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TESE

Extração de própolis com óleo e sua incorporação em partículas obtidas por gelificação iônica

Aline Ribeiro Ferreira

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UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
INSTITUTO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

EXTRAÇÃO DE PRÓPOLIS COM ÓLEO E SUA INCORPORAÇÃO EM PARTÍCULAS OBTIDAS POR GELIFICAÇÃO IÔNICA

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Sob a orientação da Professora

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Tese submetida como requerimento parcial para obtenção do grau de **Doutor em Ciência e Tecnologia de Alimentos**, no Programa de Pós-graduação em Ciência e Tecnologia de Alimentos, área de concentração em Tecnologia de Alimentos.

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RESUMO GERAL

FERREIRA, Aline Ribeiro. **Extração de própolis com óleo e sua incorporação em partículas obtidas por gelificação iônica**. 2024. Tese (Doutorado em Ciência e Tecnologia de Alimentos). 2024. Instituto de Tecnologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ. 2024.

Os produtos apícolas são muito utilizados por suas habilidades terapêuticas e capacidade em atenuar inflamações por meio da elevação da resposta imunológica. Eles apresentam significativa quantidade de compostos funcionais e protegem contra danos oxidativos, porém esses compostos são facilmente degradados. A gelificação iônica é uma técnica de encapsulação usada para proteção desses compostos bem como sua veiculação. Desta forma, este estudo tem por objetivo analisar compostos bioativos e capacidade antioxidante da própolis e influência do método de extração e solvente extrator sobre eles, assim como encapsular própolis e geleia real pelo método de gelificação iônica. Sendo assim, a tese foi dividida em 3 capítulos, onde no Capítulo I foi realizada uma revisão sobre as propriedades terapêuticas de produtos apícolas e a influência da pandemia de COVID-19 no mercado brasileiro destes. O capítulo II teve como objetivo avaliar a extração de própolis com óleo de canola como alternativa ao extrato etanólico de própolis. A comparação dos extratos foi feita por suas características físicas e propriedades funcionais, como quantidade de composto fenólicos, flavonoides e capacidade antioxidante. O extrato oleoso apresentou maior quantidade de compostos fenólicos quando comparado ao extrato alcoólico, porém menor rendimento de extração. O extrato alcoólico obteve vantagem no teor de flavonoides, capacidade antioxidante por DPPH e FRAP (Ferric Reducing Antioxidant Power). Durante a vida útil dos extratos, o extrato alcoólico obteve uma menor perda de compostos fenólicos e capacidade antioxidante, porém obteve uma maior perda de flavonoides. O estudo de digestão gastrointestinal simulada *in vitro* apresentou liberação diferente em todas as fases. O extrato oleoso teve maior liberação de compostos fenólicos na fase oral, enquanto o extrato alcoólico teve liberação gradual ao longo do ensaio, atingindo sua máxima ao final da fase intestinal. No FTIR foi possível observar diferenças nos dois extratos relacionadas a compostos extraídos. Por fim, observou-se que o extrato oleoso de própolis pode ser uma alternativa ao extrato alcoólico. O capítulo III teve como objetivo encapsular o extrato oleoso de própolis e geleia real por gelificação iônica e avaliar seu rendimento, a estabilidade das partículas geradas e o perfil de liberação *in vitro*. A mistura composta por própolis e geleia real obteve 11,7 mg/g de compostos fenólicos, 0,2 mg/g de compostos flavonoides e respectivamente 2027,1 e 2202,3 ug/g de capacidade antioxidante por DPPH e FRAP. As partículas foram testadas em diferentes proporções de pectina:mistura e a proporção 80:20 pectina:mistura foi selecionada para caracterização com 92,5% de eficiência de encapsulação de compostos fenólicos e 78,7 e 36,6% de manutenção da capacidade antioxidante por DPPH e FRAP, respectivamente. No estudo da estabilidade à estocagem as partículas obtiveram uma menor perda de compostos fenólicos e capacidade antioxidante por DPPH quando estocadas em refrigeração, em comparação a mistura não encapsulada. As partículas apresentaram formato irregular e tamanhos predominando entre 1 e 1,2 mm. Durante a simulação no trato gastrointestinal foi observada maior bioacessibilidade de compostos fenólicos nas partículas em relação à mistura livre. Na análise de FTIR foi confirmada a encapsulação do extrato oleoso de própolis na partícula de pectina. Por fim, foi observado que o processo de gelificação iônica produziu partículas estáveis, com eficiente manutenção da capacidade antioxidante e melhor bioacessibilidade quando comparadas ao extrato livre.

Palavras-chave: produtos apícolas, compostos bioativos, gelificação iônica.

GENERAL ABSTRACT

FERREIRA, Aline Ribeiro. **Extraction of propolis with oil and its incorporation into particles obtained by ionic gelation**. 2023. Tese (Doutorado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ. 2023.

Apiculture products are highly appreciated for their therapeutic properties and ability to alleviate inflammation by enhancing the immune response. They contain a significant amount of functional compounds and protect against oxidative damage; however, these compounds are easily degraded. Ionic gelation is an encapsulation technique used to protect these compounds. Thus, this study aims to analyze bioactive compounds and antioxidant capacity of propolis, examining the influence of extraction method and extracting solvent. Additionally, it aims to encapsulate propolis and royal jelly using the ionic gelation method. Therefore, the thesis is divided into three chapters. In Chapter I, a review was conducted on the therapeutic properties of apiculture products and the influence of the COVID-19 pandemic on the Brazilian market for these products. Chapter II aimed to evaluate propolis extraction using canola oil as an alternative to ethanol propolis extract. Comparison of the extracts was based on their physical characteristics and functional properties. The oily extract showed a higher amount of phenolic compounds but a lower extraction yield. The alcoholic extract had an advantage in terms of flavonoid content, DPPH, and FRAP antioxidant capacity. During the shelf life of the extracts, the alcoholic extract experienced less loss of phenolic compounds and antioxidant capacity but had a higher loss of flavonoids. The in vitro simulated gastrointestinal digestion study showed different release patterns in all phases. In Chapter III, the objective was to encapsulate the oily propolis extract and royal jelly using ionic gelation and evaluate its yield, particle stability, and in vitro release profile. The mixture of propolis and royal jelly obtained 11.7 mg/g of phenolic compounds, 0.2 mg/g of flavonoids, and 2027.1 and 2202.3 μg/g of DPPH and FRAP antioxidant capacity, respectively. Particles were tested at different pectin:mixture ratios, and the 80:20 pectin:mixture ratio was selected for characterization with 92.5% encapsulation efficiency of phenolic compounds and 78.7% and 36.6% maintenance of DPPH and FRAP antioxidant capacity, respectively. In the stability study, particles showed a lower loss of phenolic compounds and DPPH antioxidant capacity when stored in refrigeration compared to the non-encapsulated mixture. The particles had an irregular shape, with sizes predominantly between 1 and 1.2 mm. During the gastrointestinal tract simulation, higher bioaccessibility of phenolic compounds in particles was observed compared to the free mixture. FTIR analysis confirmed the encapsulation of the oily propolis extract in the pectin particle. Finally, it was observed that the ionic gelation process produced stable particles, with efficient maintenance of antioxidant capacity and improved bioaccessibility compared to the free extract.

Keywords: apiculture products, bioactive compounds, ionic gelation.

SUMÁRIO

1 INTRODUÇÃO GERAL

A microencapsulação é um processo utilizado a fim de proteger compostos sensíveis, como sólidos, líquidos ou gasosos, no qual ocorrem a formação de micropartículas por um material encapsulante que recobre o ingrediente ativo ou núcleo, possibilitando sua manutenção no interior da microestrutura. A técnica possibilita a proteção do ingrediente contra condições adversas do meio gerando uma maior estabilidade, com aumento da vida útil e liberação controlada do encapsulado em condições estabelecidas. É possível mascarar aspectos sensoriais desagradáveis de diversos ingredientes e melhorar manipulação e estocagem de alimentos (ASSADPOUR, JAFARI, 2019; FANG, BANDHARI, 2010; SOUZA et al 2018).

Uma das técnicas de encapsulação branda e simples é a gelificação iônica, que se baseia na capacidade de polissacarídeos aniônicos formarem gel na presença de íons como o cálcio. Esse método busca obter razoáveis níveis do ativo encapsulado e partículas de diferentes formas e tamanhos, sem requerer uso de solventes orgânicos ou temperaturas e pH extremos, assim apresenta baixo custo em comparação à outras técnicas, como spray dryer, coarcevação e spray chilling. Uma de suas características intrínsecas é a porosidade da matriz obtida que pode determinar a liberação da substância encapsulada (HOLKEM et al, 2015; NEMATI et al, 2019).

Vários compostos bioativos podem ser encapsulados na matriz. Eles vêm despertando interesse na indústria, comunidade científica e sociedade, pois são substâncias com potencial biológico. Os compostos antioxidantes são um conjunto heterogêneo de substâncias que tem sua atividade ligada à benefícios à saúde do ser humano ao retardarem as reações de degradação oxidativa por um ou mais mecanismos, como complexação de metais ou inibição de radicais livres. Esses compostos podem ser encontrados em diversos alimentos de forma natural, sendo alguns exemplos destes os produtos das abelhas, como o mel, a própolis e a geleia real (ETCHEPARE et al, 2015; KAVUMARCI, TAN, 2019).

Os produtos das abelhas são alimentos comumente comercializados com apelo à melhoria da saúde do ser humano, e apresentam um teor considerável de compostos bioativos, em especiais os antioxidantes, sendo muito usados como complemento alimentar funcional. A própolis é um produto adstringente formado principalmente por resinas e bálsamos de plantas. Já a geleia real é um produto fabricado pela secreção das glândulas hipofaríngeas das abelhas, rica em vitaminas e compostos antioxidantes. Estes produtos apícolas são frequentemente utilizados misturados em forma de compostos por produzirem efeitos sinérgicos funcionais (BEZERRA, 2018; OSÉS et al, 2016; YEUNG, ARGUELLES, 2019).

Além de apresentarem compostos funcionais significativos, os alimentos das abelhas são estudados por suas atividades antimicrobiana, anti-inflamatória e anticancerígena. Estas atividades estão por diversas vezes atribuídas à presença de propriedades específicas dos produtos, como pH e osmolaridade, e presença de compostos antioxidantes específicos, como é o caso dos compostos fenólicos e dos flavonoides. Quando ingeridos, estes compostos necessitam chegar em seu sítio de ação, porém são sensíveis a diversos fatores externos, como luz, temperatura e oxigênio, além de apresentarem uma alta solubilização e degradação em condições gástricas, o que pode gerar uma alta taxa de metabolização e rápida eliminação do organismo humano. Logo, sua eficácia depende diretamente de sua bioacessibilidade, estabilidade e bioatividade, e sua utilização em forma de encapsulados pode minimizar os problemas ou maximizar sua eficácia (BURATTI et al, 2007; FANG, BHANDARI, 2010; LEVYA-JIMENEZ et al, 2018).

Para ser utilizada em processos alimentícios, a própolis crua é extraída por um solvente de grau alimentício, mais comumente álcool, e por processo tecnológico adequado, gerando um produto líquido, o extrato de própolis. Dependendo do solvente utilizado e da característica dos elementos solúveis extraídos, diferentes atividades biológicas podem ser encontradas. Apesar

das inúmeras propriedades dos extratos alcoólicos, a presença de álcool gera inconvenientes, como sabor desagradável, alta sensibilidade a seu consumo e limitações em processos. O extrato de própolis feito com óleo de canola é uma alternativa ainda pouco estudada (CARVALHO et al, 2011; FAO, 2016; KUBILIENE et al, 2015; PRZYBYLEK et al, 2019; PUJIRAHAYU et al, 2014).

Este estudo teve como objetivo inicial realizar a revisão bibliográfica, como introdução geral, abordando aspectos teóricos relacionados a produtos das abelhas e suas propriedades, além da técnica de encapsulação por gelificação iônica, e a partir disto foram gerados 3 capítulos na forma de artigo. O CAPÍTULO I intitulado "Therapeutic action of bee products and the covid-19 pandemic influence in Brazilian marker of honey and propolis" se refere a uma revisão da literatura sobre as propriedades terapêuticas de produtos apícolas, principalmente mel e própolis, e a influência da pandemia de COVID-19 no mercado brasileiro deles. O CAPÍTULO II – "Propolis oil extract as an alternative means to the alcoholic extract" apresenta a extração da própolis com álcool etílico refinado ou óleo de canola, a caracterização dos extratos obtidos e a comparação entre os dois métodos de extração. O CAPÍTULO III intitulado "Particles characterization incorporated with propolis and royal jelly obtained by ionic gelation" consiste da produção e caraterização das partículas incorporadas de própolis e geleia real obtidas por gelificação iônica quanto ao seu rendimento, eficiência de encapsulação, teor de compostos fenólicos, capacidade antioxidante por DPPH e FRAP, e morfologia, além de apresentar a estabilidade dos compostos bioativos presentes na partícula durante o armazenamento a temperatura ambiente e de refrigeração, e o perfil de liberação *in vitro*.

2 JUSTIFICATIVA

Há uma tendência mundial que demonstra que os alimentos não são apenas vistos como fonte de nutrientes e de apelo sensorial, mas também como funcionalidade e saúde para os indivíduos. A mudança de estilo de vida da população tem gerado um aumento na procura de alimentos funcionais na intenção de incorporar compostos bioativos na dieta, de forma a assegurar um menor risco de desenvolvimento de doenças, principalmente as causadas por danos oxidativos como câncer, aterosclerose, entre outras (STOJANOVIC et al, 2011; WATERHOUSE, 2019).

A alta instabilidade de compostos bioativos ainda se apresenta como uma barreira na absorção deles na dieta humana. São altamente suscetíveis a fatores internos e externos, como luz, temperatura, oxigênio e pH, além de apresentarem uma alta solubilização e degradação em condições gástricas, o que acaba gerando uma baixa bioacessibilidade no trato gastrointestinal (ASSADPOUR, JAFARI, 2019; STOJANOVIC et al, 2011).

Os compostos fenólicos são as principais substâncias funcionais presentes nos alimentos das abelhas, responsáveis por parte de suas propriedades benéficas. Auxiliam a amenizar o estresse oxidativo e consequentes danos celulares, sendo eficientes na redução de risco de doenças. Podem atuar como sequestradores de radicais livres, quelantes de metais, entre outros. Apesar disto, para que possam exercer seus efeitos biológicos devem ser absorvidos e alcançar o tecido-alvo em concentrações efetivas (ALVAREZ-SUAREZ et al, 2009; ETCHEPARE et al, 2015).

A gelificação iônica externa é um método de microencapsulação físico-químico que permite a obtenção de micropartículas com características estáveis. O método de gotejamento permite a formação instantânea de estruturas de hidrogéis que são insolúveis em água, deixando o composto ativo disperso por toda a matriz. A produção de esferas com ausência de solventes orgânicos torna a técnica simples e promissora especialmente para microencapsular compostos

ativos termossensíveis de interesse. Além disso, não necessita de condições rigorosas de temperatura durante o processo e apresenta um baixo custo (HOLKEM, CODEVILLA, MENEZES, 2015; MOURA et al, 2018; OZKAN et al, 2019).

A disponibilização de partículas contendo altas concentrações de compostos bioativos pode permitir o desenvolvimento de formulações alimentícias inovadoras que possam prover saudabilidade e mascarar sabores indesejáveis de matérias-primas. Além disso, amplia a possibilidade de produção em escala industrial e melhoria da estabilidade e vida de prateleira dos produtos (ASSADPOUR, JAFARI, 2019; WATERHOUSE, WATERHOUSE, 2019).

3 REVISÃO BIBLIOGRÁFICA

3.1 Produtos das Abelhas

As abelhas são insetos voadores conhecidos por seu importante papel na polinização, prática fundamental para garantir alta produtividade e qualidade de frutos em culturas agrícolas. Pertencentes à classe *Insecta* e ordem *Hymenoptera*, são produtoras de uma variedade de alimentos considerados importantes para a saúde humana. Além do mel, elas fornecem própolis, geléia real, pólen, cera, entre outros. Esses produtos variam bastante em sua formação química de acordo com a espécie de plantas usadas pelas abelhas, a localização geográfica, estação do ano e fatores ambientais (GONÇALVEZ et al, 2005; TAPIA-GONZALEZ et al, 2019; VIUDA-MARTOS et al, 2008; YEUNG et al, 2019).

Os produtos das abelhas são relatados por apresentarem diversos benefícios à saúde. Diversos destes já foram relatados por sua ampla gama de propriedades promotoras de saúde, como atividade antibacteriana, antioxidante e eliminadora de radicais livres. Efeitos anti-inflamatórios, anticancerígenos e imunoestimulantes são frequentemente demonstrados, com relatos de benefícios comprovados em condições médicas como queimaduras, mucosites associadas à radioterapia, artrite reumatoide e gastroenterite. Testes de susceptibilidade e avaliação antimicrobiana contra diversas linhagens de bactérias e fungos patogênicos são constantemente estudados por demonstrarem satisfatória eficácia (CIULU et al, 2016; MUNSTEDT et al, 2019).

A apiterapia é uma técnica muito utilizada pela medicina alternativa para fortalecer o equilíbrio do sistema imunológico a partir dos produtos colhidos, transformados e segregados pelas abelhas. A utilização de produtos naturais fornece elementos que irão defender o organismo de elementos externos, ajudando o tratamento complementar de disfunções orgânicas e emocionais. A técnica é uma das formas de medicina natural mais antigas da humanidade e explora a mistura de vários produtos e a exploração sinérgica deles (KAVURMACI, TAN, 2019; MOUHOUBI-TAFININE, OUCHEMOUKH, TAMENDJARI, 2016; SFORCIN et al, 2017).

O crescimento significativo da apiterapia nos últimos anos representou um aumento dos estudos a seu respeito. O Brasil apresentou o primeiro lugar em publicações sobre o assunto, seguido dos EUA, China e Japão. No entanto, quando se trata de aceitação dos produtos apícolas, muitos pacientes não consideram uma opção viável, muitas vezes devido à intolerância ao sabor e quantidade. (MUNSTEDT et al, 2019; SENEL, DEMIR, 2018).

O Brasil ocupa posições de destaque em produção e exportação de produtos apícolas, sendo as condições climáticas e geográficas e as características de vegetação pontos favoráveis desse sistema de produção. A própolis brasileira é muito cobiçada no mercado internacional para alimentação e produção de medicamentos, sendo amplamente aplicado nas indústrias farmacêuticas e alimentícias em todo o mundo. Estudos relatam que ao diminuir a concentração

de um antibiótico e incluir a própolis em um tratamento, a ação contra bactérias se apresenta melhor do que com o antibiótico usado de forma isolada. A introdução desses produtos naturais tanto na alimentação diária quanto em tratamentos representa uma alternativa terapêutica pelo sinergismo entre a própolis e diversos tipos de antibióticos (ORSI et al, 2012; SFORCIN et al, 2017).

Osés et al. (2016) avaliaram um produto feito a partir de mel e própolis e observaram efeitos antimicrobianos sinérgicos e um aumento da atividade anti-inflamatória quando comparado aos produtos de forma isolada. Ao estudar os benefícios dos produtos apícolas, Sun et al (2018) analisaram o efeito de própolis, geleia real e veneno de abelha em ratos hipertensos, verificando que o tratamento reduziu a pressão arterial ao diminuir os níveis séricos de angiotensina II, endotelina 1 e melhorando a estrutura do miocárdio.

