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Juciane Prois Fortes

DESENVOLVIMENTO DE HIDROMEL E ESTUDO DA SUA MATURAÇÃO UTILIZANDO *CHIPS* DE CARVALHO (*Quercus sp.*) EM DIFERENTES NIVEIS DE TOSTA

Santa Maria, RS
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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Área de Concentração em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutora em Ciência e Tecnologia dos Alimentos.**

Orientadora: Prof. Dra. Cláudia Kaehler Sautter

Santa Maria, RS

2023

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Aprovada em 17 de março de 2023.

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Cláudia Kaehler Sautter, Dra. (UFSM)
(presidente/orientador)

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Roberta Oliveira Santos, Dra. (UNISC)

Santa Maria, RS

2023

Dedico esse trabalho a minha família, aos meus pais Marlene e Amador e aos meus três irmãos, mas principalmente aos meus três companheiros diários de vida que estiveram ao meu lado sempre mesmo diante dos obstáculos André, Murilo e Miguel, amo vocês incondicionalmente.

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RESUMO

DESENVOLVIMENTO DE HIDROMEL E ESTUDO DA SUA MATURAÇÃO UTILIZANDO *CHIPS* DE CARVALHO (*Quercus sp.*) EM DIFERENTES NIVEIS DE TOSTA

AUTORA: Juciane Prois Fortes

ORIENTADORA: Prof. Dra. Cláudia Kaehler Sautter

A procura por novas bebidas fermentadas colocou o hidromel em evidência nas últimas décadas, elevando sua produção e consumo, com isso aumentando sua relevância econômica e trazendo ao pequeno produtor de mel uma alternativa de obter um derivado do mel com alto valor agregado. Atualmente os fabricantes dessa bebida estão buscando maneiras de diversificar a produção introduzindo novos aromas e sabores como forma de melhorar a qualidade sensorial e físico-química, trazendo ao mercado produtos diferenciados. Em face disso, o objetivo de trabalho foi desenvolver um hidromel com mel de diferentes origens florais e avaliar a potencialidade do uso de *chips* de carvalho (*Quercus sp*.) em diferentes níveis de tosta no processo de maturação. No primeiro experimento foram analisados os hidromeis produzidos a partir de mel multifloral, mel de flor de laranjeira e a sua mistura na proporção de 50/50. Foram analisadas a composição físico-química, os compostos fenólicos gerados por LC-ESI-QTOF-MS/MS e a aceitação através da análise sensorial. Os compostos fenólicos totais do mel multifloral e de flor de laranjeira foram de 485,84 e 471,31 mg EAG L^{-1} , respectivamente, enquanto os hidromeis tiveram uma variação de 203,40 a 223,83 mg EAG L^{-1} . A atividade antioxidante foi de 1,94 e 1,20 mM TEAC L^{-1} para os méis e 1,80 a 2,16 mM TEAC L^{-1} para os hidromeis. Os compostos fenólicos identificados nos hidromeis foram o ácido clorogênico, protocatecuico, siríngico, *p*-cumárico e flavonoides naringenina e quercetina. A análise sensorial não apresentou diferença quanto a preferência. A partir desses resultados foi possível definir o uso do mel multifloral como fonte principal para o segundo experimento, onde o hidromel foi maturado com *chips* de carvalho (*Quercus sp*.). O segundo experimento resultou no artigo publicado "Enhancement of the functional properties of mead aged with oak (*Quercus) chips* at different toasting levels". Os resultados encontrados no hidromel com adição de *chips* de carvalho mostraram um aumento na variabilidade dos compostos fenólicos, conteúdo de flavonoides e capacidade antioxidante ao longo do tempo de maturação, independente da tosta utilizada houve uma elevação da qualidade funcional da bebida. Compostos pertencentes às classes de ácidos orgânicos, ácidos fenólicos, flavonoides e taninos foram identificados por LC-ESI-MS/MS nos hidromeis após 360 dias. Os hidromeis com adição de *chips* de carvalho são mais ricos em compostos fenólicos do que o hidromel base (apenas mel). Por fim, os resultados dessa produção cientifica mostram que o hidromel é uma bebida que carrega os compostos presentes no mel e que a adição dos *chips* de carvalho (*Quercus sp*.) no seu processo de maturação pode ser uma ferramenta promissora para aumentar os compostos fenólicos e a qualidade dos hidroméis.

Palavras-chave: Hidromel. Compostos fenólicos. Envelhecimento

ABSTRACT

DEVELOPMENT OF MEAD AND STUDY OF ITS MATURATION USING OAK CHIPS (*Quercus sp.*) AT DIFFERENT TOAST LEVELS

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The search for new fermented beverages has put mead in evidence in recent decades, increasing its production and consumption, thus increasing its economic relevance and offering small honey producers an alternative to obtain a honey derivative with high added value. Currently, manufacturers of this drink are looking for ways to diversify production by introducing new aromas and flavors as a way to improve the sensory and physical-chemical quality, bringing differentiated products to the market. In view of this, the objective of this work was to develop a mead with honey from different floral origins and to evaluate the potential of using oak chips (*Quercus sp.)* at different levels of toasting in the maturation process. In the first experiment, meads produced from multifloral honey, orange blossom honey and their mixture in a 50/50 ratio were analyzed. The physical-chemical composition, the phenolic compounds generated by LC-ESI-QTOF-MS/MS and acceptance through sensory analysis were analyzed. The total phenolic compounds of multifloral and orange blossom honey were 485.84 and 471.31 mg GAE L-^{1}, respectively, while meads ranged from 203.40 to 223.83 mg GAE L^{-1}. Antioxidant activity was 1.94 and 1.20 mM TEAC L^{-1} for honeys and 1.80 to 2.16 mM TEAC L^{-1} for meads. The phenolic compounds identified in the meads were chlorogenic, protocatechuic, syringic, p-coumaric acid and flavonoids naringenin and quercetin. Sensory analysis showed no difference regarding preference. From these results it was possible to define the use of multifloral honey as the main source for the second experiment, where the mead was matured with oak chips (*Quercus sp.*). The second experiment resulted in the published article "Enhancement of the functional properties of mead aged with oak (*Quercus*) chips at different toasting levels". The results found in mead with the addition of oak chips showed an increase in the variability of phenolic compounds, flavonoid content and antioxidant capacity over the maturation time, regardless of the toast used, there was an increase in the functional quality of the drink. Compounds belonging to the classes of organic acids, phenolic acids, flavonoids and tannins were identified by LC-ESI-MS/MS in meads after 360 days. Meads with added oak chips are richer in phenolic compounds than base mead (honey only). Finally, the results of this scientific production show that mead is a beverage that carries the compounds present in honey and that the addition of oak chips (*Quercus sp*.) in its maturation process can be a promising tool to increase phenolic compounds and the quality of the meads.

Keywords: Mead. Phenolic compounds. Aging

SUMÁRIO

1 INTRODUÇÃO

O mel tem em sua composição cerca de 200 substâncias, sendo as principais os hidratos de carbono, além de minerais, proteínas, vitaminas, lipídios, ácidos orgânicos, aminoácidos, compostos fenólicos, enzimas e outros fitoquímicos, que exibem uma ampla gama de efeitos biológicos e atuam com antioxidantes naturais. Sua qualidade é determinada através das propriedades sensoriais, físicas e químicas. Essas dependem do néctar e pólen da fonte floral, da cor, do aroma, da umidade e do conteúdo em proteínas e açúcares (BIESAGA; PYRZYNSKA, 2009; PEREIRA et al., 2015).

O hidromel consiste na bebida com graduação alcóolica entre 4% e 14% em volume a 20ºC, obtida pela fermentação alcoólica de uma solução de mel de abelha, sais nutrientes e água potável. É considerado uma das bebidas mais antigas consumidas pelo homem, talvez mesmo antes do vinho, sendo provavelmente a precursor da cerveja. Seu consumo era mais difundido na antiguidade, estando ligado a datas comemorativas e rituais religiosos. Mas o desenvolvimento das civilizações e dos recursos agrícolas desencadeou a substituição do hidromel por outras bebidas como o vinho e a cerveja (RAMALHOSA et al., 2011; KRUŽÍK et al., 2022).

Na atualidade o hidromel ainda é consumido em alguns países, tais como Inglaterra, Polônia, Alemanha, Eslovênia, Portugal e sobretudo em países africanos, como a Etiópia e África do Sul. O hidromel é produzido de uma forma artesanal, e os produtores e apicultores, tal como em outros países, deparam-se com inúmeros problemas durante a sua produção (PEREIRA, 2008; SOTTIL et al., 2019).

O processo de maturação e envelhecimento e caracterizado por alterações de cor, aroma e sabor de bebidas maturadas. Essas alterações na composição e na concentração dos seus compostos, são causadas pela extração dos compostos da madeira, devido à quebra de suas macromoléculas e extração dos seus produtos, das reações ocorridas entre os compostos do destilado e da madeira, da reação que ocorre entre extrativos da madeira ou entre componentes do destilado e da evaporação dos compostos voláteis (MOSEDALE; PUECH, 1998)

O carvalho é amplamente utilizado no envelhecimento de bebidas alcoólicas, participando ativamente da qualidade sensorial do produto, devido a extração de óleos voláteis, substâncias tânicas, açúcares, glicerol e ácidos orgânicos não voláteis, compostos esses que podem promover modificação no aroma, sabor e coloração da bebida, agregando qualidades sensoriais diferenciadas (BORTOLETTO; CORRÊA; ALCARDE, 2016).

A utilização de barris de carvalho é o processo de envelhecimento mais empregado e clássico, porém tem sofrido limitações devido ao alto custo de aquisição e pela dificuldade de higienização dos recipientes, o que tem incentivado a busca por procedimentos alternativos. Dessa forma meios alternativos vem sendo utilizados na realização do processo de envelhecimento, através do uso de fragmentos da madeira em contato direto com a bebida. A utilização de aduelas, chips, aparas, e outros materiais alternativos de origem do carvalho ou de outras espécies de madeiras florestais, vêm se tornando comum para o envelhecimento de bebidas. Essa busca acabou gerando um novo aproveitamento dos pedaços de madeira que não seriam utilizados na tanoaria, tais como restos de madeira da fabrição das barricas, árvores com pequenos diâmetros ou com defeitos físicos impróprios para produção de tonéis (HERNÁNDEZ-ORTE et al., 2014; CARPENA et al., 2020).

1.2 OBJETIVO GERAL

Desenvolver um hidromel com mel de diferentes origens florais e avaliar a potencialidade do uso de *chips* de carvalho (*Quercus sp*.) em diferentes níveis de tosta na maturação do hidromel.

1.2.1 Objetivos Específicos

- Desenvolver os hidromeis com mel multifloral e de flor de laranjeira.
- Realizar o processo de maturação com *chips* de carvalho (*Quercus sp*.).
- Caracterizar físico-químicamente o hidromel base e os hidroméis maturados com os *chips* de carvalho (*Quercus sp*.) em diferentes níveis de tosta ao longo do tempo de maturação.
- Identificar os compostos fenólicos através da cromatografia líquida de alta eficiência (CLAE).

2 DESENVOLVIMENTO

2.1 REVISÃO BIBLIOGRÁFICA

2.1.1 Hidromel, história e legislação

O hidromel é uma bebida tradicionalmente obtida a partir da fermentação alcoólica de mel diluído em água mediado por leveduras. Considerada uma das bebidas mais antigas consumidas pela humanidade, era ligado a rituais religiosos e a datas comemorativas. O desenvolvimento das civilizações e recursos agrícolas causou a substituição do hidromel por bebidas como o vinho e a cerveja, porém em alguns países no norte da Europa, África do Sul e Índia onde as vinhas são escassas ou não cultivadas o consumo de hidromel mantem-se (PEREIRA et al., 2009; RAMALHOSA et al., 2011; SOTTIL et al., 2019; SUCEVEANU; ALEXA, 2021).

