times with distilled water. Then 200 μL of 95 % ethanol was added to each well. Absorbance was measured at 570 nm using a microplate reader, and biofilm inhibition was determined using the following equation:

$$\text{Antibiofilm activity } (\%) = (OD_{control} - OD_{sample}/OD_{control}) \times 100.$$

2.8. Data analysis

All analyses were performed in triplicate. The analysis of variance (ANOVA) and graphics were performed using Statistica software version 10.0. For the cytotoxicity analysis, all data were processed statistically using the software R (R Core Team, 2021) with package ggplot2 (Wickham, 2016). The the Dunnett test ($p < 0.05$) were used to compare the treatments to control.

3. RESULTS AND DISCUSSION

3.1. *Influence of sucrose concentration and pH on maximum levan production*

Based on the CCRD results and applying a multiple regression analysis, the second-order polynomial equation in terms of decoded variables was obtained to explain levan production (Y) by G. *cerinus* UELBM11:

$$Y = -391.666 + 0.253 * x_1 - 0.001 * x_1^2 + 117.857 * x_2 - 9.475 * x_2^2 + 0.056 * x_1 * x_2$$

According to equation, the linear terms for x_1 (sucrose concentration) and x_2 (initial pH) positively influenced the increase in levan production, while the negative coefficients for

the quadratic terms indicated a decrease effect on the response. Between the factors tested, initial pH had the greatest impact on levan production as given by its the high linear (117.857) and quadratic (9.475) coefficients. The analysis by ANOVA (Table 2) confirmed that linear and quadratic terms of initial pH was also significant ($p < 0.05$) as shown by low p values, $p = 0.0053$ and $p = 0.036$, respectively. For sucrose concentration, only the quadratic term was significant ($p < 0.05$). Although the linear term of the sucrose concentration and the interaction [sucrose concentration × initial pH (x_1x_2)] were not significant ($p > 0.05$), they were maintained since we considered their high contribution in the model.

Table 1. Coded and decoded levels (in the parentheses) for the experimental design and results of CCRD.

Run	Coded levels (real values)		Levan (g/L)	
	Sucrose (g/L) (x_1)	pH (x_2)	Observed	Predict
1	-1 (160)	-1 (6.0)	35.69	35.51
2	-1 (160)	+1 (7.0)	38.31	39.16
3	+1 (300)	-1 (6.0)	35.39	34.72
4	+1 (300)	+1 (7.0)	45.85	46.2
5	-1.41 (131)	0 (6.5)	33.16	32.72
6	+1.41 (329)	0 (6.5)	36.88	37.14
7	0 (230)	-1.41 (5.8)	36.88	37.46
8	0 (230)	+1.41 (7.2)	49.02	48.19
9	0 (230)	0 (6.5)	46.99	47.6
10	0 (230)	0 (6.5)	48.87	47.6
11	0 (230)	0 (6.5)	46.94	47.6

The Table 2 also provides us the quality of the model. The determination coefficient R^2 was 0.987, indicating that the model explained 98.7 % of the variability in levan production. The F-test for lack of fit ($p = 0.623$) was not significant at the 5 % level ($p > 0.05$)

indicating that the experimental data obtained fitted well with the model and can be used for predictive purpose.

Through the response surface generated by the model (Fig. 1), it was possible to obtain the optimal working conditions for the maximum production of levan. The surface and the contour curves show us that for sucrose concentration the optimum range is 230 – 270 g/L and for initial pH is between 6.7 – 7.2. The critical values calculated by the derivative of the model leads to a maximum predict production of 49.52 g/L, and correspond to a sucrose concentration of 248.46 g/L and initial pH of 6.95. When modified HS medium was prepared under these conditions to validate the model, the actual production obtained was 48.06 ± 2.88 g/L. The results showed that the experimental and the predicted values were well correlated ($p = 0.46$) suggesting that the fitted model is satisfactory and accurate.

Table 2. ANOVA for levan production by *Gluconobacter cerinus* UELBM11 ($R^2 = 0.987$ / $R^2adj = 0.974$)

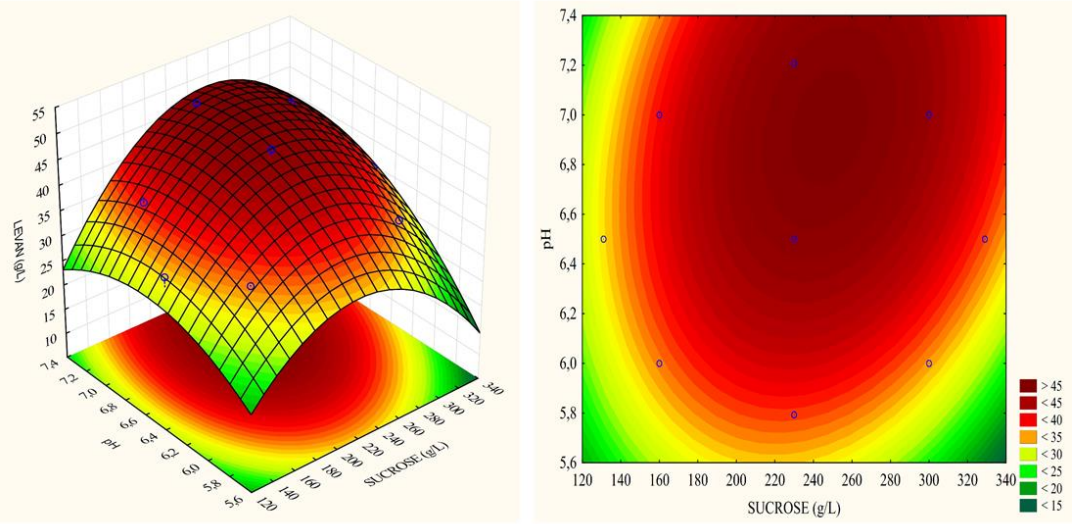
Source of variation	SS[a]	DF[b]	MS[c]	F	p value
Linear					
Sugar conc.	19.5340	1	19.5340	16.1398	0.056737
Initial pH	226.5396	1	226.5396	187.1764	0.005300*
Quadratic					
Sugar conc.	114.3719	1	114.3719	94.4988	0.010417*
Initial pH	31.6855	1	31.6855	26.1799	0.036139*
Interaction					
Sugar conc. × Initial pH	15.3664	1	15.3664	12.6964	0.070531
Lack of Fit	2.6404	3	0.8801	0.7272	0.623166
Pure error	2.4206	2	1.2103		
Total SS	382.4549	10			

[a] Sum of squares.
[b] Degrees of freedom.
[c] Mean square.
* Significant ($p < 0.05$).

Moreover, several studies (Bouallegue et al., 2020; Semjonovs et al., 2017; Silbir et al., 2014; Srikanth et al., 2015) also highlight the importance of sucrose concentration and initial pH in the synthesis of levan. Sucrose is the natural and preferred substrate of levansucrases. This enzyme catalyzes the formation and elongation of levan with an optimum in mildly acidic conditions (pH range 5–7) (Öner et al., 2016). For acetic acid bacteria, the sucrose requirement to produce levan appears to depend on the producing bacterium. For instance, for *Acetobacter xylinus*, the optimum sucrose concentrations ranged from 50 g/L to 60 g/L, and at higher concentrations the levan production drastically decreased (Srikanth et al., 2015). Similarly, this decline was also observed in our study and in optimization of levan by *G. nephelli* (Semjonovs et al., 2017) and *Tanticharoenia sakaeratensis* (Aramsangtienchai et al., 2020), however, the optimum concentrations of the substrate used were almost three times higher compared to study with *A. xylinus* (Srikanth et al., 2015). It is possible that bacteria show differences in resistance to osmotic stress and/or the accumulation of sucrose in the periplasmic space may inhibit the action of levansucrase (Aramsangtienchai et al., 2020). In the same way, an extreme initial pH can lead to metabolic stress (Srikanth et al., 2015) affecting the bacterial growth and consequently the enzymatic activity (Shaheen et al., 2017). In this work, the maximum levan production was achieved at pH 6.95. This is consistent with the optimal pH range for the activity of the enzyme levansucrase, as indicated above, and other works performed with *A. xylinus* (optimum pH 6.77) (Srikanth et al., 2015), *Bacillus lentus* (optimum pH 6.5) (Abou-taleb et al., 2015), *Bacillus subtilis* (optimum pH 7.00) (Bouallegue et al., 2020) and *Zymomonas mobilis* (optimum pH 6.00) (Silbir et al., 2014). However, studies also demonstrate that this parameter may be a non-significant variable (Semjonovs et al., 2017), as it is possible to obtain the highest levan amount at extreme values (pH 4.5), as observed for acetic acid bacterium *G. albidus* TMW 2.1191 (Ua-

Arak et al., 2017). Thus, our finds showed that the pH was an important factor for levan production by *G. cerinus* UELBM11, but may depend on the type of microorganism used. In this work, when the sucrose concentration (248.46 g/L) and initial pH (6.95) were optimized, the production of levan was approximately 3.5 times higher than in non-optimized conditions (13.89 g/L). In terms of productivity, it corresponded to 0.67 g/L.h, which was higher than those obtained by *A. xylinum* NCIM 2526 (0.11 g/L.h) (Srikanth et al., 2015), *G. nephelii* P1464 (0.66 g/L.h) (Semjonovs et al., 2017), *B. subtilis* A17 (0.11 g/L.h) (Bouallegue et al., 2020) and *Pseudomonas fluorescens* (0.11 g/L.h) (Jathore et al., 2012). This great enhancement of levan yield shows that *G. cerinus* UELBM11 is a potential microorganism for large-scale production of fructan and several industrial applications.

Fig. 1. Response surfaces and contour curves for levan production with x_1 (sucrose concentration) and x_2 (initial pH) as independent variables.



3.2. *Cytotoxic activity*

According to the World Health Organization, cancer is one of the leading causes of death worldwide, accounting for 10 million deaths in 2020 (World Health Organization, 2022). With the increase in new cancer cases, the search for natural products as therapeutic agents in treating cancer is highly desirable since they present fewer side-effects compared to conventional treatments (Queiroz et al., 2017). The potential of levan to treat hepatocellular and gastric carcinomas has been widely investigated in terms of its antineoplastic activity (Vieira et al., 2021); however, there is a lack of knowledge on effects of levan in other types of cancer cells. Therefore, in this work, the cytotoxic effect of levan from *G. cerinus* UELBM11 on SH-SY5Y neuroblastoma cells was evaluated in different concentrations (50 – 1000 µg/mL) for 24, 48 and 72 h.

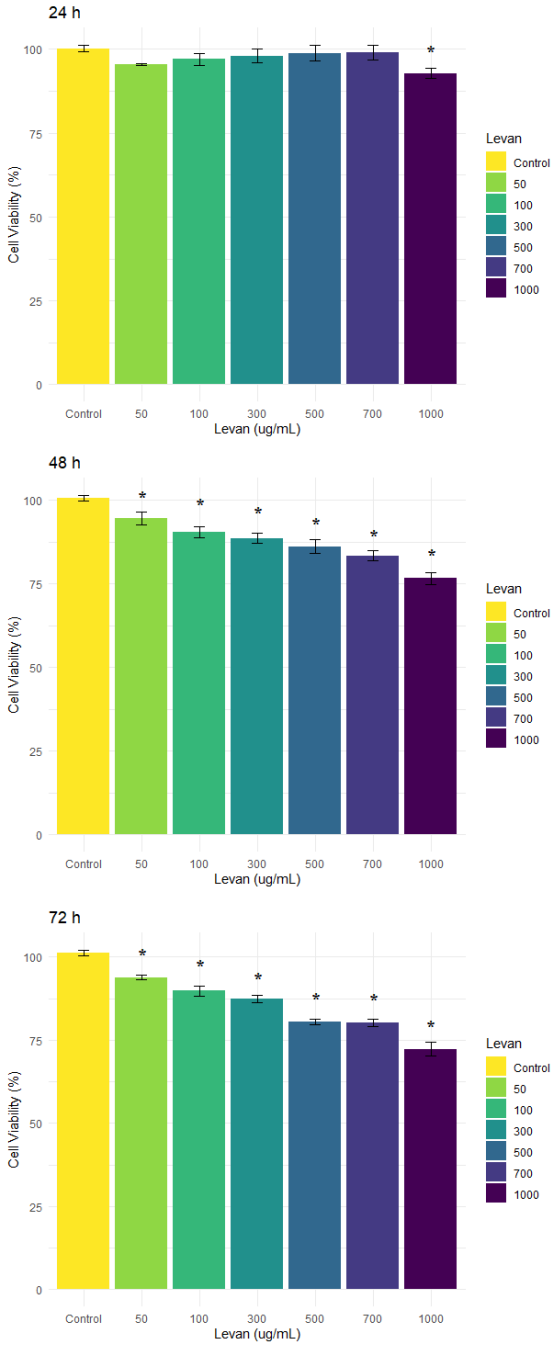
Fig. 2 shows that the viability of SH-SY5Y cells was reduced in a time - and concentration – dependent manner for 48 h and 72 h. Levan exerted little cytotoxicity at 1000 µg/mL after 24 h of treatment (~7 % non-viable cells). However, after 48 h, levan became significantly cytotoxic ($p < 0.05$) to SH-SY5Y cells from a concentration of 50 µg/mL and the maximum reduction of viability ($p < 0.05$) was observed at 1000 µg/mL (23.47 % non-viable cells). Similarly, for 72 h, the cytotoxic effect of levan was higher at 1000 µg/mL, where ~72.34 % of the cells remained viable. Unlike the study conducted by Vieira et al., (2021) that found no reduction in the viability of SH-SY5Y cells up to 96 h of treatment using levan from *Bacillus subtilis* var. natto at 1000 µg/mL. However, different from our study, the cytotoxicity of their levan was measured using a live-cell imaging system (IncuCyte ZOOM).

Several studies have reported that the cytotoxic effect of levan may vary according to the cell line tested, as well as depending on the dose, chemical structure, and molecular weight (Mw) of the polysaccharide (Abdel-Fattah et al., 2012; Sarilmiser & Oner, 2014; Yoo et al., 2004). For instance, Domżał-Kędzia et al. (2019) and Dos Santos et al. (2013) showed

that levan was not cytotoxic to human fibroblasts cell lines and Chinese hamster ovary cells when evaluated at concentrations ranging from 0.98 to 1000 μg/mL (72 h) and 80 to 400 μg/ml (24 h), respectively. Our findings for neuroblastoma cells are consistent with those of Dahech et al. (2012), who discovered that the highest cytotoxic effect (~45 %) against human hepatocellular carcinoma (HepG2) was reported at the highest dosage (200 mg/mL) of the levan over 72 hours. However, their levan concentration range (3.125 – 200 mg/mL) was significantly higher than ours. In another work, Yoo et al. (2004) showed that levans from *Microbacterium laevaniformans* (Mw 710 kDa) and *Rahnella aquatilis* (Mw 380 kDa) had efficacy against HepG2 and human stomach carcinoma (SNU-1). However, no apparent association was found between levan dose (200–1000 μg/mL) and antitumor activity in both cell lines, and similarly to study conducted by Calazans et al. (2000), they hypothesized that a particular size of levan would have a higher antitumor effect (between 600 – 800 kDa) (González-Garcinuño et al., 2018; Yoo et al., 2004).

In our previous studies (Unpublished data) identified that levan from *G. cerinus* UELBM11 has a Mw of 878 kDa. It is likely that the Mw of levan from UELBM11 positively influenced in our assay against SH-SY5Y cells, since in the study carried out by Vieira et al. (2021) the Mw was substantially smaller (8.8 kDa) and no activity was found. However, their results also showed that the treatment with 1000 μg/mL of levan induced apoptosis in SH-SY5Y cells by significantly increasing Annexin V and activating caspase 3/7 (Vieira et al., 2021). This apoptotic effect on neuroblastoma cells was associated with increased penetration into the cell due to the size of the polysaccharide (Vieira et al., 2021). In another study, Liu et al. (2012) prepared different derivatives of levan from *Paenibacillus polymyxa* EJS-3 (EPS-1, Mw 1218 kDa) by means of acetylation (AL, 260 kDa), phosphorylation (PL, 973 kDa) and benzoylation (BL, 348 kDa).

Fig. 2. Cytotoxic effects of levan (µg/mL) on human neuroblastoma SH-SY5Y cells for 24 h, 48 h and 72 h of incubation. Data represent the mean of 3 independent experiments. Differences statistically significant between controls containing only the culture medium and cells incubated with levan are represented with (*) $p < 0.05$.



The cytotoxicity of EPS-1, AL, PL and BL was evaluated in different doses on human gastric carcinoma BGC-823 cells. Although EPS-1 has higher Mw, the inhibition rates at higher dose tested (400 µg/mL) were: EPS-1 (55.37 %), AL (74.97 %), PL (95.50 %) and BL (91.67 %). This effect, particularly for PL, was linked to the negatively charged phosphate group, which has a strong affinity for immune cell receptors. In this line, Abdel-Fattah et al. (2012) also showed that sulphated levan (SL1) was more cytotoxic to HepG2 cells than "native" levan. After, this inhibition by SL1 was associated with the apoptosis induced via mitochondrial pathway by activation of caspase-9 and caspase-3 (Abdel-Fattah et al., 2012). As a result, both investigations (Abdel-Fattah et al., 2012; Liu et al., 2012) showed that structural modifications in levan can cause the antiproliferative action of tumor cells.

In this study, it was found that cytotoxic effect of levan against SH-SY5Y cells increased in a time - and concentration – dependent manner (48 h and 72 h) and it was more pronounced at 1000 µg/mL for 72 hours.

3.3. *Antimicrobial activity*

In addition to showing activity against tumor cells, levan has demonstrated anti-microbial activity against a range of food-borne pathogens and fungi (Koşarsoy Ağçeli & Cihangir, 2020; Mummaleti et al., 2022). In this study, the antimicrobial activity of the levan was evaluated against Gram-negative strains by Minimal Inhibitory Concentration (MIC) values using microdilution method. Our results showed that none of the bacteria studied exhibited sensitivity to levan, independent of the dose evaluated. Previous research has suggested that polysaccharides can disrupt the bacterial cell walls and membranes, resulting in an increase in water-soluble intracellular proteins and leakage of essential molecules, and ultimately cell death (He et al., 2010). Furthermore, polysaccharides can inhibit the bacterial

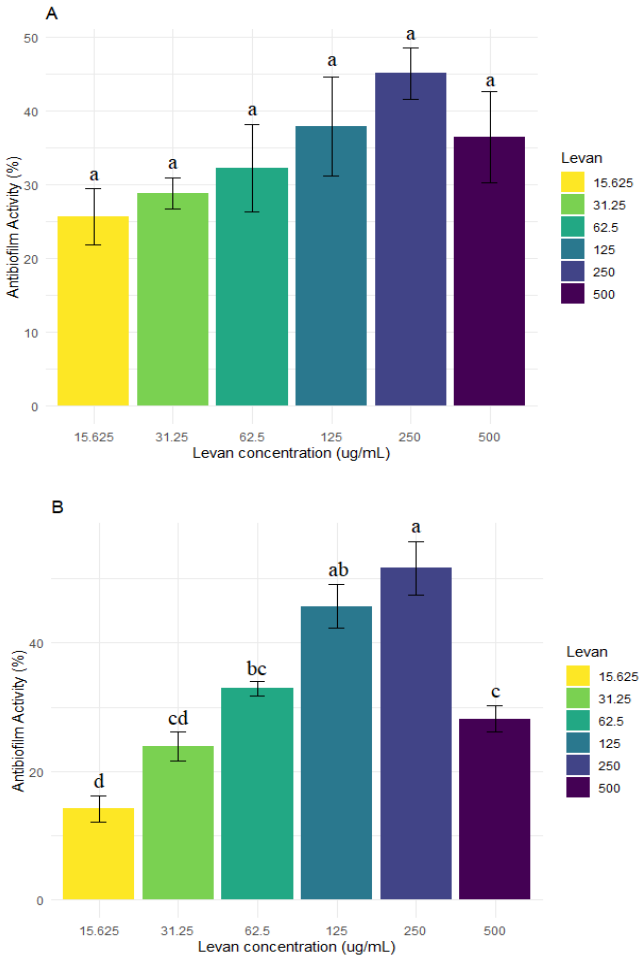
growth by obstructing nutrient entrance, and this barrier effect appears to be proportional to the increase in polysaccharide content (Li et al., 2018; Mummaleti et al., 2022). Nonetheless, our study did not corroborate these findings, since inhibition of bacterial growth was not observed even at the highest concentrations (1 %).

