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COMPOSTOS FENÓLICOS BIODISPONÍVEIS DO BAGAÇO DE OLIVA APRESENTAM EFEITO ANTIOXIDANTE EM *CAENORHABDITIS ELEGANS* E EM CÉLULAS DA MICROGLIA

Porto Alegre
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Orientador: Prof. Dr. José Claudio Fonseca Moreira
Coorientadora: Profa. Dra. Paula Rossini Augusti

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RESUMO

O bagaço de oliva é um subproduto da produção do óleo de oliva rico em compostos fenólicos que apresentam diferentes benefícios à saúde. Os compostos fenólicos mais representativos no bagaço de oliva são dos grupos secoiridoides, flavonols, ligninas, ácidos fenólicos e álcoois fenólicos. No entanto, os efeitos benéficos atribuídos ao consumo destes compostos fenólicos estão associados à sua biodisponibilidade, que normalmente é baixa (5-10%). Diante deste contexto, processos que melhoram a biodisponibilidade dos compostos fenólicos do bagaço de oliva, como técnicas de redução de tamanho, podem afetar diretamente as propriedades benéficas destes compostos. O objetido do presente trabalho foi avaliar a atividade antioxidante dos compostos fenólicos de bagaço de oliva obtidos após um processo de digestão gastrointestinal *in vitro*. O bagaço de oliva foi submetido a dois métodos de redução de tamanho: fracionamento (tamanho de partícula < 2 mm) e fracionamento seguido de micronização (tamanho de partícula < 20 μm) e as amostras foram submetidas a uma digestão gastrointestinal simulada. Os compostos fenólicos obtidos após o processo de digestão foram analisados quanto à sua composição fenólica, sua atividade antioxidante no modelo animal alternativo (*Caenorhabditis elegans*), bem como em células da microglia (BV2). O tipo de processamento afetou o perfil de compostos fenólicos biodisponíveis do bagaço de oliva, o que resultou em comportamentos diferenciados nos modelos experimentais analisados. Demonstramos pela primeira vez que os compostos fenólicos biodisponíveis do bagaço de oliva em concentrações elevadas (1,5-3 mg L^{-1}) podem ser tóxicas para células microgliais e para o nematoide. Nas células microgliais os compostos biodisponíveis do bagaço de oliva apresentaram efeito antioxidante (0,03- 1,5 mg L^{-1}), independente do tamanho de particula. Além disso, os compostos fenólicos biodisponíveis do bagaço de oliva apresentaram capacidade de prevenir o estresse oxidativo induzido por peróxido de hidrogênio no *C. elegans*. No entanto, este efeito foi dependente do tamanho de partícula. Menores tamanhos de partícula atenuaram o estresse oxidativo em concentrações de 0,03 e 0,15 mg L^{-1}, enquanto que maiores tamanhos de partícula apresentaram este efeito no nematoide nas concentrações de 0,15 e 0,30 mg L^{-1}. Os efeitos antineuroinflamatórios e antioxidantes observados nos ensaios *in vivo* e *in vitro* podem ser atribuídos aos diferentes compostos fenólicos presentes no bagaço de oliva. Além disso, diferentes tamanhos de partículas resultaram em perfis fenólicos diferentes, o que pode justificar os diferentes efeitos observados nos experimentos. Por fim, este estudo apresenta resultados extremamente relevantes para garantir a segurança

e buscar uma possível aplicação alimentícia do bagaço de oliva, visando o aproveitamento de sua riqueza em termos de compostos fenólicos.

Palavras-chave: Sub-produtos alimentícios; Digestão gastrointestinal; Biodisponibilidade; Células microgliais; *Caenorhabditis elegans*; Neuroinflamação;