De acordo com a Instrução Normativa nº 34 de 29 de novembro 2012 (BRASIL, 2012), que regulamenta os padrões de identidade e qualidade para hidromel, o termo hidromel é usado para designar a bebida com graduação alcóolica entre 4% e 14% em volume a 20ºC, obtida pela fermentação alcoólica de uma solução de mel de abelha, sais nutrientes e água potável. No que diz respeito ao sabor aparência final pode ser classificada como seco e suave ao paladar, similar aos vinhos tradicionais ou ainda classificado como doce e encorpado. Pode-se também permitir que a fermentação continue após o engarrafamento e assim se obter um hidromel similar aos vinhos espumantes (GUPTA; SHARMA, 2009).

No norte Europeu, região onde a vinha não encontrava as condições necessárias para o seu desenvolvimento, o consumo de hidromel foi bastante popular até o vinho ser importado a baixo custo de regiões do sul. Na atualidade o hidromel ainda é consumido em alguns países, tais como Inglaterra, Polônia, Alemanha, Eslovênia e sobretudo em países africanos, como a Etiópia e África do Sul. Em Portugal, o hidromel é produzido de uma forma artesanal, e os produtores e apicultores, tal como em outros países, deparam-se com inúmeros problemas durante a sua produção (PEREIRA, 2008).

A literatura reporta poucos estudos sobre a produção de hidromel, isso demonstra a necessidade na realização de novas pesquisas que visem a padronização dos parâmetros essenciais para a obtenção de um produto com qualidade. O hidromel representa uma boa opção para aumentar a renda dos produtores de mel, permitindo o desenvolvimento de uma bebida pouco conhecida em alguns países, mas com grande potencial comercial, devido a busca atual

do mercado por produtos inovadores. O decreto nº 6.871, de 4 de junho de 2009, traz todas as normas necessárias para a regulamentação do hidromel no Brasil.

2.1.2 Qualidade físico-química do hidromel

A composição do hidromel é semelhante a do mel utilizado em sua produção, incluindo compostos como açúcares, ácidos, vitaminas, antioxidantes e minerais. Sua qualidade irá depender de seus parâmetros físico-químicos, com maior relevância o conteúdo de açúcares redutores, ácidos orgânicos e compostos fenólicos. Composição essa que depende de fatores como fermentação, armazenamento e maturação da bebida (SAMANIEGO-SÁNCHEZ; MARÍN-GARCÍA; QUESADA-GRANADOS, 2020).

Os parâmetros mais estudados nos últimos anos na produção de hidromel foram taxa de fermentação, efeito da temperatura, nutrientes adicionados e estirpes de leveduras utilizadas (NAVRÁTIL; ŠTURDÍK; GEMEINER, 2001; PEREIRA et al., 2009; RAMALHOSA et al., 2011). Porém poucos estudos relatam a composição química e sua importância na qualidade final do hidromel (PEREIRA et al., 2009; KRUŽÍK et al., 2022).

O mosto de hidromel é caracterizado pelo baixo pH e por uma combinação de ácidos que têm origem no mel, os quais podem influenciar a taxa de fermentação, que no hidromel depende, sobretudo, da variedade do mel, da estirpe da levedura, da composição do meio de cultura e do pH extracelular (NAVRÁTIL; ŠTURDÍK; GEMEINER, 2001; RAMALHOSA et al., 2011).

Com o objetivo de determinar o meio ideal de fermentação, Mendes-Ferreira et al. (2010) realizaram um estudo para avaliar a influência da suplementação do mosto de hidromel pela adição de tartarato de potássio e ácido málico no crescimento da levedura e atividade fermentativa, bem como na produção de compostos voláteis do aroma, os resultados mostraram que a adição dos ácidos orgânicos não afetou o crescimento e o perfil de fermentação, contudo melhorou a quebra dos açúcares proporcionando a geração de compostos aromáticos mais interessantes.

Os compostos fenólicos estão amplamente distribuídos no reino vegetal, sendo constituídos como parte do metabolismo secundário das plantas. Por serem mais ácidos, os polifenóis são oxidados antes que a maioria das moléculas presentes nas plantas, tendo ação antioxidante, dessa forma protegendo a integridade de seu organismo formador. Devido a ampla distribuição desses compostos na natureza sua transmissão para o mel se dá facilmente através das abelhas (AKALIN; BAYRAM; ANLI, 2017; PITA-CALVO; VÁZQUEZ, 2017).

O perfil de compostos fenólicos presentes no hidromel depende da origem botânica do mel, porém pode ser fortemente influenciado pelos ingredientes adicionados. Svecová e colaboradores (2015) ao analisarem 22 amostras de hidromeis naturais e com adição de diferentes tipos de ingredientes, concluíram que os hidromeis naturais compostos apenas por mel e água, continham uma quantidade relativamente inferior de compostos fenólicos, quando em comparação com os que tinham adição de sucos de frutas, nozes ou extratos de ervas.

2.1.3 Problemas associados a produção de hidromel

Os produtores de hidromel encontram algumas dificuldades no processo de produção dessa bebida, devido aos baixos níveis de substâncias nitrogenadas e minerais presentes no mel, indispensáveis para a multiplicação das leveduras, além do pH ácido do caldo fermentativo que afeta a evolução do processo. Assim a identificação e eliminação dos fatores que diminuem a atividade celular podem tornar o processo de produção mais rápido, reduzindo, assim, os custos de produção (NAVRÁTIL; ŠTURDÍK; GEMEINER, 2001; PEREIRA, 2008).

As leveduras usadas na produção de hidromel são normalmente, as estirpes utilizadas na produção de vinho, cerveja e espumantes. Existem diversas estirpes diferentes de leveduras enológicas, que são majoritariamente de *Saccharomyces cerevisiae*. No entanto, a maior parte destas leveduras enológicas não estão adaptadas às condições presentes no mosto de mel, como os níveis de açúcar elevados, valores de pH baixos e concentrações reduzidas de nitrogênio (PEREIRA, 2008; PEREIRA et al., 2015). De todos os nutrientes assimilados pelas leveduras durante a fermentação, os compostos nitrogenados, são quantitativamente os mais importantes, depois dos compostos carbonados, pois são essenciais para o crescimento e metabolismo das leveduras (PEREIRA et al., 2015; SCHWARZ et al., 2020, 2021).

Pereira et al. (2009) realizaram estudo para avaliar o uso de leveduras selecionadas na preparação de hidromel, submetendo cepas de leveduras a condições de estresse em diferentes níveis de etanol (5 a 20%). Observaram também a resistência das leveduras ao dióxido de enxofre em diferentes concentrações (100, 250 e 500 mg L^{-1}), e induziram ao choque osmótico as leveduras expostas a meio contendo 40% de açúcares. As leveduras selecionadas foram comparadas com as leveduras não submetidas ao estresse, utilizando dois tipos diferentes de méis, um claro, pobre em minerais e um escuro, rico em nutrientes. Após o acompanhamento do processo fermentativo e a caracterização dos hidroméis, os autores chegaram à conclusão que, a qualidade do mel, o preparo do mosto e a suplementação com nutrientes são as variáveis mais importantes na produção de hidromel.

Devido ao elevado teor de açúcares no mel, o processo fermentativo é bastante lento, sendo a variedade do mel, a estirpe da levedura, os nutrientes disponíveis e o pH do meio variáveis importantes que afetam a produção e qualidade do produto (NAVRÁTIL; ŠTURDÍK; GEMEINER, 2001).

Os atrasos e problemas nas fermentações, bem como a produção de sabores indesejados, são alguns dos problemas encontrados na produção de hidromel, normalmente associados com a incapacidade de resposta das leveduras para se adaptar às condições de stress desfavoráveis ao seu crescimento. Além da qualidade da matéria prima, pH, temperatura, teor de açúcares e disponibilidade de nutrientes, a taxa de inoculação das leveduras é fundamental no processo de obtenção de hidromel (RAMALHOSA et al., 2011; MILESKI, 2016; MARGAOAN et al., 2020).

2.1.4 Envelhecimento de bebidas alcoólicas

O processo de envelhecimento em barricas de madeira é um sistema complexo que envolve numerosas reações fundamentadas principalmente pela extração de moléculas da madeira e aeração controlada do líquido alcoólico. Fenômenos de migração de constituintes da madeira, evolução de compostos fenólicos, aeração/oxidação, estabilização da cor, sabor e o surgimento do caráter amadeirado, contribuem para a riqueza e complexidade do buquê aromático (CARPENA et al., 2020; KRÜGER; ALBERTI; NOGUEIRA, 2022).

A composição química das bebidas alcoólicas é modificada através de sua estocagem em recipientes de madeira, o processo de envelhecimento intensifica a cor e eleva a complexidade de aroma, composição fenólica e diminuição da adstringência, dessa forma elevando a aceitação e agregando valor de mercado dessas bebidas (CARDELLO; FARIA, 1998; KRÜGER; ALBERTI; NOGUEIRA, 2022). A maturação de vinhos em barricas de carvalho é uma prática comum aplicada por vinícolas, por ser um processo complexo, vários compostos podem se combinar produzindo alterações sensoriais, deste modo, a bebida fermentada adquire uma maior complexidade aromática devido ao resultado da extração de compostos voláteis presente nos barris (CARPENA et al., 2020).

A madeira do barril não é um material inerte, mas sim um meio natural que confere as bebidas durante o processo de envelhecimento um grande número de compostos provenientes da degradação de macromoléculas da madeira (hemicelulose, celulose, lignina e tanino). São extraídos pela bebida, principalmente óleos voláteis, substâncias tânicas, aldeídos fenólicos, glicídios, açúcares, glicerol, ácidos orgânicos não voláteis e esteróides que modificam as

características sensoriais da bebida (CARDELLO; FARIA, 1998; SOLAR; CASTRO; GUERRERO, 2021).

O envelhecimento de bebidas alcoólicas destiladas em tonéis de madeira também é amplamente aplicado pela indústria, em função das conhecidas melhorias sensoriais conferidas às bebidas, pois as bebidas recentemente destiladas em geral apresentam características sensoriais pouco apreciadas por parte dos consumidores (DA SILVA et al., 2012). O envelhecimento da aguardente em tonéis de madeira promove diminuição significativa do sabor alcoólico e da agressividade da bebida, com simultâneo aumento da doçura e do sabor de madeira, proporcionando uma efetiva melhora sensorial do produto (CARDELLO; FARIA, 1998).

Outra tendência no mercado das bebidas envelhecidas em madeira são as cervejas artesanais, este tipo de processo passou a ser utilizado por diversas micro cervejarias sendo apresentado como um diferencial no mercado cervejeiro. O envelhecimento da cerveja pode ser feito em barris novos ou barris que foram utilizados para maturar outros tipos de bebidas, sendo o mais comum o uso de barris maturados com uísque. As cervejas maturadas em barris que já foram utilizados na maturação de outras bebidas incorporam algumas notas de sabores da bebida anteriormente armazenada. Por outro lado, inúmeras reações ocorrem pelo contato da bebida com a madeira, incluindo a extração de compostos como ácidos fenólicos e aldeídos, oxidação e esterificação da lignina, responsáveis por notas aromáticas, sabor e cor (WYLER et al., 2015; PONTES GUIMARÃES et al., 2020; KOCIJAN et al., 2021).

2.1.5 Composição química da madeira

A composição química do carvalho tem influência direta sobre a bebida. Os componentes da parede celular do carvalho possuem uma estrutura celular hierárquica com composição lignocelulósicas principalmente celulose, hemicelulose e lignina. Essas macromoléculas, polissacarídeos (celulose e hemicelulose) e polifenóis (lignina) fornecem a madeira a eficiência no transporte de massa com água e nutrientes, oportuniza a regulação de fluidos e transporte iônico em micro e nanoescala, além de suas propriedades térmicas e luminosas (CHEN et al., 2020, 2022).