Byun et al. (2014) also investigated the inhibitory effect of different levan compounds (high-Mw 3×10^6 Da, low-Mw 5×10^4 Da and difructose dianhydride IV-DDIV) on the growth of spoilage and pathogenic bacteria by MIC. They reported that low-Mw levan had MICs of around 1 % against most bacteria tested, which were lower than those of high-Mw levan and DDIV. Only for *S. enterica* ser. Typhimurium, the low-molecular-weight levan had a higher MIC (1–3 %) than high-molecular-weight levan (MIC, 1 %). Although the authors have attributed this inhibition to reduction in water activity and/or induction of osmotic stress, Gokmen et al. (2020) showed that levans from *Zymomonas mobilis* NRRL B-14023 and *Bacillus subtilis* (commercial levan) did not reduce the water activity even at the highest levan concentration (4 %). In addition, similar to our results, it was not possible to determine the MIC values, since the growth of pathogens was observed at all concentrations evaluated (5 – 40 mg/mL) (Gokmen et al., 2020). On the other hand, studies conducted by Ağçeli & Cihangir (2020) and Mummaleti et al. (2022) reported that levan had an antimicrobial effect against *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *S. abony*, and *Staphylococcus aureus.* They found a maximum zone of inhibition between 100 and 1000 µg/mL using the disk diffusion approach. We hypothesized that this difference between the methods employed can be one of the factors that contributed for this disparity in results.

3.4. *Antibiofilm assay*

The inhibitory effects of different levan concentrations on bioflm formation by two food pathogens were studied. As shown in Fig. 3 (A and B), levan inhibited biofilm formation of *S. enterica* ser. Typhimurium and *S. sonnei* at all concentrations tested.

Fig. 3. Effects of levan from *G. cerinus* UELBM11 at various concentrations on biofilm formation by *Salmonella enterica* subspecies *enterica* serotype Typhimurium (A) and *Shiguella sonnei* (B). (a,b,c,d): Different letters indicate significant differences ($p < 0.05$).



Even with increasing levan concentration, these activities (25.67 % - 45.05 %) for *S. enterica* ser. Typhimurium showed no significant differences ($p > 0.05$). In *S. sonnei*, on the other hand, the inhibitory effects were not completely dose-dependent. Levan showed the maximum antibiofilm activity at 250 µg/mL (51.58 ± 7.26 %), and the least effects were

observed at 15.125 µg/mL (14.21 ± 3.47 %) and 500 µg/mL (28.19 ± 3.60 %). Similar to our study, Xu et al. (2020) demonstrated that the inhibition of *S. enterica* ser. Typhimurium biofilm was independent of the concentration of EPS from *Lactobacillus casei* NA -2 (31.25 to 500 µg/mL), although they showed lower inhibition values (about 10 %) than our work (25.67 % - 45.05 %).

On the other hand, Wang et al. (2015) showed that the inhibitory activities of EPS from *L. plantarum* YW32 on biofilm formation by *E. coli* O157, *S. flexneri* CMCC (B), *S. aureus* AC1 and *S. enterica* ser. Typhimurium S50333 were concentration dependent. At a higher concentration than in our study (5.0 mg/mL), EPS showed higher inhibition of biofilm formation by *S. aureus* AC1 (45.13 %), *S. flexneri* CMCC (B) (44.67%), and *S. enterica* ser. Typhimurium S50333 (44.04 %), while only 12.71 % was seen for biofilm formed by *E. coli* O157. Using levan from *Enterococcus faecium* MC-5, Tilwani et al. (2021) also observed the same profile. The highest inhibition effects of levan at 2 mg/mL were demonstrated for *L. monocytogenes* biofilm (72.36 ± 2.63 %), followed by *S. enterica* (68.32 ± 3.36 %), *S.* Typhi (66.77 ± 3.75 %), and *Vibrio anguillarum* (63.85 ± 2.35 %). In another study, levan from *Leuconostoc pseudomesenteroides*-CM at 1 mg/mL inhibited biofilm formation of *E. coli* (90 %) and *Enterococcus faecalis* (88 %) (Abid et al., 2018).

These findings suggest that antibiofilm activity varies according to the pathogen under investigation as well as EPS concentration and source. The mechanism of inhibition of this activity, however, does not appear to be due to an antimicrobial effect, as levan from *G. cerinus* UELBM11 did not inhibit *S. enterica* ser. Typhimurium or *S. sonnei* at any of the previously studied concentrations (0.078-10 mg/mL). Levan may exert their antibiofilm effects by altering bacterial cell surfaces, preventing bacterial cells from adhering to surfaces at first, or by acting as signaling molecules to suppress genes involved in biofilm formation

(Tilwani et al., 2021; Wang et al., 2015). As a result, levan from *G. cerinus* UELBM11 can be used as a food-grade adjunct in a variety of foods and applied in food industries to inhibit pathogen biofilm formation.

4. CONCLUSION

Our study showed that RSM is a useful approach for large-scale levan production, since it promoted an increase of approximately 3.5 times in levan yield when compared to non-optimized conditions (13.89 g/L). Furthermore, our results also suggest that levan may be a promising natural agent against SH-SY5Y cancer cells. Further studies are required to investigate which pathways are involved in tumor cell growth inhibition, in addition to the role of molecular mass for such activity. Although our results did not show efficacy in inhibiting selected microorganisms, levan shows promise as a natural and non-toxic alternative for controlling biofilms in food processing environments and can also be used as an additive in new product development.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

Abdel-Fattah, A. M., Gamal-Eldeen, A. M., Helmy, W. A., & Esawy, M. A. (2012). Antitumor and antioxidant activities of levan and its derivative from the isolate *Bacillus subtilis* NRC1aza. *Carbohydrate Polymers*, *89*(2), 314–322. https://doi.org/10.1016/j.carbpol.2012.02.041

Abid, Y., Casillo, A., Gharsallah, H., Joulak, I., Lanzetta, R., Corsaro, M. M., Attia, H., & Azabou, S. (2018). Production and structural characterization of exopolysaccharides

from newly isolated probiotic lactic acid bacteria. *International Journal of Biological Macromolecules*, *108*, 719–728. https://doi.org/10.1016/j.ijbiomac.2017.10.155

Abou-taleb, K., Abdel-Monem, M., Yassin, M., & Draz, A. (2015). Production, purification and characterization of levan polymer from *Bacillus lentus* V8 Strain. *British Microbiology Research Journal*, *5*(1), 22–32. https://doi.org/10.9734/bmrj/2015/12448

Aramsangtienchai, P., Kongmon, T., Pechroj, S., & Srisook, K. (2020). Enhanced production and immunomodulatory activity of levan from the acetic acid bacterium, *Tanticharoenia sakaeratensis*. *International Journal of Biological Macromolecules*, *163*, 574–581. https://doi.org/10.1016/j.ijbiomac.2020.07.001

Bouallegue, A., Casillo, A., Chaari, F., La Gatta, A., Lanzetta, R., Corsaro, M. M., Bachoual, R., & Ellouz-Chaabouni, S. (2020). Levan from a new isolated *Bacillus subtilis* AF17: Purification, structural analysis and antioxidant activities. *International Journal of Biological Macromolecules*, *144*, 316–324. https://doi.org/10.1016/j.ijbiomac.2019.12.108

Byun, B. Y., Lee, S. J., & Mah, J. H. (2014). Antipathogenic activity and preservative effect of levan (β-2,6-fructan), a multifunctional polysaccharide. *International Journal of Food Science and Technology*, *49*(1), 238–245. https://doi.org/10.1111/ijfs.12304

Calazans, G. M. T., Lima, R. C., De França, F. P., & Lopes, C. E. (2000). Molecular weight and antitumour activity of *Zymomonas mobilis* levans. *International Journal of Biological Macromolecules*, *27*(4), 245–247. https://doi.org/10.1016/S0141-8130(00)00125-2

Cockerill, F. R., Wikler, M. A., Alder, J., Dudley, M. N., Eliopoulos, G. M., Ferraro, M. J., Hardy, D. J., Hecht, D. W., Hindler, J. A., Patel, J. B., Powell, M., Swenson, J. M., Thomson, R. B., Traczewski, M. M., Turnidge, J. D., Weinstein, M. P., Zimmer, B. L., 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard — Ninth Edition. In *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*; Approved Standard - Ninth Edition.

Dahech, I., Belghith, K. S., Belghith, H., & Mejdoub, H. (2012). Partial purification of a *Bacillus licheniformis* levansucrase producing levan with antitumor activity. *International Journal of Biological Macromolecules*, *51*(3), 329–335. https://doi.org/10.1016/j.ijbiomac.2012.04.030

de Moura Escobar, S. J., Simone, M., Martin, N., de Oliveira Ribeiro, C. A., Martinez, G. R., Winnischofer, S. M. B., Witting, P. K., & Rocha, M. E. M. (2019). Cytotoxic effects of 4′-hydroxychalcone on human neuroblastoma cells (SH-SY5Y). *Toxicology in Vitro*, *61*(May 2018), 104640. https://doi.org/10.1016/j.tiv.2019.104640

Domżał-Kędzia, M., Lewińska, A., Jaromin, A., Weselski, M., Pluskota, R., & Łukaszewicz, M. (2019). Fermentation parameters and conditions affecting levan production and its potential applications in cosmetics. *Bioorganic Chemistry*, *93*(December 2018), 102787. https://doi.org/10.1016/j.bioorg.2019.02.012

Donot, F., Fontana, A., Baccou, J. C., & Schorr-Galindo, S. (2012). Microbial

exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. *Carbohydrate Polymers*, *87*(2), 951–962. https://doi.org/10.1016/j.carbpol.2011.08.083

Dos Santos, L. F., Bazani Cabral De Melo, F. C., Martins Paiva, W. J., Borsato, D., Corradi Custódio Da Silva, M. D. L., & Colabone Celligoi, M. A. P. (2013). Characterization and optimization of levan production by *Bacillus subtilis* NATTO. *Romanian Biotechnological Letters*, *18*(4), 8413–8422.

Erkorkmaz, B. A., Kırtel, O., Ateş Duru, Ö., & Toksoy Öner, E. (2018). Development of a cost-effective production process for *Halomonas* levan. *Bioprocess and Biosystems Engineering*, *41*(9), 1247–1259. https://doi.org/10.1007/s00449-018-1952-x

Esawy, M. A., Amer, H., Gamal-Eldeen, A. M., El Enshasy, H. A., Helmy, W. A., Abo-Zeid, M. A. M., Malek, R., Ahmed, E. F., & Awad, G. E. A. (2013). Scaling up, characterization of levan and its inhibitory role in carcinogenesis initiation stage'. *Carbohydrate Polymers*, *95*(1), 578–587. https://doi.org/10.1016/j.carbpol.2013.02.079

Gamal, A. A., Abbas, H. Y., Abdelwahed, N. A. M., Kashef, M. T., Mahmoud, K., Esawy, M. A., & Ramadan, M. A. (2021). Optimization strategy of Bacillus subtilis MT453867 levansucrase and evaluation of levan role in pancreatic cancer treatment. *International Journal of Biological Macromolecules*, *182*, 1590–1601. https://doi.org/10.1016/j.ijbiomac.2021.05.056

Gokmen, G. G., Silbir, S., Goksungur, Y., & Kisla, D. (2020). The effect of microbial levan on the growth of microorganisms: inhibitory or stimulatory? *Fresenius Environmental Bulletin*, *29*(3), 1648–1654.

González-Garcinuño, Á., Tabernero, A., Domínguez, Á., Galán, M. A., & Martin del Valle, E. M. (2018). Levan and levansucrases: Polymer, enzyme, micro-organisms and biomedical applications. *Biocatalysis and Biotransformation*, *36*(3), 233–244. https://doi.org/10.1080/10242422.2017.1314467

Han, J., Feng, H., Wang, X., Liu, Z., & Wu, Z. (2021). Levan from *Leuconostoc citreum* BD1707: production optimization and changes in molecular weight distribution during cultivation. *BMC Biotechnology*, *21*(1), 14. https://doi.org/10.1186/s12896-021-00673-y

He, F., Yang, Y., Yang, G., & Yu, L. (2010). Studies on antibacterial activity and antibacterial mechanism of a novel polysaccharide from *Streptomyces virginia* H03. *Food Control*, *21*(9), 1257–1262. https://doi.org/10.1016/j.foodcont.2010.02.013

Hestrin, S., & Schramm, M. (1954). Synthesis of cellulose by *Acetobacter xylinum*. II. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *The Biochemical Journal*, *58*(2), 345–352. https://doi.org/10.1042/bj0580345

Hövels, M., Kosciow, K., & Deppenmeier, U. (2021). Characterization of a novel endo-levanase from *Azotobacter chroococcum* DSM 2286 and its application for the production of prebiotic fructooligosaccharides. *Carbohydrate Polymers*, *255*(August 2020). https://doi.org/10.1016/j.carbpol.2020.117384

Idogawa, N., Amamoto, R., Murata, K., & Kawai, S. (2014). Phosphate enhances levan production in the endophytic bacterium *Gluconacetobacter diazotrophicus* Pal5. *Bioengineered*, *5*(3), 173–179. https://doi.org/10.4161/bioe.28792

Jathore, N. R., Bule, M. V., Tilay, A. V., & Annapure, U. S. (2012). Microbial levan from *Pseudomonas fluorescens*: Characterization and medium optimization for enhanced production. *Food Science and Biotechnology*, *21*(4), 1045–1053. https://doi.org/10.1007/s10068-012-0136-8

Kazak Sarilmiser, H., & Toksoy Oner, E. (2014). Investigation of anti-cancer activity of linear and aldehyde-activated levan from *Halomonas smyrnensis* AAD6T. *Biochemical Engineering Journal*, *92*, 28–34. https://doi.org/10.1016/j.bej.2014.06.020

Koşarsoy Ağçeli, G., & Cihangir, N. (2020). Nano-sized biopolymer levan: Its antimicrobial, anti-biofilm and anti-cancer effects. *Carbohydrate Research*, *494*(June), 108068. https://doi.org/10.1016/j.carres.2020.108068

Li, W., Yu, S., Zhang, T., Jiang, B., & Mu, W. (2015). Recent novel applications of levansucrases. *Applied Microbiology and Biotechnology*, *99*(17), 6959–6969. https://doi.org/10.1007/s00253-015-6797-5

Li, X. L., Thakur, K., Zhang, Y. Y., Tu, X. F., Zhang, Y. S., Zhu, D. Y., Zhang, J. G., & Wei, Z. J. (2018). Effects of different chemical modifications on the antibacterial activities of polysaccharides sequentially extracted from peony seed dreg. *International Journal of Biological Macromolecules*, *116*, 664–675. https://doi.org/10.1016/j.ijbiomac.2018.05.082

Liu, J., Luo, J., Ye, H., & Zeng, X. (2012). Preparation, antioxidant and antitumor activities in vitro of different derivatives of levan from endophytic bacterium *Paenibacillus polymyxa* EJS-3. *Food and Chemical Toxicology*, *50*(3–4), 767–772. https://doi.org/10.1016/j.fct.2011.11.016

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*(1–2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Mummaleti, G., Sarma, C., Kalakandan, S. K., Gazula, H., Sivanandham, V., & Anandharaj, A. (2022). Characterization of levan produced from coconut inflorescence sap using *Bacillus subtilis* and its application as a sweetener. *Lwt*, *154*(October 2021), 112697. https://doi.org/10.1016/j.lwt.2021.112697

Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. A.-C. (2016). *Response surface methodology: Process and Product Optimization Using Designed Experiments* (4th ed.). Wiley series in probability and statistics established.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry*, *153*(2), 375–380. https://doi.org/10.1016/s0021-9258(18)71980-7

Ni, D., Xu, W., Bai, Y., Zhang, W., Zhang, T., & Mu, W. (2018). Biosynthesis of levan

from sucrose using a thermostable levansucrase from *Lactobacillus reuteri* LTH5448. *International Journal of Biological Macromolecules*, *113*, 29–37. https://doi.org/10.1016/j.ijbiomac.2018.01.187

Öner, E. T., Hernández, L., & Combie, J. (2016). Review of Levan polysaccharide: From a century of past experiences to future prospects. *Biotechnology Advances*, *34*(5), 827–844. https://doi.org/10.1016/j.biotechadv.2016.05.002

Queiroz, E. A. I. F., Fortes, Z. B., da Cunha, M. A. A., Sarilmiser, H. K., Barbosa Dekker, A. M., Öner, E. T., Dekker, R. F. H., & Khaper, N. (2017). Levan promotes antiproliferative and pro-apoptotic effects in MCF-7 breast cancer cells mediated by oxidative stress. *International Journal of Biological Macromolecules*, *102*, 565–570. https://doi.org/10.1016/j.ijbiomac.2017.04.035

R Core Team (2021). *R: A language and environment for statistical computing. R Foundation for Statistical Computing.* https://www.r-project.org/.

Semjonovs, P., Shakirova, L., Broks, R., Kistkins, S., & Zikmanis, P. (2017). Influence of Environmental Factors on Extracellular Fructan and Oligosaccharide Production by *Gluconobacter nephelii. Research Journal of Microbiology*, *12*(1), 33–41. https://doi.org/10.3923/jm.2017.33.41

Semjonovs, P., Shakirova, L., Treimane, R., Shvirksts, K., Auzina, L., Cleenwerck, I., & Zikmanis, P. (2016). Production of extracellular fructans by *Gluconobacter nephelii* P1464. *Letters in Applied Microbiology*, *62*(2), 145–152. https://doi.org/10.1111/lam.12521

Serrato, R. V., Meneses, C. H. S. G., Vidal, M. S., Santana-Filho, A. P., Iacomini, M., Sassaki, G. L., & Baldani, J. I. (2013). Structural studies of an exopolysaccharide produced by *Gluconacetobacter diazotrophicus* Pal5. *Carbohydrate Polymers*, *98*(1), 1153–1159. https://doi.org/10.1016/j.carbpol.2013.07.025

Shaheen, S., Aman, A., Ul Qader, S. A., & Siddiqui, N. N. (2017). Hyper-production of levansucrase from Zymomonas mobilis KIBGE-IB14 using submerged fermentation technique. *Pakistan Journal of Pharmaceutical Sciences*, *30*(6), 2053–2059.