ABSTRACT

The olive pomace is a by-product of producing olive oil and is rich in phenolic compounds that present different health benefits. The most representative phenolic compounds in olive oil contain two secoiridoid groups, flavonols, lignins, phenolic acids and phenolic alcohols. However, the beneficial effects attributed to the consumption of these phenolic compounds are associated with their bioavailability, which is normally low (5-10%). Given this context, processes that enhance the bioavailability of phenolic compounds from olive pomace, such as size reduction techniques, can directly affect the beneficial properties of these compounds. The objective of this work was to evaluate the antioxidant and anti-neuroinflammatory activities of phenolic compounds from olive pomace obtained after an *in vitro* gastrointestinal digestion process. The olive pomace was subjected to two size reduction methods: fractionation (particle size < 2 mm) and fractionation followed by micronization (particle size < 20 μm), and the samples were subjected to a simulated gastrointestinal digest. The phenolic compounds obtained after the digestive process were analyzed regarding their phenolic composition, their antioxidant activity in the alternative animal model (*Caenorhabditis elegans*), as well as in microglial cells (BV2). The type of processing affected the profile of bioavailable phenolic compounds from olive pomace, which resulted in differentiated behaviors in the experimental models analyzed. We demonstrate for the first time that bioavailable phenolic compounds from olive pomace in high concentrations (1.5-3 mg L^{-1}) can be toxic to microglial cells and nematoid. In the microglial cells, the bioavailable compounds from olive pomace have antioxidant (0.03-1.5 mg L^{-1}) effect, regardless of particle size. Furthermore, bioavailable phenolic compounds from olive pomace have the capacity to prevent oxidative stress induced by hydrogen peroxide in *C. elegans*. However, this effect was dependent on particle size. Smaller particle sizes attenuate oxidative stress in concentrations of 0.03 and 0.15 mg L^{-1}, while larger particle sizes present this nematoid effect in concentrations of 0.15 and 0.30 mg L^{-1}. The anti-neuroinflammatory and antioxidant effects observed in *in vivo* and *in vitro* tests can be attributed to the different phenolic compounds present in the olive pomace. Furthermore, different particle sizes will result in different phenolic profiles, which may explain the different effects observed in the experiments. Finally, this study presents extremely relevant results to guarantee safety and search for a possible food application of olive pomace, aiming at the exploitation of its richness in terms of phenolic compounds.

Key words: Food by-products; Gastrointestinal digestion; Bioavailability; Microglia cells; *Caenorhabditis elegans*; Neuroinflammation;

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1. APRESENTAÇÃO

A fim de facilitar a compreensão, a tese está organizada em diferentes seções.

A seção INTRODUÇÃO consta de uma revisão sucinta da literatura sobre os temas trabalhados nesta tese.

Na seção METODOLOGIA constam informações sobre a amostra utilizada, processo de digestão gastrointestinal *in vitro* e protocolos experimentais usados no presente trabalho.

A seção RESULTADOS está apresentada sob a forma de dois artigos já publicados e um manuscrito em processo de preparação para submissão. As seções Materiais e Métodos, Resultados, Discussão, Conclusão e Referências Bibliográficas, encontram-se nos próprios artigos e representam a íntegra deste estudo.

O item DISCUSSÃO apresenta interpretações e comentários gerais sobre os trabalhos científicos aqui incluídos.

As REFERÊNCIAS BIBLIOGRÁFICAS referem-se somente às citações que aparecem nos itens REVISÃO BIBLIOGRÁFICA e DISCUSSÃO.

2. INTRODUÇÃO

A produção de oliva (*Olea europaea L.*) tem crescido ao longo dos anos, se estendo para áreas de cultivo não tradicional. A maior área de cultivo e produção de oliva se concentra na bacia do Mediterrâneo, responsável por 95 % das olivas cultivadas no mundo inteiro. No Brasil, o cultivo de olivas ainda é recente e abrange os estados de Minas Gerais, São Paulo, Santa Catarina, Paraná e Rio Grande do Sul (Borges et al., 2017). Após o período de colheita, a maior parte das olivas é destinada para a produção do azeite, o restante é comercializado para consumo *in natura* (Simonato et al., 2019).

O processo produtivo para a obtenção do azeite gera grande quantidade de um subproduto considerado de elevado valor agregado devido a presença de compostos fenólicos, o bagaço de oliva. O bagaço de oliva consiste em um subproduto sólido oriundo do processamento da oliva para a produção de azeite (Difonzo et al., 2021). Atualmente a maior utilidade deste bagaço é para a recuperação do óleo residual, no entanto este processo é dispendioso e necessita de maior tempo e energia para sua extração. Desta maneira, o bagaço de oliva torna-se um problema para a indústria beneficiadora de azeite, uma vez que devido a presença de compostos fitotóxicos em sua composição, este subproduto não pode ser liberado no meio ambiente (Berbel & Posadillo, 2018). Diante disto, a busca por alternativas sustentáveis para o uso do bagaço de oliva têm sido alvo de estudos, sendo uma das principais alternativas a extração de moléculas de interesse como os compostos fenólicos (Difonzo et al., 2021).