A celulose corresponde aproximadamente 40% da massa de madeira de carvalho seca, sendo um componente majoritário nos vegetais. Consiste em um polímero linear de alto peso molecular, com unidades repetidas de β- D-glicose, o que corresponderia a um monossacarídeo de seis carbonos com cinco grupos hidroxílicos alcoólicos ligados e um aldeído. Considerado

principal componente da parede celular de vegetais, tem este papel devido a suas propriedades químicas e físicas e também por sua estrutura supramolecular. Sua estrutura continua inalterada sob temperaturas de até 250°C, pois geralmente não é sensível a ação do calor durante a confecção dos tonéis utilizados na tanoaria (CHEN et al., 2020).

A hemicelulose é composta por heteropolissacarideos de cadeia curta com estrutura ramificada amorfa, podendo ser resultado da polimerização de vários tipos de açúcares, sendo os mais comuns: D-glicose, D-manose, D-galactose, D-xilose, D-arabinose. Na prática da tanoaria, as hemiceluloses, após passarem pelo processo de queima da madeira, são as principais precursoras de moléculas voláteis odorantes (CHEN et al., 2022).

Como terceira substância macromolecular componente na madeira, temos a lignina com suas moléculas de formação totalmente diferente dos polissacarídeos. A lignina é incluída no grupo dos polifenóis e é formada por um sistema aromático composto de unidades de fenil-propano. A lignina encontra-se presente nas paredes celulares, sendo a maior quantidade localizada nas paredes primárias além de ser responsável pelas propriedades mecânicas da madeira (CHEN et al., 2022).

Temos ainda um grupo de compostos que não participam da forma estrutural da madeira, compostos com baixo grau ou até ausência de polimerização. São substâncias solúveis em solventes orgânicos neutros e água, ou que sejam arrastáveis por vapor de água, denominadas extrativos da madeira. Estes extrativos possuem grande influência no envelhecimento de bebidas alcoólicas por possuírem solubilidade em meio hidroalcoólico e, mesmo que contribuam pouco na massa da madeira, possuem grande influência nas propriedades sensoriais, como cor, aroma, gosto, textura, entre outros (BURGUER; RICHTER,1991).

2.1.6 Processos de transformação da madeira

A madeira para uso na tanoaria deve ser resistente e fornecer compostos às bebidas, com a finalidade de elevar a qualidade de suas características sensoriais. Seu processamento inclui uma série de etapas que influenciam a sua qualidade final, em especial os tratamentos térmicos a que esta é sujeita, como a secagem e a tosta. Em ambos os processos há modificação na estrutura e na composição química final da madeira que irá ficar em contato com a bebida durante o seu processo de envelhecimento (AMPESE; 2011).

A secagem é uma etapa importante na fabricação das barricas na tanoaria, podendo ser realizada em estufas ou ao ar livre, sujeita as mudanças atmosféricas. Em estufas, o processo é

rápido e a retirada da umidade da madeira acontece de forma competente, porém sem ocorrer a cura, o que deixa a madeira com gosto vegetal, características grosseiras, taninos agressivos e amargos. No segundo caso o processo é mais complexo, pois além da secagem temos o processo de cura, que consiste num conjunto de processos que ocorre quando exposto as mudanças atmosféricas, com um efeito sensorial positivo. A madeira é cortada em tábuas, a partir do tronco de uma árvore, é empilhada e exposta ao ar livre. Substâncias tânicas, no caso do carvalho a vescalagina e a castalagina, moléculas complexas, mas fáceis de degradar pela simples ação do sol, os monômeros liberados são lavados pela chuva. Este decaimento tânico na superfície provoca uma migração de outros compostos do interior para a superfície repetindo o processo de degradação e lixiviação (RIBÉREAU-GAYON, P. et al, 2006).

Durante o tratamento térmico, a composição de taninos sofre fortes modificações, pois são sensíveis a altas temperaturas. Aproximadamente 75% da vescalagina e 25% da castalagina presentes na madeira de carvalho sofrem degradação, reduzindo notavelmente o gosto de madeira verde. Além de outros compostos que sofrem degradação impactando principalmente nos aromas (RIBÉREAU-GAYON, P. et al, 2006; MARTÍNEZ-GIL et al., 2020).

O tanoeiro na fabricação dos barris segue as características solicitadas pelo enólogo, pois as barricas podem ser *in natura* ou tostadas. Com o auxílio de um maçarico as paredes internas do recipiente podem receber diferentes níveis de tostas, que variam do grau leve ao intenso, pois os diferentes níveis de tostas geram distintas características sensoriais de sabor e aroma. A classificação dos níveis de tosta vai variar de acordo com o tempo de exposição ao calor e a temperatura alcançada. A tosta fraca tem duração de 30 minutos e a temperatura atinge de 120 a 130 ºC; a tosta média pode variar de 35 a 40 minutos com temperatura entre 160 a 190 ºC e a tosta forte onde a madeira fica exposta ao calor durante 45 minutos e alcança uma temperatura de 200 a 210ºC (CADAHÍA et al., 2001; CHATONNET; ESCOBESSA, 2007).

Na tosta há uma significativa formação de fenóis voláteis oriundos da degradação da lignina como o guaiacol (fumaça) e vanilina (baunilha). A degradação de lipídeos como o ácido octanóico e outras estruturas mais complexas pode dar lugar a lactonas, como a metiloctalactona, responsável pelo aroma de côco. Na celulose observamos uma diminuição de seu grau de polimerização e uma produção de oligômeros que conduzem a formação de substâncias do tipo furano, como o metil-furfural (amêndoas tostadas). As hemiceluloses, se reagrupam formando poliosídios complexos que são parcialmente degradados durante a queima, formando furfural (amêndoas), entre outros compostos (AMPESE, 2011).

2.1.7 Uso de diferentes tecnologias no envelhecimento de bebidas alcoólicas

A tecnologia de envelhecimento de bebidas é onerosa e demorada para quem utiliza este tipo de processo, devido à necessidade de se recorrer à indústria de tanoaria e ao tempo de amadurecimento que a bebida destilada ou fermentada irá ser submetida. Com o objetivo de resolver essas desvantagens foram desenvolvidas novas técnicas para acelerar o processo de envelhecimento e uma delas consiste na adição de fragmentos de madeira, condicionados nos tanques de inox utilizados para manter as bebidas alcoólicas durante a sua produção e maturação, sendo definida como uma ferramenta enológica essencial para uma extração aromática rápida (DEL ÁLAMO et al., 2008; AMADO, 2014; SOLAR; CASTRO; GUERRERO, 2021).

A utilização de sistemas alternativos para envelhecimento de bebidas alcóolicas tem conseguido apresentar resultados semelhantes aos verificados no envelhecimento em barril convencional, através do uso de *chips* de carvalho em contato direto com a bebida. Os sistemas alternativos demonstram aumento na quantidade de vários compostos extraídos da madeira, resultando em atributos sensoriais relacionados aos comparados com os sistemas tradicionais. Com a diminuição no tempo de maturação, pois o tempo de contato necessário é relativamente menor para extração dos compostos que se deseja incorporar à bebida, há redução de custos no processo o tornando economicamente sustentável (OBERHOLSTER et al., 2015; GRANJA-SOARES et al., 2020).

Os *chips* são definidos como lascas de madeiras de tamanhos variáveis, com espessura geralmente inferior a 2 mm adicionadas em contato direto com a bebida que se deseja agregar valor sensorial durante seu processo de maturação. Existem outras formas de apresentação dos chips de carvalho, geralmente em "cubos" ou "grãos de carvalho", "pó de carvalho", ou simplesmente "granulados" (DEL ÁLAMO et al., 2008). Os *chips* representam o método mais rápido e mais econômico para aumentar a estrutura dos vinhos. A sua grande densidade de contato, confere logo a partir da primeira semana um aumento da estrutura e permite uma melhor extração aromática em vinhos aromaticamente menos intensos. Dosados em pequenas quantidades os *chips* permitem mascarar os defeitos herbáceos e de adstringência (PIZARRO et al., 2014). O amadurecimento dos vinhos com o uso de *chips* de carvalho é bem comum nos países da Europa, no entanto quando utilizado este procedimento o mesmo deve ser descrito no rótulo do produto, através do decreto (Regulamento N. 1507/2006 de 11 de outubro de 2006).

De acordo com o estudo de Bortoletto (2013), em amostras de vinho maturadas com lascas provenientes de diferentes aplicações de tostas, foi possível analisar que na aplicação da

tosta fraca, prevaleceram os ácidos gálico e vanílico. Com tostas médias, a predominância continuou apenas para ácido gálico e o furfural. Com a tosta alta, as madeiras apresentaram altas concentrações de marcadores derivados da degradação térmica da lignina.

Além dos chips de carvalho existem outras formas alternativas de envelhecimento de bebidas alcoólicas, a utilização de aparas de madeira já é amplamente conhecida, processo com eficácia utilização durante a vinificação e amadurecimento de diferentes bebidas. Classificadas como fragmentos maiores de madeira (em torno de 10 x 50 mm) que também são colocadas diretamente com o produto destilado ou fermentado, resultando numa bebida mais afinada para os consumidores (SUVAC, 2013).

Segundo o regulamento técnico europeu, as aparas podem ser deixadas no estado natural ou serem tostadas de modo ligeiro, médio ou forte, mas não devem ter sofrido combustão, incluindo a superfície, nem estar carbonizadas a ponto de carvão, assim como não deve se fragmentar facilmente ao toque. Também não devem ter sofrido tratamentos químicos, enzimáticos ou físicos, além do aquecimento. Não lhes deve ser adicionado qualquer produto destinado para aumentar o seu poder aromatizante natural ou os seus compostos fenólicos extraíveis. As aparas não devem liberar substâncias que possam resultar riscos para a saúde. Na rotulagem do produto utilizado deve ser mencionado a origem da espécie ou espécies botânicas de carvalho e a intensidade da tosta aplicada (UNIÃO EUROPÉIA, 2006).

Além dos sistemas alternativos já citados, existem também as aduelas de madeira, conhecidos também como *staves*. Amado (2014) realizou um experimento utilizando aduelas para o processo de envelhecimento de aguardentes vínicas, as quais foram colocadas diretamente nos tanques de inox em contato direto com o destilado para adquirir melhores características sensoriais, o estudo concluiu que apesar da superfície de contato das aduelas ser menor que dos tonéis de carvalho, quando distribuídas de forma uniforme em todo o volume do tanque, a bebida que está em contato com o material de envelhecimento adquiri os compostos de aroma e sabor de forma mais rápida, pois o meio fica saturado rapidamente, ao contrário das barricas, que a aguardente fica em contato apenas com as paredes do barril, incorporando os compostos lentamente.

No entanto, os vinhos envelhecidos com métodos alternativos raramente têm os mesmos compostos das bebidas maturadas nos barris, pois no envelhecimento em barricas de madeira ocorre a microxigenação da bebida fermentada. Visto que o oxigênio tem influência direta na composição dos compostos fenólicos e indireta no efeito de algumas características sensoriais como a cor, a adstringência e o aroma, aspetos que determinam a qualidade de um vinho, resultante do papel importante que o oxigênio tem nas reações de oxidação, condensação e

polimerização que ocorre no processamento dos vinhos envelhecidos em madeira (MCCORD, 2003; OBERHOLSTER et al., 2015; GÓMEZ-PLAZA; BAUTISTA-ORTÍN, 2018; GRANJA-SOARES et al., 2020).