Silbir, S., Dagbagli, S., Yegin, S., Baysal, T., & Goksungur, Y. (2014). Levan production by Zymomonas mobilis in batch and continuous fermentation systems. *Carbohydrate Polymers*, *99*, 454–461. https://doi.org/10.1016/j.carbpol.2013.08.031

Sokollek, S. J., Hertel, C., & Hammes, W. P. (1998). Cultivation and preservation of vinegar bacteria. *Journal of Biotechnology*, *60*(3), 195–206. https://doi.org/10.1016/S0168-1656(98)00014-5

Somogyi, M. (1952). Notes on sugar determination. *The Journal of Biological Chemistry*, *195*(1), 19–23. https://doi.org/10.1016/s0021-9258(19)50870-5

Srikanth, R., Siddartha, G., Sundhar Reddy, C. H. S. S., Harish, B. S., Janaki Ramaiah, M., & Uppuluri, K. B. (2015). Antioxidant and anti-inflammatory levan produced from Acetobacter xylinum NCIM2526 and its statistical optimization. *Carbohydrate Polymers*, *123*, 8–16. https://doi.org/10.1016/j.carbpol.2014.12.079

Tilwani, Y. M., Lakra, A. K., Domdi, L., Yadav, S., Jha, N., & Arul, V. (2021). Optimization and physicochemical characterization of low molecular levan from *Enterococcus faecium* MC-5 having potential biological activities. *Process Biochemistry*, *110*(May), 282–291. https://doi.org/10.1016/j.procbio.2021.08.021

Ua-Arak, T., Jakob, F., & Vogel, R. F. (2017a). Fermentation pH modulates the size distributions and functional properties of *Gluconobacter albidus* TMW 2.1191 levan. *Frontiers in Microbiology*, *8*(MAY), 1–11. https://doi.org/10.3389/fmicb.2017.00807

Ua-Arak, T., Jakob, F., & Vogel, R. F. (2017b). Influence of levan-producing acetic acid bacteria on buckwheat-sourdough breads. *Food Microbiology*, *65*, 95–104. https://doi.org/10.1016/j.fm.2017.02.002

Vieira, A. M., Zahed, F., Crispim, A. C., de Souza Bento, E., França, R. de F. O., Pinheiro, I. O., Pardo, L. A., & Carvalho, B. M. (2021). Production of levan from *Bacillus subtilis* var. natto and apoptotic effect on SH-SY5Y neuroblastoma cells. *Carbohydrate Polymers*, *273*(June), 118613. https://doi.org/10.1016/j.carbpol.2021.118613

Wang, J., Zhao, X., Yang, Y., Zhao, A., & Yang, Z. (2015). Characterization and bioactivities of an exopolysaccharide produced by *Lactobacillus plantarum* YW32. *International Journal of Biological Macromolecules*, *74*, 119–126. https://doi.org/10.1016/j.ijbiomac.2014.12.006

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis.* (Second edi). Springer-Verlag.

World Health Organization. (2022). *Cancer*. Retrieved April 15, 2022, from https://www.who.int/news-room/fact-sheets/detail/cancer

Xu, X., Peng, Q., Zhang, Y., Tian, D., Zhang, P., Huang, Y., Ma, L., Dia, V. P., Qiao, Y., & Shi, B. (2020). Antibacterial potential of a novel: *Lactobacillus casei* strain isolated from Chinese northeast sauerkraut and the antibiofilm activity of its exopolysaccharides. *Food and Function*, *11*(5), 4697–4706. https://doi.org/10.1039/d0fo00905a

Yoo, S. H., Yoon, E. J., Cha, J., & Lee, H. G. (2004). Antitumor activity of levan polysaccharides from selected microorganisms. *International Journal of Biological Macromolecules*, *34*(1–2), 37–41. https://doi.org/10.1016/j.ijbiomac.2004.01.002

5.4. ARTIGO CIENTÍFICO4[1]

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Natália Norika Yassunaka Hata[a]*; Mariana Assis de Queiroz Cancian[a]; Lycio Shinji Watanabe[b]; Francisco de Assis Marques[c]; Renan Reisdörfer Schorr[c]; Rodrigo Vassoler Serrato[d]; Daniele Sartori[e]; Rebeca Priscila Flora Catarino[a]; Fernando Teruhiko Hata[f]; Wilma Aparecida Spinosa[a]

[a]Departamento de Ciência e Tecnologia de Alimentos / Centro de Ciências Agrárias / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil; [b]Departamento de Química / Centro de Ciências Exatas / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil; [c]Departamento de Química / Centro de Ciências Exatas / Universidade Federal do Paraná – UFPR, Centro Politécnico, 81531-990, Curitiba, PR, Brazil; [d]Departamento de Bioquímica e Biologia Molecular / Universidade Federal do Paraná – UFPR, Centro Politécnico, 81531-990, Curitiba, PR, Brazil; [e]Departamento de Bioquímica e Biotecnologia / Centro de Ciências Exatas / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil; [f]Departamento de Agronomia / Centro de Ciências Agrárias / Universidade Estadual de Londrina - UEL, Rodovia Celso Garcia Cid, Km 380, 86057-970, Londrina, PR, Brazil
*Corresponding author. E-mail address: naty_ea@hotmail.com

Influence of levan-producing acetic acid bacterium in the gluconic fermentation of grape juice

ABSTRACT

Gluconobacter cerinus UELBM11 is an acetic acid bacterium isolated from Brazilian grapes capable of producing levan with bioactive properties. Since grapes contain various health-promoting nutrients and phytochemicals, this study aimed to introduce UELBM11 into sucrose-enriched grape juice to verify its ability to produce levan *in situ* and to evaluate the effects of gluconic fermentation on the physicochemical, microbiological, and biochemical parameters of the fermented beverage. As expected, UELBM11 adapted well to the grape beverage and was able to consume sucrose and produce 5.7 g/L of levan when cultured at 30 ºC for 24 h. The fermented beverage had a pH of 3.03, a total titratable acidity of 2.67 %, and no change in viscosity was observed. Fermentation increased the bioavailability of phenolic compounds and preserved the anthocyanin content. FRAP results showed that the fermented beverage increased its antioxidant activity from 388.03 to 460.21 (mg vitamin C equivalent/L) ($p<0.05$), whereas the ABTS method revealed no changes in antioxidant activity. Although fermentation reduced the volatile compound content, new compounds were formed. Gluconic fermentation with UELBM11 is a promising approach to promote natural, clean diet that incorporates the bioactive properties of grapes and levan to meet the demand of different consumer categories.

Keywords: *Gluconobacter*, grapes, bioactivity, levan, fermentation

1. INTRODUCTION

Today, consumers are showing a growing interest in foods that not only satisfy hunger, but also prevent diet-related diseases and improve mental health [1]. According to the World Health Organization and the Food and Agriculture Organization, fruits are an essential component of a healthy diet [2]. The fifth most cultivated fruit in the world is grapes. Brazil produces more than 1.4 million tons annually, making it the fifteenth largest producer in the world [3]. The grape is an excellent and cheap source of phenolic compounds, organic acids, amino acids, vitamins, melatonin, and carbohydrates [4]. As well as its derivative products, has demonstrated potent antioxidant, anticarcinogenic, antibacterial, anti-diabetic, and anti-inflammatory activities, as well as cardioprotective, hepatoprotective, and neuroprotective properties [5]. Thus, grapes and their derivatives are frequently used as a medium for the development of functional foods [4].

Fermentation based on fruits and vegetables has further improved the nutritional value and functionality of foods and beverages. Given the growing consumer demand for natural, healthy alternatives with fewer additives and lactose-free products, fermentation has become a promising tool [6–9]. For example, gluconic fermentation by acetic acid bacteria (AAB) is a process that converts glucose to gluconic acid and maintains the concentration of bioactive compounds and antioxidant activity in beverages [10,11]. There is evidence that gluconic acid and its derivatives are potentially useful as bowel control agents, may help prevent cardiovascular disease, and may protect against strong oxidants [12]. Many AAB also synthesize exopolysaccharides (EPS) [6,13,14], which can be produced *in situ* in foods without the need for EPS isolation and labeling on food packages [8].

Levan is one of the EPS produced by AAB that has special properties and potential applications, especially in the food and pharmaceutical industries [15]. Levan can be used in

food and feed as a potential candidate for prebiotics [16], bread texture agent [13], emulsifying, fat replacer, and stabilizing agent [15,17]. In the medical field, levan is one of the macromolecules that has shown a wide range of biological activities, such as antioxidant [18], anti-inflammatory [18], and antitumor activities [15], and others. In our previous study, AAB were isolated from grapes and five levan-producing bacteria belonging to the genus *Gluconobacter* were found. Among them, *G. cerinus* UELBM11 stood out for producing a significant amount of levan under non-optimized conditions. In addition, the levan produced showed strong antioxidant activity. AAB, which are known to be the main spoilage agents in some fermented beverages, are widely distributed in fruits such as grapes [19]. In the present study, it was proposed to introduce the levan-producing bacterium UELBM11 into grape juice supplemented with sucrose to show its advantages in beverage production and to promote natural and clean label diet. Therefore, this study evaluated kinetic parameters related to gluconic fermentation, the content of levan produced *in situ*, as well as the total phenolic content, antioxidant activity and the composition of volatiles present in the beverage.

2. MATERIAL AND METHODS

2.1. Bacterial strain, storage and propagation

Gluconobacter cerinus UELBM11 (GenBank accession No. ON149510) was isolated from grape (*Vitis vinifera* L.) and stored in 20 % malt extract (m/v) broth at -20 ºC. The bacterial strain was routinely streaked on MYP agar containing mannitol (25 g/L), yeast extract (5.0 g/L), peptone (3.0 g/L) and agar (10 g/L) (Sokollek; Hertel; Hammes, 1998). Inoculum was prepared from *G. cerinus* UELBM11 culture maintained for 72 h on MYP agar and transferred to a 250 mL Erlenmeyer flask containing 100 mL of HS medium (Hestrin & Schramm, 1954). After cultivation at 30 °C on a rotary shaker (120 rpm) (TECNAL-4200)

until 0.6 of absorbance (600 nm) (Thermo Electron Corporation®, Spectronic Genesys 6®, EUA), the cells were washed and the density spectrophotometrically adjusted to 0.7, which corresponds to 2.5×10^8 CFU/mL.

2.2. Grape juice preparation

Frozen grape pulp (blend of Brazilian grapes varieties Isabel, Concord, and Bordeaux) was purchased on local market. The nutritional content of the pulp as stated on the packaging was (per 100 g): energy (51 kcal), total fat (0 g), saturated fat (0 g), trans fat (0 g), sugar (12 g), protein (0.7 g), and sodium (0 mg). The juice was prepared according to the manufacturer's instructions (100 g of pulp: 200 mL water) using a blender and supplemented with commercial sucrose until the total soluble solids reached 15.0 ºBrix. Then, the mixture was adjusted to pH 6.0 by adding drops of 5.0 M NaOH, distributed in Erlenmeyer flasks and pasteurized at 65 ºC for 30 min, and allowed cool to room temperature.

2.3. Grape juice fermentation

Fermentation experiments were conducted in Erlenmeyer flasks containing 100 mL of pasteurized grape juice. The standardized inoculum (1 %; v/v) was added to flasks and the fermentation process was performed at 30 ºC for 24 h under agitation at 100 rpm. Chemical and microbiological analyses were performed on samples collected at 0, 6, 12, 18, and 24 h. Pasteurized grape juice (non-microbial) served as the control. All fermentations were carried out in triplicate.

2.4. Viable cells count, pH, total titratable acidity (% TTA) and viscosity

Fermented grape juice was serially diluted with 0.1 % peptone solution and subsequently plated in triplicate on MYP agar plates (drop plate method). The plates were incubated for 48 h at 30 °C, and the viable cell counts recorded as Log CFU/mL. The pH of grape juices was measured during the fermentation periods using a pH meter. Total titratable acidity, expressed as percentage of gluconic acid, was determined by titration with 0.1 M NaOH [20]. The viscosity of the grape beverages was measured by a digital viscometer Brookfield at 25 ºC.

2.5. Isolation, purification of levan and quantification of sugars (sucrose, reducing sugars and levan) from grape juices

Levan was isolated from the fermented grape juice by ethanol precipitation method according to Serrato et al. [21] with some modifications. In summary, cells and beverage particles were removed by centrifugation (Eppendorf, Germany) at 5000 rpm for 20 min and the supernatant obtained was precipitated with cold ethanol 99 % (3:1, v/v) and left overnight at -20 °C. The precipitate was recovered by centrifugation, dried at 50 °C, redissolved in ultrapure water, dialyzed (MWCO 14 kDa) (Sigma-Aldrich, USA) for at least 48 h at 4 °C and lyophilized. The sample was subjected to acid hydrolysis with 0.5 M oxalic acid for 1 h at 100 °C [22] and levan concentration (based on the amount of monomers fructose) was determined by high performance liquid chromatography (HPLC) as described earlier in Bueno et al. [23]. Reducing sugars content were quantified by Somogyi and Nelson method [24,25] and sucrose quantified by enzymatic assay kit (Sigma, Ireland) in a 96-well plate using a microplate reader. The analyses were performed in triplicate.

2.6. Total phenolic content (TPC)

Total phenolic content of the fermented baverage was determined by the Folin–Ciocalteu method [26] with modifications. Briefly, the beverage was appropriately diluted (100 µL) and mixed with 0.5 mL of diluted 4-fold Folin–Ciocalteau reagent. After, 0.375 mL of 10 % sodium carbonate solution (m/v) was used to neutralize the mixture. After 2 h of incubation in the dark at room temperature, the absorbance of the mixture was measured at 750 nm. Gallic acid was used as a standard (16-56 mg/L), and the results were expressed in mg of gallic acid equivalents per L of beverage (mg GAE/L). All experiments were carried out in triplicate.

2.7. Monomeric anthocyanin content

The determination of the monomeric anthocyanin content was carried out according to Lee et al. [27]. In this method, an aliquot of the centrifuged sample (400 µL) was added to 3.60 mL of pH 1.0 buffer (KCl, 0.025 mol/L). After, another aliquot of 400 µL of the sample was homogenized with 3.60 mL of pH 4.5 buffer (CH_3CO_2Na, 0.40 mol/L). Absorbance was measured in a spectrophotometer at $\lambda = 510$ nm and $\lambda = 700$ nm. Results were calculated using the equation:

$$\text{Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L)} = (A \times MV \times DF \times 1000)/\varepsilon \times l$$

where $A = (A_{520nm} - A_{700nm})_{pH\ 1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5}$; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; l = path length in cm; e = 26900 molar extinction coefficient, in L/mol·cm for cyd-3-glu; and 10^3 = factor for conversion from g to mg. The results were obtained from three replicates.

2.8. Determination of antioxidant activity

2.8.1. Assay of ABTS free radical scavenging activity

The radical scavenging activity 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS •+) was determinated based on the method of Re et al. [28] with some modifications. Firstly, $ABTS^{\bullet+}$ was generated by reacting 7 mM ABTS solution and 140 mM potassium persulfate for 16 h in the dark. Before analysis, ABTS•+ solution was diluted with 20 mM monobasic potassium phosphate (pH 7.4) to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Then, an aliquot of the sample (50 µL) was mixed with 1000 µL of diluted $ABTS^{\bullet+}$ solutions. The absorbance was measured at 734 nm after 6 min of initial mixing. A standard curve of vitamin C (5-70 mg/L) was used for estimation of unknown concentrations. The results were expressed as mg vitamin C equivalent/L extract (mg VcE/L). All experiments were performed in triplicates.

2.8.2. Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to the method described by Benzie and Strain [29] with some modifications. FRAP reagent was freshly prepared by mixing 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 20 mM ferric chloride and 300 mM acetate buffer at pH 3.6 in the ratio of 1:1:10 (v/v). The mixture was maintained at 37 ºC for 30 min before use. After, aliquots of appropriately diluted samples (0.1 mL), ultrapure water (0.15 mL) and FRAP solution (1 mL) were homogenized and kept in dark for 30 min. The absorbance was read at 593 nm using a UV–Vis spectrophotometer. A standard curve of vitamin C (5-32 mg/L) was used for estimation of unknown concentrations. All experiments were performed in triplicates. Results were expressed as mg vitamin C equivalent/L extract (mg VcE/L).

2.9. Determination of volatile compounds

Analysis of volatile compounds in fermented (T24 h) and unfermented (T0 h) grape juice was performed in a headspace solid-phase microextraction (HS-SPME) gas chromatoghaphy (Model GC2010Plus, Shimadzu, Quito, Japan) coupled to a triple quadrupole mass spectrometry (Model TQ 8040) (GC–MS/MS) operating with an ionization voltage of 70 eV. An aliquot of 2 g of fermented sample was added to 20 mL vial and equilibrated at 60 °C for 10 min without stirring. An automatic injector (model AOC – 5000 Plus) with a PDMS/DVB(50/30 μm) fiber (Supelco, Bellefonte, USA) was used to capture the volatile compounds. Volatile compounds were absorbed onto the fiber for 10 min. After equilibrium, the fiber was desorbed at 250 °C for 3 min, and the injector was operated in splitless mode. Helium was used as the carrier gas at 1.02 mL/min. Separation of volatiles was performed using a DB-5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness; Agilent Technologies, Santa Clara, California, EUA). Temperature programming was set at 60 °C initially followed by an increase to 250 °C at 3 °C/min. The mass spectra were compared with the National Institute of Standards and Technology (NIST) mass spectra library [30], Adams' libraries of mass spectral data [31], and Human Metabolome Database (HMDB) [32]. Arithmetic index (AI) values of samples were calculated using a homologous series of n-alkanes C_7H_{16}-$C_{30}H_{62}$ and compared with literature data. For this analysis, only the T0 h and T24 h grape beverages (inoculated) were used.

2.10. Statistical analysis

All measurements were carried out in triplicates and the values were reported as mean ± standard deviation. Data was analyzed using one-way Analysis of variance (ANOVA) and Tukey test using at 5 % level of significance.

3. RESULTS AND DISCUSSION

3.1. Viable cells count, pH, total titratable acidity (% TTA) and viscosity

The changes in the number of viable cells and pH during the growth of *G. cerinus* UELBM11 with grape juice sucrose as substrate are shown in Fig. 1A. With a total duration of 24 h, it can be seen that half of the time was spent in the lag phase, as no significant variation ($p>0.05$) in the number of viable cells was detected during this time interval (0h-12h; ~ 8 log CFU/mL). Despite the necessity for this period of adaptation to grape juice, pH decreased rapidly ($p<0.05$) from 5.6 (T0 h; after pasteurization) to 3.76 (T12 h), and the nutrient differences compared to the HS medium do not appear to have caused enough bacterial stress to reduce the viability of *G. cerinus* UELBM11.