3.1.1 Mel

Dos produtos apícolas, o mel é o alimento mais reconhecido por sua relevância histórica, cultural e econômica. É definido como o produto alimentício fabricado por abelhas melíferas a partir do néctar de flores ou de secreções procedentes de partes vivas das plantas que as abelhas recolhem, transformam, combinam com as substâncias próprias delas para armazenar e deixar madurar nos favos das colmeias. É gerada uma complexa mistura de carboidratos (70-80%), água (10-20%) e outros componentes minoritários como enzimas, ácidos orgânicos, minerais, proteínas, compostos fenólicos, entre outros (BRASIL 2000; KRSTONOSIC et al, 2019).

Os méis podem ser classificados em monoflorais quando provêm de uma espécie botânica, com predominância de no mínimo 45% de pólen de uma única flor, ou poliflorais quando são originários de várias fontes florais. Os componentes do mel estão diretamente relacionados a sua fonte floral e origem geográfica. Os compostos fenólicos são os principais metabólitos secundários das plantas, logo, seu conteúdo no mel é bastante variável de acordo com sua origem botânica. Podem ser encontrados no mel ácidos fenólicos e derivados, como ácido cumárico, gálico, sérico, entre outros, e flavonoides, como luteolina, quercetina, entre outros (ÁVILA et al, 2019; BELAY et al, 2017; KRSTONOSIC et al, 2019).

O mel apresenta uma expressiva atividade antibacteriana contra uma ampla gama de microrganismos em diferentes ambientes, se apresentando tão potente quanto diversos antibióticos existentes. Já foi demonstrada atividade antimicrobiana sobre *Staphylococcus* sp., *Bacillus* sp., *Escherichia coli, Pseudomonas aeruginosa, Salmonella* sp. e leveduras. Essa atividade está relacionada a diversos fatores químicos como alta acidez, baixa atividade de água, alta pressão osmótica e presença de componentes fitoquímicos e substâncias voláteis. Além disso, apresenta um sistema glicose-oxidase que em presença de oxigênio e água converte glicose em ácido glucônico e peróxido de hidrogênio, substâncias de relevante ação antioxidante que afetam o envoltório de microrganismos. O peróxido de hidrogênio, entretanto, é facilmente destruído pelo calor ou presença de catalase (ELBANA et al, 2014; FYFE et al, 2017; MCLOONE, WARNOCK, FYFE, 2016; TAVAKOLI et al, 2017; OSES et al, 2017).

A alta concentração de açúcar do mel causa um estresse osmótico em células microbianas, e o baixo pH desfavorece o crescimento de microrganismos, auxiliando no seu poder antimicrobiano. Porém, estudos já confrontaram amostras de méis com soluções de açúcar, e concluíram que a atividade antimicrobiana foi efetiva apenas em méis, sugerindo que o mecanismo de inibição de crescimento bacteriano não está relacionado somente ao efeito osmótico (CARNWATH et al, 2013; MCLOONE, WARNOCK, FYFE, 2016).

Diversos estudos (Alvarez-Suarez et al, 2009; Rosa et al, 2011) tem relatado à forte correlação entre as propriedades antioxidantes e a presença de compostos fenólicos em méis. No entanto, Culun et al (2016) afirmam que alguns cuidados devem ser tomados para analisar as propriedades dos méis, como uma padronização de amostragem adequada, além de

observarem que o padrão fenólico está relacionado à autenticidade e frescor das amostras, pontuando o hidroximetilfurfural como importante parâmetro para comprovar sua estabilidade frente a um estresse térmico e fotoquímico, com consequente perda nutricional e funcional. De acordo com os procedimentos analíticos publicados, os méis devem ser purificados por meio de uma etapa de extração logo antes da análise fenólica. Mouhoubi-Tafinine, Ouchemoukh e Tamendjari (2016) analisaram amostras de mel em relação à sua capacidade antioxidante e verificaram que a água permite uma maximização da extração de polifenóis.

3.1.2 Própolis

A própolis é uma complexa mistura de compostos que as abelhas produzem a partir de bálsamos e resinas (50%) e ceras (30%), além de óleos essenciais, polens, entre outros. É usado nas colmeias como proteção contra predadores e como isolante térmico. Além de ser frequentemente estudado por seus efeitos antibacterianos, anti-inflamatórios e antioxidativos, a própolis também tem demonstrado efeitos hipotensivos e cardioprotetores. É bastante utilizado não só na medicina como em formulações alimentares funcionais e até em cosméticos (BRUSCHI et al, 2017; SILVA et al, 2015; SUN et al, 2017).

A própolis contém quantidades consideráveis de compostos funcionais, com mais de 150 tipos de polifenóis, e mesmo com origens distintas e composição diversa, exibem uma conformação fenólica parecida, assim como efeitos antimicrobianos comparáveis. O extrato proveniente da própolis contém menores quantidades de compostos fenólicos, sendo exigido o mínimo de 0,5% em legislação, enquanto na própolis bruta é exigido o mínimo de 5,0%. Tem sido demonstrado que esses compostos estão diretamente relacionados com diversas propriedades biológicas da própolis. Além disso, estudos sugerem que os flavonoides desempenham papel importante na atividade antioxidante dos extratos, atuando de forma sinérgica com hidroxiácidos e sesquiterpenos, embora outros fatores também possam estar envolvidos (BRUSCHI et al, 2017; DE-MELO et al, 2014; SILVA et al, 2006).

Entretanto, considera-se muito difícil a utilização da própolis bruta na produção industrial de alimentos. Somando ao fato do interesse na pureza dos princípios ativos da própolis, geralmente é feito seu fracionamento para posterior incorporação em líquidos. Os solventes mais utilizados em sua extração são etanol, óleo ou água. Devido à sua complexidade química, não existe um único solvente que solubilize a amostra bruta em sua totalidade, sendo apenas capazes de extrair um determinado grupo de compostos (KESKIN, KESKIN, KOLAYLI, 2019; MACHADO et al, 2019; POBIEGA, KRASNIEWSKA, GNIEWOSZ, 2019).

O método de extração pode influenciar na atividade do extrato resultante, com rendimento e seletividade de alguns compostos diretamente afetados. A própolis é mais comumente extraída em sua forma hidroalcoólica devido à sua baixa solubilidade em água, pela presença de substâncias apolares. A extração é geralmente realizada pela imersão da própolis no solvente por até 60 dias. Um inconveniente é a formação de um filme insolúvel de ceras e resinas que adere à parede do sistema digestivo e dificulta a absorção dos componentes bioativos, além da rejeição do extrato sensorialmente por presença de álcool etílico e reações adversas ao seu uso com possíveis contraindicações. O extrato oleoso é uma alternativa viável que torna o extrato mais agradável para alguns, com mascaramento do sabor desagradável, além de facilitar sua absorção pela compatibilidade entre glicerídeos e biomembranas (MELLO, PETRUS, HUBINGER, 2009; SILVA et al; 2017).

A própolis pode se apresentar de diversos tipos, de acordo com a florada e região na qual é originado. Existem 13 grupos diferentes de própolis disponíveis no mercado brasileiro atualmente, e essa grande variação se deve à biodiversidade do país. Sua cor varia de verde à marrom e vermelho. A própolis verde é o mais comum, proveniente principalmente da planta

alecrim-do-campo (*Baccharis dracunculifolia),* predominante no Sudeste brasileiro. Diversos estudos têm demonstrado o potencial biológico da própolis verde, com propriedades antiparasitárias e antioxidantes (MACHADO et al, 2015; Silva et al, 2017; PINTO, PRADO, CARVALHO, 2011).

A própolis de zonas tropicais, especialmente a brasileira, tem sido objeto de muitos estudos devido à sua composição química diferenciada quando comparada aos países de zona temperada. A própolis verde brasileira contém derivados prenilados do ácido cumárico e grande quantidade de flavonoides, muitos dos quais não se apresentam em amostras da Ásia, Europa e América do Norte. A Artepelina C é a substância em maior abundância na própolis brasileira e possui significativas propriedades antioxidantes e efeitos antitumorais, com inibição da peroxidação lipídica e ação quimiopreventiva na carcinogênese do cólon, além de efeito antileucêmico (BRUSCHI et al, 2017; LUSTOSA et al, 2008; MACHADO et al, 2015; SIMOES-AMBROSIO et al 2010).

Buratti, Benedetti e Cosio (2007) compararam a capacidade antioxidante de mel, própolis e geleia real, concluindo que a própolis apresentou a maior atividade dentre os produtos apícolas, seguida da geleia real e do mel. Além disso, a própolis bruta apresentou uma maior atividade quando comparada ao extrato hidroalcoólico. Ao estudarem diversos compostos apícolas, Ozkok e Silici (2017) observaram que a mistura de geleia real, mel, própolis e pólen apresentou a maior atividade antioxidante.

3.1.3 Geleia Real

A geleia real é um alimento funcional bem-conceituado para a promoção da saúde humana, considerada um eficiente nutracêutico. Produzida pelas glândulas hipofaringeanas das abelhas operárias, é composta por água (60-70%), proteínas (9-18%) e açúcares (10-16%), além de pequenas quantidades de vitaminas, lipídeos, sais e aminoácidos livres. Suas principais proteínas são responsáveis pelo desenvolvimento, longevidade e reprodução das abelhas (PARK et al, 2019; RAMANATHAN, NAIR, SUGUNAN, 2018).

Além de ser extremamente nutritiva, também apresenta significativa atividade antimicrobiana, em grande parte relacionada às proteínas, sendo a maioria delas classificadas como Principais Proteínas da Geleia Real (MRJP), assim chamadas por participarem de diversos processos e serem consideradas o principal fator que direciona o desenvolvimento da rainha das abelhas. As MRJPs representam 82 a 90% das proteínas totais e atuam de forma parecida à peptídeos antimicrobianos. Apresenta uma proteína antibacteriana chamada royalisina que atua em diversas bactérias, principalmente Gram-positivas. Além das proteínas, apresenta distintos componentes bioativos como o ácido 10-hidroxi-2-decenóico (10-HDA), principal componente da fração lipídica e bastante raro na natureza, servindo como marcador biológico. Por fim, outros compostos contribuem para seu efeito antioxidante como flavonoides. É qualificada como um eficiente nutracêutico (BEZERRA et al, 2018; PARK et al, 2019; RAMANATHAN, NAIR, SUGUNAN, 2018).

Diversos estudos atestam o potencial antienvelhecimento, anti-inflamatório, antidiabético e antimutagênico da geleia real. Tem sido usada amplamente em várias doenças como câncer, diabetes e hipertensão. É redutora do estresse oxidativo, além de melhorar o status glicêmico e o perfil lipídico de pacientes com diabetes mellitus. Também apresenta um importante efeito hipocolesterolêmico pela ligação de suas proteínas aos ácidos biliares no intestino. Jenkhetkan et al, (2017) demonstraram que o consumo de geleia real estimulou a proliferação de hepatócitos em ratos e promoveu a antiapoptose, resultando na sobrevivência celular e longevidade (BEZERRA et al, 2018; KHAZAEI, ANSARIAN, GHANBARI, 2017; MALEKI et al, 2019).

A comercialização da geleia real deve ser feita, de acordo com a legislação brasileira, de forma a manter a conservação do produto, em uma temperatura não superior a 4°C, por ser altamente susceptível à luz e ao calor e sofrer oxidação em contato direto com o ar. As condições de armazenamento são ponto crítico para manter inalterada suas características. Como exceção, pode ser comercializada de forma liofilizada, ou adicionada em pequena quantidade em compostos, embora não exista uma legislação atual específica para o último. Porém, de acordo com a Portaria SDA n° 795, de 10 de maio de 2023, a geleia real só poderá ser veiculada em mel na proporção mínima de 0,2% (BRASIL, 2000; KOSHIO, ALMEIDA-MURADIAN, 2003).

Sabatini et al. (2009) estudaram a estabilidade das proteínas e lipídeos da geleia real durante o armazenamento a 4°C por 10 meses, não apresentando alterações significativas na quantidade total, no entanto, quando armazenada em temperatura ambiente, obteve um aumento no conteúdo de lisina e prolina, sugerindo uma atividade proteolítica em temperaturas desfavoráveis. Já Chen e Chen (1995) relataram que temperaturas inadequadas afetam diretamente o ácido 10-HDA, que apesar de ser termoestável tem sua atividade bactericida diminuída.

3.2 Processos de Microencapsulação

O conceito de nutrição mudou significativamente nos últimos anos. Uma nutrição adequada passou de uma dieta com nutrientes suficientes à necessidade do corpo para um interesse em produtos naturais usados para promover a saúde e reduzir o risco de doenças. A microencapsulação surgiu então como um conjunto de técnicas que permitem um desenvolvimento de formulações com conteúdo protegido e liberação modificada, com o intuito de atuar num determinado local em tempo e velocidade específicas. Permite então a proteção de diversos compostos e é empregada em diversas áreas, como alimentícia e farmacêuticas, com o objetivo de proteger o material encapsulado de condições adversas do meio e liberar diretamente no sítio de ação em quantidade e momento adequados (FRASCARELI et al, 2012; MOURA et al, 2018; YEUNG, ARGUELLES, 2019).

A técnica de microencapsulação consiste em um material sólido, líquido ou gasoso que é cercado por um material de revestimento, criando uma partícula. O tamanho das partículas pode ser macro, micro ou nano e tem influência nas propriedades sensoriais das aplicações alimentares. A morfologia das partículas depende diretamente do material da parede e do núcleo, assim como da técnica empregada (AGUIAR, ESTEVINHO, SANTOS, 2016; FANG, BANDHARI, 2010; FRASCARELI et al, 2012).

A microencapsulação tem sido utilizada com o intuito de melhorar a utilização de compostos bioativos em formulações alimentícias, aumentando sua biodisponibilidade no trato gastrointestinal, reduzindo a sensibilidade destes compostos às condições adversas como temperaturas elevadas e presença de luz e oxigênio. Além de melhorar o tempo de retenção dos nutrientes nos alimentos e retardar os processos de degradação como a oxidação, a microencapsulação também é capaz de mascarar o odor ou mau gosto, além de evitar interações indesejáveis com uma matriz alimentar. Outras vantagens incluem a facilidade de manuseio, concentração adequada e dispersão uniforme (AGUIAR, ESTEVINHO, SANTOS, 2016; FANF, BHANDARI, 2010)

Existem várias técnicas disponíveis para microencapsular um composto, sendo que a escolha da técnica mais adequada é dependente das propriedades químicas e físicas do núcleo e do material de revestimento, assim como da aplicação final pretendida (FANG, BHANDARI, 2010).

3.2.1 Gelificação Iônica

A gelificação iônica é uma técnica crescente e promissora que apresenta características simples e reprodutíveis. Consiste em um método físico-químico rápido para se obter micropartículas na ausência de condições drásticas, minimizando perdas de atividade biológica de um composto a ser encapsulado. Apresenta diversas vantagens como o baixo custo e metodologia branda. Sua metodologia consiste em lançar uma solução contendo um polímero (material de parede) e o material de recheio por gotejamento em uma solução contendo íons, ocorrendo então a solidificação por gelatinização iônica. Quando duas macromoléculas de cargas opostas são misturadas formam-se complexos de polieletrólitos resultados de interações eletrostáticas entre os íons (LOPEZ-CORDOBA, DELADINO, MARTINO, 2014; MENEZES et al, 2015).

Por não utilizar temperaturas elevadas, solventes orgânicos, agitação vigorosa ou condições extremas de pH, a gelificação iônica se apresenta como um método adequado para a encapsulação de compostos bioativos, que apresentam instabilidade química e dificuldades associadas à sensibilidade em condições de processamento. Além disso, tais compostos são suscetíveis a degradação sob condições gástricas, não chegando ao sistema intestinal. Suas limitações estão entre reações de autoxidações, epimerização, instabilidade em variações de pH e conteúdo gástrico e baixa disponibilidade (LOPEZ-CORDOBA, DELADINO, MARTINO, 2014; MOURA et al, 2018).

O objetivo da técnica de gelificação iônica é criar partículas insolúveis em água, de forma a retardar a liberação de substâncias. Para modular a permeabilidade das partículas geralmente é adicionado um polímero de revestimento ou realizada uma dupla emulsão, principalmente para proteção de bioativos hidrofílicos, uma vez que o processo de gelificação iônica geralmente é aplicado para compostos hidrofóbicos ou de baixa solubilidade (LOPEZ-CORDOBA, DELADINO, MARTINO, 2014; MOURA et al, 2018).

A não toxicidade e biodegradabilidade de polissacarídeos naturais os tornam ideais para microencapsular uma variedade de compostos ativos. A pectina é frequentemente utilizada no método de gelificação iônica, por uma de suas principais propriedades biofuncionais, a capacidade de formar géis em presença de íons cálcio divalentes. É um hidrocolóide industrialmente importante por conter muitos grupos hidroxilas e servir como polieletrólito. Além disso, pode ser obtida através da extração de resíduos de frutos cítricos e são classificadas de acordo com seu grau de metilação, que pode influenciar na capacidade de hidratação e de formar gel. Partículas de pectina de baixo teor de metoxilação são muito utilizadas pela técnica de gelificação iônica como material de revestimento. Os géis formados são termo reversíveis e sua força pode aumentar com a elevação da concentração de cálcio, porém reduz com aumento da temperatura e acidez (BELSCAK-CVITANOVIC et al, 2015; MENEZES et al, 2015).

A pectina tem diversos aspectos benéficos à saúde e estudos indicam que é hidrolisada no trato gastrointestinal, interage com o colesterol na regulação da homeostase do colesterol hepático e no metabolismo de lipoproteínas. Wang et al (2013) encapsularam óleo de canola enriquecido com quercetina utilizando tanto alginato quanto pectina como materiais de parede, obtendo bons resultados de ambos com preservação do encapsulado, demonstrando boas opções para encapsular esse tipo de substância, que pode ser diretamente consumida ou utilizada como ingrediente funcional em alimentos. Moura et al. (2018) utilizaram pectina como material de parede para a encapsulação de pigmento, resultando em cápsulas com boa resistência térmica, íntegras em matriz de bala submetida à temperatura de 80 °C. Partículas com pectina podem ser usadas para proteger substâncias adicionadas a produtos que passem por processamento térmico (MUZZARELLI et al, 2012).

3.3 Encapsulação dos Produtos de Abelha

Tanto o mel quanto a própolis podem ser ferramentas terapêuticas úteis e sua preservação se faz importante. Estudos mostram que mesmo com variação do perfil de compostos fenólicos encontrados em própolis, baixos teores de flavonoides ainda apresentam atividade antioxidante. Já em mel, mesmo com sua capacidade antioxidante atribuída ao sinergismo entre seus compostos fenólicos, foi observado que apenas um composto isolado é capaz de produzir efeitos antioxidantes e antimicrobianos. O tempo de vida útil desses alimentos apícolas é relativamente alto, porém compostos bioativos apresentam grande instabilidade em condições adversas. Nagai et al (2001) analisaram amostras de méis comerciais e observaram que a atividade antioxidante diminuía gradativamente com o tempo, o que pode ser devido à degradação de seus compostos, que são sensíveis a temperaturas elevadas e condições de armazenagem inadequadas. O mesmo acontece com a própolis. Porém, estudos mostram que quando estocado em temperaturas de 4°C sua atividade antimicrobiana não é reduzida por até 4 anos. Logo, processos de microencapsulação podem se apresentar eficientes para manter a estabilidade dos produtos apícolas (BONVEHI, COLL, JORDA, 1994; LEVYA-JIMENEZ et al, 2019; OZDAL et al, 2019; PINTO, PRADO, CARVALHO, 2011; SAVOIA 2012; SIMOES-AMBROSIO et al, 2010; SFORCIN et al, 2017; YEUNG, ARGUELLES, 2019).