2.2 ARTIGO 1

HIDROMEIS COM MÉIS BRASILEIROS DE DIFERENTES ORIGENS BOTÂNICAS: ELABORAÇÃO E CARACTERIZAÇÃO FENÓLICA

MEADS WITH BRAZILIAN HONEYS FROM DIFFERENT BOTANICAL ORIGINS: ELABORATION AND PHENOLIC CHARACTERIZATION

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ESTRATO: A4

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Meads with brazilian honeys from different botanical origins: Elaboration and phenolic characterization

Abstract - Due to the increased demand for differentiated beverages in the market, new products appear in order to meet consumer demand. This present study aimed to evaluate the phenolic compounds present in mead prepared with multifloral and unifloral orange blossom honey and the mixture of these two honeys as well as their sensory quality through acceptance and preference. Multifloral honey and orange blossom honey from the south and midwest regions of Brazil, respectively, were used in the preparation of meads. Phenolic compounds and antioxidant capacity were analyzed in mead. LC-ESI-QTOF-MS/MS was used to analyze the chemical profile of meads. The meads were submitted to acceptance and ordering tests regarding preference. Total phenolic compounds of multifloral and orange blossom honey were 485,84 e 471,31 mg GAE L^{-1} , respectively, while the meads had a variation of 203,40 a 223,83 mg L^{-1} . The antioxidant activity was 1,94 e 1,20 mM TEAC L^{-1} for honeys and 1,80 a 2,16 mM TEAC L^{-1} for meads. The phenolic compounds identified in meads were chlorogenic acids, protocatechuic, syringic, p-coumaric, naringenin and quercetin. Sensory analysis showed no difference regarding the preference. It is concluded that botanical origin of honey determines the final mead composition.

Index terms: multifloral honey, orange blossom honey, phenolic compounds, sensory analysis.

Hidromeis com méis brasileiros de diferentes origens botânicas: Elaboração e caracterização fenólica

Resumo - Devido ao aumento da demanda por bebidas diferenciadas no mercado, novos produtos surgem com o intuito de atender a demanda do consumidor. O presente estudo teve como objetivo avaliar os compostos fenólicos presentes no hidromel elaborado com mel multifloral e unifloral de flor de laranjeira e a mistura desses dois méis, bem como sua qualidade sensorial através da aceitação e preferência. Mel multifloral e mel de flor de laranjeira das regiões sul e centro-oeste do Brasil, respectivamente, foram utilizados na preparação de hidroméis. Os compostos fenólicos e a capacidade antioxidante foram analisados no hidromel. LC-ESI-QTOF-MS/MS foi usado para analisar o perfil químico dos hidroméis. Os hidroméis foram submetidos a testes de aceitação e ordenação quanto à preferência. Os compostos fenólicos totais dos méis multifloral e de flor de laranjeira foram de 485,84 e 471,31 mg EAG L^{-1}, respectivamente, enquanto os hidroméis variaram de 203,40 a 223,83 mg EAG L^{-1}. A atividade antioxidante foi de 1,94 e 1,20 mM TEAC L-1 para méis e 1,80 a 2,16 mM TEAC L^{-1} para hidroméis. Os compostos fenólicos identificados nos hidromel foram ácidos clorogênico, protocatecuico, siríngico, *p*-cumárico, naringenina e quercetina. A análise sensorial não mostrou diferença quanto à preferência. Conclui-se que a origem botânica do mel determina a composição final do hidromel.

Termos para indexação: mel multifloral, mel de flor de laranjeira, compostos fenólicos, análise sensorial.

Introduction

Honey is a food rich in nutrients, with main compounds as carbohydrates, minerals, proteins, vitamins, lipids, organic acids, amino acids, enzymes, in addition to other phytochemical compounds (KHALIL et al., 2011; AFROZ et al., 2014). They still have a wide range of phenolic acids and flavonoids responsible for their antioxidant potential. The flavor, color and other physical-chemical properties that determine honey quality come from non-volatile compounds that include sugars and phenolic compounds. The amount of these compounds may vary with the floral and geographic origin of the honey (KHALIL et al., 2011; CIANCIOSI et al., 2018; VASIĆ et al., 2019).

Due to its high availability, the variety of multifloral honey, derived from different types of flowers, has greater prominence in the market. While orange blossom honey is among the most important unifloral honeys in the world, due to its differentiated sensory characteristics such as specific color, aroma and flavor (GAO et al., 2020; SERAGLIO et al., 2021).

Mead is a beverage obtained through the fermentation of honey, water and yeast, with or without nutrient salts addition, and an alcoholic strength between 4 - 14 %. It is considered the first fermented beverage discovered by man (KAHOUN et al., 2008; MORALES; ALCARDE; DE FRANCESCHI DE ANGELIS, 2013; ADAMENKO et al., 2018; PEEPALL et al., 2019). Its production may represent an economic alternative for honey producers, as it originates a product with high added value and with probable commercial potential (PEREIRA et al., 2015).

Mead composition is varied, and is directly linked to the type of honey used and technological processes involved, such as fermentation, maturation, storage and consumption (KAHOUN et al., 2008; ŠVECOVÁ et al., 2015; AKALIN; BAYRAM; ANLI, 2017; KAHOUN; ŘEZKOVÁ; KRÁLOVSKÝ, 2017). Mead has in its composition sugars, vitamins, organic acids, minerals and phenolic compounds (ŠVECOVÁ et al., 2015; AKALIN; BAYRAM; ANLI, 2017).

Phenolic compounds are widely distributed in tplants, being constituted as part of secondary metabolism of plants, having in their group an aromatic ring with one or more hydroxyl substituents, including their functional groups (DELGADO; ISSAOUI; CHAMMEM, 2019). Due to the wide distribution of these compounds in nature, their transmission to honey occurs through bees (*Apis mellifera)* (AKALIN; BAYRAM; ANLI, 2017). The protective health effects attributed to phenolic compounds are associated to their antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, antimicrobial properties, among other biological Properties (KIOKIAS; PROESTOS; OREOPOULOU, 2020)

Among the phenolic compounds present in mead, the main ones are gallic, caffeic, chlorogenic, ferulic, p-coumaric and syringic acid, as well as flavonoids such as chrysin, galangin, hesperidin, kaempferol, quercetin and naringenin. The presence of these individual compounds in mead is directly affected by honey's floral, geographical origin, and seasonality (ŠVECOVÁ et al., 2015).

This work aimed to evaluate the phenolic compounds and sensory perception of consumers in meads made with Brazilian honeys from different botanical origins.

Material and Methods

Analytical reagents

HPLC-grade methanol used for the mobile phase was obtained from Merck (Darmstadt, Germany). HPLC-grade acetonitrile and formic acid used for the mobile phase were obtained from Sigma-Aldrich (St. Louis,MO, USA). HPLC-grade water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). ABTS (2,20-azino-bis (3 ethylbenzothiazoline) 6-sulfonic acid) was obtained from Sigma Aldrich (St. Louis, MO, USA).

Experimental design

The experiment was completely randomized with three mead treatments (100% multifloral honey, 100% orange blossom honey and 50% multifloral honey and 50% orange blossom mixture) in six replications.

Samples acquirement

To produce the mead we used multifloral honey from an apiary located in Santiago city in the central region of Rio Grande do Sul, Southern Brazil. The orange blossom honey was purchased from the apiary located in the city of Rio Claro, in the east-central region of the state of São Paulo. The mead was prepared with multifloral honey and water until it reached 21 Brix; sulfited at 50 ppm and bottled in 700 mL then it was inoculated with 200 mg L^{-1} of the Saccharomyces baianus yeast strain and 300 mg L^{-1} of nutrients (NUTRISTART®). Fermentation was carried out in a polyethylene fermenter with a capacity of 5 L with the system maintained under anaerobic conditions through the bater seal at aconstant temperature of 20 ºC, and it was monitored daily by measuring the total soluble solids content and initial and final density. The end of the fermentation process occurred with the cessation of carbon dioxide evolution followed by the stabilization of total soluble solids and the stabilization of density. The mead was then stabilized for 16 days at 16 ºC.

Physicochemical analysis

The physicochemical analyses performed on honey were pH, total acidity and reducing and non-reducing sugars, while in mead the analyses of pH, total acidity, total sugars, reducing and non-reducing sugars and alcohol content were performed. The pH was determined by potentiometric method with pH meter (DM 22 Digimed®). Titratable total acidity (TTA) was determined by a methodology described in (IAL, 2008). The methodology used for the determination of reducing and non-reducing sugars was described by Lane & Eynon. The alcohol content was determined by distillation in an electronic distiller of Gibertini ™.

Total phenolic content

The total phenolic compounds in each mead sample were quantified with spectrophotometry through the redox reaction with the Folin–Ciocalteu reage (SINGLETON; ROSSI, 1965) nte . The readings of the samples were performed in triplicate of the absorbances in a UV–visible spectrophotometer (FEMTO 600 plus) at a wavelength of 765 nm after they were left to rest for two hours at room temperature. The phenolic compounds content was calculated by interpolating the absorbance of the samples against the calibration curve constituted with a standard of gallic acid (0 - 80mg L^{-1}), and the results are expressed in milligrams of gallic acid equivalent per liter of honey and mead (mg GAE L^{-1}).

Antioxidant capacity determination

The antioxidant capacity of meads was determined with the ABTS method using the method described by RE et al. (1999). The absorbance readings of the samples were taken 6 min after the reaction in a UV–visible spectrophotometer (FEMTO® 600 plus) at 750 nm. The ABTS concentration was calculated from a calibration curve using Trolox the standard (0–0.2 mM TEAC L^{-1}). Readings were performed in triplicate, and the results are expressed as mM of Trolox equivalent antioxidant capacity per liter (mM TEAC L^{-1}).

Purification procedure in SPE C18

Samples were purified prior to performing the LC-ESI-QTOF-MS/MS analysis. Sample purification was performed according to the method described by Rodriguez-Saona; Wrolstad (2001) with adaptations (BOCHI; GODOY; GIUSTI, 2015). The mead samples (6 mL) were placed in a rotary evaporator (Büchi, Essen, Germany) at 35 ºC for five minutes to remove the alcohol present in the sample. Afterward, the sample was loaded into C-18 solid phase extraction (SPE) cartridges (cartridges SPE-C18, Strata C18-E, Phenomenex), previously activated with methanol and conditioned with acidified water (0.1% v/v formic acid). The polar compounds were washed with two volumes of aqueous formic acid solution (0.1% v/v). Fewer

150 polar phenolic compounds were eluted with two volumes of ethyl acetate (3 mL). The ethyl
151 acetate fraction was dried on a rotary evaporator and made up to a known volume (1 mL) with
152 acidified methanol (0.1% v/v formic acid) and acidified water (0.1% v/v formic acid) (200 +
153 800 µL). All fractions were analyzed directly as purified fractions in a chromatograph.

154 **Phenolic compound identification by LC-ESI-QTOF-MS/MS**

155 The method to identify the phenolic compounds was based on Quatrin et al. (2019). The liquid
156 chromatography (LC) instrument (Shimadzu, Kyoto, Japan) was connected in series to a DAD
157 detector (SPD-M20A) and a mass spectrometer (MS) with a Quadrupole-Time-of-Flight
158 (QTOF) analyzer and an electrospray ionization source (ESI) (Bruker Daltonics, micrOTOF-Q
159 III, Bremen, Germany). A 20 µL sample was injected into a reversed-phase column (C-18
160 Hypersil Gold, 5 µm particle size, 150 mm, 4.6 mm; Thermo Fisher Scientific, Waltham, MA,
161 USA). Mobile phase A for this method consisted of ultrapure water with formic acid acidified
162 methanol (95:5:0.1 v/v); mobile phase B was acetonitrile and formic acid (99.9:0.1 v/v). The
163 ESI conditions were a capillary voltage of -4500 V (negative), nebulizer gas pressure at 30 psi,
164 dry gas at 11 mL min^{-1}, and gas temperature at 310 ºC. The MS/MS experiments were
165 performed in a full scan range of 100–1800 m/z of all fragments formed from 3 major parent
166 ions per second. The LC solutions software (Version 3, Shimadzu, Kyoto, Japan) was used to
167 process the data obtained. The tentative identification of compounds was based on the combined
168 information of elution order, ultraviolet–visible (UV–Vis) spectra, and mass spectrometry
169 fragmentation patterns. These data were compared to literature data and public databases
170 (PubChem, KEGG, MassBank of North America (MoNA), ChemSpider, Phenol-Explorer, and
171 FooDB).