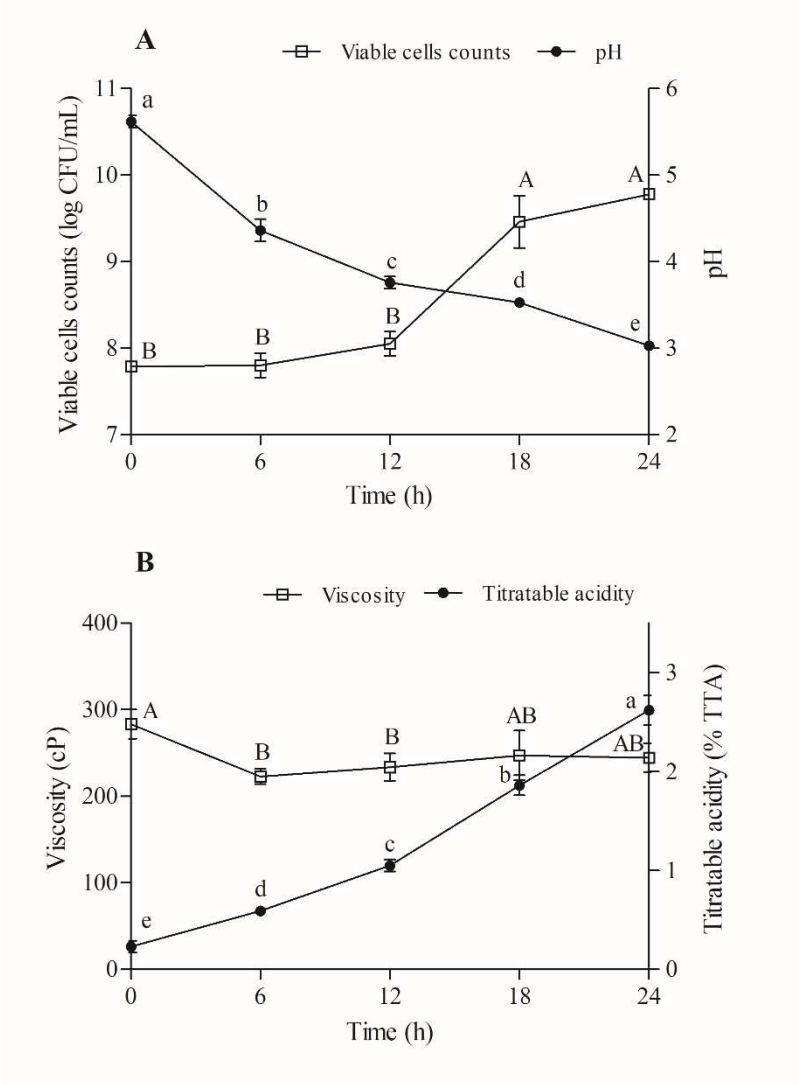
After that, a significant increase in cell viability (logarithmic phase) was observed (T12 h: 8.05 log CFU/mL to T18 h: 9.46 log CFU/mL) ($p<0.05$) and finally, the stationary phase was observed between 18 h and 24 h, lasting until 30 h of the evaluated period (data not shown). Meanwhile, the pH of the fermented juice decreased slightly to 3.03 after 24 h of fermentation. It is worth noting that *G. cerinus* UELBM11 is an aerobic bacterium isolated from grapes, which may have contributed to its greater adaptation to the sugary juice and the decrease in pH. This adaptation to the medium can also be attributed to the initial pH of the unfermented grape juice of 5.6 (after pasteurization). This value is consistent with descriptions of the genus *Gluconobacter*, where the optimal pH for growth is 5.0-6.0 [33] with growth observed at pH of 3-4 to the most strains [34].

Agitated culture is also important for the growth of obligate aerobes such as *G. cerinus* UEL BM11. Under these conditions, the oxygen transfer rate increases, and oxygen is generally used to generate ATP by oxidizing carbon sources such as sugars to organic acids [6]. It is possible to observe that the bacterium was able to grow by partially oxidizing the

added sucrose and probably the reducing sugars (Fig. 1A and Fig. 2A). As a result, pH levels decreased due to the accumulation of organic acids produced by the bacteria as part of their energy metabolism and growth. Other carbohydrates, vitamins, minerals, and edible fibers [35] in the juice may have also contributed to the increase in cell numbers. The pH values of the control (non-microbial) remained unchanged ($p>0.05$) during the study period (SMTable1). Corresponding to the pH changes, the profile of % TTA (expressed as gluconic acid) in the fermented beverage increased significantly from 0.23 % (T0 h) to 2.67 % (T24 h) ($p<0.05$) (Fig. 1B). The % TTA of the control (non-microbial) remained unchanged ($p>0.05$) during the study period (SM Table).

Gluconobacter species are typically found in sugary, sucrose-rich environments and are specialized in the oxidation of glucose to gluconic acid via membrane-bound dehydrogenases [36]. In our study, *G. cerinus* UEL BM11 most likely converted glucose in grape juice to gluconic acid, resulting in the observed increase in acidity. Sainz et al. [37] compared glucose metabolism in AAB species (*Gluconobacter* and *Acetobacter* strains) and concluded that strains of the genus *Gluconobacter* produced higher amounts of gluconic acid and would therefore be best suited for the production of a non-alcoholic fermented beverage from strawberries. Gluconic acid is a non-volatile, non-toxic, mild organic acid naturally occurring in fruits, plants, wine, kombucha, and honey and has a soft, mild, and refreshing sourness [38,39]. It is an approved food additive (E574) by the European Food Safety Authority (EFSA) and is listed as a GRAS (Generally Recognized As Safe) additive by the US Food and Drug Administration [39]. In addition, recent studies have indicated that this acid may have human and animal health benefits, which has promoted its use as a prebiotic in the food industry [12].

Fig. 1. Changes in (A) Viable cells count and pH and (B) Viscosity and total titratable acidity during grape-juice-sucrose fermentation by *Gluconobacter cerinus* UELBM11.



In contrast to the sharp increase in % TTA, the viscosity of the grape juice medium with sucrose remained practically constant during the 24 h fermentation (inoculated and control) (Fig. 1B and SMTable1). During the first twelve hours of fermentation, the initial viscosity of the beverage (283.20 cP) slightly decreased (6 h, 222.93 cP; 12 h, 233.60 cP) ($p < 0.05$), but remained constant from 12 h until the end of fermentation (244.53 cP). Although *in situ* formation of levan was detected during the fermentation process (Fig. 2B), our results showed that its presence did not lead to a noticeable increase in viscosity, which could be due

to its low concentration in the sample. Similar results were observed by Juvonen et al. [7] during fermentations with levan-producing *Lc. mesenteroides* E-91461T using liquid carrot samples (5 % sucrose; w/w). The researchers found that the viscosity of the sample increased compared to the non-started samples, but compared to other fermentation samples producing low-branched dextran (BAL), it was the least viscous sample (producing only 0.2 % levan; w/w). In another study by Han et al [40], the remarkable increase in viscosity (0.00 cP to 451 cP) in tomato juice medium (15 % sucrose) fermented by *Leuconostoc mesenteroides* BD1710 was attributed to the high sucrose concentration of the medium, which led to a pronounced production of dextran (32.15 g/L). However, the rheological properties of EPS, such as viscosity, are influenced not only by its concentration but also by its composition, structure, size, and charge [7].

Levan, for example, has a very low intrinsic viscosity compared to other polysaccharides of comparable molecular weight due to its spherical and compact molecular conformation and the presence of lateral branches [41]. Uncharged EPS, such as levan, are a promising source of functional hydrocolloids with unique technological and functional properties that can be used as food additives or formed *in situ* in foods [42]. Although the rheological properties of fermented grape juice were not affected by *in situ*-produced levan, its presence in the beverage may already indicate that it would have health-promoting properties.

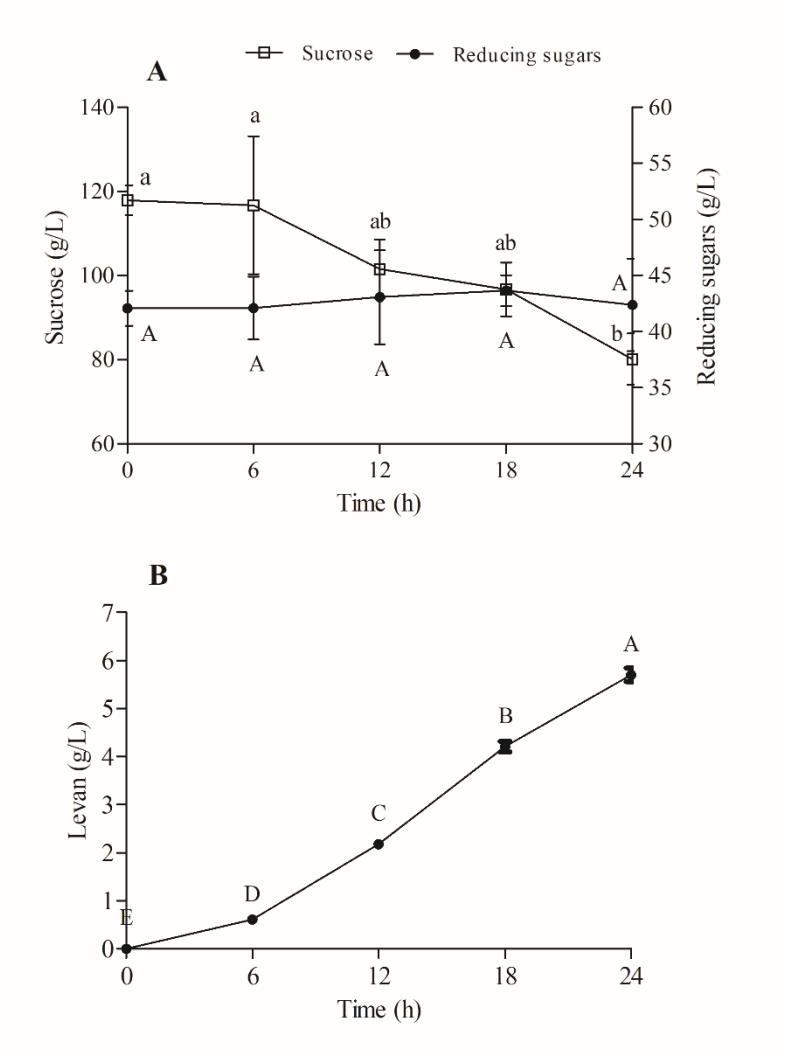
3.2. Sugars determination (sucrose, reducing sugars and levan)

The primary sugars of the beverage consumed during fermentation, sucrose and reducing sugars, and levan produced by *G. cerinus* UELBM11 were monitored and shown in Fig. 2 (A and B). Low concentrations of reducing sugars were present in the control and T0 h

(inoculated) samples (~4.0 %), and the total amount of sucrose added to promote fructan production was nearly 11.5 % (w/v). The sucrose concentration and reducing sugars of the control (non-microbial) remained unchanged ($p > 0.05$) during the study period (SMTable2).

During fermentation, sucrose concentration decreased ($p < 0.05$) from 117.89 g/L (T0 h) to 80.11 g/L (T24 h), while levan production increased ($p < 0.05$) compared with the start of fermentation (T0 h), reaching a maximum production of 5.70 g/L (T24 h). This yield (5.70 g/L; T24 h) corresponded to approximately 5 % sucrose added to the unfermented grape juice. The enzyme levanasucrase (EC 2.4.1.10), whose main substrate is sucrose, catalyzes the synthesis of levan [15]. Levanasucrase can catalyze the hydrolysis of sucrose to glucose and fructose as well as transfer fructose residues to a variety of acceptor molecules (water, sucrose, FOS, glucose and fructose) via transfructosylation reactions [15]. Levanasucrase is abundant in AAB (*Acetobacteraceae*) and occurs in only one gene copy in all strains of the genus *Gluconobacter* [43]. During fermentation of the grape beverage, levanasucrase most likely utilized the energy of glycosidic linkage of sucrose to polymerize fructose, while the released glucose was oxidized to gluconic acid or used for other metabolic purposes [43]. *Gluconobacter* can also produce 5-oxofructose in small amounts by oxidation of fructose [44]. In this study, it was also observed that the reducing sugar content remained constant throughout the fermentation ($p>0.05$). This suggests that there was a balance between the consumption of reducing sugars (such as glucose and fructose) for *G. cerinus* UEL BM11 growth and the production of metabolites (organic acids and polysaccharides), as well as the release of glucose and fructose into the medium by levansucrase. During fermentation, this is demonstrated by an increase in % TTA, a decrease in pH, and an increase in levan production.

Fig. 2. Changes in (A) Sucrose and reducing sugars and (B) levan yield by *Gluconobacter cerinus* UELBM11 cultivated in the grape juice supplemented with sucrose.



The highest levan productions observed between T18 h and T24 h also coincided with the cell growth of *G. cerinus* UBM11 in the stationary phase (T18 h to T24 h), indicating that the levan polymerization reaction occurred mainly in this phase and thus levan was a secondary metabolite, as described by other authors [18,45]. To the best of our knowledge, this is the only study reporting *in situ* production of levan from AAB in fruit beverages.

For baking purposes, levan has been formed *in situ* in the unconventional sourdoughs by AAB such as *Kozakia baliensis* DSM 14400 and *Neoasaia chiangmaiensis* NBRC 101099 [13]. *K. baliensis,* for instance, produced up to 49 g/kg EPS in sucrose-enriched spelt dough, whereas *N. chiangmaiensis* produced 24-29 g/kg EPS in spelt and whole wheat doughs [13]. Previous study with *G. albidus* TMW 2.1191 and *K. baliensis* NBRC 16680 on gluten-free sourdough production using molasses as a natural source of sucrose showed that *G. albidus* produced more levan than *K. baliensis* [6]. The highest concentrations of levan in doughs of *G. albidus* were 14.85±3.92 g/kg flour after 48 hours and 10.96±2.24 g/kg flour in doughs of *K. baliensis* after 54 hours [6]. The authors confirmed that levan has a positive effect on bread quality; however, they highlighted that an appropriate balance between strong acidification caused by acetic acid and levan production (amount and structure) must be maintained [6].

For BAL, Juvonen et al. [7] reported that *Leuconostoc mesenteroides* E-91461T produced 0.2 % (w/w) levan using liquid carrot with 5 % sucrose (w/w). Similar to our work, Eckel et al. (2019) produced dextran *in situ* in apple or grape juices supplemented with sucrose and obtained yields of 2.5 to 8.5 g/L using *Lactobacillus hordei* TMW 1.1907 from water kefir. In another work, Han et al [40] demonstrated that *Lc. citreum* BD1707, when cultured at 30 °C for 96 h, could synthesize more than 28 g/liter of levan in the tomato juice-sucrose medium (15 %; w/v). Subsequently, the response surface methodology (RSM) was used to optimize production, and the authors obtained 34.86 g/L of levan yield at optimal cultivation conditions of 26 °C and 200 rpm for 112 h in tomato juice supplemented with 172 g/L sucrose and an initial pH of 6.12 [46]. Although levan production in grape juice was not optimized in this study, the initial pH (5.6 after pasteurization), shaking speed (100 rpm), temperature (30 °C), cultivation time (24 h), and added sucrose (117.89 g/L) were adjusted based on preliminary experiments and the literature [15,18]. The yield of levan (24 h; 5.7 g/L) obtained

in situ in the grape beverage was not as pronounced as in previous work [6,7,13,40,46], but it is high enough to exert health-promoting effects such as antioxidant, antitumor, and antibiofilm activity, as reported in our previous experiments (unpublished data).

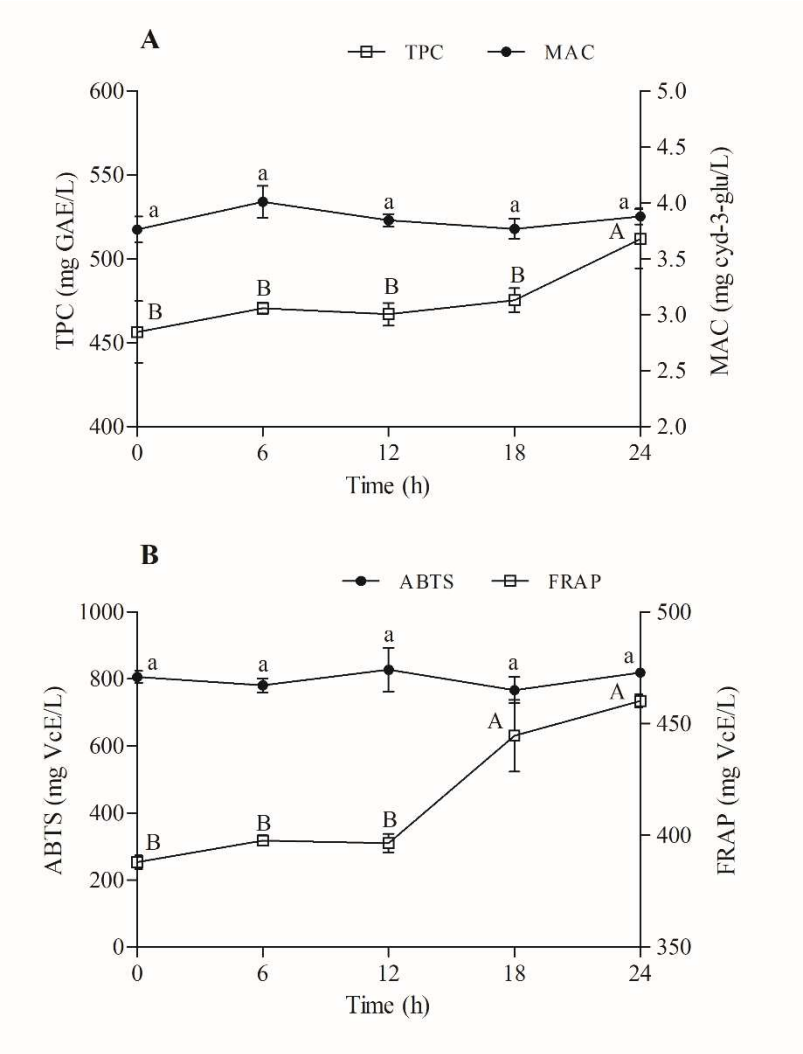
3.3. Total phenolic compounds, anthocyanin content and antioxidant activities

Phenolic compounds are known for their nutritional and health benefits, especially for their ability to act as potent antioxidants [47]. Therefore, the effect of fermentation with *G. cerinus* UELBM11 on TPC, anthocyanin content, and antioxidant activity of grape juice was studied (Figs. 3 - AB).

The control showed no significant changes in TPC, as can be seen in SMTable3, while the fermented sample (T24 h) had a significantly higher concentration (511.99 mg GAE/L) compared to the other fermentation times ($p<0.05$) (Fig. 3A). On the other hand, TPC in grape juice gradually decreased during LAB fermentation (*L. plantarum* 21802 and *L. brevis* 6239), indicating that phenolic compounds were oxidized and degraded during the process [4]. The same decrease patterns were observed in sweet lemon juice fermentation with *L. plantarum* LS5 [48], apple juice fermentation with *L. plantarum* ATCC14917 [49], and cabbage juice fermentation with different LAB strains [50].

Our results suggest that gluconic fermentation by UELBM11 increases the bioavailability of phenolic compounds and leads to their release and conversion into more active forms, as these bioactive molecules may be present in cell wall-bound, glycosylated or polymerized forms in food matrices [51]. These findings are supported by Ahmed et al. [52] who reported an increase in TPC during fermentation from various types of kombucha. The authors attributed this increase to the metabolic transformation of the beverage components by the enzymes of the kombucha microbial consortium.