A tecnologia de microencapsulação pode servir para proteger compostos sensíveis responsáveis pela capacidade antioxidante dos produtos apícolas desejáveis, com objetivo de: proteger o material do núcleo da degradação, reduzindo sua sensibilidade ao ambiente externo, como no caso da geleia real altamente instável à luz e ao calor; reduzir a taxa de transferência do material do núcleo para o ambiente externo; modificar as características físicas do material para facilitar o manuseio; adaptar a liberação do material do núcleo no tempo e local desejados; mascarar sabor indesejado do material do núcleo, como no caso da própolis com forte sabor e aroma (FANG, BHANDARI, 2010; FRATINI et al, 2016; KESKIN, KESKIN, KOLAYLI, 2019; SABATINI et al, 2009).

Sansone et al. (2011a) e Sansone et al. (2011b) avaliaram a capacidade antioxidante de compostos bioativos microencapsulados, como por exemplo a quercetina, observando que a atividade permaneceu inalterada por um tempo, até uma redução final de no máximo 10% em 12 meses. Já Nori et al (2011) estudaram extratos de própolis encapsulados por coacervação complexa observando efetividade para a preservação dos compostos fenólicos e flavonoides, que se mostraram estáveis durante 6 meses de armazenamento em diferentes temperaturas (ambiente e refrigerada), com preservação também de sua atividade antioxidante e antimicrobiana.

Azam e Amim (2018) incorporaram mel a um hidrogel com alginato de sódio, resultando em um filme com boas propriedades para ser usado como material curativo. Segundo Bonifacio et al. (2018), a viscosidade única do mel ajuda a aprimorar os recursos mecânicos de hidrogel, além de superar simultaneamente os riscos de contaminação com sua potente atividade antimicrobiana, auxiliando no reparo da cartilagem. Já Keskin, Keskin e Kolayli (2019) encapsularam um extrato etanólico de própolis com alginato como material de parede e obtiveram resultados satisfatórios, com a liberação dos componentes bioativos atingindo a parte superior do intestino grosso resultando em um aumento da biodisponibilidade desses componentes. Atualmente, ainda há carência de trabalhos que abranjam a metodologia de encapsulação por gelificação iônica em produtos apícolas.

4 CONCLUSÃO

A partir desta revisão, foi possível observar características bioativas de compostos apícolas e as possíveis vantagens obtidas ao encapsular esses alimentos, além do entendimento de etapas importantes que podem influenciar esse processo, como o método de extração, o solvente utilizado e a técnica escolhida.

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CAPÍTULO I

THERAPEUTIC ACTION OF BEE PRODUCTS AND THE COVID-19 PANDEMIC INFLUENCE IN BRAZILIAN MARKER OF HONEY AND PROPOLIS

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ABSTRACT

The global pandemic caused by the novel coronavirus is a severe concern worldwide. Strategies that stimulate the immune system have been sought to alleviate the complications associated with the disease. Bee products are highly appreciated for their therapeutic abilities and have attracted attention for being a natural and effective therapy in mitigating inflammation by increasing the immune response. Due to this reputation, there was a considerable increase in the consumption of honey and propolis during this period, with a consequent increase in its commercial value in the Brazilian market and a significant growth in the amount of exported honey. Thus, future research should be carried out to enhance the use of these products preventively or in combination with conventional therapy.

Keywords: bee products, immune response, pandemic, coronavirus, Brazilian market.

1 INTRODUCTION

Popularly known for their therapeutic properties, bee products are foods with nutritional and medicinal properties, with antibacterial, antifungal, antiviral and anti-inflammatory effects. Despite the benefits associated with these products, their consumption in Brazil is not significant compared to other countries. While in Europe honey consumption reaches 1.5 kg per capita, it does not reach 100 grams among Brazilians. In addition to the low income level, this fact is also due to the lack of knowledge about the product properties. However, the bee products market in Brazil assumed a prominent role during the novel coronavirus pandemic, even in the face of the economic instability experienced (GOMES e SANTOS, 2016; SABBAG e NICODEMO, 2011; VIDAL, 2021).

The growing market for these products follows the trend in search of healthy habits that are gaining ground in the daily lives of people worldwide. In addition, natural products have been increasingly applied for healing. Bee products have been used in traditional and modern medicine recently and are considered functional foods for promoting health benefits. Apitherapy uses bee products whose main objective is to guide the organism to create immunological defense barriers. They are also used as a complementary treatment in combination with various medications (BERRETTA et al., 2017; NAGGAR et al., 2021; RAHMAN et al., 2014).

In this context honey and propolis stand out as products of greater value for the Brazilian market. According to the Food and Agriculture Organization of the United Nations (FAO), in 2021 the production of 2,108,564 tons of honey worldwide was estimated, and Brazil was the 10th largest honey producer. According to data from the Municipal Livestock Survey (PPM) linked to the Brazilian Institute of Geography and Statistics (IBGE) collection network, it was estimated that honey production in 2019 was close to 55 thousand tons. Regarding propolis, the Micro and Small Business Support Service (SEBRAE, 2018) estimated that the average annual production is 150 tons. These products are often consumed raw or as syrups and compounds. The regional characteristics are reflected in a wide range of distinguished products, which makes Brazil competitive and highlighted in the international scenario (GOMES e SANTOS, 2016).

According to the Ministry of Agriculture, Livestock and Food Supply (MAPA), “Honey is the naturally sweet and viscous substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which honeybees collect, transform, and combine with specifics substances of their own, store and leave in the hive to ripen and mature.” Honey is a sweet food with unique characteristics and is widely used as a food supplement due to the high amount of carbohydrates present, in addition to several vitamins (B1, B2, B3, B5, B6, C, D, E and Biotin), minerals and antioxidant compounds. It is naturally viscous and can be presented in crystallized form, with the formation of tiny sugar crystals by a natural separation of glucose, without altering its quality and nutritional value. The diverse composition of honey has already shown benefits in several studies. Administered alone or in combination with conventional therapy, it has been shown to be helpful in the treatment of chronic diseases associated with oxidative stress and inflammation state (BRASIL, 2000; NAGGAR et al., 2021; RAHMAN et al., 2014; SILVA et al., 2016).

Propolis is “the product derived from resinous, gummy and balsamic substances, collected by bees from sprouts, flowers and plant exudates, in which the bees add salivary secretions, wax and pollen for the final elaboration of the product”. The word propolis is derived from the Greek where “pro-” means in defense, and “polis-” means the city, due to being used by bees for the defense of the hive. The product is considered a natural antibiotic and rich in

phenolic compounds and flavonoids responsible for many pharmacological properties and high antioxidant capacity, acting against infections in our body. In addition, it has been proven to boost immunity and protect the body against different types of viruses. The crude propolis, so called after harvested in its original solid form, is commonly extracted with alcohol until it forms a liquid named propolis extract (BERRETTA et al., 2017; BRASIL, 2001; NAGGAR et al., 2021).

Although studies are conducted on the therapeutic effects of bee products, there is still much to be understood about their action mechanisms. This review focuses on the main therapeutic effects of honey and propolis and their constituents, as well as their potential against the coronavirus. In addition, the influence of the pandemic on the commercialization of these products in the national market is reported.

2 METHODOLOGY

The present study was developed from a bibliographic review that sought publications on the therapeutic activity of bee products like honey and propolis. With a qualitative approach, the th articles were searched rough the Scielo, Scopus, Web of Science and PubMed databases. For data collection, the keywords “honey”, “propolis”, “functional”, “activity”, “benefits”, “antiviral”, “COVID-19” and “Sars-CoV-2” were used. There was no delimitation of the period of publication in the research, which was carried out in November 2022.

Subsequently, a survey of data on the production of honey and propolis in Brazil was carried out through the IBGE website. The effect of the COVID-19 pandemic on the honey and propolis export market was analyzed using the Trademap website and the Agrostat database. Finally, an interview was conducted to assess the Brazilian market for these products with the bee products processing unit of the company “Fumel Commercial e Industrial LTDA” in the State of Rio de Janeiro.

3 DISCUSSION

3.1 Bee Products and Their Compositions

The concern with the increase of diseases brought the need for better maintenance of the body and started a search for foods that could add nutritional and medicinal values to the daily diet. The bee products have become the object of study because they have benefits for human health. They are considered functional foods for their ability to improve health and for being able to contribute to the excellent maintenance of the body and decrease the risk of diseases, whether bacterial, viral or tumoral. The presence of vitamins and polyphenols is responsible for maintaining a nutritional balance and the integrity of the individual's health. (MENEZES, 2022; FEITOSA et al., 2020, STOPIN, 2020).

The honey has several components that may be responsible for its therapeutic action, and despite being a saturated solution of sugars and water, it has a diverse composition attributed to its floral source, which gives it high complexity. Its bioactive potential is directly linked to the presence of phenolic acids and flavonoids, some of which have already been identified and named, such as cinnamic, caffeic, ferulic, coumaric, chrysin and kaempferol acids. In addition, it presents several other compounds, such as sugars, organic acids, amino acids, vitamins and minerals. More than 400 different volatile compounds have been found in

honey (ABEDI et al., 2021; AL-HATAMLEH et al., 2020; HOSSAIN et al., 2020; SEEDI et al., 2020).

In addition to honey, propolis is a highly sought-after food for treatment due to its effects on organisms. The therapeutic functions of propolis are numerous and justify its potential in developing beneficial products for human health. Its chemical composition is directly related to the resins and balsams of plant origin used by bees to produce it. The main groups found in addition to resin are waxes and essential oils. More than 300 chemical components have already been identified in propolis. Currently, in Brazil, propolis production focuses on three main types: green, red, and brown, and this type of variation occurs according to the botanical origin and chemical composition that reflects on the biological properties and specific benefits associated with the product. Like honey, propolis also has phenolic compounds and flavonoids that are important for its therapeutic function, as well as microelements and vitamins. Several studies demonstrate that propolis has various properties: antioxidant, anti-inflammatory, antimicrobial, antiviral, immunomodulatory, and antiparasitic, among others (BERRETTA et al., 2017, PRZYBYLEK et al., 2019; RIPARI et al., 2021).

3.1.1 The polyphenols

Bee products have phenolic compounds in their composition, also called polyphenols. Its basic definition is characterized by being bioactive substances with the presence of hydroxyls linked to aromatic rings characterized by their antioxidant and anti-inflammatory potential (FURLAN, 2016). They are often found linked to compounds such as esters and glycosides, but food processing can affect their structure causing a beneficial or harmful effect on the product (ARNOSO et al., 2019).

Phenolic compounds are divided into subgroups and flavonoid compounds are frequent objects of study, mainly responsible for substances antioxidant and anti-inflammatory activity. Flavonoids are one of the most important and diverse substances present in products of plant origin (MACHADO, 2008). Its functional properties are associated with antiviral, antitumor, anti-inflammatory, antioxidant, and hormonal activities, making this polyphenol the subject of studies in the medicine and pharmacology (SANTOS et al., 2017; DA SILVA et al., 2015).

The presence of antiviral activity of flavonoids was tested by studies involving the dengue virus, Ebola, Zika and Chikungunya viruses and demonstrated a potential inhibitor of the enzymes responsible for virus replication (MISHRA et al., 2020; QIU et al., 2016; ZANDI et al., 2011; ZOU et al., 2020). In addition, some recent studies are evaluating the ability of these compounds to prevent the proliferation of the SARS-CoV-2 virus (JO et al., 2020, KHAZEEI TABARI et al., 2021).

As they are not synthesized by the body, flavonoids need to be consumed by high content foods. Bee products, such as pollen and propolis, have many flavonoids and become options to be consumed during the treatment of diseases, such as in a COVID-19 infection (BERRETTA et al., 2020; GULER e KARA, 2020; LIMA et al., 2021; ARUNG et al., 2022).

3.1.2 Vitamins

Vitamins are organic compounds, not commonly synthesized by human cells, which participate in various processes and functions within the organism. Vitamins are essential for maintaining a healthy body and have a probable potential for reducing symptoms and diseases sequelae, such as those caused by the SARS-CoV-2 virus, since a nutritional imbalance has shown being one of the causes of a deficit in the immune system. (BOMFIM e GONÇALVES, 2020). Bee products are mainly rich in vitamins of type A, complex B and C (ABEDI et al., 2021; MAGNAVACCA et al., 2021; PRZYBYLEK et al., 2019).

Vitamin A, known as retinol, participates in several bodily functions, one of which is strengthening the immune system. Retinol deficiency can affect organs such as lung tissues,

making a person more susceptible to diseases such as the SARS-CoV-2 virus (ALLEGRA et al., 2020). In turn B complex vitamins are responsible for maintaining a balance in the immune system. Vitamin B2, or riboflavin, is associated with the part of several systems such as the respiratory cycle (MALPAGA et al., 2021). Vitamin B6 has anti-inflammatory potential, and its deficiency is linked to low immunity (UELAND et al., 2017). A study carried out by Fiocruz foundation revealed the effectiveness of vitamin B12 in regulating inflammatory processes during COVID-19 infection (CASSIANO et al., 2022), while vitamin B6 showed the ability to reduce the number of severe cases of COVID-19 in a study carried out by the University of Hiroshima in Japan (KUMRUNGSEE et al., 2020). Despite their importance, the human body cannot produce enough of them and therefore depends on external means to obtain these vitamins. The B complex vitamins are easily obtainable and are primarily found in low-value foods. They are also found in products such as royal jelly, honey, propolis, and their consumption has the potential to be used to reduce the reactions generated during infection of the SARS-CoV-2 virus (OLAITAN et al., 2007; SOUZA, 2015; BEIGMOHAMMADI et al., 2020; SANTANA et al., 2021; DARAND et al., 2022).

Vitamin C, or ascorbic acid, is a water-soluble vitamin not stored by the body, requiring its replacement with greater constancy. Its oral form of consumption is associated with studies of various diseases and is responsible for part of immune modulation (CAVALARI e SANCHES, 2018). Vitamin C deficiency may be linked to increased infections mainly in the respiratory tract. Its replacement in the body is easily accessible since it is found in various foods, such as animal and vegetable foods, and bee products, such as honey. Despite not being in high concentrations, bee foods are also a source of vitamin C consumption and have the potential to be used during the treatment of COVID-19 infections (FILHO et al., 2017; KIM e YEOM, 2020, DE JESUS et al., 2021).

3.2 Therapeutic Effects

For a long time, natural products have been investigated as alternative medicines for treating infections and inflammatory diseases (PELVAN et al., 2022). Apicultural products, such as honey and propolis, are made by bees for their use, derived from plants. In addition to being used for food, these products demonstrate beneficial potential when used together in health treatments. These benefits have already been used in natural medicine by ancient peoples, such as the ancient Egyptians, Assyrians, Chinese, Greeks, and Romans, to treat illnesses (ZUMLA e LULAT, 1989).

Physical and chemical factors are responsible for cellular aging in the human body, such as the activity of free radicals, which cause pathological conditions such as cardiovascular diseases and cancer. Antioxidant agents defend the human body from free radicals. For example, there are enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, constituting an efficient defense system against oxidation. When the production of radicals exceeds the response capacity of the enzymatic system, vitamins are presented as the second line of the body defense. Vitamins C and E can eliminate and inactivate these radicals through oxidation. If both defense mechanisms are overcome, significant damage occurs at the cellular level (ABEDI et al., 2021; ZARATE et al., 2018).

Several studies demonstrate that bee products modulate the body's immune system and have antioxidant properties by inhibiting free radicals that result from cellular metabolism, collaborating with the reduction of the risk of cardiovascular diseases. In addition, they have therapeutic effects against various diseases such as diabetes, neurological and cancer diseases, among others (ABEDI et al., 2021; AL-HATAMLEH et al., 2020; GOMES et al., 2022; NAGGAR et al., 2021; PATEL, 2016).

Honey is a product capable of eliminating wound infections, suppressing the inflammatory process, and the growth of the epithelium. It also proves to be very useful in

treating chronic diseases associated with oxidative stress and inflammation. In addition, it has anticarcinogenic potential through the induction of apoptosis in several types of cancer cells by depolarization of the mitochondrial membrane. Many chemotherapeutic agents are used as apoptosis inducers, which makes honey a potent anticancer agent, in addition to the advantage of not having the side effects of these chemotherapeutic agents (AHMED e OTHMAN, 2013; HOSSAIN et al., 2020; KALEDIENE et al., 2021).

Recent studies have been done on the effects of honey in the management of obesity-related disorders, and it has been found that honey improves glycemic control and lipid profile, with consequent protection against neurodegeneration and endothelial dysfunction. As a metabolic disorder characterized by visceral adiposity that leaves the adipose tissue hypertrophic with the production of pro-inflammatory mediators, obesity is an dangerous disease. Therefore, the effect of honey as an anti-inflammatory can be an important ally in the management of disorders related to this disease (SAMAT et al., 2017; TERZO, MULE e AMATO, 2020).

Arshad, Lin, and Yahaya (2020) verified the influence of the use of honey in the brains of rats evaluating the glycemic index, the level of oxidative stress, and cognitive performance in animals. With this product, blood glucose was normalized, and serum levels of triglycerides and LDL were reduced. In addition, an effect on the level of anxiety and memory was verified, improving the learning potential and reducing anxiety levels. There was protection of neurons from damage and cell death by metabolic aggressions and oxidative stress.

It has already been demonstrated that honey is effective against several viral infectious diseases by inhibiting the entry of the virus into the host cell and its consequent replication. Honey components can also modulate signaling cascades necessary for virus replication and attachment. The reduction of acute respiratory symptoms has also been reported using honey. Recommended by the National Institute for Health and Care Excellence (NICE) as a treatment for cough caused by upper respiratory tract infection, honey can alleviate the main symptom of COVID-19 (ABEDI et al., 2021; NAGGAR et al., 2021; SULAIMAN et al., 2011).

The antiviral effect of honey has also been observed on several enveloped viruses such as HIV, influenza virus, herpes simplex, and varicella virus. Behbahani et al. (2014) and Watanabe et al. (2014) reported that honey efficiently inhibited the replication of the HIV-1 and influenza viruses, respectively. Hashemipour et al. (2014) evaluated the effects of honey on herpes simplex virus type 1 (HSV-1) in an extrasomatic environment and observed an inhibition of HSV-1 and a decrease in the viral load. Thus, honey may benefit patients with COVID-19, as SARS-CoV-2 is an enveloped virus. Kalediene et al. (2021) obtained favorable results in the evaluation of the antiviral effect of honey in vitro, with inhibition of SARS-CoV-2 dependent on the concentration of the product. With the use of honey, it is possible to improve comorbidities and antiviral responses (HOSSAIN et al., 2020).

Propolis contains a high concentration of elements that interfere with the maturation and replication of several types of viruses and reduce the exaggerated inflammatory response. The Inhibition of viral replication of strains such as poliovirus, herpes simplex, and adenovirus have been reported. Encouraging results were also obtained in animal models with a reduction of inflammatory cytokines IL-6 and TNF-α after the use of propolis, with significant immunoregulatory activity (NAGGAR et al., 2021, RIPARI et al., 2021).