O bagaço de oliva desperta interesse em estudos de diferentes áreas relacionadas a saúde, medicina e nutrição, pois apresenta em sua composição uma grande quantidade de compostos bioativos, como os compostos fenólicos, tocoferóis, fitoesteróis e fibras. Estes compostos, especialmente os compostos fenólicos apresentam diferentes efeitos benéficos para a saúde humana, como ação como antioxidante, anti-inflamatória, anticâncer, anti-hipertensiva, neuroprotetora e antidiabétes (Angeloni et al., 2017). Dentre os compostos bioativos do bagaço de oliva, os compostos fenólicos são os mais representativos. Ao longo do processo de produção do azeite de oliva, cerca de 98 % dos compostos fenólicos presentes na oliva permanecem do bagaço, devido a suas características hidrofílicas, o que torna este subproduto de elevado valor agregado (Simonato et al., 2019).

Os compostos fenólicos são produto do metabolismo secundário das plantas quando em condições estressoras. O bagaço de oliva é rico em compostos fenólicos dos grupos flavonols, secoiridoides, ácidos fenólicos, álcoois fenólicos e ligninas. A

oleuropeína é o composto secoiridóide mais representativo no bagaço de oliva, seguido dos álcoois fenólicos, tirosol e hidroxitirosol. Uma grande variedade de ácidos fenólicos está presente no bagaço de oliva, em especial os ácidos vinílico, cafeico, *p*-cumárico, ferulico e gálico. O pinoresinol e seus derivados são as ligninas mais comuns no bagaço de oliva, enquanto rutina, apigenina e luteolina são os compostos flavonols mais representativos (Altindiş et al., 2020; Angeloni et al., 2017; Difonzo et al., 2021).

A principal característica destes compostos é sua capacidade de impedir a formação e/ou remover espécies reativas (ER). Através da remoção das ER os compostos fenólicos atuam no balanceamento do estresse oxidativo no organismo humano. O estresse oxidativo, por sua vez, pode desencadear diferentes patologias como por exemplo doenças cardiovasculares e câncer (Difonzo et al., 2021).

As propriedades benéficas atribuídas aos compostos fenólicos presentes no bagaço de oliva é dependentem da sua biodisponibilidade no organismo. Em sua maioria, os compostos fenólicos presentes no bagaço de oliva são rapidamente metabolizados e absorvidos no trato gastrointestinal. Diante deste contexto, técnicas que visam aumentar biodisponibilidade dos compostos fenólicos do bagaço de oliva têm sido desenvolvidas (Speroni et al., 2019). O processo que simula a digestão gastrointestinal é uma das técnicas emergentes que resulta na obtenção de frações biodisponíveis do bagaço de oliva. No entanto, esta técnica necessita de preparo de amostra prévio, visando a redução do tamanho das partículas. Partícula menores são capazes de aumentar a área de contato com os ácidos, enzimas e solventes necessários para a realização do processo de digestão gastrointestinal (Sefrin et al., 2021a). As frações biodisponíveis obtidas a partir do bagaço são ricas em compostos fenólicos e podem ser utilizadas na formulação de alimentos funcionais (Angeloni et al., 2017; Mushtaq et al., 2020).

Diante do exposto anteriormente, este projeto se justifica pela utilização de amostras de compostos fenólicos biodisponíveis do bagaço de oliva, com a finalidade de compreender seus efeitos neuroprotetor e antioxidante em células e em modelo vivo *Caenorhabditis elegans*. Além disso, este estudo destaca a importância do desenvolvimento de técnicas sustentáveis para o aproveitamento do bagaço de oliva. Desta maneira, ao tornar os compostos fenólicos presentes no bagaço de oliva biodisponíveis, podemos auxiliar na destinação adequada deste subproduto da produção do azeite, tornando o bagaço de oliva um produto de alto valor agregado.