172 **Sensory analysis**

173 The meads were submitted to affective acceptance and ordering tests regarding preference
174 (IAL, 2008; BALOGU; TOWOBOLA, 2017). The evaluators were recruited locally, on a

completely voluntary basis, and they were informed verbally and through the Free and Informed Consent Form (TCLE) of the objectives, benefits and risks of participating in the research, as well as being informed about the total confidentiality of the data. A 30 mL sample of the drink was offered at a temperature of 4 ºC (± 2 ºC) in transparent plastic cups with a capacity of 50 mL, all coded with three digits in random order. Acceptance attributes were evaluated using a seven-point hedonic scale (1 = I really disliked; 7 = I really liked), where the attributes of color, aroma, flavor and overall acceptance were analyzed. For the ordering test, the evaluators ordered the samples from most preferred to least preferred. Sensory analyzes were carried out in a laboratory suitable for this type of analysis, in individual booths, with adequate lighting, free of odors and noise. Tests were conducted with 102 untrained adult tasters of both sexes. The project was recognized by the National Research Ethics Committee (CONEP/MS), being approved (CAAE 58889316.3.0000.5346) in its ethical and methodological aspects, complying with the Guidelines established in Resolution Nº 466, of December 12, 2012, of the National Health Council.

Statistical analysis

All analytical results were selected for an analysis of variance (ANOVA). The comparison of posthoc mean analysis was performed with a Tukey's test at 5% error probability using Statistica 9.0 software (StatSoft, Tulsa, OK, USA). The result of the sensory analysis of preference was submitted to the Friedman test using the Newel-McFarlane table (1987) and acceptability by ANOVA at the 5% level of significance.

Results and Discussion

The multifloral honey analyzed in this study showed greater interference from the floral origin in the total phenolic compounds, being 485,84 GAE kg^{-1} (Table 1). Comparatively, the results of these Brazilian honeys are highly relevant, because, Khalil et al. (2011) found values between 15,21 and 52,63 mg GAE kg^{-1} in honeys from Malaysia and Lachman et al., (2010) found 83,6

and 242,5 mg GAE kg^{-1} in Czech honeys, values lower than those found in our study, while Pontis et al. (2014) found levels ranging from 250 to 509 mg GAE kg^{-1} in Brazilian honeys, corroborating those found in this study.

We can observe that meads presented relevant contents regarding the total phenolic compounds in their composition of 203,40 a 223,83 mg GAE L^{-1} (Table 1). In relation to honey of origin there was a reduction of 44 % in multiflower honey torespective mead and 43,15 % in orange blossom honey for its mead. If one takes into account the approximate dilution of honey in 3 times for mead elaboration, it can be inferred that fermentation process preserves the phenolic compounds in their majority. This fact may be due to the acidic nature of mead (54,6 and 54,0 meq L^{-1}), which confers a pH of 3,27 to 3,57, as well as an alcohol content of 10,6 ºGL, factors that are favorable for the solubilization and preservation of these compounds (Table 1). Mead with multifloral honey showed a significant difference in relation to same with orange blossom honey, this is possibly related to the more diverse flora that multifloral honey presents and because it is a darker honey, as also observed by Estevinho et al. (2008).

In fermentation process, it was observed that the orange blossom honey contained an interfering potential, since the multifloral honey has a higher total sugar content compared to the orange blossom honey, however the orange blossom mead has a higher content of total residual sugars. Regarding the antioxidant power, the mixed mead showed the highest activity followed by the multifloral mead (Table 1). This demonstrates that there is a synergistic interaction between the honeys, which is possibly related to their differentiated composition. Recently, many studies link the antioxidant activity of honey with its content of phenolic compounds (MEDA et al., 2005; ESTEVINHO et al., 2008) and consequently with its botanical origin (BERTONCELJ et al., 2011). This was observed in the present study, as the meads that contained multifloral honey in their composition showed greater antioxidant activity.

Phenolic compounds are often found in plants as they come from their secondary metabolism. Bees, by collecting pollen, transfer many of these compounds to honey and consequently these phytochemicals will be present in mead.

Among the identified phenolic compounds, only chlorogenic acid and quercetin were found in all samples. Syringic acid was present only in mead with multifloral honey. p-coumaric acid and naringenin were only present in mead with orange blossom honey. Protocatechuic acid was identified in multifloral and mixed mead, which indicates its presence in multifloral honey only. Kahoun et al. (2017) identified protocatechuic acid, syringic acid and p-coumaric acid in traditional meads. Adamenko et al., (2018) and collaborators quantified only p-coumaric and chlorogenic acids in mead samples. The phenolic compounds in mead originate from the honey used, and from other ingredients that may have been added, phenolic acids are the main bioactive compounds in honey and consequently in mead. These results suggest that phenolic acids are stable during the fermentation process (ŠVECOVÁ et al., 2015; AKALIN; BAYRAM; ANLI, 2017; KAHOUN; ŘEZKOVÁ; KRÁLOVSKÝ, 2017). The Brazilian flora is varied, generating honeys with distinct phenolic compounds, with a predominance of phenolic acids such as 3,4-dihydroxybenzoic, salicylic, caffeic, chlorogenic, p-coumaric, ferulic, gallic, syringic acids and flavonoids, including isorhamnetin, kaempferol, luteolin , naringenin, pinobanksin, quercetin and rutin (SERAGLIO et al., 2016). .

The sensory analyzes carried out in this study were carried out with the aim of verifying acceptance and ordering in terms of tasters' preference in view of the general characteristics of the meads. For the acceptance test, the attributes of color, odor, flavor and overall appearance were evaluated (Table 3 and Table 4).

For the attributes of color, aroma and flavor, the average scores obtained were between 4 and 6 corresponding to the terms “indifferent” and “liked” considering the seven-point structured hedonic scale. In these attributes, all meads differed from each other, with multiflower mead

being the least appreciated among tasters, and orange mead the most appreciated in all individual attributes. Orange blossom honey has a unique and striking flora, which has given its respective mead more defined characteristics in terms of color, aroma and flavor. In the two other meads (multifloral and mixed), the flowering of the multifloral honey may have interfered in the distinction of these attributes because it is diversified (Table 3).

The data obtained demonstrate that in the individual attributes the floral source showed a greater acceptance for mead with orange blossom honey, this honey is considered one of the best unifloral honeys in the world, in addition to the appreciated flavor, the floral aroma is exclusive to this type of honey , characterized by its light color, intense aroma, mild flavor and creaminess (TETTE et al., 2017), while mead with multifloral honey has a diverse flora in its composition. When we observe the global acceptance, we see that the orange blossom and the mixed mead present the highest scores, not differing from each other, which is probably attributed to these two meads having orange blossom honey in their composition.

Considering the number of samples tested and the number of tests applied, according to the Newel-McFarlane table, the critical difference between the ordering totals was 34, as the results were not greater than 34, there was no statistical difference for the preference at the 5% significance level. However, even with no significant difference, the highest sum of orders obtained was for orange mead, mixed mead and multifloral mead, respectively. This result may be due to the fact that the drink is not commonly consumed by tasters and, therefore, the less impactful affective memory for this type of analysis (Table 4).

Conclusions

1 The total phenolic compounds of meads that had multifloral honey in their composition showed higher levels compared to those that had orange blossom honey.

2 As antioxidant capacity, meads that had multifloral honey in their composition, even showing greater antioxidant potential, did not differ from orange mead.

3 The phenolic compounds identified in meads show that, regardless of the honey used in its production, they have a varied phenolic composition, which makes it a beverage that carries the benefits present in honey.

4 Meads with orange blossom honey had a higher global acceptance than meads with multifloral honey, demonstrating that the mixture of these two honeys with different characteristics is beneficial for the production of mead.

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Tables

Table 1- Physicochemical characterization, total phenolic compounds and antioxidant activity in honey and mead.

Samples	Total Sugars ($g\ Kg^{-1}$ / $g\ L^{-1}$)	pH	Total acidity ($meq\ Kg^{-1}$/ $meq\ L^{-1}$)	alcohol content (^{o}GL)	Total Polyphenols ($mg\ GAE\ Kg^{-1}$ / $mg\ GAE\ L^{-1}$)	ABTS ($mM\ TEAC\ Kg^{-1}$ / $mM\ TEAC\ L^{-1}$)
Multifloral honey	72,59	3,94	3,65	n.a.	485,84	1,94
Orange blossom honey	67,57	3,98	4,08	n.a.	471,31	1,20
Multiflower mead	29,71 b	3,57 a	54,56 b	10,6 a	218,38 a**	2,04 ab
Orange mead	36,33 a	3,27 c	54,00 b	10,6 a	203,40 B	1,80 b
Mixed mead	29,71 b	3,43 b	59,67 a	10,6 a	223,83 A	2,16 a

n.a = Not applicable. ** Different lowercase letters in the same column correspond to significant differences of each mead by Tukey's test ($p \leq 0.05$). Meq = milliequivalent

Table 2 Identification of phenolic compounds in mead with multifloral honey, orange blossom honey and mixture of multifloral honey and orange blossom honey by LC-ESI-QTOF-MS/MS

RT (min)	Tentative Indentification	Molecular Formula	Monoisotopic Mass	m/z [M-H]- experimental	m/z [M-H]- correction	Error (ppm)	MS^2 product ions (-) (m/z)	Multiflower mead	Orange mead	Mixed mead
9.1	chlorogenic acid	$C_{16}H_{17}O_9$	354,0951	353,0877	354,0950	0,3	191,0574	X	X	X
9.3	protocatechuic acid	$C_7H_5O_4$	154,0266	153,0193	154,0266	0,2	109,0376	X		X
10.8	syringic acid	$C_9H_9O_5$	198,0528	197,0455	198,0528	0,2	111,0182/125,0360/140,0247	X		
11.1	*p*-coumaric acid	$C_9H_8O_3$	164,0473	163,0400	164,0473	0,4	119,0518		X	
18.4	Naringenin	$C_{15}H_{12}O_5$	272,0685	271,0612	272,0685	0,0	125,0266/197,0639/225,0540/253,0480		X	
18.7	Quercetin	$C_{15}H_9O_7$	302,0427	301,0353	302,0426	0,2	151,0175/107,0253/116,0828/121,0426	X	X	X

X= Indicates the presence of the compound in the sample.

Table 3 - Mean scores of sensory attributes evaluated in the mead acceptance test.

Sensory Attributes	Samples		
	Multiflower mead	Orange mead	Mixed mead
Color	5,36 [c]	5,51 [a]	5,42 [b]
Aroma	4,97 [c]	5,54 [a]	5,07 [b]
Flavor	4,76 [c]	5,35 [a]	5,04 [b]
Global Acceptance	5,12 [b]	5,41 [a]	5,30 [a]

Means with equal letters on the same line do not differ significantly from each other by Tukey's test $p \leq 0.05$.

Table 4 - Differences between total sum pairs of preference ordering test for meads.

	Samples		
	Multiflower mead	Orange mead	Mixed mead
Total sum	127 [a]	148 [a]	135 [a]
Difference c I		21	8
Difference x II			29

Means with equal letters on the same line do not differ significantly from each other by the Newel-McFarlane test $p \leq 0.05$

2.3 ARTIGO 2

MELHORIA DAS PROPRIEDADES FUNCIONAIS DO HIDROMEL ENVELHECIDO COM LASCAS DE CARVALHO (QUERCUS) EM DIFERENTES NÍVEIS DE TOSTA.

ENHANCEMENT OF THE FUNCTIONAL PROPERTIES OF MEAD AGED WITH OAK (QUERCUS) CHIPS AT DIFFERENT TOASTING LEVELS.