The host cell receptors responsible for mediating SARS-CoV-2 infection are angiotensin-converting enzyme 2 (ACEA2) and transmembrane serine protease 2 (TMPRSS2), which imply overexpression of PAK 1, the main pathogenic kinase. The abnormal activation of this kinase can cause a wide range of disorders, such as lung inflammation, fibrosis, and immune system suppression. Propolis has components that cause inhibition of ACE2, TMPRSS2 and PAK1 signaling pathways (BERRETTA et al., 2020; MARUTA e He, 2020; WU et al., 2021).

3.3 Brazilian Bee Products

The bee products produced in Brazil are known worldwide for their biological properties and are widely accepted in the international market. Honey and propolis stand out as the most manufactured bee products in the country and come mainly from Apis mellifera bees, with a small part from native bees. Brazil is a leader in research on bee products, and many types of propolis stand out for their botanical origin. In addition, it has a favorable climate where bees collect propolis all year round. The development of research has proven the effectiveness of honey and propolis against various types of microorganisms, serving as a natural antibiotic, in addition to having anti-inflammatory, immunomodulatory properties, among others (PRZYBYLEK et al., 2019; SILVEIRA et al., 2021; VIDAL, 2021). Table 1 and Table 2 cite examples of these studies.

Table 1. Therapeutic activities found in propolis and Brazilian honeys

Type	Biological activity	References
Honey	Anti-inflammatory	Biluca et al. (2020); Silva et al. (2020)
	Antimicrobial	Ávila et al. (2019); Mendonça et al. (2020); Alcântara et al. (2021); Nascimento et al. (2021); Vieira et al. (2022)
	Antioxidant	Ávila (2019); Lopes (2019); Biluca et al. (2020); Mendonça et al. (2020); Alcântara et al. (2021); Vieira et al. (2022)
Brown Propolis	Antimicrobial	Gomes et al. (2016); Picoli et al. (2016); Sousa (2019); Vieira et al. (2022); De Brito (2022)
	Antioxidant	Cisilotto et al. (2018); Sousa, (2019); Marcucci et al. (2020); Rubinho et al. (2020); Vieira et al. (2022)
Green própolis	Antibacterial	Fiordalisi et al. (2016); Vasconcelos et al. (2019); De Lima et al. (2020): Do Nascimento et al. (2020)
	Anti-inflammatory	Santos et al. (2017); Pimentel et al. (2022)
	Antioxidant	Salgueiro et al. (2016); Zhao et al. (2016); Marcucci et al. (2020)
Red própolis	Antibacterial	Freires et al. (2016); Silva et al. (2017); Rufatto et al. (2018); Silva et al. (2018); Martins et al. (2019); Do Nascimento et al. (2019)
	Anticancer	Freires et al. (2016); Silva et al. (2018); Banzato et al. (2020)
	Anticarcinogenic	Freires et al. (2016)
	Antifungal	Freires et al. (2016); Sobreira et al. (2020), Reis et al. (2021)
	Anti-inflammatory	Freires et al. (2016); Banzato et al. (2020)
	Antioxidant	Freires et al. (2016); Silva et al. (2017); Silva et al. (2018); Marcucci et al. (2020)
	Antiproliferative	Freires et al. (2016); Squarisi et al. (2020)
	Immunomodulator	Freires et al. (2016); Santiago et al. (2023)
Propolis from *mimosa tenuiflora*	Antioxidant	Ferreira et al. (2017); Marcucci et al. (2020)

Table 2. Antiviral activities found in bee products.

Propolis	Antiviral activities	Kwon et al. (2020), Silva-Beltrán et al. (2020); Ozarowski et al. (2023)
	Anti-SARS-CoV	Miryan et al. (2020); Fiorini et al. (2021); Lima et al. (2021); Silveira et al. (2021); Hidayat et al. (2022)
	Anti-HIV	Silva et al. (2019); Mojarab et al. (2020); Triyono et al. (2021)
Honey	Anti-SARS-CoV	El Sayed et al. (2020); Hashem (2020); Tantawy (2020)

The therapeutic properties of bee products may vary according to their chemical composition and geographic origin. Its components are directly related to these properties, and many of these compounds have already been found. One of the most studied types of compounds is the phenolic compounds. Examples of compounds frequently found in bee products include caffeic acid, cinnamic acid, coumaric acid, ferulic acid, chrysin, quercetin and hesperetin. Propolis is rich in polyphenols and terpenoids, and steroids (ANDRADE et al., 2017; AVILA et al., 2019; AMARANTE et al., 2019; YEN et al., 2017).

Fischer et al. (2010) studied fractions rich in phenolic compounds from a green propolis extract as adjuvants in formulating a vaccine against swine herpesvirus and observed an increase in humoral and cellular immune responses, with a potentiation in vaccine performance. Prenylated compounds such as Artepilin C are the main substances responsible for the adjuvant activity. Amarante et al. (2019) used green propolis obtained in the São Paulo state and observed antimicrobial activity against several strains tested, emphasizing the higher concentration of coumaric and cinnamic acids.

Apitherapy is an alternative medicine practice that aims to promote health using bee products due to their therapeutic benefits. They can be used in the treatment or prevention of diseases. In 2018, apitherapy was included by the Ministry of Health in the list of practices made available in the National Policy for Integrative and Complementary Practices (PNPIC) in the Unified Health System (SUS) (Sener e Delmir, 2018; Silveira et al., 2021).

Although honey represents most exported bee products, propolis is more valuable from an economic point of view. Green propolis from Minas Gerais state and red propolis from Brazilian mangroves are certified by the National Institute of Industrial Property (INPI) with an Indication of Origin, guaranteeing that these products have specific characteristics according to their origin and are recognized internationally for them (VIDAL, 2021).

3.4 COVID-19 and Bee Products

The SARS-CoV-2 virus was responsible for the emergence of a pandemic-scale disease in 2019: COVID-19. This virus, known for presenting sets of spike proteins (S), has crown shapes that bind to the receptor proteins of human cells, making the cell replicate its RNA strands and thus producing the viral DNA (UZUNIAN, 2020). This different format in the viral protein is the main reason for the high rate of contagion of the disease since previously the viruses of the Coronaviridae family could not carry out this connection because they did not have a binding protein that connected to cell receptors (BENVENUTO et al., 2020). The mutation altered the shape of the binding proteins and made the disease responsible for high mortality rates.

This threat to global health has made scientific studies focus on understanding this disease: ways to fight the virus, reduce contagion and treat the disease. According to the World Health Organization, the SARS-CoV-2 virus is capable of causing symptoms similar to those

caused by flu and colds, but the biggest concern was related to the rate of hospitalizations and deaths due to the strong symptoms in the respiratory tract, causing pneumonia and respiratory failure. As a result, studies have turned to several fields that could present positive rates in treating symptoms of the COVID-19 disease, aiming to reduce cases of hospitalizations and deaths.

Although there are no variety of studies on the effect of bee products on the new coronavirus, the antiviral activity of these products has been reported several times. This factor served as the basis for research that addressed the use of bee products in the treatment of COVID-19, in addition to evaluating the action of its compounds as inhibitors of SARS-CoV-2 viral replication (CLEMENTI et al., 2021; PITSILLOU et al., 2021). Dilokthornsakul et al. (2022) points out that despite their great potential, the effect of honey or propolis can be different according to the variants, since SARS-CoV-2 mutates its spike protein, one of the possible targets of bee products.

The study carried out by Hashem (2020) evaluated the performance of honey and propolis active compounds in inhibiting the main protease of Sars-CoV-2, essential for processing polyproteins and virus life cycles. The study was made in silico and the cited bee products were efficient as antiviral agents, highlighting six main compounds that showed binding energy with the active site of the protease receptor, with a consequent inhibition of virus replication. It was also studied by Berretta et al. (2020) the anti-inflammatory and immunoregulatory effect of propolis by inhibiting the production of IL-1 beta in macrophage cultures, an important pathway in autoimmune diseases, raising the hypothesis that the use of propolis can minimize effects in patients with COVID-19 who have a strong inflammatory reaction resulting from the infectious process.

Silveira et al. (2021) performed a clinical study from the treatment of hospitalized adult patients with COVID-19 with standardized green propolis extract as an adjuvant therapy for 7 days. Two different dosages of propolis were used according to each group, in addition to a control group without treatment with the extract. Monitoring them for 28 days, it was observed that the hospitalization length was shorter in the groups which propolis extracts were used, and in the group with a higher dose there was a lower rate of acute kidney injury ompared to the other two. Furthermore, no adverse effects were observed and the inclusion of propolis in the treatment only resulted in clinical benefits for patients. The study by Matoso and Matoso (2021) using green propolis demonstrated that symptomatic patients whose diagnosis had confirmed the COVID-19 infection had improvements in their clinical condition with a decrease in symptoms in a short time, in addition to changes in the clinical examination that indicated a decrease in viral load.

One of the possible explanations for the improvement in the clinical picture in several studies may be linked to the potential of flavonoids present in propolis, since they have the potential to bind with the virus proteins and consequently inhibit it. Flavonoids such as quercetin and kaempferol can reach the main protein responsible for reproducing the RNA of the virus and blocking its process (Clementi et al., 2021; Pitsillou et al., 2021). Shaldam et al. (2021) evaluated the ability of bee products to prevent the functioning of main protease (Mpro) and RNA Depentend RNA Polymerase (RdRp) viral enzymes from the SARS-CoV-2 virus. The high affinity of these enzymes to phenolic compounds and terpenes honey and propolis can inhibit virus. Furthermore, the main compounds present that can carry out more promising connections in the places where COVID-19 is active were presented, with emphasis on ellagic acid, hesperetin and kaempferol in the inhibition of RdPd. In contrast, in the Mpro the more active compounds were artepelin C, ellagic acid, hesperetin, kaempferol and quercetin. Jaim et al. (2021) also corroborated these results in a study on flavonoids and their binding energy required to prevent the proliferation of the SARS-CoV-2 virus.

Pelvan et al. (2022) developed a spray with propolis and essential oils for oral use in order to help treat people infected with COVID-19 or be used prophylactically. The antiviral activity and cytotoxicity of the spray were analyzed against the severe acute respiratory syndrome of SARS-CoV-2, with a potent virus inhibitory effect and identification of 43 types of phenolic compounds, also with anti-inflammatory and analgesic activities, proving to be an important supplement for the prevention of infection.

More studies are needed to investigate the mechanism of action of honey, propolis and their constituents in inhibiting SARS-CoV-2. Besides their potential viral replication inhibitor, they can serve as a adjuvant treatment and reduce clinical symptoms. Bee products have been recommended by agencies such as the National Institute for Health and Care Excellence (NICE) and Public Health England (PHE) for treating respiratory illnesses. As it is one of the main symptoms of patients with COVID-19, these products show significant potential in treating the disease (SHALDAM et al., 2021; DILOKTHORNSAKUL et al., 2022).

3.5 Bee Products Market During the Pandemic

The apiculture is responsible for exploiting various bee products, such as honey, propolis, wax, bee pollen, royal jelly and apitoxin. As the easiest product to exploit, honey has the greatest sales possibilities (SOUZA and BENDINI, 2022). Propolis comes next as the second largest bee product traded in Brazil, however the difficulty in disclosing data on its trade is significantly greater.

During the pandemic, some beekeeping activities were negatively affected globally scale. The apicultural research has been most significantly affected across the world. Studies report a decrease in research activities such as field experiments and laboratories, a reduction in the amount of research output due to lack of data collection, as well as decreased participation of beekeepers in projects and meetings such as workshops and conferences (DALL'OLLIO et al., 2020). However, according to the scale of production and reports from the beekeepers, apicultural activities related to production did not show a drop. Lau et al. (2022) evaluated apicultural operations during the COVID-19 pandemic based on reports from beekeepers in the United States, observing the impact only on extension and research, without affecting food production.

The consumption of bee products, although still modest, has been increasing in Brazil. The COVID-19 pandemic may have contributed to boosting its sales due to the population's search for therapeutic options. To encourage the consumption of propolis by the population, the State of São Paulo launched Bill No. 328 on 05/08/2020 to exempt the payment of the Tax on Circulation of Goods and Services (ICMS) on the sale of propolis with the aim of facilitate access to the product, increase demand and consumption, strengthen the population's immune system, and help fight infections in the face of the crisis, consequently reducing the number of hospital admissions.

In an interview with Nelson Victor de Oliveira Filho, the owner of the company Fumel Comercial e Industrial Ltda and president of the Beekeeping Federation of the State of Rio de Janeiro (FAERJ), the amount paid to the beekeeper in the purchase of a kilo of honey was increased by more than 45%, when comparing December 2019 with May 2021, while raw propolis increased by 65% and propolis extract by 160%. According to the interviewee, in addition to the high demand generated during the COVID-19 crisis, the off-season also influenced the product value. Regarding the industry's sales volume, there was a significant increase, around 105% for honey and 85% for propolis extract, in quantity, when comparing the month of December 2019 with May 2021.

Regarding the world market, there was an increase in the amount of bee products exported by Brazil during the pandemic. In the years leading up to the pandemic, there was a drastic drop in the country's dollar billing in the export market of bee products, leading to a

44% decrease from 2017 to 2019 compared to the annual average. However, with the pandemic crisis, it was possible to observe a significant increase in the number of kilos exported. From 2019 to 2020, an increase of 52% was observed when considering the exported weight, while in exported value the growth was 40%. At the peak of the pandemic, from 2020 to 2021, an increase of only 3% in the exported amount was observed, while the exported value increased by 64%, demonstrating an appreciation of products that may be related to high demand. In 2022 Brazil had a 22% drop in the export quantity of bee products when compared to 2021, with the improvement of the pandemic crisis, however, the data show that the trend of the product appreciation continued (AGROSTAT, 2022; TRADEMAP, 2021).

Brazil presented a honey production around 55,828 thousand tons in 2021, obtaining a prominent position in the world ranking as the 10th largest producer. Rio Grande do Sul was the state responsible for the largest amount produced. The country also stood out in 2022 as the 4th largest honey exporter, representing 6% of world sales in exported value. The biggest importer of Brazilian honey was the United States. The value of exported honey has increased recently, with a selling price of US$3.7 per kg in 2022. The valuation on the kg of honey from 2019 to 2022 was 64%, even with the drop in the amount exported in 2022 compared to 2021. A lower supply of the domestic market can be observed with the growing honey export compared to the production, related to lower prices on domestic sales, around R$15.3 per kg, and low productivity to meet total demand. On a scale of exported bee products, honey represented 94% of the exported value by Brazil in 2022. In terms of quantity, honey represented 99%. Therefore, the other bee products were observed to have greater added value (AGROSTAT, 2022; IBGE, 2021; TRADEMAP, 2021).

Given this, there is a positive image associated with bee products by consumers, which has proven to be important for the heating up of bee products and derivatives productive sector, and should continue to bring encouraging results for the segment in the coming months. However, it should be noted that despite the nutritional character and its proven differentiated therapeutic properties, the World Health Organization (WHO) and the Ministry of Health reiterate that there is still no medicine, substance or specific food that can cure or prevent infection by the new coronavirus (AL-HATAMLEH et al., 2020; PORTAL DO GOVERNO DO ESTADO DO MATO GROSSO DO SUL).

4 CONCLUSIONS

Bee products are traditionally used because of their nutritional and medicinal values benefit the human body. With the arrival of the COVID-19 pandemic, natural alternatives are sought to strengthen the immune system and alleviate the disease's symptoms without causing side effects. There was an increase in the consumption of honey and propolis during the pandemic period, making the Brazilian market increase its sales and its commercial value. Not only sales to the domestic market but also the amount of honey exported had a significant growth. Several studies have been carried out and open new paths for using bee products as adjuvants in the treatment of various diseases, including COVID-19. However, its effects on inhibiting SARS-CoV-2 still need to be further investigated.

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CAPÍTULO II

PROPOLIS OIL EXTRACT AS AN ALTERNATIVE TO THE ALCOHOLIC EXTRACT

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ABSTRACT

Propolis is a substance with important biological activities, partly responsible for its number of bioactive compounds. It is commonly extracted with ethanol, but the resulting extract has several disadvantages. The present study aimed to evaluate the propolis extraction with canola oil as an alternative to the ethanol extract of propolis. The comparison of the extracts was made by their physical characteristics and functional properties. The oil extract showed a higher number of phenolic compounds, but a lower extraction yield. The alcoholic extract obtained an advantage in the content of flavonoids, antioxidant capacity by DPPH and FRAP. During the extracts shelf life, the alcoholic extract had a smaller loss of phenolic compounds. In the in vitro simulated gastrointestinal digestion, the oil extract had a greater release of phenolic compounds in the oral phase, while the alcoholic extract had a gradual release throughout the trial, reaching its maximum at the end of the intestinal phase. In FTIR it was possible to observe differences in the two extracts related to extracted compounds. Finally, it was observed that the propolis oil extract can be an alternative to the alcoholic extract, but it needs more studies that optimize the extraction of the bioactive compounds.

Keywords: propolis, canola oil, bioactive compounds, bioaccessibility, FTIR.

1 INTRODUCTION

Propolis is a nutritional product produced from balsamic, resinous, and gummy substances collected from plants by bees that add salivary secretions, wax, and pollen for its final elaboration. It is used by bees for its antimicrobial properties, being recognized as a natural product GRAS (Generally Recognized as Safe), for benefits brought to our body, such as antibacterial, antioxidant, antifungal activities, among others. Its effects are directly linked to its composition, which can vary according to flowering and region (FAO, 2016; Przybylek & Karpinski, 2019; Rivero et al, 2021; Aziz et al, 2022).

Propolis is produced from its extraction by a food-grade solvent, most commonly alcohol, and by an appropriate technological process, generating a liquid product of varied colors, the propolis extract. This extract is composed of soluble elements from crude propolis. There is no standard technique or time for this extraction, and the final composition of the product may vary according to the legislation of each country. To be marketed in Brazil, propolis must meet specific requirements in accordance with the Technical Regulation of Identity and Quality of Propolis Extract, which establishes the identity and minimum quality parameters that propolis extract must meet to be marketed national and internationally. However, this regulation is limited to few types of solvents used in the preparation of the extract. Depending on the solvent used, different biological activities can be found (Brasil, 2001; FAO, 2016; Pujirahayu et al, 2014; Przybylek & Karpinski, 2019).

The chemical composition of propolis is complex and represented by several constituents, and its pharmacological activity is attributed to phenolic compounds, including flavonoid compounds. These compounds are frequently studied, but their studies have been limited to ethanol extracts of propolis. Despite the numerous properties of these extracts, the presence of alcohol generates inconveniences, such as an unpleasant taste, high sensitivity to its consumption and limitations in processes. New studies have emerged in search of propolis extracts with low or zero alcohol content, and that can be used in specific applications (Carvalho et al, 2011; Kubiliene et al, 2015; Przybylek & Karpinski, 2019; Svecnjak et al, 2020).

The propolis oil extract presents itself as an alternative to the alcoholic extract because it preserves the propolis characteristics and can be used in various technological processes. The propolis extract made with canola oil is a possibility that has yet to be studied. The effectiveness of the oil in extracting the main active compounds from propolis, although not very well known, has already been demonstrated through the identification of compounds and their direct relationship with pharmacological activities (Carvalho et al, 2011; Finger et al, 2013; Kubilene et al, 2015). Despite this, no studies were found on the stability of compounds in this type of extract in a simulated gastrointestinal simulation and in the analysis of its Fourier transform infrared spectroscopy (FTIR).