Fig. 3. Changes in: (A) total phenolic compounds and monomeric anthocyanin content and (B) antioxidant activity using ABTS and FRAP method during grape juice fermentation by *Gluconobacter cerinus* UELBM11.



Anthocyanins, which are phenolic compounds, are potent antioxidants that impart a fruity color to beverages and influence their taste and chemical composition [35,53]. The data in Figure 3A show that the content of anthocyanins was maintained during gluconic fermentation, as it remained stable until the end of fermentation. However, in the control (non-microbial), this content gradually decreased over time, reaching a final value of 3.33 mg/L (from an initial value of 3.76 mg/L) (SMTable3). These results are in agreement with those of

Hornedo-Ortega et al. [10] who observed no significant changes in anthocyanin compounds during gluconic fermentation of strawberry beverages with *G. japonicus*. According to the authors, the bacterium did not interact with the anthocyanins, and the decrease in pH after gluconic fermentation probably stabilized the compounds. Our results also show that gluconic fermentation mediated by UELBM11 is an innovative method to preserve anthocyanin content.

The antioxidant capacity of phenolic compounds is attributed to their ability to donate hydrogen or electrons to oxidizing species and scavenge free radicals, forming stable intermediate radicals, thus preventing many disorders and metabolic diseases [35,54]. In our study, the ABTS and FRAP methods were used to evaluate the antioxidant activity of grape juices (Figure 3B). The results of the ABTS assay in the control and fermented samples showed no significant differences in the values of antioxidant activity throughout the fermentation ($p>0.05$) (SMTable3). This result is consistent with the findings of Li et al. [49] who reported that the ABTS scavenging activity of apple juice fermented with *L. plantarum* ATCC14917 remained stable ($p<0.05$) from 24 h to 72 h. Although they used different methods (DPPH method and ORAC assay) from ours, Hornedo-Ortega et al. [10] concluded that gluconic fermentation maintained antioxidant activity in strawberry beverage. On the other hand, the results of FRAP show that the fermented samples had significantly higher antioxidant activity after 18 h (444.66 mg vitamin C equivalent/L) and 24 h (460.21 mg vitamin C equivalent/L) than the other fermentation times (0 h: 388.03 mg vitamin C equivalent/L; 6 h: 397.71 mg vitamin C equivalent/L; 12 h: 396.54 mg vitamin C equivalent/L) ($p<0.05$). Several authors have also reported an increase in antioxidant activity of fruit-based fermented beverages using the FRAP method [55–57]. These results simply

reflect the effects of gluconic fermentation on TPC content, which most likely contributed to the release of phenolic bonds and consequently to the enhancement of antioxidant activity.

In addition, the radical scavenging effects must also be attributed to the levan produced *in situ* in the beverage. In our previous study (unpublished data), the ability of levan from *G. cerinus* UELBM11 to scavenge ABTS and hydroxyl radicals was investigated. Levan from *G. cerinus* showed ABTS and hydroxyl radical scavenging abilities at higher evaluated concentrations of nearly 50 % (0.9 mg/mL) and 71 % (2.9 mg/mL), respectively. In the present study, *G. cerinus* UELBM11 produced 5.7 g/L of levan from fermented grape beverage (T24 h). However, because substantial dilutions were required for analysis, the scavenging activity of the levan produced *in situ* was most likely not detected. In addition to the bioactive molecules present in the grape and synthesized during fermentation, levan undoubtedly provided an additional scavenging effect in the beverage.

3.4. Fermented grape juice volatile compounds

The volatile components of fruit beverages play an important role in the palatability of the consumer [47]. These components consist of low molecular weight molecules derived from pools of fatty acids, amino acids, and carbohydrates [58]. Grapes, for example, contain several families of compounds responsible for their aroma, including terpenes, norisoprenoids, phenylpropanoids, and straight-chain alcohols, aldehydes, ketones, acids, esters, and lactones derived from fatty acids [59]. As free volatile compounds, they contribute directly to the aroma profile of beverages, while when bound to sugars in the form of glycosides, these compounds have no odor; however, upon hydrolysis, these compounds can volatilize and become active odor molecules [60,61].

Several reports [4,62,63], have documented the volatile compound profiles of unfermented and fermented fruit juices using LAB strains; however, studies related to fruit gluconic fermentation by AAB are still scarce or nonexistent. In this study, the volatile compounds in unfermented (T0 h) and fermented (T24 h) grape juices were qualitatively characterized by headspace solid-phase microextraction (HP-SPME) coupled to GC-MS /MS. The beverage chromatograms can be seen in Fig. 4. A total of 44 volatile compounds were found in the beverages, with aldehydes, esters, alcohols, and terpenoids being the most abundant (Table 1 and Fig. 5). In terms of relative abundance, the group of alcohols was the most abundant in the unfermented sample, accounting for 15.35 %, while terpenoids were the most abundant in the fermented sample, accounting for 13.74 % (Fig. 5). Other classes of volatile compounds, such as hydrocarbons, ketones, aromatic compounds, carboxylic acids, phenols, arylpropanoids, lactones and compounds with mixed functions, were found in relatively low concentrations in both grape juices.

Grape juice contains carbohydrates and alcohols that can be partially oxidized to aldehydes, alcohols, and acids by *Gluconobacter* species via cytoplasmic membrane-bound dehydrogenase systems [33]. Table 1 shows that several volatiles normally present in T0 h juice disappeared after fermentation. Nevertheless, new compounds not present in T0 h were observed in the fermented juice, including nonanal, 1-isobutyl-4-isopropyl-3-isopropyl-2,2-dimethyl succinate, 3-octanol, 2,2,4,6,6-pentamethyl-3-heptene, 2,2,6-trimethyloctane, ethylbenzene, isovaleric acid, octanoic acid, nonanoic acid, butyrolactone, and phenoxyethanol. In addition, nine compounds could not be identified in the fermented sample, representing about 31% (relative abundance) of the total compounds found in the sample (Fig. 5 and Table 1).

Fig. 4. Chromatograms of (A) unfermented - T0 h and (B) fermented - T24 h grape juices. Peak numbers are referred in Table 1.

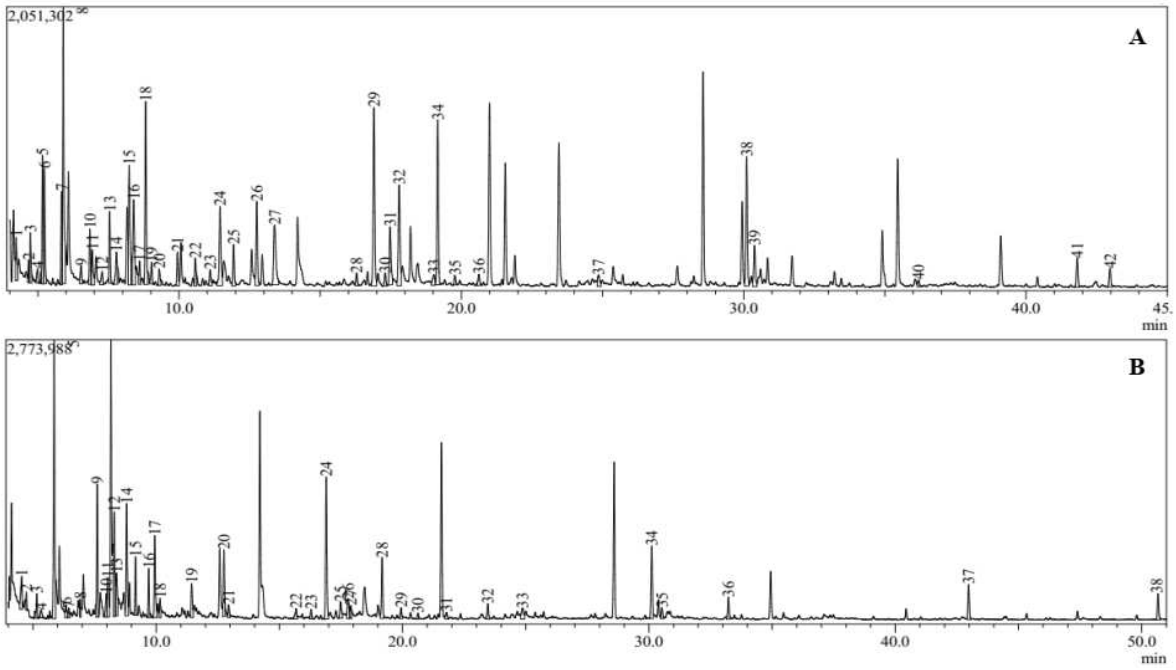


Table 1. Volatile compounds in grape juices before and after fermentation.

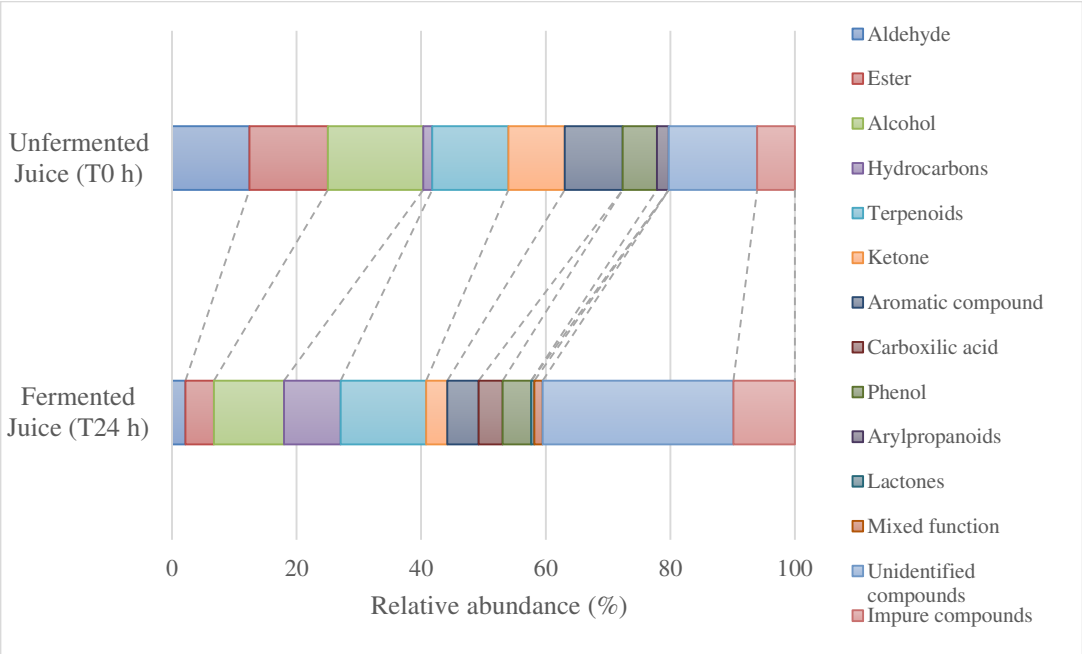
Groups		Compounds	T0 h		T24 h		Odor Descriptor	References
Peak Number								
T0 h	T24 h		R_T[a] (min)	RA[b]	R_T[a] (min)	RA[b]		
Aldehydes								
13	-	(*E*)-2-Heptenal**	7.54	2.2	-	n.d.	Fatty, green	[64]
14	-	Benzaldehyde**	7.78	1.42	-	n.d.	Sweet, bitter, almond, cherry	[65]
19	-	Octanal**	9.025	0.83	-	n.d.	Fat, soap, lemon, green	[66]
22	-	Phenylacetaldehyde**	10.575	1.06	-	n.d.	Flowery, rose	[67]
23	-	(*E*)-2-Octen-1-al**	11.1	0.69	-	n.d.	Green, nut, fat	[67]
30	-	Decanal*	17.3	0.55	-	n.d.	Soap, orange peel, tallow	[66]
32	26	2,5-Dimethylbenzaldehyde**	17.795	4.96	17.795	1.59	Almond-like	[68]
35	-	(E)-2-Decenal*	19.77	0.39	-	n.d.	Fatty, green	[64]
40	-	(*E*)-Amyl cinnamaldehyde*	36.185	0.3	-	n.d.	Jasmine	[69]
-	21	Nonanal*	-	n.d.	12.945	0.55	Fat, floral, green, lemon	[70]
Esters								
3	2	(*E*)-2-Butenoic acid ethyl ester**	4.73	1.58	4.725	0.8	Tropical fruit	[70]
9	-	Methyl hexanoate**	6.53	0.76	-	n.d.	Fruit, fresh, sweet	[66]
18	-	Ethyl hexanoate*	8.815	6.6	-	n.d.	Apple peel, fruit	[66]
25	-	2-Propenyl hexanoate*	11.93	1.59	-	n.d.	Pineapple	[70]
42	37	Di-sec-butyl phthalate**	42.97	0.84	42.98	2.32		
-	36	1-Isobutyl 4-isopropyl 3-isopropyl-2,2-dimethylsuccinate**	-	n.d.	33.225	1.46	Sweat	[66]
41	-	Isopropyl myristate**	41.81	1.24	-	n.d.	Odorless compound	[71]
Alcohols								

1	-	2,3-Butanediol*	4.225	1.6	-	n.d.		
6	-	1-Hexanol*	5.225	3.44	-	n.d.	Banana, flower, grass, herb	[70]
15	-	1-Octen-3-ol*	8.23	5.0	-	n.d.	Cucumber, earth, fat, floral, mushroom	[70]
21	17	2-Ethyl-1-hexanol**	9.945	1.28	9.94	4.88	Green, rose	[70]
27	-	Phenethyl alcohol**	13.38	4.03	-	n.d.	honey, spice, rose, lilac	[66]
-	14	3-Octanol**	-	n.d.	8.805	6.4	Citrus, moss, mushroom, nut, oil	[70]
Hydrocarbons								
2	-	2,4-Dimethylheptane**	4.675	0.65	-	n.d.		
17	-	2,2,4,6,6-Pentamethylheptane**	8.6	0.79	-	n.d.		
-	15	2,2,4,6,6-Pentamethyl-3-heptene***	-	n.d.	9.16	2.8		
-	9	2,2,6-Trimethyloctane**	-	n.d.	7.61	6.25		
Terpenoids								
26	20	Linalol**	12.75	3.28	12.75	4.02	Flower, lavender	[66]
28	23	Terpinen-4-ol**	16.29	0.52	16.286	0.56	Turpentine, nutmeg, must	[66]
29	24	alpha-Terpineol**	16.9	7.7	16.905	9.16	Pine, terpenic, lilac, citrus, woody, floral	[65]
33	-	Carvone*	19.025	0.69	-	n.d.	Mint, basil, fennel	[66]
Ketones								
11	8	4-Methyl-2-heptanone**	6.92	1.53	6.92	0.98		
16	-	6-Methyl-5-hepten-2-one*	8.39	3.8	-	n.d.	Citrus, mushroom, pepper, rubber, strawberry	[70]
24	19	Acetophenone*	11.45	3.72	11.445	2.43	Must, flower, almond	[66]
Aromatic compounds								
7	-	Styrene**	5.84	2.69	-	n.d.	Sweet, floral	[72]I
34	28	m-Di-tert-butylbenzene**	19.16	6.65	19.175	3.81		
-	3	Ethylbenzene**	-	n.d.	5.155	1.2		
Carboxilic acid								

-	1	Isovaleric acid*	-	n.d.	4.545	2.44	Sweat, acid, rancid	[66]
-	29	Nonanoic acid*	-	n.d.	19.945	0.75	Green, fat	[66]
-	22	Octanoic acid*	-	n.d.	15.68	0.69	Faint, fruity-acid odour	[69]
Phenols								
38	34	2,4-Di-tert-butylphenol**	30.105	5.47	30.115	4.56		
Arylpropanoids								
39	-	Eugenol acetate*	30.38	1.9	-	n.d.	Floral	[70]
Lactones								
-	7	Butyrolactone**	-	n.d.	6.452	0.53	Faint, sweet, caramel	[73]
Mixed function								
-	27	Phenoxyethanol**	-	n.d.	19.175	1.32	Roses	[74]
Unidentified compounds[#]		-						
8,10,12,31	5,6,12,16,18, 25,32,35,38			14.18		30.65		
Impure compounds[##]		-						
4,5,20,36,37	4,10,11,13,30,31,33			6.07		9.85		

[a]The retention time of aroma compounds in column
[b]Relative area
n.d.: Not detected
T0 h: Unfermented grape juice
T24 h: Fermented grape juice
References: *[31]; **[30]; ***[32]
#Sum of the Unidentified compounds
##Sum of the Impure compounds

Fig. 5. Relative abundance of volatile compound groups presents in grape juices



The amount of alcohols and aldehydes in fermented grape juice was significantly lower than in unfermented grape juice. According to the literature, alcohols are responsible for the characteristics of "banana," "flower," "cucumber," "earth," "fat," "honey," "spice,25" "citrus," "mushroom," "nut," and other notes (Table 1). Of the alcohols originally present in the T0 h juice, only 2-ethyl-1-hexanol was found after fermentation, while 3-octanol was detected only in the fermented beverage. This decrease in alcohol content could be attributed to membrane-bound pyrroloquinoline quinone (PQQ)-dependent alcohol dehydrogenase (ADH) and membrane-bound aldehyde dehydrogenase (ALDH) in *Gluconobacter*. ADH (ethanol:ubiquinone oxidoreductase) is responsible for the conversion of ethanol to acetaldehyde, but has broad substrate specificity encompassing primary short-chain alcohols up to 1-hexanol (with the exception of methanol) [75]. Nevertheless, 1-octen-3-ol (secondary alcohol) and longer-chain primary alcohols such as 2-ethyl-1-hexanol and phenethyl appear to have been consumed during juice fermentation.

According to Gómez-Manzo et al. [76], ADH oxidizes not only alcohols but also aldehydes; some ADHs from AAB oxidize aldehydes at a rate comparable to that of alcohols. ALDH (acetaldehyde:ubiquinone oxidoreductase), in turn, is the complex that catalyzes the oxidation of acetaldehyde to acetic acid. It is also capable of oxidizing primary short-chain aldehydes, with the exception of formaldehyde [75]. In our study, all the aldehydes found had six to ten carbons. Of the aldehydes present in the unfermented beverage, only 2,5-dimethylbenzaldehyde was found after fermentation, while nonanal was observed only in the fermented beverage. Although (*E*)-2-heptenal, benzaldehyde, octanal, phenylacetaldehyde, (*E*)-2-octen-1-al, decanal, 2,5-dimethylbenzaldehyde, (*E*)-2-decenal, and (*E*)-amyl cinnamaldehyde are not short-chain aldehydes, we assume that ADH and ALDH oxidized them to acids, as indicated by the presence of short- (< 6 carbons; isovaleric acid) and medium- (6 to 12 carbons; nonanoic and octanoic acids) chain fatty acids [59] in fermented juice.

For instance, Molinari et al. [77] reported that 2-phenylethanol (or phenethyl alcohol) can be oxidized by AAB, including *Acetobacter* and *Gluconobacter* species, to produce both acid and phenylacetaldehyde. In our study, 2-phenylethanol and phenylacetaldehyde were detected in the unfermented juice but not in the fermented sample, suggesting that UELBM11 may be able to oxidize these compounds. It was also observed that LAB-induced juice fermentation led to a decrease in aldehydes and an increase in acids [4,78]. This fact was explained by the unstable nature of aldehydes in food matrices, which can be easily converted to alcohols or acids during LAB fermentation [4]. Previous research has shown that aldehydes with six to ten carbons can contribute to particular and characteristic green, greasy, or tallow aromas at low concentrations, but can also lead to rancid, painty, or other unpleasant flavors when present at high concentrations due to their low threshold [79,80]. In addition, high

concentrations of aldehydes have been associated with poor consumer acceptance of beverages [4].