2.1 Olivicultura

A bacia do Mediterrâneo é a região de cultivo tradicional de oliveiras, com uma produção de 3 milhões de toneladas na safra de 2017/2018, representando 95% dos olivais presentes no mundo. Nos últimos anos, a produção de oliva (*Olea europaea L.*) e azeite de oliva tem se estendido para além do Mediterrâneo chegando a regiões de cultivo não tradicional no hemisfério sul. No Brasil, a olivicultura está sendo gradativamente inserida nas regiões sul e sudeste do país, resultando em áreas de cultivo com aproximadamente 500 ha, especialmente nos estados de Minas Gerais, São Paulo, Santa Catarina, Paraná e Rio Grande do Sul (Borges et al., 2017).

A produção de oliva é dependente da adaptação das diferentes cultivares às condições climáticas (índice de chuvas, temperatura, umidade relativa do ar, entre outros) e geográficas (altitudes e latitudes). Além disso, a composição e qualidade do azeite e dos derivados da oliva está associada ao cultivar, maturação do fruto e práticas agrícolas (Borges et al., 2017). As temperaturas de crescimento da oliveira variam entre 15 e 20 °C, porém a temperatura ótima para o crescimento da azeitona é de 40 °C. A oliveira é extremamente resistente à seca, e o crescimento da azeitona é favorecido em solos franco-arenosos com concentrações de nitrogênio, potássio e fósforo equilibradas (Mushtaq et al., 2020). Estas condições afetam a concentração de compostos com propriedades benéficas presentes no azeite. Mais de 200 compostos químicos diferentes podem ser encontrados no azeite, dentre eles se incluem carotenóides e compostos fenólicos das subclasses dos álcoois fenólicos, ácidos fenólicos, flavonóides, ligninas e secoridoides (Angeloni et al., 2017).

A produção do azeite gera grande quantidade de um subproduto de alto valor agregado, o bagaço de oliva, sendo produzidas de 0,5 a 0,6 toneladas de bagaço para cada tonelada de azeitona processada. O bagaço de oliva é o sub-produto sólido com elevado teor de umidade (62 %) proveniente da extração do azeite virgem, seu descarte representa um grande problema para os produtores de azeite (Difonzo et al., 2021; Simonato et al., 2019). No entanto, o bagaço de oliva torna-se interesse de estudos devido ao seu conteúdo de celulose, hemicelulose, lignina e pectinas, que apresentam baixa digestibilidade e conteúdo energético, bem como seu conteúdo de compostos fenólicos (Simonato et al., 2019).

A maior parte dos compostos fenólicos presentes na oliva fica retida no bagaço (cerca de 98 %), uma vez que estes compostos em sua maioria são hidrossolúveis. O alto conteúdo de compostos fenólicos, em especial tirosol e oleuropeína, que representam a

maior fração fenólica livre no bagaço de oliva, torna de extrema importância a busca por técnicas que auxiliem na extração e aumento da biodisponibilidade dos compostos fenólicos presentes neste subproduto (Simonato et al., 2019; Torić et al., 2020).

Devido a presença de componentes fitotóxicos o descarte do bagaço de oliva no meio ambiente não é recomendado, por se tratar de um poluente. Além disso, o bagaço de oliva apresenta odor forte e textura pastosa, características que dificultam seu manuseio e transporte. No entanto, a utilização deste subproduto para geração de energia elétrica em usinas de biomassa ou em sistemas de aquecimento doméstico, gera um aproveitamento de aproximadamente 80 % (Berbel & Posadillo, 2018).

Na agricultura, o bagaço de oliva pode ser utilizado para alimentação animal. No entanto, uma das limitações é seu baixo conteúdo de proteínas biodisponíveis, além do alto teor de gordura. Estes fatores limitam a utilização do bagaço para alimentação animal, sendo considerado um suplemento alimentar que deve constituir em no máximo 10 % da dieta total dos animais (Berbel & Posadillo, 2018). A possível utilização do bagaço de oliva como adubação de solo é outra alternativa de utilização deste subproduto, sendo capaz de aumentar a disponibilidade de potássio no solo. Outro uso menos comum do bagaço é como absorvente de metais pesados no tratamento de água contaminada (Lanfranchi et al., 2016). Desta maneira, alternativas que agreguem valor ao bagaço de oliva têm sido alvo de estudos, uma vez que se trata de um subproduto de alto valor agregado devido a presença de diferentes compostos com potencial benéfico para a saúde humana.