The aim of this study is to evaluate the extraction efficiency of an alternative propolis extract made with canola oil compared to an alcoholic extract by determining its properties, stability of bioactive compounds, gastrointestinal release profile and infrared FTIR spectroscopy.

2 MATERIALS AND METHODS

Raw propolis samples were donated by the company Fumel Comercial e Industrial LTDA. from the state of Rio de Janeiro and stored at -180 ºC until use. The propolis used was

of the green type and obtained in the Southeast region of Brazil. The solvents used for extraction were of the brand Liza canola oil and 96º grain alcohol.

For the alcoholic extract preparation, according to the FAO methodology (2016), the propolis was cleaned with the removal of dirt and broken into pieces, to be weighed 30 g and placed in containers in contact with 70 g of refined ethyl alcohol in 70% concentration. The vials were closed and stored in the dark at room temperature and manually shaken once a day. After 10 days, the extract was filtered with Whatman qualitative filter paper.

To obtain the propolis oil extract, the methodology described by patent registration No. PI 1000615-0 A2 of 09/20/2011 was adapted. The crude propolis was ground in an industrial blender to obtain powdered propolis, which was mixed in 95% ethanol at a ratio of 2 g to 1 mL. The mixture was maintained under constant mechanical stirring for 48 hours at room temperature. After 48 hours, an emulsifier (polysorbate) was added in the proportion of 1 g for 50 g of propolis powder, and canola oil, in the proportion of 20 mL for each 15 g of propolis, gradually, in a rate of 5% of total amount per minute. Agitation was maintained for another 12 hours, then a addition of glycerides was carried out to reach a ratio of 100:15 between the extraction mixture and the propolis powder. After a new period of 12 h in constant agitation, the oil extract was filtered through filter paper.

The pH of the propolis extracts were measured in a potentiometer according to the methodology of Adolfo Lutz Institute (Brasil, 1985).

The dry extract content was quantified according to the methodology of the Official Methods for the Analysis of Products of Animal Origin of MAPA (2022). 5 mL of the samples were transferred to capsules, which were weighed, and the mass noted. After that, the capsules were placed in an oven at 102 ºC for 2 hours. They were then transferred to a desiccator and, after cooling, weighed, repeating the procedure until the weight was constant. The calculation was done as follows:

$$\text{Dry extract (g/100 mL)} = \frac{m2 - mo}{5} \cdot 100$$

Where mo is the mass of the capsule in grams; m2 is the mass of the capsule + the dried mass of the sample in grams; and 5 is the volume of the sample in milliliters.

The content of total phenolic compounds (TPC) was determined according to the methodology of Swain & Hillis (1959). The extracts were obtained by diluting 0.1g of propolis extracts in 100 mL of methanol. The samples were prepared and placed in test tubes covered with aluminum foil, where 1 mL of extract, 1 mL of Folin Ciocalteu solution (10%) and 10 mL of distilled water were added and left to rest for 3 minutes. Subsequently, 1.5 mL of Na2CO3 solution (10% p p-1) was added and homogenized in the vortex. After being kept in the dark for 2 hours, the reading was performed in a spectrophotometer at 725 nm, and the results were expressed in mg equivalent of GAE g-1 of sample, from the standard curve of calibration of gallic acid. The blank was prepared from 1 mL of water and 1 mL of Folin Ciocalteu.

The content of flavonoid compounds was determined according to the methodology by Buriol et al. (2019). For mixture analysis, 0.3 g of samples were dissolved in 100 mL of methanol. 500 uL of this solution were added to a 5 mL volumetric flask with 250 uL of aluminum chloride (5% in methanol) and the volume was later adjusted with methanol. After 30 minutes in the dark, absorbances were measured at 425 nm. For the analytical curve, methanolic solutions of concentration between 1 and 75 ug/mL of quercetin standard were prepared and treated identically to the extracts analysis.

The antioxidant capacity measured by the FRAP method (Ferric Reducing Antioxidant Power) was performed according to the methodology described by Thaipong et al. (2006). 0.1 g of extracts were diluted in 100 mL of methanol. An aliquot of 90 µL of the extract was diluted

in 270 µL of distilled water, in sealed tubes, then 2.7 mL of FRAP reagent (25 mL of 0.3 M acetate buffer + 2.5 mL of TPTZ solution 10 mM + 2.5 mL of 20 mM aqueous ferric chloride solution) previously prepared was added. The tubes were homogenized and placed in a water bath at 37° C/30 minutes, then cooled to room temperature. The reading was taken at 595 nm. As the blank, the FRAP reagent was used. The results were expressed in µg equivalents (µmol TE g-1) of sample, from the standard Trolox calibration curve.

For the analysis of the antioxidant capacity of the extract evaluated by DPPH 0,05 g of extracts were diluted in 10 mL of methanol. Antioxidant capacity was carried out according to the methodology described by Rufino et al. (2010). 2.85 mL of methanolic solution of DPPH radical (0.06 mM, whose absorbance should be close to 700 when read at 515 nm) and 150 µL of each sample were homogenized in the vortex and stored protected from light for 60 minutes. The quantification was performed in a spectrophotometer at 517 nm. As the blank, methanol was used. The results were expressed in µmol Trolox equivalent/g sample, from the standard Trolox calibration curve.

A stability study of the bioactive compounds of the extracts was carried out, in the absence of light, for a period of 60 days, with an evaluation interval of 10 days. The samples were stored in closed plastic pots at room temperature and periodically evaluated for the content of phenolic compounds, flavonoids and antioxidant capacity evaluated by DPPH and FRAP.

The methodology used to evaluate the release profile of bioactive compounds under gastrointestinal conditions follows the standardized method for simulated gastrointestinal digestion of food (Brodkorb et al, 2019). To simulate the oral phase, 1g of the sample was added to 1 mL of simulated saliva fluid for 2 minutes at 37º C, and part of the samples were preserved. For the gastric phase, the remaining part of the samples were used with the addition of 2 mL of simulated gastric fluid together with pepsin of final concentration 2000 U mL-1 and gastric lipase (60 U mL-1), with pH adjusted to 3 with HCl. After incubation at 37º C for 2 hours, the gastric digestion samples were preserved. To simulate the intestinal phase, the rest of the samples were used, and 4 mL of simulated intestinal fluid were added together with bile (10 mM bile salts) and pancreatin (trypsin activity of 100 U mL-1), with pH adjustment with NaOH to 7, and incubated for another 2 hours at 37º C. In each phase, after the incubation with rotation, the resulting samples were ultracentrifuged and filtered with Whatman filter paper and stored in glass jars protected from light until further quantification.

The spectra of propolis extracts and canola oil were evaluated by FTIR according to the methodology by Belscak-Cvitanovic et al. (2016), with adaptations. The spectroscopy in the infrared region was performed in the waveband between 4000 and 600 cm-1, using the Perkin Elmer FT-IR Spectrometer equipment with Origin software help.

The analyzes were performed in triplicate. These results were submitted to analysis of variance (ANOVA) and comparison of averages was performed by Tukey's test at the 5% level of significance.

3 RESULTS AND DISCUSSION

The propolis oil extract was studied as an alternative to alcoholic extract. The Table 1 presents the yield and pH of the propolis extracts, as well as the dry extract content of the propolis alcoholic extract.

Table 1. Characterization of propolis extracts

Analysis	PAE*	POE*
pH	5,1 ±0,04	3,7 ±0,1
Coloring	Dark brown	Yellowish brown
Dry extract (%)	12,4 ±0,2	-

*AEP – propolis alcoholic extract; OEP – propolis oil extract

The samples ranged from yellowish-brown to dark brown. The pH of the propolis oil extract was 3.7 while the alcoholic extract had an average of 5.1. These results are consistent with Pujirahayu et al. (2014), who found pH 5.4 in alcoholic samples of propolis and staining similar to the present study. The dry extract of the alcoholic sample was 12.4%. There is a minimum of 11% of dry extract required by Brazilian legislation in the product to be marketed, which must be specified on the label. This content is related to the amount of total soluble solids extracted and is directly related to the number of bioactive compounds (Brasil, 2001; Barbeira et al, 2013).

The results for the analyzes of functional compounds and antioxidant capacity of propolis extracts were presented (Table 2). The oil extract obtained 10.3 mg/g of total phenolic compounds, which is significantly higher than the amount obtained by the propolis alcoholic extract. However, the pure canola oil, which also contains phenolic compounds, was analyzed to discount the final yield value in the extracts. Therefore, only 28% of the phenolic compounds in the propolis oil extract come from crude propolis.

Table 2. Characterization of propolis extracts regarding their phenolic compounds, flavonoids and antioxidant capacity by DPPH and FRAP

Extracts	Phenolics[1]	Flavonoids [2]	FRAP[3]	DPPH[3]
Alcoholic Extract of Propolis	7,18 ± 0,06	8,4 ± 0,20	28,98 ± 0,62	14,29 ± 0,09
Propolis oil extract	10,31* ± 0,30	0,23 ± 0,00	15,39 ± 1,00	0,68 ± 0,68

7.33 phenolic compounds from canola oil + 2.98 phenolics compounds from propolis; [1]mg of gallic acid/g of sample; [2]mg of quercetin/g of sample; [3]mmol of trolox/g of sample.

The chemical content of propolis depends on its origin, the type of bee producer and the seasonality of collection. The concentration of propolis in the extract is also a factor that directly influences the number of functional compounds. Other factors can influence, such as the type of solvent and the extraction process, as well as your experimental variables. The solvent selection is generally selected by the end use purpose of the extract (Carvalho et al, 2011; Finger et al, 2013; FAO, 2016). Buriol et al. (2009) observed that when extracting propolis compounds with canola oil, the resulting extract was related to the extraction time variable, and the increase in yield was proportional to the number of days the liquid was in contact with the solvent. After 90 days of extraction, the values of phenolic compounds and flavonoids in the oil extract had a significant increase, approaching the values of the compared alcoholic extract. Kubilene et al. (2015) compared extracts of propolis extracted with oil with the traditional alcoholic extract and did not find significant differences in the content of phenolic compounds in predefined concentrations. In addition, the oil extract had an advantage in inhibiting the growth of several microorganisms tested and by the HPLC method it was possible to observe different flavonoids extracted due to the lower polarity of the oil. The great diversity of extraction methods used in biological assays makes it difficult to compare results. There is no consensus standard for the

calibration curve, in addition to a lack of regulation on standardized procedures (Pujirahayu et al, 2014; Osés et al, 2020).

The flavonoid compounds in the present study obtained a lower extraction yield in the oil extract. Oldoni et al. (2015) analyzed samples of propolis alcoholic extract and found lower values, with 2.4 to 5.7 mg/g of phenolic compounds and 1.8 mg/g of flavonoids. Pujirahayu et al. (2014) found low amounts of flavonoids in propolis oil extract when compared with the traditional alcoholic extract. The difference is probably due to the properties of ethanol, which is an organic solvent capable of dissolving most of the propolis contents. Furthermore, the oil is little used for extraction of flavonoid compounds, although it is a solvent capable of extracting some types of non-polar flavonoids (Pujirahayu et al, 2014). Schmidt et al. (2014) observed that vegetable oils were not efficient in extracting large amounts of flavonoids from propolis, when comparing extracts made with canola oil and alcoholic extracts, however oil extracts were efficient in inhibiting a sarcoma, indicating that there may be other compounds responsible for antitumor activity. Carvalho et al. (2011) obtained propolis extracts from canola oil and identified several phenolic compounds such as flavonoids and phenolic acids, demonstrating that the vegetable oil was able to extract important natural compounds, including those responsible for antitumor activities.

The DPPH free radical scavenging method is one of the most popular techniques for assessing antioxidant capacity by radical scavenging. The alcoholic extract of propolis showed significantly higher values in antioxidant capacity by DPPH. These results corroborate those of Kubilene et al. (2015), who evaluated alcoholic, aqueous and oil extracts of propolis and observed that the alcoholic extract had a higher antioxidant capacity by DPPH than the others. Regarding the antioxidant capacity by FRAP, a method based on the reduction of Fe3+ to Fe2+, the alcoholic extract showed an advantage, with activity 1.8 times greater than the oil extract. The values of the present study were higher than those found by Morais et al. (2021), in ethanolic extracts also from the Brazilian region.

There are few data on antioxidant activity in oil extracts of propolis, such as the extract made with canola oil. In addition, data comparison between propolis studies is hampered by the lack of harmonization of analytical extraction procedures and common reference standards, in addition to the lack of a common expression of results. Published data are insufficient to estimate the actual antioxidant activity of propolis. When comparing the extracts in the present study, the lower antioxidant capacity of the propolis oil extract may be related to the absence of some bioactive compounds, such as flavonoids, which are considered one of the main components responsible for the antioxidant potential of the products. Another factor that can influence the antioxidant activity is the concentration of the solvent. According to Osés et al. (2020), the higher the ethanol concentration, the greater the anti-radical activity of propolis extracts, which can be affected by the pH of the solvent used (Aziz et al, 2022). Finally, oxidation is a complex process with different mechanisms, and there is no exclusive method capable of determining the total antioxidant capacity of extracts (Asem et al, 2019).

A study was carried out to analyze the stability of phenolic compounds, flavonoids, and maintenance of the antioxidant capacity of propolis extracts for 60 days (Figure 1). The propolis oil extract had a significant loss of phenolic compounds after 40 days. At the end of the 60 days, losses totaled 10%. Already the alcoholic extract of propolis showed a loss of 3% at the end of the same period. The content of flavonoid compounds showed a greater loss in the alcoholic extract of propolis at the end of 60 days, around 24%, while in the oil extract the loss was only 12%. Regarding the antioxidant activity by DPPH, there was a significant loss of 27% for the oil extract of propolis. As for the alcoholic extract, there was a significant loss already at 30 days of storage, with a total of 20% after 60 days. For the antioxidant capacity by FRAP, the loss in the oil extract was 29%. The alcoholic extract had only 8% of loss in the same period studied, not being significant.

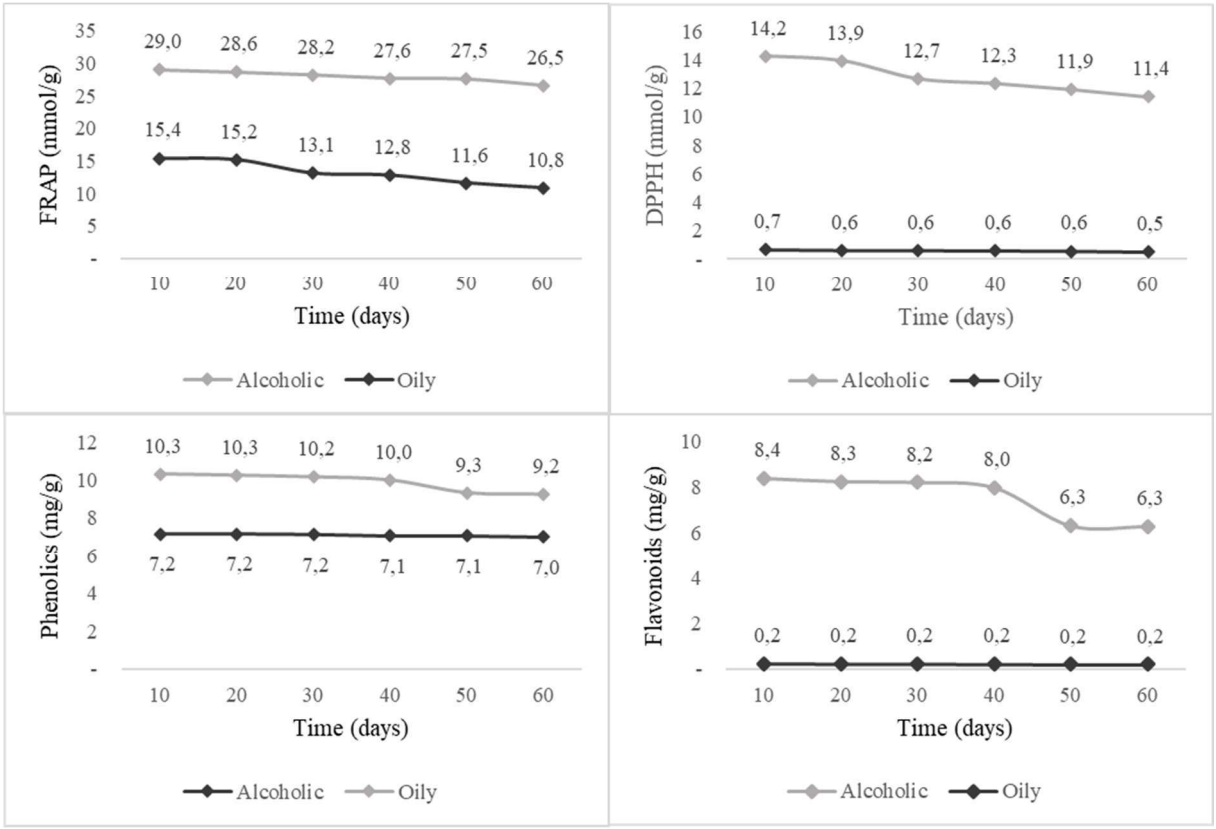

Figure 1. Evaluation of the stability of phenolic compounds and the antioxidant capacity of propolis extracts over a period of 60 days.

Flavonoids and phenolic compounds are important in propolis for their ability to scavenge free radicals, in addition to protecting lipids and vitamins from being destroyed in the oxidative process. However, these compounds are highly sensitive to external factors such as light, oxygen and temperature, and can be degraded during extraction or storage (Moura et al, 2018; Zarate et al, 2018). In general, propolis is quite stable, and factors such as proper storage after extraction are important to protect its compounds. Studies show that over 12 months in proper storage, propolis maintains its antibacterial activities, and that alcoholic extracts can be stored longer (FAO, 2016; Hagar et al, 2021). According to Kawakita et al (2015), when observing the stability of phenolic compounds in ethanolic extracts of propolis during a period of 7 months under certain conditions, there was not a significant loss, and its antibacterial activity was preserved. No data were found on the stability of active compounds in propolis oil extract.

The antioxidant activity of propolis is linked to the synergistic effect of phenolic compounds and non-phenolic antioxidant substances. Therefore, there may be a direct relationship between the antioxidant capacity of extracts and their number of active compounds, as well as their losses (Osés et al, 2020). Extrinsic factors are responsible for affecting the stability of compounds in storage, and consequently their antioxidant activity. Variations in time and temperature conditions must be controlled to protect antioxidant compounds, preventing their loss or decomposition (Hagar et al, 2021).

The digestion of propolis extracts in oral, gastric and intestinal phases was evaluated (Figure 2).