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Article

Enhancement of the Functional Properties of Mead Aged with Oak (*Quercus*) Chips at Different Toasting Levels

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Abstract: Consumers increasingly prefer and seek functional beverages, which, given their characteristics, provide important bioactive compounds that help prevent and treat chronic diseases. Mead is a traditional fermented alcoholic beverage made from honey solution. The aging process of mead with oak chips is innovative and bestows functional characteristics to this beverage. Thus, in this study, we sought to develop and characterize a novel functional beverage by combining the health benefits of honey with the traditional aging process of alcoholic beverages in wood. Phenolic compounds, flavonoids, and antioxidant capacity were analyzed in mead using oak chips at different toasting levels and aged for 360 days. LC-ESI-QTOF-MS/MS was used to analyze the chemical profile of different meads. Over time, the aging process with oak chips showed a higher total phenolic and flavonoid content and antioxidant capacity. Eighteen compounds belonging to the classes of organic acids, phenolic acids, flavonoids, and tannins were identified in meads after 360 days. Our findings revealed that the addition of oak chips during aging contributed to *p*-coumaric, ellagic, abscisic, and chlorogenic acids, and naringenin, vanillin, and tiliroside significantly impacted the functional quality of mead.

Keywords: polyphenols; honey; functional beverage; characterization; alcoholic beverage; fermented beverage; phenolic compounds; antioxidant; functional foods; beneficial effects

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1. Introduction

The increased prevalence of noncommunicable diseases in recent years has made consumers increasingly aware of healthy and natural diets [1]. Consequently, the food and beverages industry faces new challenges in designing functional foods. Among the different types of functional foods, beverages are the most acceptable due to logistic facilities, their distribution, and the ease of incorporating bioactive compounds as functional ingredients [2]. Furthermore, functional beverages have gained more market shares over the last decade [3].

Recently, wine and beer, two of the most popular alcoholic beverages, have been identified as functional beverages, and the benefits of their moderate consumption have been widely supported by the scientific community [4]. The main source of the beneficial potential of consuming these beverages is phenolic compounds. After consuming phenolic-compound-rich foods, such as functional beverages, the colon is the leading site of microbial fermentation. Intestinal microbiota transforms phenolic compounds into phenolic acids or

lactone structures, which produce metabolites with biological and antioxidant activity, and evidence suggests that these metabolites have health benefits for humans [3,5,6].

Mead or honey wine is a beverage traditionally produced by diluting honey in water and yeast and may present some variations through the addition of fruit or fruit juice, herbs, or spices [7]. Fermentation and maturation/aging are the two most time-consuming processes in mead production, often lasting a few days to months [8]. The final composition of the mead will depend on the type of honey used, the ingredients added, and the fermentation and storage conditions [9]. Mead has been produced since ancient times, especially in Eastern European countries; however, it is currently not as popular as other alcoholic beverages, highlighting the need for further research on this beverage and its potential functionality [10].

Honey is widely known for its health-promoting biological characteristics such as its anti-inflammatory, antiviral, antifungal, and antitumor properties [11]. Among the bioactive descriptors in honey, phenolic compounds, organic acids, carbohydrates, amino acids, proteins, minerals, vitamins, and lipids stand out [11,12], which directly influence the chemical and sensory characteristics of the mead [8,9,12]. Nevertheless, the composition of honey is quite variable and relies on the floral source and seasonal and environmental conditions, in addition to the processing and storage techniques used [9,11].

The aging process of alcoholic beverages in wood provides various changes in the composition and concentration of compounds in beverages [13,14]. Such modifications may be noticeable by changes in the beverages' taste, color, and aroma. In addition to sensory changes, many phenolic compounds are acquired or elevated during maturation [13,15]. Martinez et al. (2008) tested different quenching methods on the chemical composition of American (*Quercus alba*) and French (*Quercus petraea*) oak, and their findings showed the evolution of ellagitannins, a low molecular weight phenolic compound, and volatile compounds regarding oak species and the tempering method [16]. The natural process in the open air was considered superior to the artificial and mixed drying methods, as it showed greater effectiveness in reducing excess ellagitannins. In addition, the evolution of the aromatic potential of the wood was more positive, reaching higher concentrations of compounds such as volatile phenols, phenolic aldehydes, furanic compounds, and *cis*- and *trans*-methyl-γ-octalactones. The wood toasting process takes place after tempering, where the wood is subjected to temperatures in the range of 150–240 °C for a certain period, according to the desired toasting level. In this step, thermal degradation reactions occur, transforming nonvolatile precursors into active aromatic volatile compounds [17].

Due to technological advances in the maturation/aging area of alcoholic beverages, alternative systems using wooden barrels to carry out this process have emerged. Staves, chips, shavings, and other alternative sources of extraction of compounds from oak or other species of forest wood have become common for the maturation and aging process of alcoholic beverages, generating a new use of wood residues in the cooperage [15,18].

Thus, this study aimed to develop a functional mead with multifloral honey and submit it to the aging process with oak chips (*Quercus*), a wood commonly used for the maturation and aging of alcoholic beverages that can provide bioactive compounds. In addition, we sought to evaluate the phenolic compounds content and antioxidant capacity after fermentation and identify the phytochemical compounds in mead aged with oak chips at two toasting levels.

2. Results and Discussion

Functional beverages can be a valuable component of the human diet given their ability to provide essential hydration and important bioactive compounds for maintaining health and/or contributing to preventing and treating chronic diseases [1,2,5,6]. Mead is a beverage that has been gaining more and more notoriety over time, although there are few studies on it; hence, this study is unprecedented as it presents data from meads aged with oak chips, a process so far only used for other types of alcoholic beverages [13] and which can improve the functionality of this beverage. In this study, the antioxidant capacity,

total phenolic and flavonoid content, and characterization of phytochemical compounds of meads subjected to a 360-day aging period with oak chips (*Quercus*) at different toasting levels were determined.

Honey is a source of numerous biologically active compounds, including phenolic and volatile compounds, peptides, proteins, amino acids, enzymes, and minerals, which can be transferred to the mead during production [12]. Among all the substances, our attention was given to the phenolic compounds, which contribute to the mead functional quality. This phytochemical group includes flavonoids, tannins, and phenolic acids, which are natural antioxidants that play significant roles in the human body due to their capability to inhibit free radicals, which may cause cell damage, leading to chronic diseases [2,3,19]. Beverages are considered good dietary sources of phenolic compounds; moreover, the phenolic compounds in beverages are highly bioaccessible because they pass directly into intestinal fluids [5], making this study highly innovative in carrying out the aging process of mead with oak chips and monitoring it over time.

At time zero, all elaborated meads had a similar content of phenolic compounds, which came from the honey used (Figure 1). In the base mead (BM), these values remained constant for up to 180 days and then decreased with advancing maturation time. Meads with chips addition showed higher total phenolic compound levels until the aging period of 180 days. This increase may be related to the extraction of phenolic compounds from oak into mead throughout the maturation process. Canas et al. (2019) observed higher total phenolic compounds in wine spirits aged for 180 days with oak staves [20]. This increase was due to the combination of the thermal degradation of lignin and increased wood permeability during thermal treatment. Mead is still poorly studied, although due to its characteristics similar to young white wines, aging time may have provided oxidative reactions and/or condensation between the mead compounds with some wood molecules, reducing the phenolic compounds after 270 days of aging.

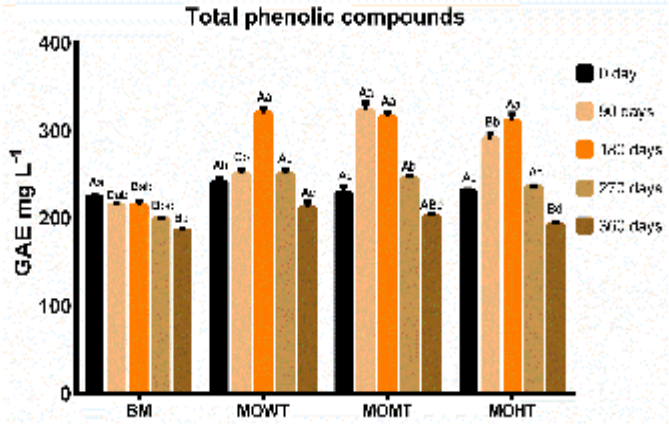

Figure 1. Composition of total phenolics (mg GAE L^{-1}) of meads aged with oak (*Quercus*) chips. Data are presented as means ± SEM ($n = 5$). Lowercase letters indicate significant differences over time within the same experimental group ($p \leq 0.05$). Capital letters indicate significant differences between experimental groups within the same time ($p \leq 0.05$). GAE: gallic acid equivalent; BM: base mead; MOWT: mead aged with oak chips without toasting; MOMT: mead aged with oak chips at medium toasting; MOHT: mead aged with oak chips at high toasting.

Furthermore, flavonoids gradually increased in meads with oak chips throughout the aging period, with a maximum content at 270 days (Figure 2). We observed that the concentration of this class of phenolic compounds varied from 10.6 mg to 20.5 mg CAE L^{-1}. Regardless of the toasting degree, the oak chips increased the mead flavonoids concentra-

tions, which may have contributed to increasing the antioxidant capacity throughout the aging process (Figure 3) since these compounds have high free radical scavenging potential, as described elsewhere [8,19].

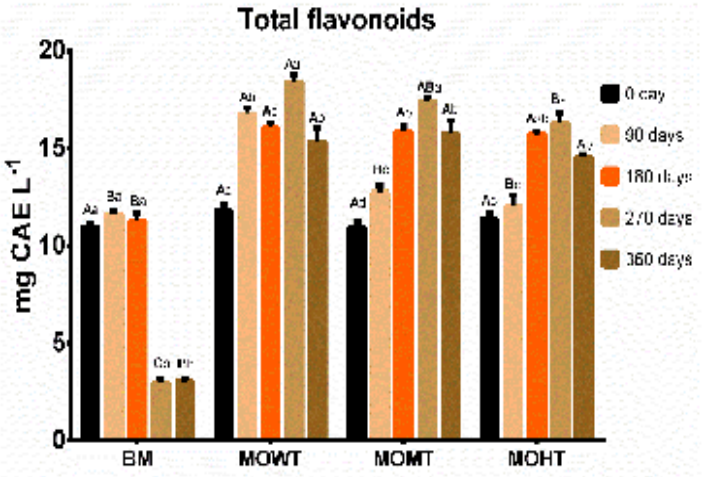

Figure 2. Composition of total flavonoids (mg CAE L^{-1}) of meads aged with oak (*Quercus*) chips. Data are presented as means ± SEM ($n = 5$). Lowercase letters indicate significant differences over time within the same experimental group ($p \leq 0.05$). Capital letters indicate significant differences between experimental groups within the same time ($p \leq 0.05$). CAE: catechin equivalent; BM: base mead; MOWT: mead aged with oak chips without toasting; MOMT: mead aged with oak chips at medium toasting; MOHT: mead aged with oak chips at high toasting.

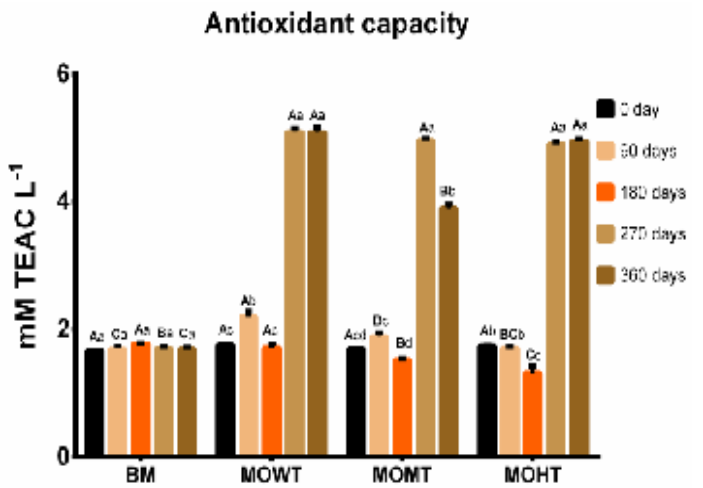

Figure 3. Antioxidant capacity (mM TEAC L^{-1}) of meads aged with oak (*Quercus*) chips. Data are presented as means ± SEM ($n = 5$). Lowercase letters indicate significant differences over time within the same experimental group ($p \leq 0.05$). Capital letters indicate significant differences between experimental groups within the same time ($p \leq 0.05$). TEAC: Trolox equivalent antioxidant capacity; BM: base mead; MOWT: mead aged with oak chips without toasting; MOMT: mead aged with oak chips at medium toasting; MOHT: mead aged with oak chips at high toasting.