Esters were the second most important volatile compounds in the unfermented grape beverage (12.61 %). These compounds are mainly derived from the esterification reaction, in which alcohols and fatty acids are converted to esters. In beverages, esters may have a positive effect on enhancing sweet-fruity aroma notes [59]. In this study, a total of 6 volatile esters were identified in unfermented grape juice, of which only (*E*)-2-butenoic acid ethyl ester and di-sec-butyl phthalate were detected after fermentation with UELBM11. However, one new ester, namely 1-isobutyl-4-isopropyl-3-isopropyl-2,2-dimethylsuccinate was formed during fermentation.

In plants, terpenoids are synthesized enzymatically from acetyl-CoA and pyruvate from the carbohydrate pools. From this family, the monoterpenes (C10) have influenced the flavor profile of most fruits and aroma at different levels [58]. In the present study, the C10 members, including linalool, terpinen-4-ol, alpha-terpineol, and carvone, had a relative abundance of about 12 % in unfermented grape juice. It is well known that monoterpenes are the main aromatic compounds responsible for the distinctive "floral" aroma of Muscat grapes and the products made from them [81]. These compounds are abundant especially in grapes and must in the form of glycosidic conjugated forms, where they can be hydrolyzed by enzymes or acids to release the free forms [4]. Several studies have reported an increase in terpenes after fermentation with LAB as a result of hydrolysis of their conjugated precursors in grape juice and wine by β-glycosidases from LAB [4,82,83]. In this work, the acids formed during fermentation do not appear to have hydrolyzed these precursors to the point of increasing terpene content. Moreover, hydrolysis by β-glycosidases from UELBM11 would not be possible because, to our knowledge, the enzyme gene has not been detected in

Gluconobacter, but only in cellulose-producing bacteria such as *Komagataeibacter* species [84]. Although carvone was not detected in the fermented grape juice, monoterpenes were still the most abundant in this sample (13.74 %). In addition to their significant contribution to the aroma of grape beverages, studies have shown that monoterpenes possess a number of pharmacological properties, including antifungal, antibacterial, antioxidant, anticancer, and antispasmodic activity. They may also be useful for the treatment and prevention of a number of cancers, including breast, skin, lung, colon, and prostate cancers [81].

In unfermented grape juice, 3 ketones were found, including 4-methyl-2-heptanone and 6-methyl-5-hepten-2-one, which are short-chain methyl ketones (C5-C11), highly potent aroma molecules found in numerous plants [58], and acetophenone, which contributes to must, flower, and almond aroma (Table 1). After fermentation, only 6-methyl-5-hepten-2-one was not detected.

Based on Fig. 4, it is possible to see that the abundance of hydrocarbons in the fermented sample increased when compared to T0 h (from 1.44 to 9.05 %), however, little is known about how AAB synthesizes and degrades these compounds. Among these compounds, 2,4-dimethylheptane was one of the volatile compounds identified by Kaškoniene et al. [85] in a fresh honey sample from Lithuania. The highly branched alkane 2,2,4,6,6-pentamethylheptane was found in ginseng root oils and in the essential oil of *Anthemis montana* (*Asteraceae*) [86]. Junqueira et al. [72] also demonstrated the formation of 2,2,6-trimethyloctane by bacterial and fungal communities during the fermentation of Colombian coffee beans.

In this study, among the aromatic compounds found in the unfermented (styrene and m-di-tert-butylbenzene) and fermented (m-di-tert-butylbenzene and ethybenzene) grape juices, styrene was detected as one of the volatile organic compounds in Cannonau wines [87],

and fresh (unfermented) and fermented Colombian coffee beans [72]. Tang et al. [88], on the other hand, reported that the presence of styrene and ethylbenzene in foods was primarily due to migration from polymeric packaging materials.

The phenol detected in our samples, namely 2,4-di-tert-butylphenol, was also detected in white wine by Oliveira et al.[89]. This compound was isolated from pomegranate and sweet potato extracts and has been shown to have antioxidant and neuron-protective properties [90]. Apart from these properties, 2,4 di-tert-butylphenol also has antifungal, antitumor, and anti-inflammatory activities [91,92].

Eugenol acetate, an arylpropanoid that contributes to the "floral" character of grapes, was not detected in the fermented juice; however, butyrolactone (faint, sweet, caramel-like aroma) and phenoxyethanol (roses) (Table 1) appeared as new aromatic compounds in the sample. Butyrolactone is a flavoring agent that has been found naturally in foods such as wine, coffee, tropical fruits, teas, and various beans [93]. 2-Phenoxyethanol has also been found in grape musts from five different red *Vitis vinifera* cultivars grown in Spain [94], and in fruit wines made from gabiroba pulp, an exotic tropical fruit from Brazil [95].

4. CONCLUSION

In this work, it was found that *G. cerinus* UELBM11 was able to produce levan (5.7 g/L) in sucrose-enriched grape juice. The bacterium showed great adaptability to this substrate, as evidenced by the increase in its viability and total titratable acidity and the decrease in pH and sucrose concentration. Although the change in viscosity of the fermented beverage could not be demonstrated, it was found that the gluconic fermentation by UELBM11 could increase the bioavailability of phenolic compounds and preserve anthocyanin content. In addition, an increase in the antioxidant activity of the beverage was

detected by the FRAP method, although no changes were observed by the ABTS method. The analysis of volatile compounds showed that gluconic fermentation can reduce the volatile components of grape juice, but at the same time form new compounds and preserve compounds such as 2,4-di-tert-butylphenol and most monoterpenes, which can have an impact on the aroma of the beverage and human health. Gluconic fermentation with UELBM11 is a promising approach to promote natural, clean diet that incorporates the bioactive properties of grapes and levan to meet the demand of different consumer categories.

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6. REFERENCES

[1] D. Donno, F. Turrini, Plant foods and underutilized fruits as source of functional food ingredients: Chemical composition, quality traits, and biological properties, Foods. 9 (2020) 7–10. https://doi.org/10.3390/foods9101474.

[2] T.I. Year, Fruit and vegetables – your dietary essentials, 2020. https://doi.org/10.4060/cb2395en.

[3] FAO, https://www.fao.org/faostat/en/#rankings/countries_by_commodity, (2020).

[4] B. Wu, J. Liu, W. Yang, Q. Zhang, Z. Yang, H. Liu, Z. Lv, C. Zhang, Z. Jiao, Nutritional and flavor properties of grape juice as affected by fermentation with lactic acid bacteria, Int. J. Food Prop. 24 (2021) 906–922. https://doi.org/10.1080/10942912.2021.1942041.

[5] M. Nassiri-Asl, H. Hosseinzadeh, Review of the Pharmacological Effects of *Vitis vinifera* (Grape) and its Bioactive Compounds 1197, Phyther. Res. 23 (2009) 1197–1204. https://doi.org/10.1002/ptr.2761.

[6] T. Ua-Arak, F. Jakob, R.F. Vogel, Influence of levan-producing acetic acid bacteria

on buckwheat-sourdough breads, Food Microbiol. 65 (2017) 95–104. https://doi.org/10.1016/j.fm.2017.02.002.

[7] R. Juvonen, K. Honkapää, N.H. Maina, Q. Shi, K. Viljanen, H. Maaheimo, L. Virkki, M. Tenkanen, R. Lantto, The impact of fermentation with exopolysaccharide producing lactic acid bacteria on rheological, chemical and sensory properties of pureed carrots (*Daucus carota* L.), Int. J. Food Microbiol. 207 (2015) 109–118. https://doi.org/10.1016/j.ijfoodmicro.2015.04.031.

[8] V.P.L. Eckel, R.F. Vogel, F. Jakob, In situ production and characterization of cloud forming dextrans in fruit-juices, Int. J. Food Microbiol. 306 (2019) 108261. https://doi.org/10.1016/j.ijfoodmicro.2019.108261.

[9] M.A. Álvarez-Fernández, R. Hornedo-Ortega, A.B. Cerezo, A.M. Troncoso, M.C. García-Parrilla, Determination of Nonanthocyanin Phenolic Compounds Using High-Resolution Mass Spectrometry (UHPLC-Orbitrap-MS/MS) and Impact of Storage Conditions in a Beverage Made from Strawberry by Fermentation, J. Agric. Food Chem. 64 (2016) 1367–1376. https://doi.org/10.1021/acs.jafc.5b05617.

[10] R. Hornedo-Ortega, S. Krisa, M. Carmen García-Parrilla, T. Richard, Effects of gluconic and alcoholic fermentation on anthocyanin composition and antioxidant activity of beverages made from strawberry, LWT - Food Sci. Technol. 69 (2016) 382–389. https://doi.org/10.1016/j.lwt.2016.01.070.

[11] M.A. Álvarez-Fernández, R. Hornedo-Ortega, A.B. Cerezo, A.M. Troncoso, M.C. García-Parrilla, Effects of the strawberry (fragaria ananassa) purée elaboration process on non-anthocyanin phenolic composition and antioxidant activity, Food Chem. 164 (2014) 104–112. https://doi.org/10.1016/j.foodchem.2014.04.116.

[12] A.M. Cañete-Rodríguez, I.M. Santos-Dueñas, J.E. Jiménez-Hornero, A. Ehrenreich, W. Liebl, I. García-García, Gluconic acid: Properties, production methods and applications—An excellent opportunity for agro-industrial by-products and waste bio-valorization, Process Biochem. 51 (2016) 1891–1903. https://doi.org/10.1016/j.procbio.2016.08.028.

[13] M. Hermann, H. Petermeier, R.F. Vogel, Development of novel sourdoughs with in situ formed exopolysaccharides from acetic acid bacteria, Eur. Food Res. Technol. 241 (2015) 185–197. https://doi.org/10.1007/s00217-015-2444-8.

[14] F. Jakob, S. Steger, R.F. Vogel, Influence of novel fructans produced by selected acetic acid bacteria on the volume and texture of wheat breads, Eur. Food Res. Technol. 234 (2012) 493–499. https://doi.org/10.1007/s00217-011-1658-7.

[15] E.T. Öner, L. Hernández, J. Combie, Review of Levan polysaccharide: From a century of past experiences to future prospects, Biotechnol. Adv. 34 (2016) 827–844. https://doi.org/10.1016/j.biotechadv.2016.05.002.

[16] C. Liu, S. Kolida, D. Charalampopoulos, R.A. Rastall, An evaluation of the prebiotic potential of microbial levans from *Erwinia* sp. 10119, J. Funct. Foods. 64 (2020). https://doi.org/10.1016/j.jff.2019.103668.

[17] R. Srikanth, C.H.S.S.S. Reddy, G. Siddartha, M.J. Ramaiah, K.B. Uppuluri, Review on production, characterization and applications of microbial levan, Carbohydr. Polym. 120 (2015) 102–114. https://doi.org/10.1016/j.carbpol.2014.12.003.

[18] R. Srikanth, G. Siddartha, C.H.S.S. Sundhar Reddy, B.S. Harish, M. Janaki Ramaiah, K.B. Uppuluri, Antioxidant and anti-inflammatory levan produced from *Acetobacter xylinum* NCIM2526 and its statistical optimization, Carbohydr. Polym. 123 (2015) 8–16. https://doi.org/10.1016/j.carbpol.2014.12.079.

[19] M.J. Valera, F. Laich, S.S. González, M.J. Torija, E. Mateo, A. Mas, Diversity of acetic acid bacteria present in healthy grapes from the Canary Islands, Int. J. Food Microbiol. 151 (2011) 105–112. https://doi.org/10.1016/j.ijfoodmicro.2011.08.007.

[20] I.A.L. IAL, 1ª Edição Digital, Métodos Físicos-Quimicos Para Análise Aliment. 9 (2008) 453–460.

[21] R. V. Serrato, C.H.S.G. Meneses, M.S. Vidal, A.P. Santana-Filho, M. Iacomini, G.L. Sassaki, J.I. Baldani, Structural studies of an exopolysaccharide produced by *Gluconacetobacter diazotrophicus* Pal5, Carbohydr. Polym. 98 (2013) 1153–1159. https://doi.org/10.1016/j.carbpol.2013.07.025.

[22] P. Semjonovs, L. Shakirova, R. Treimane, K. Shvirksts, L. Auzina, I. Cleenwerck, P. Zikmanis, Production of extracellular fructans by *Gluconobacter nephelii* P1464, Lett. Appl. Microbiol. 62 (2016) 145–152. https://doi.org/10.1111/lam.12521.

[23] R.S. Bueno, J.B. Ressutte, N.N.Y. Hata, F.C. Henrique-Bana, K.B. Guergoletto, A.G. de Oliveira, W.A. Spinosa, Quality and shelf life assessment of a new beverage produced from water kefir grains and red pitaya, Lwt. 140 (2021). https://doi.org/10.1016/j.lwt.2020.110770.

[24] N. Nelson, A photometric adaptation of the Somogyi method for the determination of glucose, J. Biol. Chem. 153 (1944) 375–380. https://doi.org/10.1016/s0021-9258(18)71980-7.

[25] M. Somogyi, Notes on sugar determination., J. Biol. Chem. 195 (1952) 19–23. https://doi.org/10.1016/s0021-9258(19)50870-5.

[26] V.L. Singleton, J.A.J. Rossi, Colorimetry to total phenolics with phosphomolybdic acid reagents, Am. J. Enol. Vinic. 16 (1965) 144–58. http://garfield.library.upenn.edu/classics1985/A1985AUG6900001.pdf.

[27] J. Lee, R.W. Durst, R.E. Wrolstad, Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study, J. AOAC Int. 88 (2005) 1269–1278. https://doi.org/10.1093/jaoac/88.5.1269.

[28] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (1999) 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.

[29] I.F.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay, Anal. Biochem. 239 (1996) 70–76. https://doi.org/10.1006/abio.1996.0292.

[30] William E. Acree, Jr. Phase transition enthalpy measurements of organic and organometallic compounds. in NIST Chemistry WebBook, NIST Standard Reference Database Number 69, Eds. P.J. Linstrom and W.G. Mallard, National Institute of Standards and Technology, Gaithersburg MD, 2, (n.d.). https://doi.org/10.18434/T4D303, (accessed 11 July 2022).

[31] R.P. Adams, Identification of essential oil components by gas chromatography / mass spectroscopy Robert P. Adams, J. Am. Soc. Mass Spectrom. 8 (1997) 1–698. https://doi.org/10.1016/s1044-0305(97)00026-3.

[32] Wishart DS, Guo AC, Oler E, et al., HMDB 5.0: the Human Metabolome Database for 2022. Nucleic Acids Res. 2022. Jan 7;50(D1):D622–31. 34986597, https://hmdb.ca.

[33] M. Sievers, J. Swings, *Gluconobacter* , Bergey’s Man. Syst. Archaea Bact. (2015) 1–9. https://doi.org/10.1002/9781118960608.gbm00884.

[34] J.M. Guillamón, A. Mas, Acetic Acid Bacteria, Mol. Wine Microbiol. (2011) 227–255. https://doi.org/10.1016/B978-0-12-375021-1.10009-8.

[35] L. Ayed, S. Ben Abid, M. Hamdi, Development of a beverage from red grape juice fermented with the Kombucha consortium, Ann. Microbiol. 67 (2017) 111–121. https://doi.org/10.1007/s13213-016-1242-2.

[36] U. Deppenmeier, A. Ehrenreich, Physiology of acetic acid bacteria in light of the genome sequence of *Gluconobacter oxydans*, J. Mol. Microbiol. Biotechnol. 16 (2008) 69–80. https://doi.org/10.1159/000142895.

[37] F. Sainz, D. Navarro, E. Mateo, M.J. Torija, A. Mas, Comparison of d-gluconic acid production in selected strains of acetic acid bacteria, Int. J. Food Microbiol. 222 (2016) 40–47. https://doi.org/10.1016/j.ijfoodmicro.2016.01.015.

[38] D. Laureys, S.J. Britton, J. De Clippeleer, Kombucha Tea Fermentation: A Review, J. Am. Soc. Brew. Chem. 78 (2020) 165–174. https://doi.org/10.1080/03610470.2020.1734150.

[39] S. Ramachandran, S. Nair, C. Larroche, A. Pandey, Gluconic Acid, Elsevier B.V., 2016. https://doi.org/10.1016/B978-0-444-63662-1.00026-9.

[40] J. Han, F. Hang, B. Guo, Z. Liu, C. You, Z. Wu, Dextran synthesized by *Leuconostoc mesenteroides* BD1710 in tomato juice supplemented with sucrose, Carbohydr. Polym. 112 (2014) 556–562. https://doi.org/10.1016/j.carbpol.2014.06.035.

[41] E.C. de Siqueira, J. de S. Rebouças, I.O. Pinheiro, F.R. Formiga, Levan-based nanostructured systems: An overview, Int. J. Pharm. 580 (2020). https://doi.org/10.1016/j.ijpharm.2020.119242.

[42] C.S. Hundschell, A.M. Wagemans, Rheology of common uncharged

exopolysaccharides for food applications, Curr. Opin. Food Sci. 27 (2019) 1–7. https://doi.org/10.1016/j.cofs.2019.02.011.

[43] F. Jakob, C. Gebrande, R.M. Bichler, R.F. Vogel, Insights into the pH-dependent, extracellular sucrose utilization and concomitant levan formation by *Gluconobacter albidus* TMW 2.1191, Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol. 113 (2020) 863–873. https://doi.org/10.1007/s10482-020-01397-3.

[44] J.C. Barbe, G. De Revel, A. Joyeux, A. Bertrand, A. Lonvaud-Funel, Role of botrytized grape micro-organisms in SO_2 binding phenomena, J. Appl. Microbiol. 90 (2001) 34–42. https://doi.org/10.1046/j.1365-2672.2001.01200.x.

[45] A. Haddar, M. Hamed, A. Bouallegue, R. Bastos, E. Coelho, M.A. Coimbra, Structural elucidation and interfacial properties of a levan isolated from *Bacillus mojavensis*, Food Chem. (2020) 128456. https://doi.org/10.1016/j.foodchem.2020.128456.

[46] J. Han, H. Feng, X. Wang, Z. Liu, Z. Wu, Levan from *Leuconostoc citreum* BD1707: production optimization and changes in molecular weight distribution during cultivation, BMC Biotechnol. 21 (2021) 14. https://doi.org/10.1186/s12896-021-00673-y.

[47] R. Kaprasob, O. Kerdchoechuen, N. Laohakunjit, D. Sarkar, K. Shetty, Fermentation-based biotransformation of bioactive phenolics and volatile compounds from cashew apple juice by select lactic acid bacteria, Process Biochem. 59 (2017) 141–149. https://doi.org/10.1016/j.procbio.2017.05.019.

[48] S.M.B. Hashemi, A. Mousavi Khaneghah, F.J. Barba, Z. Nemati, S. Sohrabi Shokofti, F. Alizadeh, Fermented sweet lemon juice (*Citrus limetta*) using *Lactobacillus plantarum* LS5: Chemical composition, antioxidant and antibacterial activities, J. Funct. Foods. 38 (2017) 409–414. https://doi.org/10.1016/j.jff.2017.09.040.