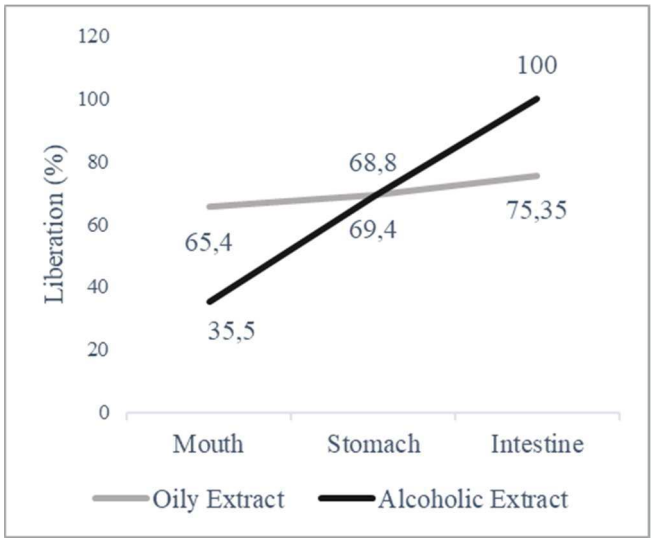

Figure 2. The release of propolis extracts in simulated gastrointestinal system

The propolis oil extract released approximately 65% of phenolic compounds in the oral phase and 4% in the gastric phase, while in the intestinal phase the release was less than 6% during the analysis period. In the alcoholic extract of propolis, the release was approximately 35% in the mouth and 33% in the stomach, while in the intestine it was 33%, reaching the maximum release of the phenolic compounds available in the extract at the end of the analysis period. A greater bioaccessibility of the propolis oil extract occurred in the oral phase, while in the gastric and intestinal phases it declined considerably, and at the end of the analysis period a complete release of the available phenolic compounds was not observed. Zhang et al. (2018) analyzed samples of free propolis and observed that only 33% of its compounds diffused in the simulated gastrointestinal tract within 6 hours of analysis. Yen et al. (2017) analyzed propolis extracts produced with different solvents (ethanol, glycerol, and water) and observed that the maximum amount of total phenolics was observed in the oral digestive phase, followed by the intestinal phase, and in smaller amounts in the gastric phase. In addition, the oral digestive product showed better antioxidant activity, attributing the greater bioaccessibility of the compounds in this phase.

The alcoholic extract did not obtain a significant difference in the release of phenolic compounds in the analyzed digestion phases, being gradually released throughout the test. At the end of the review period, all its content had been released. When analyzing the in vitro simulated gastrointestinal digestion of ethanolic samples of propolis obtained by maceration, González-Montiel et al. (2022) also found no significant difference in the release of phenolic compounds in the gastric and intestinal phases. Ozdal et al. (2019) observed an increase in bioaccessibility in the gastric and intestinal phases, when compared to the oral phase, in propolis samples from different sources, with the highest values achieved at the end of the intestinal phase. In addition, release variations were observed between the analyzed samples, concluding that the content and profile of phenolic compounds can influence the bioaccessibility of the product.

FTIR is an infrared spectroscopy technique used to analyze functional groups corresponding to the chemical bonds of constituents present in the sample. The propolis spectra can be complex according to its chemical composition that varies significantly depending on its origin, in addition to the extraction technique and solvent used. The identification of absorption bands and allocation of functional groups is varied due to the large number of organic compounds and molecular vibrations (Abdullah et al, 2020; Svecnjak et al, 2020). The present study analyzed the alcoholic extract of propolis, as well as the oil extract and its

respective solvent, to identify possible correlations between them (Figure 3). The samples showed different vibrational spectra at certain points, which can be attributed to different compounds. It was possible to observe that the propolis samples presented divergent spectra in the region between 3500 and 2850 cm^{-1}.

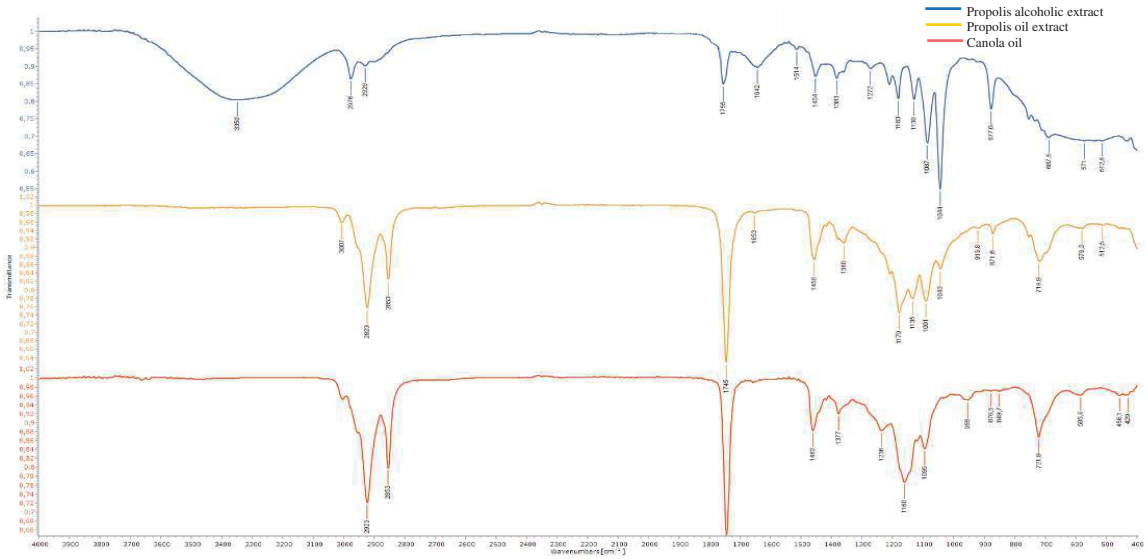

Figure 3. FTIR spectra of propolis and canola oil extracts

In the alcoholic extract of propolis, a broad band peaking at 3350 cm^{-1} was observed, resulting from the stretching of the OH groups, such as phenolic compounds, or due to the presence of moisture in propolis, which could not be observed in the oil extract. The course between 2976 and 2929 cm^{-1} is derived from methyl groups with asymmetric and symmetric CH2 elongations (Hajinezhad et al, 2019; Razavizadeh & Niazmand, 2020). The band at 1755cm^{-1}, originating from C=O bonds of carboxylic acids, can be observed at a lower intensity when compared to the oil extract and canola oil. A wider band can be observed in the alcoholic extract, with a peak at 1642 cm^{-1}, linked to the C=C stretch of flavonoids. This band appears at a lower intensity in the oil extract. According to Mot et al. (2011), the band in this range is directly related to the antioxidant capacity of the extract. A slight band at 1515 cm^{-1} of the samples is related to vibrations of C-C bonds and elongation of aromatic rings and flavonoids, appearing more prominently in the alcoholic extract compared to the oil extract, while in the oil it is absent (Li et al, 2021). According to Mot et al. (2011) this band is specific for propolis extracts, despite having a low correlation with antioxidant activity. The most prominent band at 1087 cm^{-1} in the alcoholic extract may be related to aromatic rings. The band at 1044 is very prominent in the alcoholic extract and has little intensity in the oil extract, corresponding to the elongation of the C-O-C ether of the pyranose ring. The band at 877 cm^{-1} corresponding to the off-plain angular deformation of the aromatic C-H bond is more intense in the alcoholic extract when compared to the oil extract. A slight band at 687 cm^{-1} of the alcoholic extract is related to OH bonds of phenolic groups and alcohols (Hajinezhad et al, 2019; Abdullah et al, 2020; Razavizadeh & Niazmand, 2020; Nascimento et al, 2022).

The spectrum from the oil extract of propolis presents bands similar to the spectrum of canola oil, since its composition is predominantly oil. The peaks in the 3007 and 2853 cm^{-1} ranges have bands with similar intensity in both samples. In the spectrum of the propolis oil extract, two large peaks at 2923 and 1745 cm^{-1} are also present in canola oil and are attributed to the presence of CH2 and C=O elongations, respectively. Moura et al. (2018) analyzed canola oil spectra and found similar peaks at 2924 and 1743 cm^{-1}. The band with peaks at 1458 cm^{-1}

in canola oil is also present in the other samples, but to a lesser extent in the alcoholic extract, and is related to bending vibrations of C-H groups and aromatic elongation, attributed to flavonoid compounds (Hajinezhad et al, 2019; Razavizadeh & Niazmand, 2020).

In the range between 1454 and 1044 cm-1 it is possible to observe differences between all samples, with shifting of peaks and changes in intensity. The peak at 1383 cm^{-1} in the alcoholic extract, related to C-O bonds of carboxylic acids, is shifted to 1360 cm^{-1} in the oil extract (Abdullah et al, 2020). The band present at 1183 cm^{-1}, attributed to the stretch of the C-O-C bond, is prominent in propolis extracts. The bands at 1130 and 1135 cm-1, present in alcoholic and oil extracts respectively, are not present in canola oil, and come from C-O ester bonds. A broad band peaking at 1160 cm^{-1} in canola oil is related to C=C alkenes binding (Hegazi et al, 2019). At 721 cm^{-1}, canola oil presents an intense band that is also present in the propolis oil extract. In this absorption range, deformation of the OH group of phenols may occur (Svecnjak et al, 2020).

The propolis oil extract showed characteristic bands of a canola oil spectral profile. However, it was possible to observe changes in intensity, as well as displacements of some bands. Some bands were present in the spectrum of alcoholic extract and oil extract, but not in canola oil, suggesting that these compounds come from extracted propolis.

4 CONCLUSIONS

1. The content of phenolic compounds is higher in the propolis oil extract, while the levels of flavonoids and the antioxidant capacity by DPPH and FRAP are lower than those of the alcoholic extract.

2. In the stability study of bioactive compounds in the propolis oil extract, the loss of flavonoid compounds is smaller when compared to the loss of the alcoholic extract.

3. During simulated gastrointestinal digestion, the oil extract shows a greater release of phenolic compounds in the oral phase, while the alcoholic extract shows similar release in the oral, gastric, and intestinal phases.

4. In the FTIR analysis, differences are observed in the propolis extracts related to the different compounds extracted according to the type of solvent.

5. The results demonstrate the feasibility of oil extraction, but also the need for further studies on formulations and processes to obtain greater extraction yields of bioactive substances, in addition to a more detailed chemical study to identify different substances that can be extracted from the oil, and which are responsible for the biological activities of propolis.

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CAPÍTULO III

PARTICLES CHARACTERIZATION INCORPORATED WITH PROPOLIS AND ROYAL JELLY OBTAINED BY IONIC GELATION

Enviado para: Carpathian of Food Science and Technology

ABSTRACT

Bee products are foods that have a significant amount of functional compounds, which protect against oxidative damage, but are easily degraded. Ionic gelation is an encapsulation technique used to protect these compounds. The objective of this work was to encapsulate propolis and royal jelly by ionic gelation and to evaluate its yield, the particles stability and *in vitro* release profile. The mixture composed of propolis and royal jelly contained 11.7 mg/g of phenolic compounds, 0.2 mg/g of flavonoid compounds and respectively 2027.1 and 2202.3 ug/g of antioxidant capacity by DPPH and FRAP. The particles were tested in different proportions of pectin:mixture and the proportion 80:20 pectin:mixture was selected for characterization with 92.5% of encapsulation efficiency of phenolic compounds and 78.7 and 36.6% of maintenance of the antioxidant capacity by DPPH and FRAP, respectively. In the storage stability study, the particles showed a lower loss of phenolic compounds and antioxidant capacity by DPPH when stored under refrigeration, compared to the non-encapsulated mixture. The particles showed an irregular shape and sizes predominantly between 1 and 1.2 mm. During the simulation in the gastrointestinal tract, a greater bioaccessibility of phenolic compounds in the particles was observed in relation to the free mixture. The FTIR analysis confirmed the encapsulation of the propolis oil extract in the pectin particle. Finally, it was observed that the ionic gelation process produced stable particles, with efficient maintenance of antioxidant capacity and better bioaccessibility when compared to the free extract.
Keywords: Bee products, antioxidants, phenolics, ionic gelation.

1 INTRODUCTION

Microencapsulation is a process used to protect sensitive compounds in which microparticles are formed by an encapsulating material that covers the active ingredient and protects against adverse environmental conditions, generating greater stability, with increased shelf life and the controlled encapsulated release under established conditions. One of the mild and simple encapsulation techniques is ionic gelation, which relies on the ability of anionic polysaccharides to form gels in the presence of ions such as calcium. This method aims to obtain reasonable levels of the active encapsulated and particles of different shapes and sizes, without the need to use organic solvents or extremes temperature and pH, and it has a low cost compared to other techniques. One of its intrinsic characteristics is the porosity of the obtained matrix, which can determine the release of the encapsulated substance (ASSADPOUR and JAFARI, 2019; LI et al., 2021; NEMATI et al., 2019; WISSAM and SAMER, 2019).

Several bioactive compounds can be encapsulated in the matrix. These have been arousing interest in the industry, scientific community and society, as these are substances with biological potential. Bee-derived products are foods commonly marketed with an appeal to improve human health, and have a considerable content of bioactive compounds, especially antioxidants, being widely used as a functional food supplement. Propolis is astringent and formed mainly by resins and plant balms. Its extraction is commonly done with alcohol, but its resulting extract has some disadvantages such as a strong aftertaste and limitations of application in the industry. Propolis oil extract has been studied as an alternative to alcoholic extract. Royal jelly, on the other hand, is a gelatinous product manufactured by the secretion of the hypopharyngeal glands of bees, rich in vitamins and antioxidant compounds and with broad bioactivity. Bee products are often used mixed because they produce functional synergistic effects (CEBI et al., 2020; CHEN et al., 2022; ETCHEPARE et al., 2015; KUBILIENE et al., 2015; OSÉS et al., 2017).

In addition to containing significant functional compounds, bee-derived foods are studied for their antimicrobial, anti-inflammatory and anticancer activities. These activities are often attributed to the presence of specific properties of the products, such as pH and osmolarity, and the presence of specific antioxidant compounds, such as phenolic compounds and flavonoids. When ingested, these compounds need to reach their action place, but they are sensitive to several factors such as temperature and light, due to their alternating double bonds, which can generate a high rate of metabolism and rapid elimination from the human body. Therefore, its effectiveness depends directly on bioavailability, stability and bioactivity, and its use in encapsulated form can minimize problems or maximize its effectiveness (ETCHEPARE et al., 2015; KAVUMARCI et al., 2019; TAN et al., 2021).

The aim of this study was to encapsulate propolis oil extract and royal jelly, using the ionic gelation method, characterize the particles obtained and evaluate them in relation to stability and release profile by in vitro simulated gastrointestinal digestion.

2. MATERIALS AND METHODS

2.1. Materials

The Propolis and royal jelly samples were donated by the company Fumel Comercial e Industrial from Rio de Janeiro state and was stored at -180º C until use. The pectin used was of low methoxylation (DuPont Danisco).

2.2. Methods

To obtain the propolis oil extract, the methodology described by patent registration No. PI 1000615-0 A2 of 09/20/2011 was adapted. The crude propolis was ground in an industrial blender to obtain powdered propolis, which was mixed in 95% ethanol at a ratio of 2 g to 1 mL. The mixture was maintained under constant mechanical stirring for 48 hours at room temperature. After 48 hours, an emulsifier (polysorbate) was added in the proportion of 1 g for 50 g of propolis powder, and canola oil, in the proportion of 20 mL for each 15 g of propolis, gradually, in a rate of 5% of total amount per minute. Agitation was maintained for another 12 hours, then an addition of glycerides was carried out to reach a ratio of 100:15 between the extraction mixture and the propolis powder. After a new period of 12 h in constant agitation, the oil extract was filtered through filter paper.

2.2.1. Preparation of the material to be encapsulated

Royal jelly was added to 0.5 g in 100 g of propolis oil extract and kept under stirring for one hour in a magnetic stirrer. The resulting extract was called a mixture.

2.2.2. pH

The pH of the propolis oil extract and royal jelly was measured using an Instrutemp brand pH meter according to the methodology of Adolfo Lutz Institute (IAL, 1985).

2.2.3. Moisture

The moisture of royal jelly was determined according to the methodology of the Adolf Lutz Institute (IAL, 1985).

2.2.4. Particle production

The particles were produced with the ionic gelation method by dripping, according to the methodology described by Skjak-braek et al. (1989) with modifications. The mixture was added to a low methoxylation pectin solution, previously solubilized in water (4%), and kept under agitation for one hour in a magnetic stirrer. The pectin: compound mass proportions were defined according to preliminary tests (60:40, 70:30, 80:20, 90:10). Each proportion was dropped into a 2% calcium chloride solution. For dripping, a sterile PVC probe with a smooth internal diameter of 2 mm and opening of 0.15 mm was used, coupled to a peristaltic dosing pump. A control particle was made without the mixture, only with pectin. After 30 min in the calcium solution, the formed particles were filtered and washed with distilled water. The process was performed in duplicate.

The dependent variables to evaluate the most suitable pectin: compound mass proportions were moisture and encapsulation efficiency. In addition, the yield of the process was evaluated by the ratio between the amount of particles produced and the amount of pectin: compound mixture used. The amount of compound pectin mixture processed was determined by the difference in bottle weight before and after processing.

2.2.5. The particle moisture

The particles moisture was determined gravimetrically by drying at 70º C, in triplicate (AOAC, 1996).

2.2.6. Particle dissolution for bioactive analysis

The particles were broken for the analysis of total phenolic compounds and antioxidant capacity. Glass tubes with EDTA (0.2 M) were used to dilute the particles (1:100), with vortex agitation for 5 minutes. The samples were transferred to glass jars and stored with protection from light until quantification.

2.2.7. Total phenolic compounds

The amount of total phenolic compounds was measured in the initial mixture (propolis extract and royal jelly) and in the obtained particles. Before analysis, 0.1g of the mixture was diluted in 100 mL of methanol. The particles were dissolved as mentioned above.

The content of total phenolic compounds (TPC) was determined according to the methodology of Swain and Hillis (1959). The samples were prepared and placed in test tubes covered with aluminum foil, where 1 mL of extract, 1 mL of Folin Ciocalteu solution (10%) and 10 mL of distilled water were added and left to rest for 3 minutes. Subsequently, 1.5 mL of Na2CO3 solution (10% p p-1) was added and homogenized in the vortex. After being kept in the dark for 2 hours, the reading was performed in a spectrophotometer at 725 nm, and the results were expressed in mg equivalent of GAE g-1 of sample, from the standard curve of calibration of gallic acid. The blank was prepared from 1 mL of water and 1 mL of Folin Ciocalteu.

2.2.8. Flavonoids

The content of flavonoid compounds was determined in the initial mixture and in the obtained particles according to the methodology by Buriol et al. (2019). For mixture analysis, 0.1 g of samples were dissolved in 10 mL of methanol. 500 uL of this solution were added to a 5 mL volumetric flask with 250 uL of aluminum chloride (5% in methanol) and the volume was later adjusted with methanol. After 30 minutes in the dark, absorbances were measured at 425 nm. For the analytical curve, methanolic solutions of concentration between 1 and 75 ug mL^{-1} of quercetin standard were prepared and treated identically to the analysis of the mixture.

2.2.9. Encapsulation Efficiency (EE)

The EE was calculated by the amount of active compounds retained in the particles, according to Equation 1:

$$EE\ (\%) = \frac{\frac{\text{mg of asset in the particle}}{\text{100 g of microparticle}}}{\frac{\text{100 mg of active added in the mixture}}{\text{100 g of mixture}}} \times 100$$

2.2.10. Antioxidant capacity by DPPH

Particles selected for best yield according to their encapsulation efficiency were used for the rest of the analyses. The initial mixture was used for further comparison. First, 0.04g of mixture was diluted in 100 mL of methanol. The particles were broken according to the methodology described above. Antioxidant capacity was carried out according to the methodology described by Rufino et al. (2010). 2.85 mL of methanolic solution of DPPH radical (0.06 mM, whose absorbance should be close to 700 when read at 515 nm) and 150 µL of each sample were homogenized in the vortex and stored protected from light for 60 minutes. The quantification was performed in a spectrophotometer at 517 nm. As the blank, methanol was used. The results were expressed in µmol Trolox equivalent/g sample, from the standard Trolox calibration curve.