The antioxidant capacity of meads depends on the raw material's chemical composition, the environmental factors that directly affect the honey production process, and the technologies used to process it [8,9]. Apart from technological processes such as fermentation and aging, these beverages' antioxidant properties and chemical composition are determined by the additives used in their manufacturing [8]. In this investigation, the antioxidant capacity was determined with a 2,2′-azino-bis (3-ethylbenzothiazoline) 6-sulfonic acid (ABTS) assay and varied from 1.31 to 5.06 mM of Trolox equivalent antioxidant capacity per liter (TEAC L^{-1}), showing a significant increase after 270 days of aging in meads with the addition of the oak chips (Figure 3). In meads, the antioxidant capacity is related to the presence of phenolic compounds, and the diversity of these compounds is directly linked to the honey used [8]. Hence, meads that only use honey and water in their composition tend to have lower compound diversity.

Nevertheless, meads with the addition of fruit juices or herbal extracts have a wider range of these phytochemicals [12]. The profile of the antioxidant capacity is a concomitant event to the behavior of the flavonoid content over time (Figures 2 and 3), especially at 270 days. A similar behavior was observed in unripened meads and other wood-aged beverages [10,21]. However, one cannot rule out the possibility that other bioactive compounds, in addition to flavonoids, contributed to the antioxidant capacity observed in the final aging period of meads with oak chips in this investigation. Phenolic compounds are found in plants as they come from their secondary metabolism. Bees, by collecting pollen, transfer many of these compounds to the honey; consequently, these phytochemicals will be present in the mead, even in smaller amounts [9,11]. The Brazilian flora is vast, generating honeys with various phenolic compounds, with a predominance of many phenolic acids such as 3,4-dihydroxybenzoic, salicylic, caffeic, chlorogenic, *p*-coumaric, ferulic, gallic, syringic acids, and flavonoids including isorhamnetin, kaempferol, luteolin, naringenin, pinobanksin, quercetin, and rutin [22].

In this study, the phenolic compounds in the meads at the end of 360 days of aging were identified through MS/MS mass spectrometry analysis by combining the chromatographic behavior and collision spectra with compounds already described in the literature. Eighteen compounds belonging to the classes of organic acids, phenolic acids, flavonoids, and tannins were tentatively identified and are listed in Table 1 and in the Supplementary Material (Figure S1).

Among the compounds identified are citric acid, protocatechuic acid, sinapyl alcohol, syringic acid, ethylvanillin, 1-(2-hydroxy-4,6-dimethoxyphenyl)-ethanone, sebacic acid, and quercetin in all samples, which are therefore compounds from the honey used in the beverage's elaboration. Adding oak chips during aging, regardless of the toasting, contributed to vanillin, *p*-coumaric acid, ellagic acid, abscisic acid, and naringenin. However, chlorogenic acid and tiliroside were only present in the meads aged with oak chips without toasting (MOWT) and meads aged with oak chips at high toasting (MOHT). Various studies have demonstrated that chlorogenic acids are partially bioavailable and potentially beneficial to human health [6]. The antioxidant and anti-inflammatory effects of coffee chlorogenic acids are responsible for, at least to a certain extent, the association between coffee consumption and the lower incidence of various degenerative and nondegenerative diseases, in addition to higher longevity [6]. 2,3-Dihydroxy-1-guaiacylpropanone was only found in the BM, and the 3-hydroxy-3-(3-hydroxyphenyl) propionic acid was found in the BM and mead aged with oak chips without toasting (MOWT).

Table 1. Phytochemical compounds detected in mead produced with multifloral honey aged with oak (*Quercus*) chips.

Tentative Indentification	RT (min)	Molecular Formula	Molecular Weight	Theoretical (*m*/*z*)	Observed (*m*/*z*)	Fragmentation Ion (*m*/*z*)	BM	MOWT	MOMT	MOHT	Reference
Citric acid	2.1	$C_6H_8O_7$	192.0270	191.0197	191.0368	111.0205	X	X	X	X	[12]
3-Hydroxy-3-(3-hydroxyphenyl) propionic acid	9.0	$C_9H_{10}O_4$	182.0579	181.0506	181.0533	121.0420/122.0480	X	X			[23]
2,3-Dihydroxy-1-guaiacylpropanone	9.2	$C_{10}H_{12}O_5$	212.0685	211.0612	211.0789	134.0499/150.0450	X				[23]
Chlorogenic acid	9.2	$C_{16}H_{18}O_9$	354.0951	353.0878	353.0899	191.0574		X		X	[22]
Protocatechuic acid	9.3	$C_7H_6O_4$	154.0266	153.0193	153.0335	109.0376	X	X	X	X	[12]
Butanedioic acid	10.6	$C_8H_{14}O_5$	190.0841	189.0768	189.0791	129.0680/127.0878/99.0934		X	X		[24]
Vanillin	10.7	$C_8H_8O_3$	152.0475	151.0403	151.0414	108.0181		X	X	X	[12]
Sinapyl alcohol	10.7	$C_{11}H_{14}O_4$	210.0892	209.0819	209.0996	137.0266	X	X	X	X	[25]
Syringic acid	10.8	$C_9H_{10}O_5$	198.0528	197.0455	197.0477	111.0182/125.0360/140.0247	X	X	X	X	[22]
Ethylvanillin	11.0	$C_9H_{10}O_3$	166.0630	165.0557	165.0583	119.0514/117.0355	X	X	X	X	[12]
p-Coumaric	11.1	$C_9H_8O_3$	164.0473	163.0401	163.0401	119.0518		X	X	X	[10]
1-(2-hydroxy-4,6-dimethoxyphenyl)-ethanone	11.4	$C_{10}H_{12}O_4$	196.0736	195.0663	195.0690	117.0337/134.0387	X	X	X	X	[26]
Ellagic acid	11.6	$C_{14}H_6O_8$	302.0063	300.9990	301.0017	229.0170/301.0018		X	X	X	[27]
Abscisic acid	17.1	$C_{15}H_{20}O_4$	264.1362	263.1289	263.1313	136.0543/203.1091		X	X	X	[22]
Sebacic acid	17.5	$C_{10}H_{18}O_4$	202.1205	201.1132	201.1302	183.1170/139.1259	X	X	X	X	[28]
Quercetin	17.7	$C_{15}H_{12}O_5$	302.0427	301.0354	301.0584	151.0175/107.0253/116.0828/121.0426	X	X	X	X	[22]
Naringenin	18.5	$C_{15}H_{10}O_7$	272.0685	271.0612	271.0632	125.0266/197.0639/225.0540/253.0480		X	X	X	[10]
Tiliroside	20.7	$C_{30}H_{26}O_{13}$	594.1373	593.1301	593.1326	121.0298/209.0480/417.0965		X		X	[29]

BM: base mead (only honey); MOWT: mead aged with oak chips without toast; MOMT: mead aged in oak chips in medium toast; MOHT: mead aged in oak chips in high toast.

During the aging process, the transformations of lignin, present in woods such as French oak, are among the most important factors that affect the quality of beverages aged in contact with this material. Lignin is a polymer that undergoes thermal degradation during the manufacturing of barrels or by hydrolysis and ethanollysis during the aging of wines and alcoholic beverages [30]. The lignin macromolecule has ramifications of coniferyl alcohols (guaiacyl compounds) and sinapyl (syringyl compounds). Coniferyl alcohol generates coniferylaldehydes, which are converted into vanillin, while sinapyl alcohol gives rise to sinapaldehyde, which is transformed into syringaldehyde and later oxidized to syringic acid. Other compounds, such as hydrolyzable tannins, present in French oak, are more soluble in hydroalcoholic solutions; its transformation into ellagic acid is very common [31], corroborating the identification of this compound only in meads with the addition of oak chips.

In the present investigation, meads aged with oak chips showed a higher phenolic compound content and diversity. It is known that these compounds may be related to physicochemical and sensory characteristics in foods [8,12]. Thus, the sensory evaluation of meads is important to know the impact of compounds on sensory aspects, including color, flavor, and astringency. The absence of a sensory evaluation of different meads is one of the limitations of this study. These analyses were scheduled to be carried out in 2020–2021, and the study had already been approved by the institutional ethics committee (CAAE no. 58889316.3.0000.5346). Unfortunately, given the restrictions imposed by the SARS-CoV-2 pandemic, it was impossible to carry out the analyses. Until now, the classic wood-aging method employed in numerous alcoholic beverages has not yet been tested in mead. Hence, despite the absence of a sensory evaluation, this study is pioneering and can contribute to essential elucidations in the field of functional beverages.

Mead is a traditional alcoholic beverage obtained by the fermentation of mead wort and popularly produced at home and in small meaderies. Different types of mead can be distinguished based on the honey-to-water ratio, the addition of spices and/or fruits, and the method of wort preparation [8,10,12]. The consumption of mead has gained popularity given the presence of its natural and high-quality bioactive compounds. As a result, mead production and consumption have remarkably increased during the past years [8]. The regular consumption of foods and beverages rich in bioactive compounds has been associated with a series of beneficial health effects [1,2], although, in the case of mead, in addition to these compounds, this beverage also has a considerable alcohol content (8–18%) [8,10,12]. The excessive and prolonged use of alcoholic beverages is significantly linked to mild symptoms such as fatigue, difficulty walking, fainting, and behavioral changes. In addition, long-term consumption is responsible for serious health problems such as depression, anxiety, impaired cognitive performance, and liver diseases (e.g., alcoholic hepatitis and liver cirrhosis), which is considered one of the main causes of death and functional disability in the world [32,33]. Given the above, the moderate consumption of mead and other alcoholic beverages is suggested.

3. Materials and Methods

3.1. Analytical Reagents

HPLC-grade methanol used for the mobile phase was obtained from Merck (Darmstadt, Germany). HPLC-grade acetonitrile and formic acid used for the mobile phase were

Table 2. Coding and characterization of treatments used in this study.

Code	Treatment
BM	Base mead—not aged with oak chips
MOWT	Mead aged with oak chips without toasting
MOMT	Mead aged with oak chips at medium toasting (170 °C for 35 min)
MOHT	Mead aged with oak chips at high toasting (200 °C for 45 min)

3.3. Samples Acquirement

To produce the mead we used multifloral honey from an apiary located in Santiago city (29.1991393′ S and 54.8644842′ W, 467 m) in the central region of Rio Grande do Sul, Southern Brazil. It has an area of 2,413 km^2 of foraging, and the climate is classified as humid subtropical, with an annual average temperature of between 18 and 20 °C and an average annual rainfall of 359 mm. For the aging of mead, we utilized oak chips (*Quercus*) purchased from WE Consultoria (Porto Alegre, Rio Grande do Sul, Brazil).

The mead was prepared with multifloral honey and water until it reached 21° Brix; then, it was inoculated with 20 hg L^{-1} of the *Saccharomyces baianus* yeast strain and 30 hg L^{-1} of nutrients (Nutristart). Fermentation was carried out in a glass fermenter with a capacity of 30 L with the system maintained under anaerobic conditions through the water seal at a constant temperature of 20 °C, and it was monitored daily by measuring the total soluble solids content and initial and final density. The end of the fermentation process occurred at 27 days with the cessation of carbon dioxide evolution followed by the stabilization of total soluble solids and the stabilization of density. The mead was then stabilized for 15 days at 16 °C. The time between fermentation and the beginning of aging was 45 days. In the last step, the mead was sulfited at 50 ppm and bottled in 300 mL bottles with the addition of 2 g L^{-1} of oak chips (*Quercus*) followed by the aging process. Aliquots were taken every 90 days for up to 360 days to analyze the content of the total phenolic compounds, total flavonoids, and antioxidant capacity. At the end of the 360 days of aging, the phenolic compounds in the different meads were identified.