2.2 Composição fenólica do óleo e do bagaço de oliva

Os compostos fenólicos compreendem um grupo extremamente variado de compostos com potencial benéfico à saúde, presentes em diversas frutas, vegetais e grãos. Estes compostos são produto do metabolismo secundário das plantas em condições estressoras durante as etapas de cultivo, e atualmente, mais de 8.000 estruturas de compostos fenólicos são conhecidas (Shahidi & Ambigaipalan, 2015; Vuolo et al., 2018). O consumo de frutas e vegetais está associado a efeitos benéficos à saúde humana tais como propriedades antioxidante, anti-carcinogênica e anti-inflamatória atribuídas a capacidade destes compostos de atuarem como agentes capazes de reduzir o estresse oxidativo. Estas propriedades benéficas estão relacionadas ao conteúdo e diversidade de compostos fenólicos presentes nestes alimentos e em seus derivados (Vuolo et al., 2018).

Dois grandes grupos caracterizam os compostos fenólicos, e são divididos de acordo com a estrutura química de suas agliconas em flavonoides e não flavonoides. Os flavonoides são a classe de compostos fenólicos mais abundante, compreendendo a mais de 6000 compostos (Vuolo et al., 2018). Este grupo de compostos apresentam baixo peso molecular e são caracterizados por apresentarem 15 carbonos distribuídos basicamente em dois anéis aromáticos ligados por três carbonos em forma de heterociclo oxigenado (C6-C3-C6), com diferentes substituições, graus de insaturação e arranjo do estrutural básico, resultando em diferentes subclasses como flavonols, flavonas, flavanols, flavanonas, secoridoides, isoflavonas e antocianidinas (Joseph et al., 2016; Tsimogiannis & Oreopoulou, 2019; Vuolo et al., 2018). Os compostos não flavonoides apresentam como estrutura básica ligações C1-C6 e C1-C3 e compreendem principalmente a dois grades grupos, os derivados do ácido hidroxibenzóico e derivados do ácido hidroxicinamico (Joseph et al., 2016; Salehi et al., 2019).

Além disso, os compostos fenólicos podem ser encontrados na forma de oligômeros e polímeros conhecidos como taninos condensados e taninos hidrolisáveis. Os taninos hidrolisáveis apresentam em sua estrutura um poliol central (em sua maioria) acilado por unidades de ácido gálico ou ácido elágico formando compostos fenólicos de alto peso molecular (Tsimogiannis & Oreopoulou, 2019).

De maneira geral, a ingestão de compostos fenólicos está associada a efeitos benéficos a saúde e pode ser afetada pelos hábitos alimentares e preferências do indivíduo. A ingestão média diária destes compostos é de aproximadamente 1 g por pessoa e, as principais fontes são frutas, vegetais, legumes e seus derivados (Shahidi & Ambigaipalan, 2015). A oliva e seus derivados como o azeite e o bagaço são exemplos de alimentos ricos nestes compostos. Os compostos fenólicos que apresentam características lipofílicas permanecem no azeite após o processamento da oliva (2 %) e os compostos fenólicos com características hidrofílicas permanecem no bagaço de oliva (98 %) (Simonato et al., 2019).

Os compostos fenólicos presentes no azeite de oliva são compostos que integram o grupo dos ácidos fenólicos, flavonols, ligninas e secoiridóides. Destes, os compostos secoiridóides são os prevalentes neste produto, representados em sua maioria pela oleuropeína que compreende a um éster heterosídico de ácido elenólico e hidroxitirosol. Além disso, o azeite pode conter compostos fenólicos simples como o hidroxitirosol e o tirosol, e ainda compostos flavonóides como a luteolina-7-*O*-glicosídeo, rutina, apigenina-7-*O*-glicosídeo e luteolina-4-*O*-glicosídeo (Martín-García et al., 2020).