2.2.11. The antioxidant capacity by FRAP

The antioxidant capacity measured by the FRAP method (Ferric Reducing Antioxidant Power) was performed according to the methodology described by Thaipong et al. (2006). 0.2 g of mixture was diluted in 10 mL of methanol and the particles were broken according to the methodology described in DPPH. An aliquot of 90 µL of the extract was diluted in 270 µL of

distilled water, in sealed tubes, then 2.7 mL of FRAP reagent (25 mL of 0.3 M acetate buffer + 2.5 mL of TPTZ solution 10 mM + 2.5 mL of 20 mM aqueous ferric chloride solution) previously prepared was added. The tubes were homogenized and placed in a water bath at 37° C/30 minutes, then cooled to room temperature. The reading was taken at 595 nm. As the blank, the FRAP reagent was used. The results were expressed in µg equivalents (µmol TE g-1) of sample, from the standard Trolox calibration curve.

2.2.12. From storage to stability study

A stability study of the bioactive compounds contained in the particles obtained by encapsulation process was carried out, at two temperatures (5º and 25° C) and in the absence of light, for a period of 60 days, with an evaluation interval of 12 days. A study was also carried out on the stability of bioactive compounds in the mixture (royal jelly + propolis) for later comparison. The samples were stored in closed plastic pots. The choice of temperatures is based on the normal commercialization temperature of the samples and on the interest in verifying whether the refrigeration temperature can alter the stability. The samples were periodically evaluated for the content of phenolic compounds and antioxidant capacity.

2.2.13. Study of the release profile in simulated gastric and intestinal conditions

The methodology used to evaluate the release profile of bioactive compounds under gastrointestinal conditions follows the standardized method for simulated gastrointestinal digestion of food (BRODKORB et al, 2019). To simulate the oral phase, 1g of the sample was added to 1 mL of simulated saliva fluid for 2 minutes at 37º C, and part of the samples were preserved. For the gastric phase, the remaining part of the samples were used with the addition of 2 mL of simulated gastric fluid together with pepsin of final concentration 2000 U mL-1 and gastric lipase (60 U mL-1), with pH adjusted to 3 with HCl. After incubation at 37º C for 2 hours, the gastric digestion samples were preserved. To simulate the intestinal phase, the rest of the samples were used and 4 mL of simulated intestinal fluid were added together with bile (10 mM bile salts) and pancreatin (trypsin activity of 100 U mL-1), with pH adjustment with NaOH to 7, and incubated for another 2 hours at 37º C. In each phase, after the incubation with rotation, the resulting samples were ultracentrifuged and filtered with Whatman filter paper, and stored in glass jars protected from light until further quantification.

2.2.14. Microstructure

The particles were analyzed using an optical microscope (Biofocus - BIO1600BA-L-BI) equipped with a camera, using DinoCapture software version 1.4.2.D. The diameter measurement of 60 particles was determined for the size distribution, from a millimeter ruler according to the scale of the generated photo.

2.2.15. Fourier transform infrared spectroscopy (FT-IR)

The individual components and particles spectra were evaluated according to the methodology of Belscak-Cvitanovic et al. (2016), with adaptations. To observe the compounds encapsulation and the presence of wall materials in the particles, the spectroscopy in the infrared region was performed in the waveband between 4000 and 600 cm-1, using the Perkin Elmer FT-IR Spectrometer equipment with Origin software help.

2.2.16. Statistical analysis

The aforementioned analyzes were performed in triplicate. These results were submitted to analysis of variance (ANOVA) and comparison of averages was performed by Tukey's test at the 5% level of significance.

3 RESULTS AND DISCUSSIONS

3.1. Characterization of Raw Materials in Terms of Yield, Moisture and pH

Propolis oil extract was studied as an alcoholic extract alternative. The Table 1 shows the pH of the propolis oil extract and royal jelly, as well as the moisture of royal jelly.

Table 1. Characterization of propolis oil extract (POE) and royal jelly

Analysis	Royal jelly	POE
Moisture (%)	65.4±0.05	-
pH	3.1±0.02	3.7±0.03

The royal jelly moisture is an important factor to analyze the extract quality that will be used in the mixture. The results obtained are in agreement with Balkanska, Zhelyazkova and Ignatova (2012), who analyzed royal jelly samples from different regions and found similar moisture values, ranging from 59.1 to 65.8%, while the pH ranged from 3.6 to 4.6.

3.2. Mixture Characterization

The royal jelly was used in a proportion of 0.5% in relation to the propolis oil extract, being termed as mixture, and presented the results according to Table 2.

Table 2. Mixture characterization regarding its phenolic compounds and antioxidant capacity

Analysis	Mixture
Phenolics*	11.7±0.16
Flavonoids**	0.2±0.0
DPPH***	2027.1±137.5
FRAP***	2202.3±157.6

*mg of gallic acid g-1 sample. **mg g-1 sample. ***umol of Trolox g-1 sample

The amount of phenolic compounds found in the mixture is similar to reported by Silici and Baysa (2020), who when extracting propolis with oil at different concentrations, achieving values from 6.3 to 20.6 mg g^{-1}, emphasizing that the chemical content of the propolis depends on its origin and extraction methods.

Schmidt et al. (2014) analyzed propolis oil extract from canola oil and found 8.2 mg g-1 of flavonoids in dry extract and 58.01 mg L^{-1} of antioxidant activity by DPPH. There are few data on antioxidant activity in propolis oil extract and its mixtures, in addition to the great diversity in extraction and analysis methods that make comparison difficult.

3.3. Particles Production of the Ionic Gelation Method

The particles produced at different pectin:mixture ratios were evaluated in relation to moisture and encapsulation efficiency, the results are shown in Table 3.

Table 3. Particles in different proportions of pectin:mixture in relation to moisture and encapsulation efficiency

Proportions	90:10	80:20	70:30	60:40
Moisture (%)	89.8 ± 1.0^{a}	84.9 ± 0.2^{b}	82.0 ± 0.7^{c}	77.7 ± 0.1^{d}
Encapsulation efficiency (%)	83.1 ± 3.9^{a}	92.3 ± 0.4^{b}	68.2 ± 1.1^{c}	54.1 ± 3.9^{d}

*Same letters represent that there was no significant difference between the averages, with 95% confidence (in line).

The particles moisture showed a statistically significant difference between the samples, inversely proportional to their amount of extract. Moura et al. (2018) found values close to the present work in anthocyanin encapsulated with pectin by ionic gelation, with approximately 83% of moisture. The analysis of this parameter is an important factor to be evaluated since the particles content can be affected due to the hydrophilic nature of the encapsulating material (OTÁLORA et al, 2018).

The encapsulation efficiencies (EE) ranged from 54.1 to 92.3%, being the highest in particles with an 80:20 proportion. The encapsulation efficiency demonstrated a significant improvement with increasing concentration of the pectin solution, peaking at an 80:20 ratio. However, beyond this point, there was a decrease in encapsulation efficiency. The reason may be attributed to the size and morphology of the particles, as well as the volume of encapsulating material in the sample. In addition to the material properties, which influence the encapsulation process by the types of molecular associations and interactions between polymers and active compounds. According to Castañón-Rodriguez et al. (2019), when encapsulating orange juice, the amount of soluble solids and the pH affected the particles stability by favoring the interactions of pectin molecules. During encapsulation, the pH of the aqueous phase affects the ionization and solubility of the active compound.

The emulsion stability can also influence the encapsulation efficiency, according to Wissam and Samer (2019), when encapsulating linseed oil with alginate, they observed an improvement in stability according to the increase in the concentration of the encapsulating material, causing a greater degree of crosslinking and higher charging efficiency. The values found in the present study were higher than those of Moura et al. (2018), by encapsulating anthocyanins with a pectin matrix by ionic gelation. The values were also higher than those of Zhang et al. (2019), who encapsulated propolis by complexing alginate with zein and obtained an encapsulation efficiency of up to 86.5%, directly related to the different techniques used, proportion of materials and particle size.

3.4. Selected Particles Characterization

Particles in the same proportion of 80:20 (pectin: mixture) were prepared for further characterization. Table 4 presents the results of the content of bioactive compounds and antioxidant capacity of the particles and the encapsulation efficiency of each one.

Table 4. Bioactive compounds characterization and antioxidant capacity of selected 80:20 particles

Analysis	Content	EE (%)[a]
Phenolics[b]	2.2±0.0	90.6±0.0
DPPH[c]	702.6±22.4	78.7±4.8
FRAP[c]	160.9±9.3	36.6±4.1

[a]EE – encapsulation efficiency. [b]mg of gallic acid g-1 sample. [c]umol of Trolox g-1 sample.

The particles obtained an encapsulation efficiency of 90.6% in relation to the phenolic compounds. Losses may occur during processing and subsequent preparation for analysis, due to the sensitivity of these compounds to external factors such as oxygen and light. The values found in the work are higher than those of Belscak-Cvitanovic et al. (2016), who encapsulated dandelion extract with both pectin and alginate and found up to 77.3% efficiency in compound retention, associating it with physical-chemical factors during encapsulation, such as the types of interactions between active compounds and carrier polymers.

Regarding the values found for antioxidant capacity maintenance, there was a variation between the analyzes performed of 78.7% for DPPH and 36.6% for FRAP. The values are

similar to the values found by Andrade et al. (2018), when encapsulating alcoholic extracts of propolis by spray drying, found FRAP values ranging from 144.87 to 396.09 ug g-1.

3.5. From Storage to Stability Study

A study was carried out using the particles and the pure mixture, aiming to analyze the phenolic compounds stability and the antioxidant capacity maintenance, at two temperatures (5º and 25º C), for 60 days. Figure 1, Figure 2 and Figure 3 show the results.

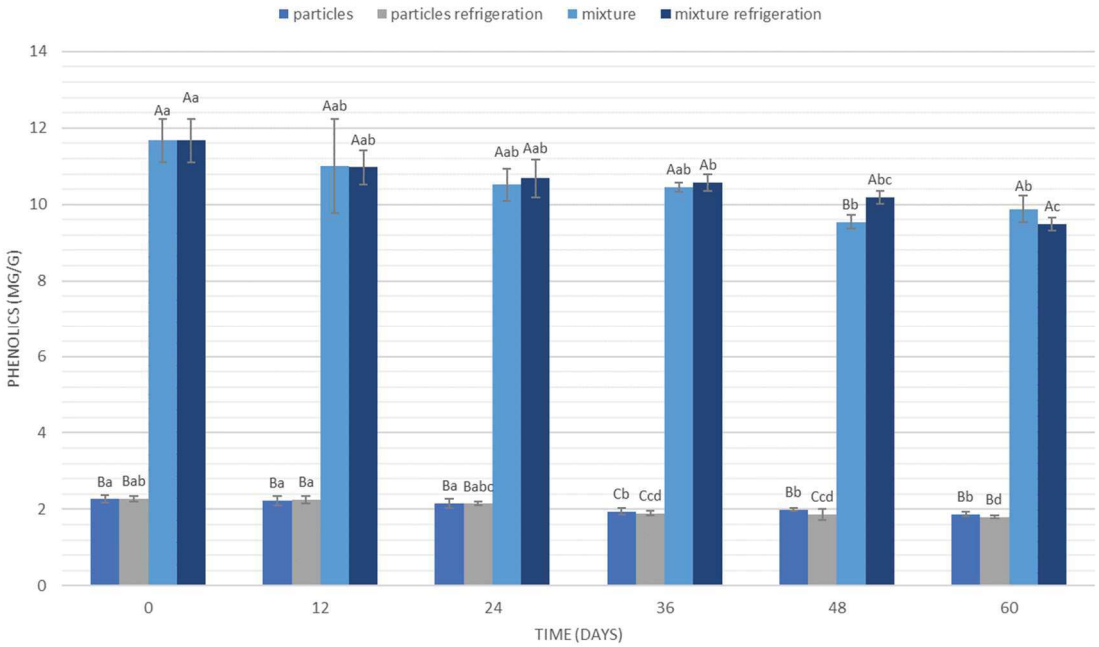

Figure 1. Evaluation of the stability of phenolic compounds in the mixture and in the particles generated by ionic gelation during a period of 60 days at room (25ºC) and refrigeration temperatures (5ºC).
* Different capital letters mean that the results differ statically from among products in the same time studied ($p<0.05$); Different lowercase letters mean that the results differ statically from the same product in different times ($p<0.05$).

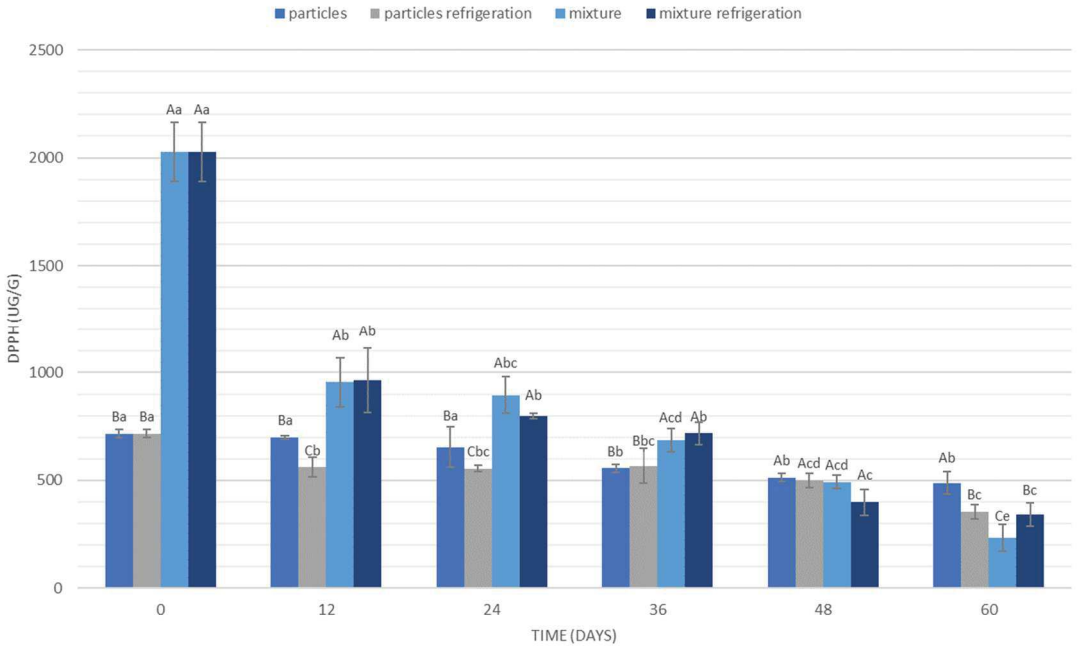

Figure 2. Evaluation of the antioxidant capacity stability in the mixture and in the particles generated by ionic gelation during a period of 60 days at room (25ºC) and refrigeration temperatures (5ºC).
* Different capital letters mean that the results differ statically from among particles and mixture in the same measured time ($p<0.05$); Different lowercase letters mean that the results differ statically from in the same product in different measured times ($p<0.05$).

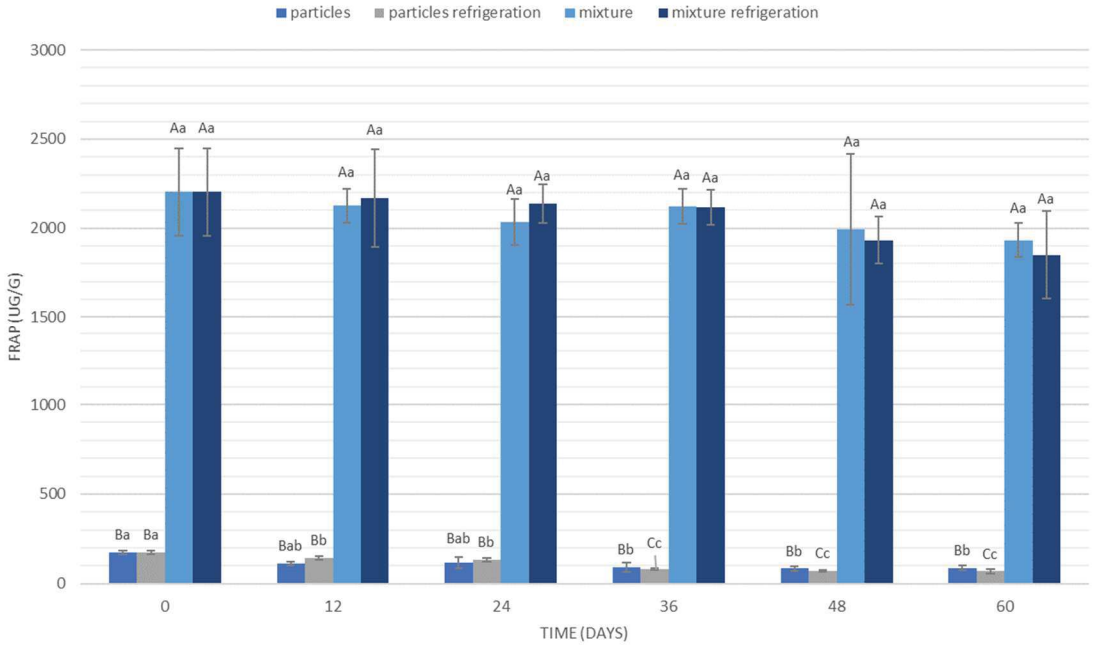

Figure 3. Evaluation of the antioxidant capacity stability in the mixture and in the particles generated by ionic gelation during a period of 60 days at room (25ºC) and refrigeration temperatures (5ºC).
* Different capital letters mean that the results differ statically from among particles and mixture in the same measured time ($p<0.05$); Different lowercase letters mean that the results differ statically from in the same product in different measured times ($p<0.05$).

The particles had a significant loss of phenolic compounds after 36 days. At the end of the 60 days, losses amounted to 19% at room temperature and 16.1% at refrigeration temperature. It was observed that increasing the storage temperature, the amount of phenolic compounds reduced. The mixture showed a significant loss of 15.3 and 18.7% at room and refrigeration temperatures, respectively. According to Ferreira et al. (2021), when observing the compounds stability in the medium over a 28 days after encapsulating beet extract by ionic gelation, they concluded that there was a significant reduction over the days due to the increase in permeability of the encapsulating material and consequent migration between the particle compounds and the medium.

Regarding the antioxidant activity by DPPH, there was a significant loss of 34.6% and 46.8%, respectively, for particles stored at room temperature and under refrigeration. As for the mixture, there was a significant loss after 12 days of storage, with a total of 88% at room temperature and 83.2% in refrigeration after 60 days. Effective encapsulation may be responsible for avoiding possible losses of bioactive compounds that would be reduced by temperature, oxygen and light during storage. The encapsulation led to greater stability of the antioxidant capacity of the mixture (OTÁLORA et al, 2018).

For the antioxidant capacity by FRAP, the loss in the particles was 44% when stored at room temperature, and 58.2% when stored under refrigeration. The mixture had a loss of 12.2% and 15.9% in ambient and refrigeration temperatures, respectively, in the same studied period. The loss of antioxidant capacity by FRAP of the mixture was not significant and the temperature had no influence on the final value. Extrinsic factors and particle composition affect the stability of compounds at different storage temperatures (FERREIRA et al, 2021; OTÁLORA et al, 2018).

The particle moisture over the 60-day storage period was also evaluated. The presented values varied around 85%, observing a stability of the particles over time, with no significant difference in moisture when comparing the initial period and the final period, after 60 days. It is an important parameter to be evaluated since the stability of particles decreases with increasing moisture content, decreasing the encapsulated material content (OTÁLORA et al, 2018).