[49] Z. Li, J. Teng, Y. Lyu, X. Hu, Y. Zhao, M. Wang, Enhanced antioxidant activity for apple juice fermented with *Lactobacillus plantarum* ATCC14917, Molecules. 24 (2019) 1–12. https://doi.org/10.3390/molecules24010051.

[50] A.K. Jaiswal, N. Abu-Ghannam, Kinetic studies for the preparation of probiotic cabbage juice: Impact on phytochemicals and bioactivity, Ind. Crops Prod. 50 (2013) 212–218. https://doi.org/10.1016/j.indcrop.2013.07.028.

[51] M. Verni, V. Verardo, C.G. Rizzello, How Fermentation Affects the Antioxidant Properties of Cereals and Legumes, Foods. 8.9 (2019) 362. https://doi.org/10.3390/foods8090362.

[52] R.F. Ahmed, M.S. Hikal, K.A. Abou-Taleb, Biological, chemical and antioxidant activities of different types Kombucha, Ann. Agric. Sci. 65 (2020) 35–41. https://doi.org/10.1016/j.aoas.2020.04.001.

[53] B. Olas, Berry phenolic antioxidants - implications for human health?, Front. Pharmacol. 9 (2018) 1–14. https://doi.org/10.3389/fphar.2018.00078.

[54] P. Cosme, A.B. Rodríguez, J. Espino, M. Garrido, Plant phenolics: Bioavailability as a key determinant of their potential health-promoting applications, Antioxidants. 9 (2020) 1–20. https://doi.org/10.3390/antiox9121263.

[55] A. Abuduaibifu, C.E. Tamer, Evaluation of physicochemical and bioaccessibility properties of goji berry kombucha, J. Food Process. Preserv. 43 (2019) 1–14. https://doi.org/10.1111/jfpp.14077.

[56] P. Darvishzadeh, V. Orsat, J.L. Martinez, Correction to: Process optimization for development of a novel water kefir drink with high antioxidant activity and potential probiotic properties from russian olive fruit (*Elaeagnus angustifolia*), Food and Bioprocess Technology, 14 (2021) 248-260. https://doi.org/10.1007/s11947-021-02583-5.

[57] X. Yang, J. Zhou, L. Fan, Z. Qin, Q. Chen, L. Zhao, Antioxidant properties of a vegetable–fruit beverage fermented with two *Lactobacillus plantarum* strains, Food Sci. Biotechnol. 27 (2018) 1719–1726. https://doi.org/10.1007/s10068-018-0411-4.

[58] W. Schwab, R. Davidovich-Rikanati, E. Lewinsohn, Biosynthesis of plant-derived flavor compounds, Plant J. 54 (2008) 712–732. https://doi.org/10.1111/j.1365-313X.2008.03446.x.

[59] A.L. Robinson, P.K. Boss, P.S. Solomon, R.D. Trengove, H. Heymann, S.E. Ebeler, Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts, Am. J. Enol. Vitic. 65 (2014) 1–24. https://doi.org/10.5344/ajev.2013.12070.

[60] H. Alem, P. Rigou, R. Schneider, H. Ojeda, L. Torregrosa, Impact of agronomic practices on grape aroma composition: a review, J. Sci. Food Agric. 99 (2019) 975–985. https://doi.org/10.1002/jsfa.9327.

[61] S. Matijašević, J. Popović-Djordjević, R. Ristić, D. Ćirković, B. Ćirković, T. Popović, Volatile aroma compounds of brandy produced from Muscat table grapevine cultivars (*Vitis vinifera* L.), Molecules. 24 (2019) 1–15. https://doi.org/10.3390/molecules24132485.

[62] X.F. Wei, X.L. Ma, J.H. Cad, X.Y. Sun, Y.L. Fang, Aroma characteristics and volatile compounds of distilled crystal grape spirits of different alcohol concentrations: Wine sprits in the Shangri-La region of China, Food Sci. Technol. 38 (2018) 50–58. https://doi.org/10.1590/fst.12117.

[63] A. Ricci, M. Cirlini, A. Levante, C. Dall'Asta, G. Galaverna, C. Lazzi, Volatile profile of elderberry juice: Effect of lactic acid fermentation using *L. plantarum, L. rhamnosus* and *L. casei* strains, Food Res. Int. 105 (2018) 412–422. https://doi.org/10.1016/j.foodres.2017.11.042.

[64] Y. Liu, C. He, H. Song, Comparison of fresh watermelon juice aroma characteristics of five varieties based on gas chromatography-olfactometry-mass spectrometry, Food Res. Int. 107 (2018) 119–129. https://doi.org/10.1016/j.foodres.2018.02.022.

[65] Z. Liang, A. Pai, D. Liu, J. Luo, J. Wu, Z. Fang, P. Zhang, Optimizing extraction method of aroma compounds from grape pomace, J. Food Sci. 85 (2020) 4225–4240.

https://doi.org/10.1111/1750-3841.15533.

[66] Terry Acree & Heinrich Arn, Flavornet and human odor space. https://www.flavornet.org/flavornet.html, 2004 (acessed 11 July 2022).

[67] Y. Wu, S. Duan, L. Zhao, Z. Gao, M. Luo, S. Song, W. Xu, C. Zhang, C. Ma, S. Wang, Aroma characterization based on aromatic series analysis in table grapes, Sci. Rep. 6 (2016) 1–16. https://doi.org/10.1038/srep31116.

[68] Fischer scientific, 2,5-Dimethylbenzaldehyde, 98%, Thermo Scientific™. https://www.fishersci.at/shop/products/2-5-dimethylbenzaldehyde-98-1/11371437/en, 2022 (acessed 11 July 2022).

[69] National Center for Biotechnology Information, PubChem. https://pubchem.ncbi.nlm.nih.gov/, 2022 (acessed 11 July 2022).

[70] The Flavor and Extract Manufacturers Association of the United States (FEMA), Flavor Ingredient Library. https://www.femaflavor.org/flavor-library, 2022 (acessed 11 July 2022).

[71] L. Sun, X. Dong, Y. Ren, M. Agarwal, A. Ren, Z. Ding, Profiling Real-Time Aroma from Green Tea Infusion during Brewing, Foods. 11 (2022) 1–17. https://doi.org/10.3390/foods11050684.

[72] A.C. de Oliveira Junqueira, G. V. de Melo Pereira, J.D. Coral Medina, M.C.R. Alvear, R. Rosero, D.P. de Carvalho Neto, H.G. Enríquez, C.R. Soccol, First description of bacterial and fungal communities in Colombian coffee beans fermentation analysed using Illumina-based amplicon sequencing, Sci. Rep. 9 (2019) 1–10. https://doi.org/10.1038/s41598-019-45002-8.

[73] FAO, Food safety and quality. https://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-flav, 2022 (acessed 11 July 2022).

[74] P. Ziosi, T. Tabanelli, G. Fornasari, S. Cocchi, F. Cavani, P. Righi, Carbonates as reactants for the production of fine chemicals: The synthesis of 2-phenoxyethanol, Catal. Sci. Technol. 4 (2014) 4386–4395. https://doi.org/10.1039/c4cy00913d.

[75] O. Adachi, T. Yakushi, Membrane-bound dehydrogenases of acetic acid bacteria, in: Acetic acid bacteria. Ecol. Physiol., Springer Nature, 2016: pp. 273–298. https://doi.org/DOI 10.1007/978-4-431-55933-7.

[76] S. Gómez-Manzo, M. Contreras-Zentella, A. González-Valdez, M. Sosa-Torres, R. Arreguín-Espinoza, E. Escamilla-Marván, The PQQ-alcohol dehydrogenase of *Gluconacetobacter diazotrophicus*, Int. J. Food Microbiol. 125 (2008) 71–78. https://doi.org/10.1016/j.ijfoodmicro.2007.10.015.

[77] F. Molinari, R. Gandolfi, F. Aragozzini, R. Leon, D.M.F. Prazeres, Biotransformations in two-liquid-phase systems - Production of phenylacetaldehyde by oxidation of 2-phenylethanol with acetic acid bacteria, Enzyme Microb. Technol. 25 (1999) 729–735. https://doi.org/10.1016/S0141-0229(99)00107-6.

[78] C. Chen, Y. Lu, H. Yu, Z. Chen, H. Tian, Influence of 4 lactic acid bacteria on the

flavor profile of fermented apple juice, Food Biosci. 27 (2019) 30–36. https://doi.org/10.1016/j.fbio.2018.11.006.

[79] J.C. Zhu, F. Chen, L.Y. Wang, Y.W. Niu, D. Yu, C. Shu, H.X. Chen, H.L. Wang, Z.B. Xiao, Comparison of Aroma-Active Volatiles in Oolong Tea Infusions Using GC-Olfactometry, GC-FPD, and GC-MS, J. Agric. Food Chem. 63 (2015) 7499–7510. https://doi.org/10.1021/acs.jafc.5b02358.

[80] J.C. Zhu, Z.B. Xiao, Characterization of the key aroma compounds in peach by gas chromatography–olfactometry, quantitative measurements and sensory analysis, Eur. Food Res. Technol. 245 (2019) 129–141. https://doi.org/10.1007/s00217-018-3145-x.

[81] C. Aubert, G. Chalot, Chemical composition, bioactive compounds, and volatiles of six table grape varieties (*Vitis vinifera* L.), Food Chem. 240 (2018) 524–533. https://doi.org/10.1016/j.foodchem.2017.07.152.

[82] H. Michlmayr, S. Nauer, W. Brandes, C. Schümann, K.D. Kulbe, A.M. Del Hierro, R. Eder, Release of wine monoterpenes from natural precursors by glycosidases from *Oenococcus oeni*, Food Chem. 135 (2012) 80–87. https://doi.org/10.1016/j.foodchem.2012.04.099.

[83] P. Hernandez-Orte, M. Cersosimo, N. Loscos, J. Cacho, E. Garcia-Moruno, V. Ferreira, Aroma development from non-floral grape precursors by wine lactic acid bacteria, Food Res. Int. 42 (2009) 773–781. https://doi.org/10.1016/j.foodres.2009.02.023.

[84] N. Tonouchi, Cellulose and other capsular polysaccharides of acetic acid bacteria, in Acetic acid bacteria, Springer, Tokyo, 2016, pp. 299-320. https://doi.org/10.1007/978-4-431-55933-7.

[85] V. Kaškoniene, P.R. Venskutonis, V. Čeksteryte, Composition of volatile compounds of honey of various floral origin and beebread collected in Lithuania, Food Chem. 111 (2008) 988–997. https://doi.org/10.1016/j.foodchem.2008.05.021.

[86] V.M. Dembitsky, Natural neo acids and neo alkanes: Their analogs and derivatives, Lipids. 41 (2006) 309–340. https://doi.org/10.1007/s11745-006-5103-9.

[87] G.L. Petretto, L. Mercenaro, P.P. Urgeghe, C. Fadda, A. Valentoni, A. Del Caro, Grape and wine composition in *Vitis vinifera* L. cv. Cannonau explored by GC-MS and sensory analysis, Foods. 10 (2021). https://doi.org/10.3390/foods10010101.

[88] W. Tang, I. Hemm, G. Eisenbrand, Estimation of human exposure to styrene and ethylbenzene, Toxicology. 144 (2000) 39–50. https://doi.org/10.1016/S0300-483X(99)00188-2.

[89] A.S. Oliveira, I. Furtado, M. de L. Bastos, P. Guedes de Pinho, J. Pinto, The influence of different closures on volatile composition of a white wine, Food Packag. Shelf Life. 23 (2020). https://doi.org/10.1016/j.fpsl.2020.100465.

[90] S.J. Choi, J.K. Kim, H.K. Kim, K. Harris, C.J. Kim, G.G. Park, C.S. Park, D.H. Shin, 2,4-Di-tert-butylphenol from sweet potato protects against oxidative stress in PC12

cells and in mice, J. Med. Food. 16 (2013) 977–983. https://doi.org/10.1089/jmf.2012.2739.

[91] K.K. Varsha, L. Devendra, G. Shilpa, S. Priya, A. Pandey, K.M. Nampoothiri, 2,4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated *Lactococcus* sp., Int. J. Food Microbiol. 211 (2015) 44–50. https://doi.org/10.1016/j.ijfoodmicro.2015.06.025.

[92] R.V.R. Nair, D.V. Jayasree, P.G. Biju, S. Baby, Anti-inflammatory and anticancer activities of erythrodiol-3-acetate and 2,4-di-tert-butylphenol isolated from *Humboldtia unijuga*, Nat. Prod. Res. 34 (2020) 2319–2322. https://doi.org/10.1080/14786419.2018.1531406.

[93] A.K. Holt, J.L. Poklis, C.O. Cobb, M.R. Peace, Identification of Gamma-Butyrolactone in JUUL Liquids, J. Anal. Toxicol. 45 (2021) 892–900. https://doi.org/10.1093/jat/bkab067.

[94] P. Canosa, J.M. Oliveira, A. Masa, M. Vilanova, Study of the volatile and glycosidically bound compounds of minority *Vitis vinifera* red cultivars from NW Spain, J. Inst. Brew. 117 (2011) 462–471. https://doi.org/10.1002/j.2050-0416.2011.tb00494.x.

[95] W.F. Duarte, D.R. Dias, J.M. Oliveira, J.A. Teixeira, J.B. de Almeida e Silva, R.F. Schwan, Characterization of different fruit wines made from cacao, cupuassu, gabiroba, jaboticaba and umbu, LWT - Food Sci. Technol. 43 (2010) 1564–1572. https://doi.org/10.1016/j.lwt.2010.03.010.

6 CONCLUSÕES

- *G. cerinus* UELBM11 exibiu uma produção expressiva de EPS (13,89 g/L) e análises realizadas por RMN, FT-IR e GC-MS confirmaram que estrutura da levana consistia principalmente de unidades de D-frutose conectadas por ligações β-(2→6) com algumas ramificações do tipo β-(2→1);
- Levana mostrou excelente propriedade térmica, podendo ser usado como um agente estabilizante em formulações cosméticas/farmacêuticas, bem como um agente carreador em alimentos. Levana pode agir protegendo os ingredientes ativos da decomposição térmica e ambiental e, consequentemente, prolongaria a vida útil do produto;
- Levana revelou uma estrutura microporosa, que permite maior capacidade de retenção de água; e faz dela um componente interessante para a produção de cosméticos. Além disso, esta propriedade sugere que levana tem potencial como espessante, geleificante, estabilizante, emulsificante e capaz de realizar ligações com a água no processamento de alimentos;
- Levana mostrou potencial para a captura de radicais livres, principalmente de radicais hidroxila, que são conhecidas por serem altamente reativas e destruir várias biomoléculas nas células. Sendo assim, levana poderia ser utilizada como um antioxidante ou aditivo natural em produtos farmacêuticos, em alimentos e cosméticos;
- A MSR é um ferramente útil para a produção de levana em grande escala, uma vez que pode promover um aumento de ~3,5 vezes na produção de levana quando comparado a condições não otimizadas (13,89 g/L);
- Ensaios indicaram que levana teve um efeito citotóxico mais pronunciado sob células de neuroblastoma SH-SY5Y quando administrado a 1000 µg/mL por 72 h, e tem potencial para ser utilizado como um novo agente terapêutico para o tratamento de câncer;
- Levana não apresenta atividade antimicrobiana contra *S. enterica* ser. Typhimurium and *S. sonnei*, mas pode controlar a formação de biofilmes desses patógenos;

- *G. cerinus* UELBM11 adaptou-se bem à bebida de uva suplementada com sacarose, sendo capaz de consumir a sacarose e produzir 5,7 g/L de levana quando cultivada a 30 ºC por 24 h;
- A fermentação glucônica mediada por *G. cerinus* UELBM11 provocou mudanças no pH, acidez titulável, mas a viscosidade manteve-se praticamente constante, mesmo com a presença de levana produzida *in situ*;
- A fermentação aumentou a biodisponibilidade de compostos fenólicos, preservou o teor de antocianinas e aumentou a atividade antioxidante da bebida de uva quando avaliada por FRAP;
- A fermentação glucônica reduziu o conteúdo de compostos voláteis, porém novos compostos foram formados. A fermentação glucônica com UELBM11 é uma abordagem promissora para promover uma dieta natural e limpa que incorpora as propriedades bioativas de uvas e da levana para atender a demanda de várias categorias de consumidores;
- O presente estudo mostrou a versatilidade das BAA e a sua capacidade em produzir diferentes metabólitos. *G. cerinus* UELBM11 é hábil em produzir levana com propriedades bioativas e funcionais que são aplicáveis principalmente nas áreas de alimentos e biomédicas.

ANEXOS

ARTIGO CIENTÍFICO 2

GRAPHICAL ABSTRACT

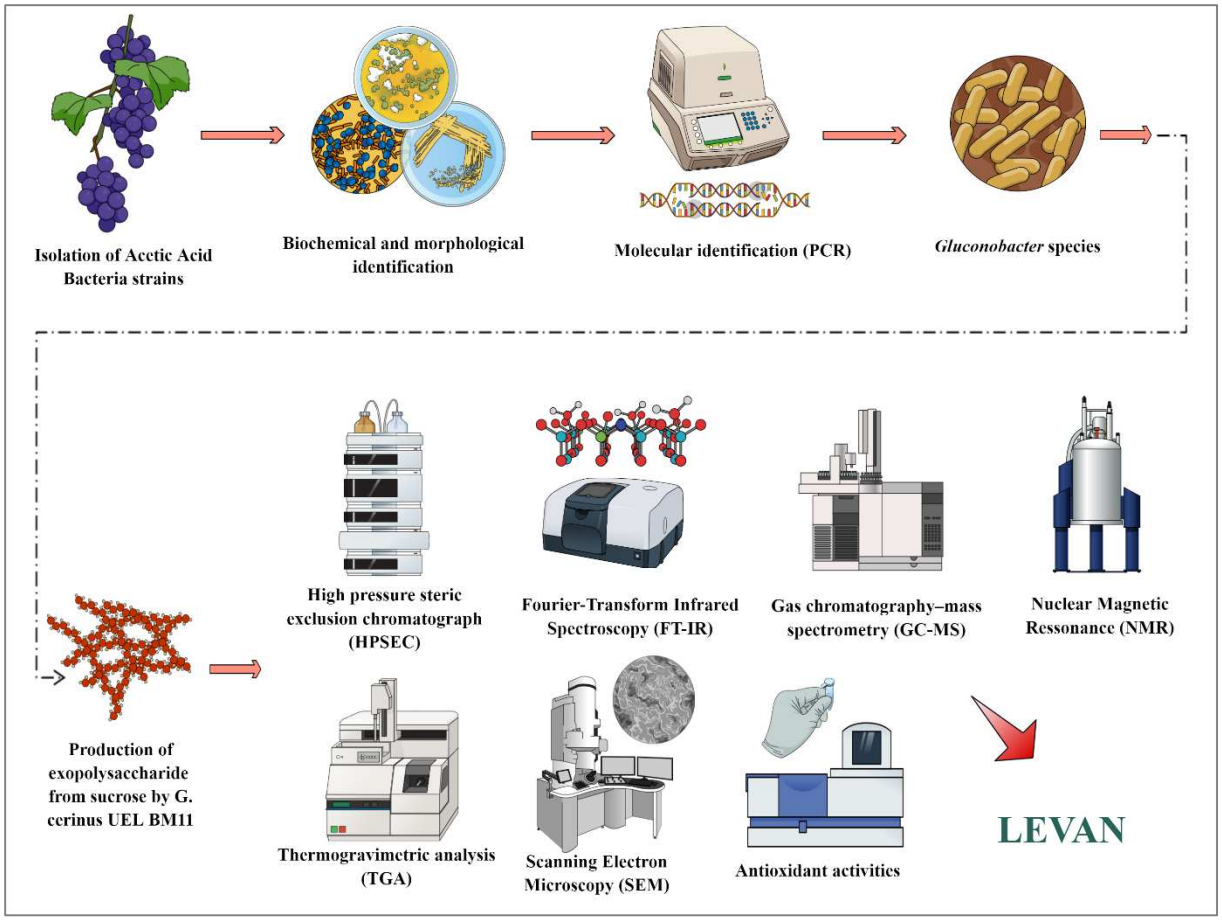


Table SM1. Morphological, physiological and biochemical characteristics of *Gluconobacter* strains with description in Bergey's Manual of Systematic Bacteriology

Test	Strains					
	G. cerinus UELBM11	*G. kondonii* UELMM4	*G. cerinus* UELBM1	*G. frateurii* UELMM2	*G. frateurii* UELBM13	BMSB
Gram stain	-	-	-	-	-	-
Cells form	rs	rs	rs	rs	rs	*
Spore formation	-	-	-	-	-	-
Production of catalase	+	+	+	+	+	+
Oxidation of ethanol to acetic acid	+	+	+	+	+	+
Oxidation of acetic acid to CO_2 and H_2O	-	-	-	-	-	-
Oxidation of lactate to CO_2 and H_2O	-	-	-	-	-	-
Acid production from D-glucose	+	+	+	+	+	+
Ketogenesis (dihydroxyacetone) from glycerol	+	+	+	+	+	+
Production of cellulose	-	-	-	-	-	-
Production of water soluble brown pigment	-	-	-	-	-	**
Production of levan-like mucous substance from sucrose	+++	+++	+++	+++	+++	-

Symbols: +, positive; -, negative; +++, strong EPS-producing activity; *, Ellipsoidal to rod-shaped cells; **, correlated with the production of 2,5-diketogluconic acid and γ-pyrones from D-glucose. Abbreviations: rs, rod shape; BMSB, Bergey's Manual of Systematic Bacteriology.