3.4. Total Phenolic Content

The total phenolic compounds in each mead sample were quantified with spectrophotometry through the redox reaction with the Folin–Ciocalteu reagent [34]. The readings of the samples were performed in triplicate of the absorbances in a UV–visible spectrophotometer (FEMTO 600 plus) at a wavelength of 765 nm after they were left to rest for two hours at room temperature. The phenolic compounds content was calculated by interpolating the absorbance of the samples against the calibration curve constituted with a standard of gallic acid (0–80 mg L^{-1}), and the results are expressed in milligrams of gallic acid equivalent per liter (mg GAE L^{-1}).

3.5. Flavonoids Content

Flavonoid compound determination was performed according to the method of Zhishen, Mengcheng, and Jianming (1999) [35]. The absorbance readings were performed in triplicate with a UV–visible spectrophotometer (FEMTO 600 plus) at 550 nm. The flavonoid concentration in the meads was calculated by interpolating the data with the calibration curve constituted with the catechin standard (0–250 mg L^{-1}), and the results were expressed in milligrams of catechin equivalent per liter (mg CAE L^{-1}).

3.6. Antioxidant Capacity Determination

The antioxidant capacity of meads was determined with the ABTS method using the method described by Re et al. (1999) [36]. The absorbance readings of the samples were taken 6 min after the reaction in a UV–visible spectrophotometer (FEMTO® 600 plus) at 750 nm. The ABTS concentration was calculated from a calibration curve using Trolox as

the standard (0–0.2 mM TEAC L^{-1}). Readings were performed in triplicate, and the results are expressed as mM of Trolox equivalent antioxidant capacity per liter (mM TEAC L^{-1}).

3.7. Purification Procedure in SPE C18

Samples were purified prior to performing the LC-ESI-QTOF-MS/MS analysis. Sample purification was performed according to the method described by Rodriguez-Saona and Wrolstad (2001) with adaptations [37,38]. The mead samples (6 mL) were placed in a rotary evaporator (Büchi, Essen, Germany) at 35 °C for five minutes to remove the alcohol present in the sample. Afterward, the sample was loaded into C-18 solid phase extraction (SPE) cartridges (cartridges SPE-C18, Strata C18-E, Phenomenex), previously activated with methanol and conditioned with acidified water (0.1% v/v formic acid). The polar compounds were washed with two volumes of aqueous formic acid solution (0.1% v/v). Fewer polar phenolic compounds were eluted with two volumes of ethyl acetate (3 mL). The ethyl acetate fraction was dried on a rotary evaporator and made up to a known volume (1 mL) with acidified methanol (0.1% v/v formic acid) and acidified water (0.1% v/v formic acid) (200 + 800 µL). All fractions were analyzed directly as purified fractions in a chromatograph.

3.8. Phenolic Compound Identification by LC-ESI-QTOF-MS/MS

The method to identify the phenolic compounds was based on Quatrin et al. (2019) [39]. The liquid chromatography (LC) instrument (Shimadzu, Kyoto, Japan) was connected in series to a DAD detector (SPD-M20A) and a mass spectrometer (MS) with a Quadrupole-Time-of-Flight (QTOF) analyzer and an electrospray ionization source (ESI) (Bruker Daltonics, micrOTOF-Q III, Bremen, Germany). A 20 µL sample was injected into a reversed-phase column (C-18 Hypersil Gold, 5 µm particle size, 150 mm, 4.6 mm; Thermo Fisher Scientific, Waltham, MA, USA). Mobile phase A for this method consisted of ultrapure water with formic acid acidified methanol (95:5:0.1 v/v); mobile phase B was acetonitrile and formic acid (99.9:0.1 v/v). The ESI conditions were a capillary voltage of −4500 V (negative), nebulizer gas pressure at 30 psi, dry gas at 11 mL min^{-1}, and gas temperature at 310 °C. The MS/MS experiments were performed in a full scan range of 100–1800 m/z of all fragments formed from 3 major parent ions per second. The LC solutions software (Version 3, Shimadzu, Kyoto, Japan) was used to process the data obtained. The tentative identification of compounds was based on the combined information of elution order, ultraviolet–visible (UV–Vis) spectra, and mass spectrometry fragmentation patterns. These data were compared to literature data and public databases (PubChem, KEGG, MassBank of North America (MoNA), ChemSpider, Phenol-Explorer, and FooDB).

3.9. Statistical Analysis

All analytical results were selected for an analysis of variance (ANOVA). The comparison of posthoc mean analysis was performed with a Tukey's test at 5% error probability using Statistica 9.0 software (StatSoft, Tulsa, OK, USA); the graphs were made using the GraphPad Prism 6.0 software (Dotmatics, San Diego, CA, USA).

4. Conclusions

The highly innovative process of using oak chips to improve mead characteristics has not yet been described in the literature. Oak chips increase phenolic compound variability in mead, their flavonoid content, and their antioxidant capacity over storage time. Our findings revealed that mead aged with oak chips as a beverage has more potential for beneficial biological activity due to the higher phenolic compound content than mead without oak chips. Therefore, the use of oak chips in the mead aging process, regardless of toasting levels, improved the functional quality of the beverage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules28010056/s1, Figure S1: Representative chromatogram of mead aged with oak chips.

Author Contributions: Conceptualization, J.P.F. and C.K.S.; data curation, J.P.F., S.S. and C.K.S.; formal analysis, J.P.F., F.W.F., J.B. and G.A.U.; funding acquisition, E.R., M.A.M. and C.K.S.; investigation, J.P.F., F.W.F., J.B. and S.S.; methodology, J.P.F., F.W.F., J.B., G.A.U., C.A.B. and M.A.M.; project administration, C.K.S.; supervision, C.A.B., E.R., S.S. and C.K.S.; validation, F.W.F., E.R. and M.A.M.; writing—original draft, J.P.F.; writing—review and editing, S.S. and C.K.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are openly available in the Harvard Dataverse at doi: 10.7910/DVN/MBWA4J.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: All meads samples are available from the authors.

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3 CONSIDERAÇÕES FINAIS

Os resultados obtidos nesse trabalho indicaram que o hidromel é uma bebida que tem boa aceitação pelo público consumidor de bebidas alcoólicas, podendo tornar-se uma fonte de renda para o pequeno produtor de mel. A origem floral do mel utilizado demonstrou influência na qualidade físico-química do hidromel, sendo o mel multifloral mais rico em compostos bioativos. Porém méis com características mais marcantes como o mel de flor de laranjeira podem ser mais atrativos ao paladar. Por fim, o hidromel é uma bebida que carrega os compostos presentes no mel e a adição dos *chips* de carvalho (*Quercus sp*.) no seu processo de maturação é uma ferramenta promissora para aumentar os compostos fenólicos e a qualidade dos hidroméis.

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APÊNDICE - PHENOLIC COMPOUND TENTATIVE IDENTIFICATION

In the analysis of phytochemical compounds in meads by LC-ESI-QTOF-MS/MS it was possible to identify 18 compounds (Figure S1). Tentative identification was performed based on retention time, elution order and MS spectra compared to literature data.

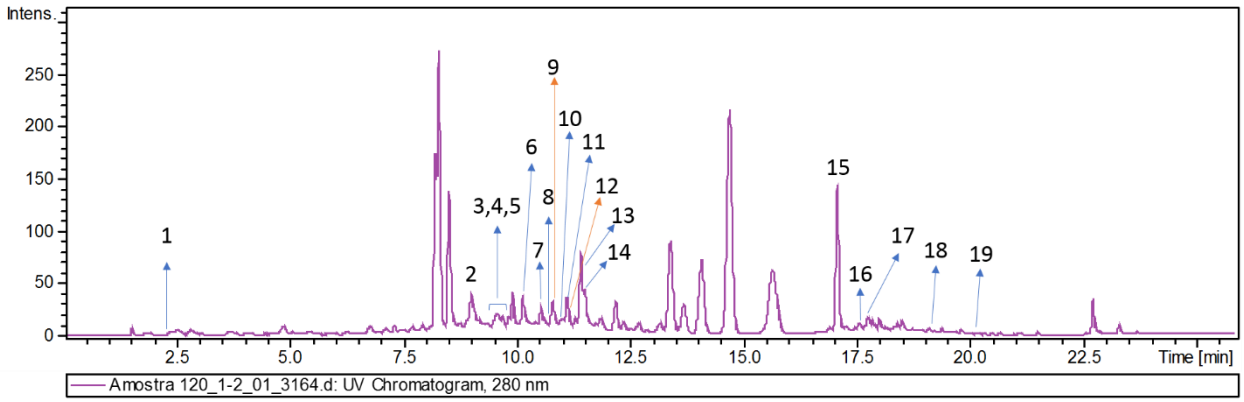

Figure S1 – Representative chromatogram of mead aged with oak chips

Compound 1 (citric acid) was detected with a precursor ion at m/z 191, which produced ion m/z 111.

Compound 2 (3-hydroxy-3-(3-hydroxyphenyl) propionic acid) was tentatively identified by a molecular ion at m/z 181 in negative ionization mode.

2,3-Dihydroxy-1-guaiacylpropanone (compound 3) exhibiting precursor ion at m/z 211 in ESI− mode.

Compound 4 identified as chlorogenic acid presented precursor ion m/z 353 and showed characteristic product ion m/z 191 which corresponds to the deprotonated quinic acid.

Compound 5 (Protocatechuic acid) was detected with a precursor ion at m/z 153.0335 [M-H]- and base peak ion at m/z 109.0376.

Compound 6 (not identified) was detected with a precursor ion at m/z 281.1609 [M-H]- , which produced MS/MS base peak ion at m/z 137.1136; 171.1336, and 189.1480.

Compound 7 butanedioic acid was detected with a precursor ion at m/z 189.0791[M-H]- which produced MS/MS base peak ion at m/z 129.0680; 127.0878 and 99.0934.

Compound (peak 8) was detected with a precursor ion at m/z 151.0414 [M-H]- with base peak ion 108.0181.

Compound 9, sinapyl alcohol was detected with precursor ion at m/z 209.0996 [M-H]- with base peak ion 137.0266.

Compound 10 (syringic acid) was detected with a precursor ion at m/z 197.0477 produced fragment íons 111.0182; 125.0360; 140.0247 m/z.

Compound 11 (ethylvanillin) was detected with a precursor ion 165.0583 and produced fragment ions at m/z 119.0514/117.0355.

Compound 12 was identified as p-Coumaric acid furnished a deprotonated molecule [MH] at 163.0401 m/z and an $[M-H]^-$ ion at 119.0518 m/z.

Compound 13 was identified as 1-(2-hydroxy-4,6-dimethoxyphenyl)-ethanone with a precursor ion at m/z 195.0690 and produced MS/MS base peak ion at m/z 117.0337l and 134.0387.

Compound 14 (ellagic acid) shows a precursor ion at 301.0017 with produced MS/MS base peak ion at 229.0170 and 301.0018.

Compound 15 (abscisic acid) was trying to identify with a precursor ion at m/z 263.1313 and produced the fragment ion at m/z 136.0543 and 203.1091.

Compound 18 (naringenin) shows a precursor ion at 271.0632 with produced MS/MS base peak ion at m/z 125.0266/197.0639/225.0540/253.0480.

Compound 19, the aglycone quercetin with m/z 301.0584 produced the fragment ion at m/z 151.0175; 107.0253; 116.0828 and 121.0426.