3.6. Microscopy

The particles microscopy is shown in Figure 4. An irregular shape is observed, with particles ranging from spherical to ovoid. According to the bimodal distribution shown in Figure 5, most of the particles were around 1 to 1.2 mm in size.

These results are in agreement with Otálora et al. (2018), when encapsulating betaxanthin using the ionic gelation technique, found irregular particles, attributing the variations to the type of encapsulating agent and the relationship between the encapsulating material amount and the encapsulated material. According to Melo et al. (2021), the chemical composition is decisive for the final diameter of the particles, as well as the molecular weight of the encapsulated material, and its size can be an advantage in the future application in food.

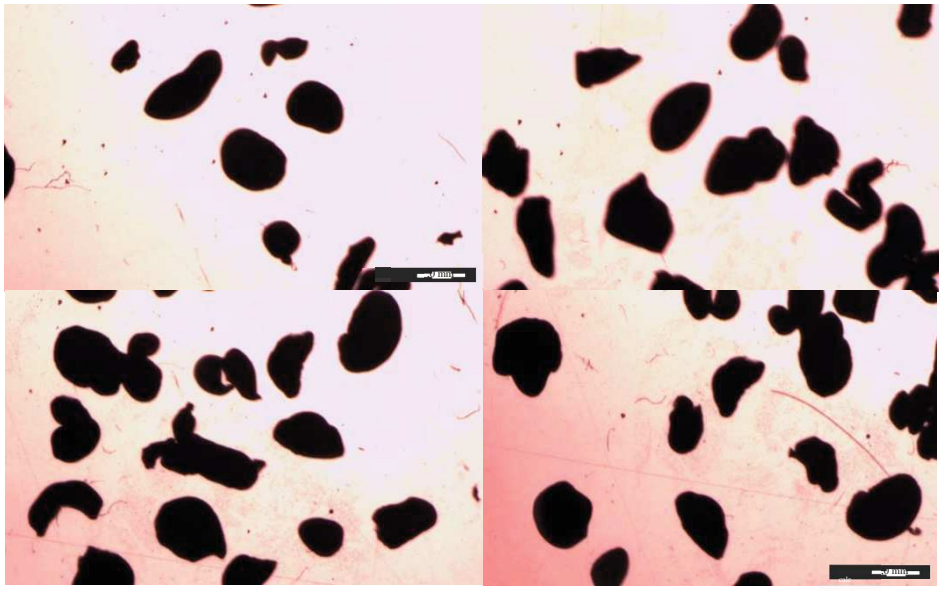

Figure 4. Particles format obtained by ionic gelation through microscopy

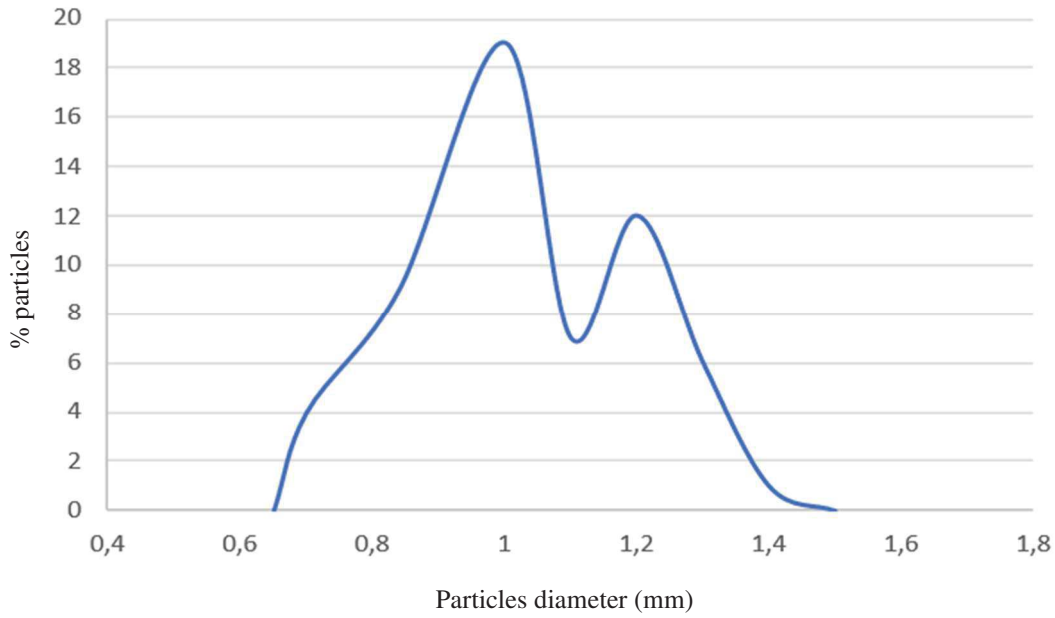

Figure 5. Bimodal distribution of particles obtained by ionic gelation

3.7. Bioaccessibility

The digestion of the particles and the mixture by the amount of phenolic compounds released in oral, gastric and intestinal phases can be seen in Figure 6.

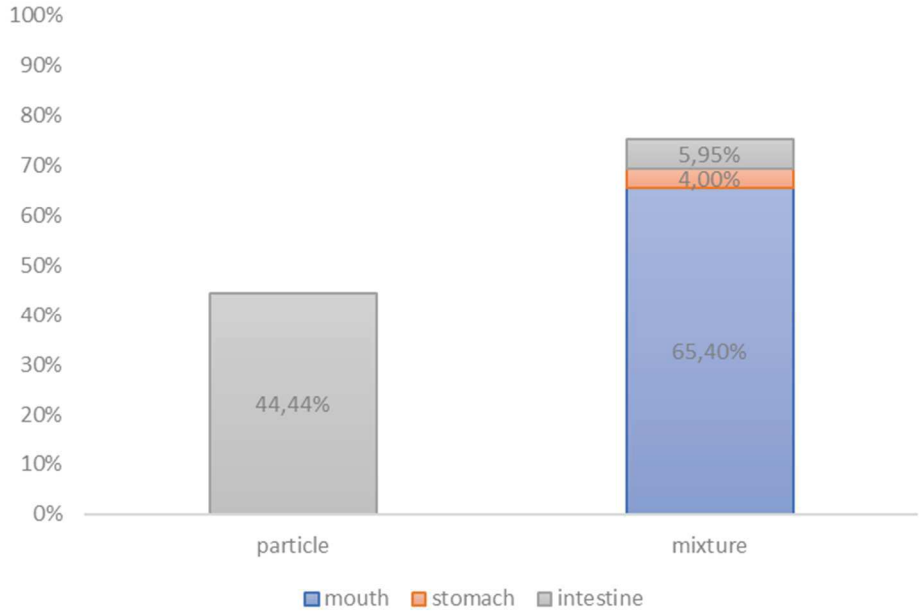

Figure 6. Release of mixture and particles in simulated gastrointestinal system

As can be seen, the mixture released 65.4% of phenolic compounds in the mouth and 4% in the stomach, while in the intestine the release was only 5.9%. As for the particle, the release in the mouth and stomach did not occur, while in the intestine the release occurred in 44.4% during the analysis period. The results show that the encapsulation increased the bioaccessibility of the phenolic compounds in the particles, when compared to the initial mixture, during in vitro simulation.

Wissam and Samer (2019) observed that linseed oil took up to 5 hours to be released almost completely into the intestinal fluid, in an alginate matrix, which, when in contact with the medium, swells and dissolves, releasing its content. Zhang et al. (2019) encapsulated propolis using alginate with different encapsulation techniques and observed an improvement of up to 80% in the bioaccessibility of phenolic compounds when compared to free propolis. The particles provide greater penetration of biological barriers and efficiency in the bioactive compounds release, delaying chemical degradation and obtaining adequate absorption in the body (MELO et al, 2021).

3.8. FTIR

The characteristics of the FTIR spectra were divided according to Figure 7, Figure 8 and Figure 9.

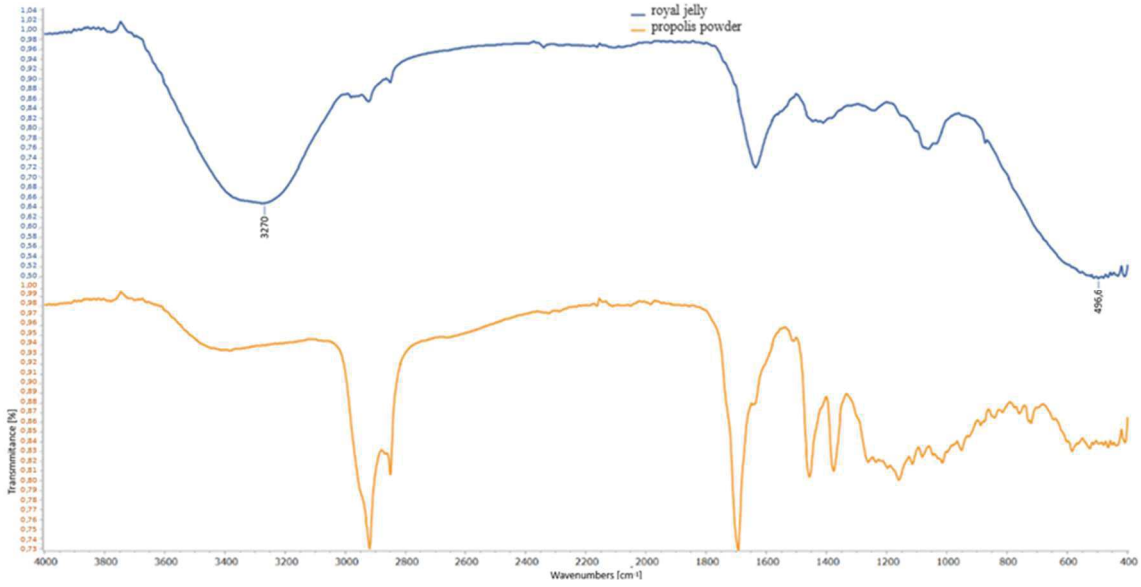

Figure 7. FTIR spectra of propolis powder and royal jelly

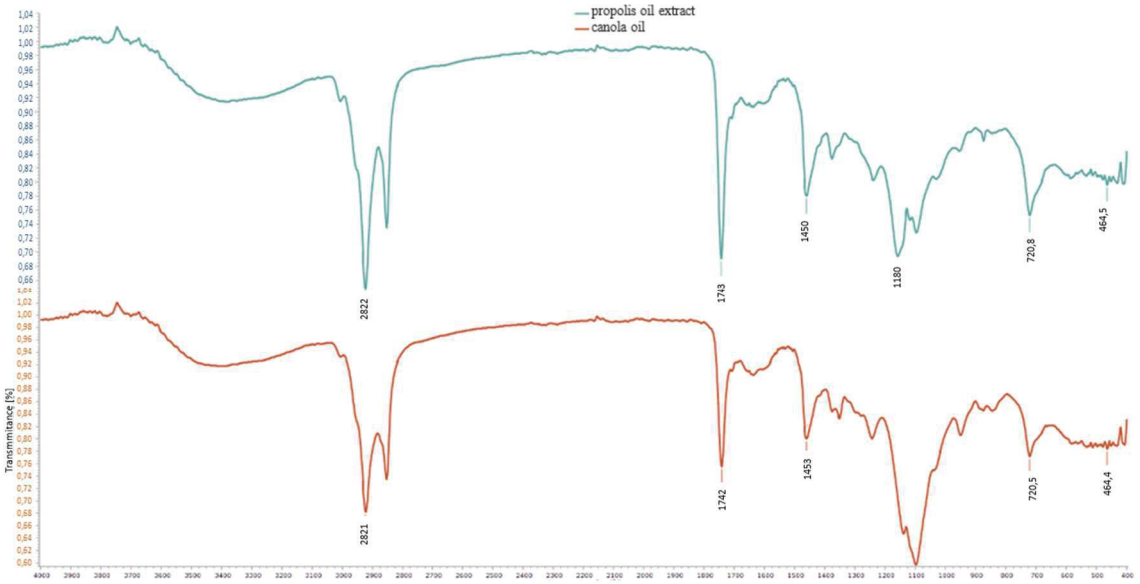

Figure 8. FTIR spectra of canola oil and propolis oil extract

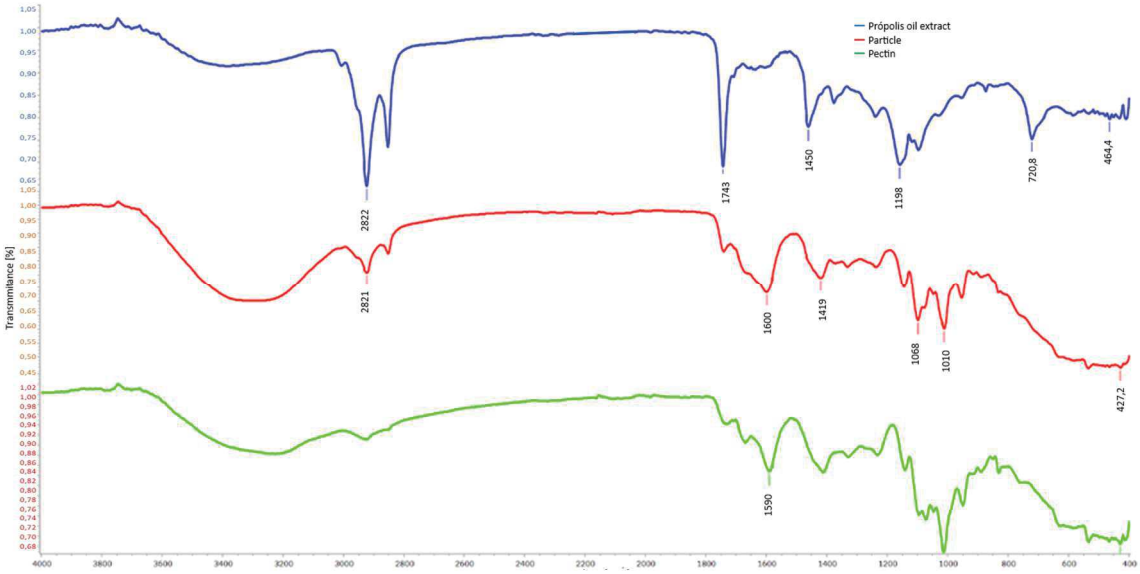

Figure 9. FTIR spectra of pectin, propolis oil extract and particles

FTIR is used to determine functional groups and types of chemical bonds present in the compounds. A broad band can be seen in royal jelly at 3270 cm^{-1}, as seen in Figure 7, resulting from the elongation of the -OH groups. A strong peak can be seen at 1635 cm^{-1}, related to the structure of soluble proteins, being widely used to assess the authenticity of the product. Two smaller peaks at 2919 and 2851 cm^{-1} represent the asymmetric and symmetric elongation of CH2, which can also be observed in the propolis powder with greater intensity. The spectral band in the absorption range between 3500-3200 cm^{-1} in propolis powder represents the stretching of -OH groups and H bond for alcohol and phenolic compounds. The large peak observed at 1695 cm^{-1} represents asymmetric C=O bending vibrations of flavonoids, and a slight band at 1510 cm^{-1} is related to the elongation of aromatic rings and flavonoids. The peaks at 1456 and 1377 cm^{-1} are related to bending vibrations of C-H groups (AZIZ et al, 2022; MELO et al, 2021; NASCIMENTO et al, 2022; WISSAM and SAMER, 2019).

The broad band in the region of 3384 cm^{-1} seen in the propolis powder spectrum (belonging to the -OH groups of phenolic compounds) also appeared in the propolis oil extract, with wave displacement and greater intensity. It was possible to observe that part of the peaks that were present in the powder spectrum were also present in the oil extract, with displacement of some peaks. In the spectrum of canola oil two large peaks at 2921 and 1742 cm^{-1} are attributed to the presence of CH2 and C=O respectively, as seen in Figure 8 (MOURA et al, 2018). These peaks are also present in the propolis oil extract in greater intensity. In the spectrum of the propolis oil extract, characteristic bands of the propolis powder were also observed, in addition to changes in intensity and displacement of some bands.

The Figure 9 presents the particles spectra and their content. The peaks between 3000 and 2800 and 1800 and 1000 cm^{-1} were used to explain the particle structure. The peaks are mostly related to the wall material of the particles, in addition to phenolic acids and flavonoids in the mixture. Both the peak at 2921 cm^{-1} represented by -OH groups and the lowest peak at 2852 cm^{-1} are present in the propolis oil extract. The band at 1741 cm^{-1} is also the result of band broadening due to the propolis oil extract. The bands observed in 1726, 1600, 1419 and 1410 cm^{-1} are present in pectin, with slight wavelength shifts in the particles. The wavelength 1419 cm^{-1} refers to ionic bonding and COO- groups with calcium ions. The particle formation can change the charge density and radius around the functional group and cause displacement (MOURA et al, 2018).

A band at 1373 cm^{-1} is related to the presence of CH groups of flavonoids in propolis powder. This same band is also observed in the oil extract and in the particle. The FTIR analysis confirm that the propolis oil extract is encapsulated in the pectin particle produced.

4. CONCLUSIONS

In this study, propolis was obtained by extraction with canola oil and mixed with royal jelly to produce a final mixture to be encapsulated. The ionic gelation method generated stable particles with high retention of phenolic compounds. The shape of the particles was irregular, requiring a patterning for greater control of the properties of particles, which can be carried out in future studies. The oil extract can be an important alternative for obtaining a non-alcoholic propolis and its generated particles with the addition of royal jelly promote stability in its shelf life, contributing to the maintenance of the antioxidant capacity and better bioaccessibility of the product with possible application in a food matrix.

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CONSIDERAÇÕES FINAIS

Os produtos apícolas são alimentos tradicionalmente utilizados devido aos seus valores nutricionais e medicinais e resultam em benefícios para o corpo humano. No capítulo I foi observado que com a chegada da pandemia da COVID-19, houve um aumento no consumo de mel e própolis durante o período de pandemia, fazendo com que o mercado brasileiro aumentasse suas vendas e seu valor comercial.

Ao comparar o extrato oleoso de própolis como uma alternativa ao extrato alcoólico no capítulo II, o teor de compostos fenólicos foi maior no extrato de óleo, apesar da maior parte desses compostos extraídos serem provenientes do óleo puro. Os teores de flavonoides e a capacidade antioxidante do DPPH e FRAP foram inferiores no extrato oleoso. Durante a digestão gastrointestinal simulada, o extrato oleoso apresenta maior liberação de compostos fenólicos na fase oral. Os resultados demonstraram a viabilidade da extração do óleo, mas também a necessidade de mais estudos sobre formulações e processos para obter maiores rendimentos de extração de substâncias bioativas, além de um estudo químico mais detalhado para identificar diferentes substâncias que podem ser extraídas do óleo, e que são responsáveis pelas atividades biológicas da própolis.

No capítulo final, a própolis foi obtida por extração com óleo de canola e misturada com geleia real para produzir uma mistura final a ser encapsulada. O método de gelificação iônica gerou partículas estáveis com alta retenção de compostos fenólicos. O formato das partículas apresentou-se irregular, necessitando de uma padronização para maior controle das propriedades das partículas.

O extrato oleoso pode ser uma importante alternativa para obtenção de própolis não alcoólica e suas partículas geradas com adição de geleia real promovem estabilidade em sua vida útil, contribuindo para a manutenção da capacidade antioxidante e melhor bioacessibilidade do produto com possível aplicação em uma matriz alimentar.