Fig. SM1. The phylogenetic tree based on 16S rDNA sequences of *Gluconobacter* species constructed by the neighbor-joining method.

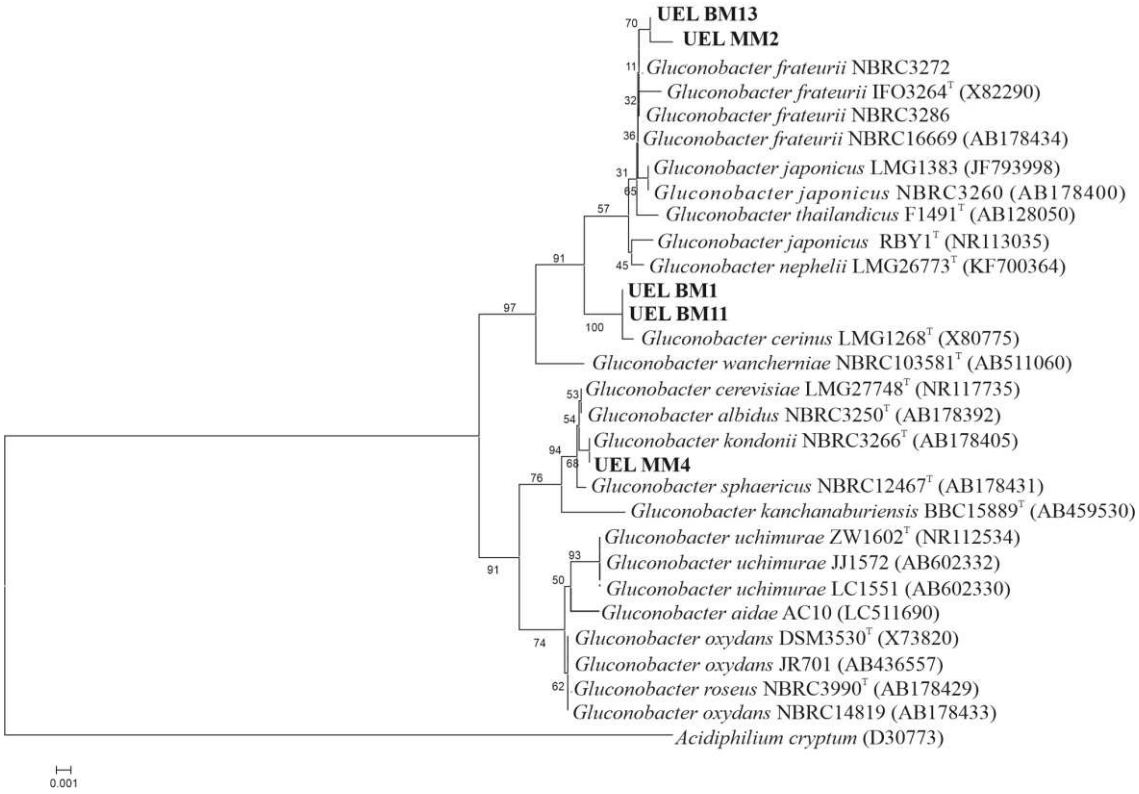
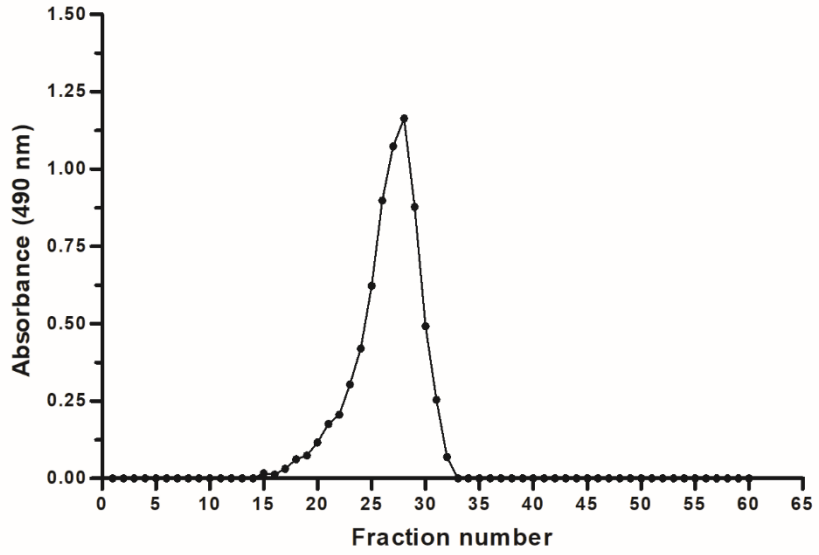
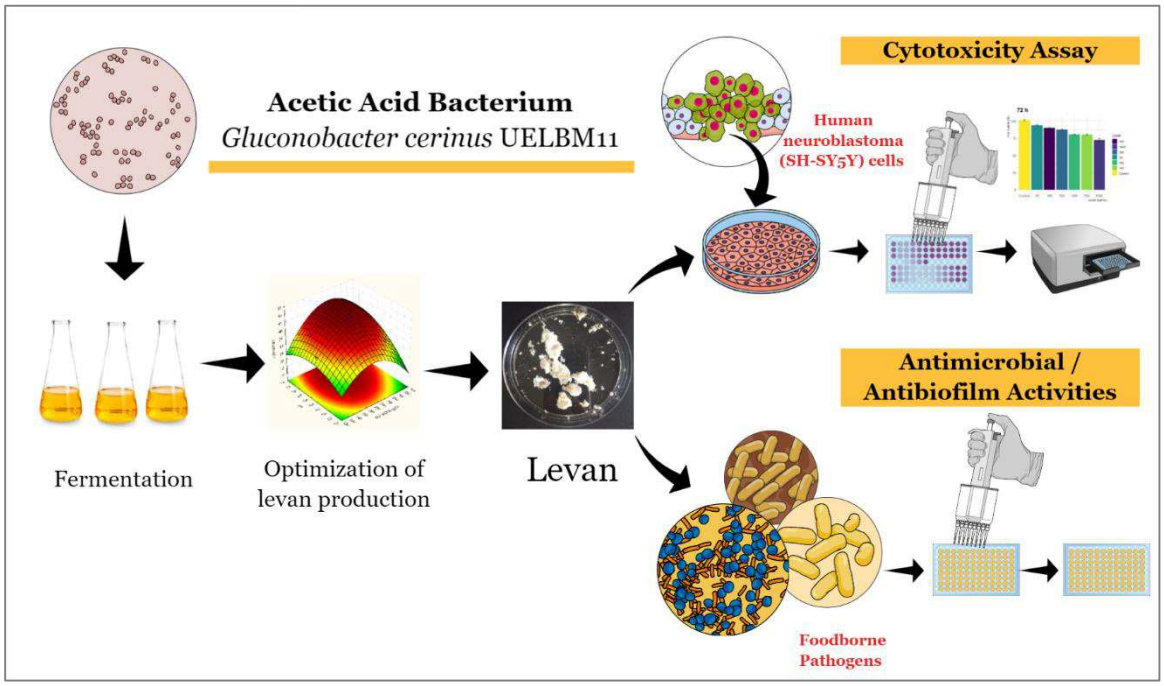


Fig. SM2. Elution profile of levan from *Gluconobacter cerinus* UELBM11 on Sepharose CL-2B column; eluted with 50 mmol/L phosphate buffer (pH 7.0) at a flow rate of 0.5 mL/min.



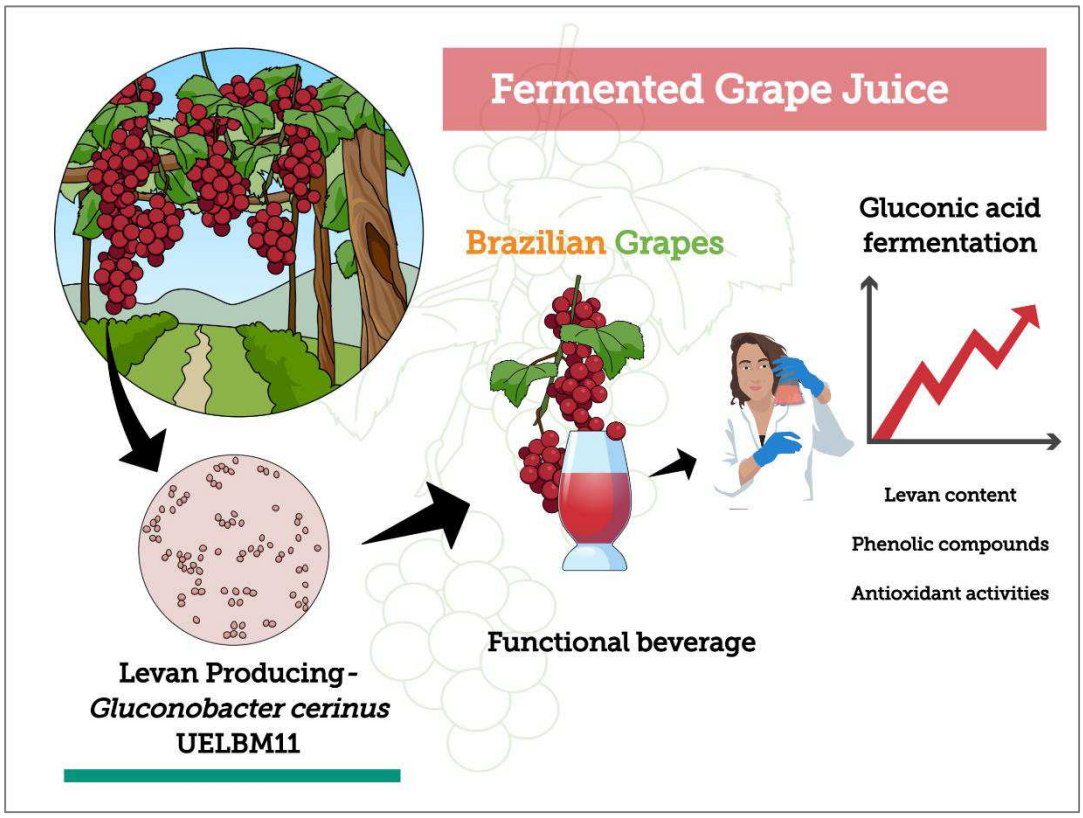
ARTIGO CIENTÍFICO 3

GRAPHICAL ABSTRACT



ARTIGO CIENTÍFICO 4

GRAPHICAL ABSTRACT



Supplementary material

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SMTable1 - Changes in viables cells count, pH, viscosity and total titratale acidity of the beverages

SMTable2 - Changes in sucrose concentration, reducing sugars, and levan yield of the beverages

SMTable3 - Changes in TPC, MAC, ABTS, and FRAP of the beverages

SMTable4 - Relative abundance (%) of volatile compounds groups present in grape juices.

SMTable1 - Changes in viable cell counts, pH, viscosity and total titratable acidity of the beverages

	Viable cells count (CFU/mL)		pH		% TTA (expressed as gluconic acid)		Viscosity (cP)	
Time (h)	CONTROL (non-microbial)	FERMENTED (inoculated)	CONTROL (non-microbial)	FERMENTED (inoculated)	CONTROL (non-microbial)	FERMENTED (inoculated)	CONTROL (non-microbial)	FERMENTED (inoculated)
0	N.A.	7.79 ± 0.02^{b}	5.62 ± 0.07^{a}	5.62 ± 0.07^{a}	0.23 ± 0.06^{a}	0.23 ± 0.06^{e}	282.67 ± 8.74^{a}	283.2 ± 16.8^{a}
6	N.A.	7.80 ± 0.14^{b}	5.63 ± 0.03^{a}	4.36 ± 0.13^{b}	0.26 ± 0.06^{a}	0.59 ± 0.00^{d}	280.93 ± 12.22^{a}	222.93 ± 8.78^{b}
12	N.A.	8.05 ± 0.14^{b}	5.68 ± 0.08^{a}	3.76 ± 0.07^{c}	0.23 ± 0.06^{a}	1.05 ± 0.06^{c}	283.10 ± 8.15^{a}	233.6 ± 15.84^{b}
18	N.A.	9.46 ± 0.30^{a}	5.73 ± 0.02^{a}	3.53 ± 0.03^{d}	0.23 ± 0.06^{a}	1.86 ± 0.10^{b}	274.63 ± 10.31^{a}	247.2 ± 28.67^{ab}
24	N.A.	9.78 ± 0.01^{a}	5.71 ± 0.05^{a}	3.02 ± 0.05^{e}	0.23 ± 0.06^{a}	2.62 ± 0.15^{a}	283.90 ± 12.66^{a}	244.53 ± 16.52^{ab}

$^{a–e}$Values with different superscripts in the same column are significantly different ($p \leq 0.05$). N.A.: Not applicable

SMTable2 - Changes in sucrose concentration, reducing sugars, and levan yield of the beverages

	Sucrose (g/L)		Reducing sugars (g/L)		Levan (g/L)	
Time (h)	CONTROL (non-microbial)	FERMENTED (inoculated)	CONTROL (non-microbial)	FERMENTED (inoculated)	CONTROL (non-microbial)	FERMENTED (inoculated)
0	114.39 ± 3.02^{a}	117.89 ± 3.56^{a}	43.54 ± 2.80^{a}	42.08 ± 1.58^{a}	N.A.	0.00 ± 0.00^{e}
6	113.52 ± 5.39^{a}	116.73 ± 16.39^{a}	44.33 ± 2.29^{a}	42.12 ± 2.80^{a}	N.A.	0.61 ± 0.03^{d}
12	115.23 ± 3.42^{a}	101.55 ± 6.99^{ab}	44.60 ± 0.77^{a}	43.08 ± 4.19^{a}	N.A.	2.18 ± 0.06^{c}
18	116.67 ± 4.19^{a}	96.70 ± 6.39^{ab}	43.44 ± 0.40^{a}	43.67 ± 1.37^{a}	N.A.	4.21 ± 0.11^{b}
24	113.11 ± 5.87^{a}	80.11 ± 6.10^{b}	43.44 ± 1.65^{a}	42.38 ± 4.12^{a}	N.A.	5.70 ± 0.14^{a}

$^{a–e}$Values with different superscripts in the same column are significantly different ($p \leq 0.05$). N.A.: Not applicable

SMTable3 - Changes in TPC, MAC, ABTS, and FRAP of the beverages

	TPC (mg GAE/L)		MAC (mg cyd-3-glu/L)		ABTS (mg VcE/L)		FRAP (mg VcE/L)	
Time (h)	CONTROL (non-microbial)	FERMENTED (inoculated)	CONTROL (non-microbial)	FERMENTED (inoculated)	CONTROL (non-microbial)	FERMENTED (inoculated)	CONTROL (non-microbial)	FERMENTED (inoculated)
0	470.08 ± 20.59^{a}	456.44 ± 18.43^{b}	3.76 ± 0.11^{a}	3.76 ± 0.11^{a}	776.71 ± 25.91^{a}	806.62 ± 17.65^{a}	403.87 ± 3.17^{a}	388.03 ± 3.05^{b}
6	462.25 ± 3.06^{a}	470.58 ± 3.42^{b}	3.52 ± 0.11^{ab}	4.01 ± 0.14^{a}	824.79 ± 43.04^{a}	780.98 ± 21.34^{a}	385.68 ± 9.16^{b}	397.71 ± 2.33^{b}
12	458.96 ± 11.37^{a}	467.05 ± 6.73^{b}	3.51 ± 0.05^{ab}	3.85 ± 0.06^{a}	799.15 ± 10.30^{a}	827.99 ± 65.40^{a}	391.84 ± 2.69^{ab}	396.54 ± 4.16^{b}
18	465.78 ± 7.44^{a}	475.38 ± 7.23^{b}	3.35 ± 0.16^{b}	3.77 ± 0.09^{a}	799.15 ± 14.45^{a}	767.09 ± 38.77^{a}	378.64 ± 9.49^{b}	444.66 ± 16.15^{a}
24	478.16 ± 21.43^{a}	511.99 ± 17.54^{a}	3.33 ± 0.10^{b}	3.88 ± 0.07^{a}	805.56 ± 16.45^{a}	819.44 ± 4.90^{a}	388.62 ± 1.34^{ab}	460.21 ± 3.05^{a}

$^{a–e}$Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

SMTable4. Relative abundance (%) of volatile compounds groups present in grape juices.

Groups	Fermented Juice (T24 h) (%)	Unfermented Juice (T0 h) (%)
Aldehydes	2.14	12.4
Esters	4.58	12.61
Alcohols	11.28	15.350
Hydrocarbons	9.05	1.440
Terpenoids	13.4	12.19
Ketones	3.41	9.05
Aromatic compounds	5.01	9.34
Carboxilic acids	3.88	0.0
Phenols	4.56	5.47
Arylpropanoids	0.0	1.9
Lactones	0.53	0.0
Mixed function	1.32	0.0
Unidentified compounds	30.65	14.18
Impure compounds	9.85	6.07