Em contrapartida, o bagaço de oliva é rico compostos fenólicos como hidroxitirosol e derivados de tirosol, seicoridoides e derivados, flavonols ligninas e ácidos fenólicos. Entre os ácidos fenólicos presentes no bagaço de oliva se destacam os ácidos cinâmico, *p*-cumárico, cafeico, ferulico e vanilico. A oleuropeína e seus derivados se destacam no grupo dos seicoridoides e rutina, apigenina, luteolina e quercetina no grupo dos flavonols (Difonzo et al., 2021; Mushtaq et al., 2020). Dentre as ligninas presentes no bagaço de oliva o composto principal é o pinoresinol (Romero-García et al., 2014). Além disso, o bagaço de oliva é rico em tocoferóis, tocotrienóis, esteróis, esqualeno e carotenóides. Destacam-se ainda neste subproduto compostos como fibras, minerais e oligossacarídeos, sendo também uma ótima fonte de ácidos graxos monoinsaturados e polinsaturados (Difonzo et al., 2021).

2.2.1. Propriedade antioxidante dos compostos fenólicos da oliva

Os compostos fenólicos presentes no bagaço de oliva quando extraídos e isolados podem ser utilizados como aditivos para obter alimentos funcionais com alto teor nutricional. Além disso estes compostos apresentam propriedades antioxidantes atuando no aumento da resistência ao estresse oxidativo (Difonzo et al., 2021).

A sobrecarga do sistema de defesas antioxidantes (enzimáticas e não enzimáticas) induzida pelo excesso de espécies reativas (ER) resulta no estresse oxidativo. O estresse oxidativo pode ser descrido como um desequilíbrio entre substâncias antioxidantes e pro-oxidantes presentes no organismo e, está associado ao desenvolvimento de diferentes doenças. Substâncias pro-oxidantes como as espécies reativas de oxigênio (EROs) e nitrogênio (ERNs) são produzidas constantemente pelo organismo humano através do metabolismo oxidativo, bioenergética mitocondrial e manutenção de funções imunológicas (Dumitrescu et al., 2018; Tan et al., 2018). A maior parte das ER é produzida nas mitocôndrias durante o processo de fosforilação oxidativa para formação de adenosina trifosfato (ATP), que é a principal fonte de energia celular. A geração de energia via formação de ATP necessita de uma grande quantidade de oxigênio (O_2) que atua como um aceptor de elétrons na cadeia transportadora de elétrons (CTE). A adição de um elétron ao O_2 resulta na formação do ânion superóxido ($^{\bullet}O^{-}_{2}$) que é o principal precursor de diferentes ER (Dumitrescu et al., 2018).

Em condições normais os níveis de $^{\bullet}O^{-}_{2}$ produzidos durante a CTE são regulados por enzimas antioxidantes como a superóxido dismutase (SOD), que convertem o $^{\bullet}O^{-}_{2}$ em peróxido de hidrogênio (H_2O_2) e oxigênio (O_2). O peróxido de hidrogênio formado é em

seguida convertido em água por catalases (CAT) e glutationa peroxidases (GPx) (Dumitrescu et al., 2018). No entanto, este processo não é perfeito e grandes quantidades de O_2 na CTE podem levar a produção excessiva de $^{\bullet}O^{-}_{2}$ que não pode ser totalmente eliminado pelas defesas antioxidantes enzimáticas. A adição de um elétron ao $^{\bullet}O^{-}_{2}$ resulta na formação do H_2O_2 e do radical hidroxil ($^{\bullet}OH$) (Pisoschi & Pop, 2015).

O radical hidroxil é extremamente reativo e o metabolismo não possui defesas antioxidantes enzimáticas para a remoção desta ER, por esse motivo é considerado uma das ER mais importantes. A geração do radical $^{\bullet}OH$ depende não apenas da quantidade de O_2, mas também da presença de íons ferro e cobre que atuam como fatores catalíticos na reação conhecida como reação de Fenton (Dumitrescu et al., 2018). Além das ER citadas anteriormente, destacam-se ainda o oxigênio singlete ($^1O_2$) e o radical peroxil ($ROO^{\bullet}$). Ambas as ER são de extrema importância para o metabolismo, uma vez que estão envolvidas na progressão, crescimento, diferenciação e morte celular. Além disso, quando geradas em excesso essas espécies podem danificar enzimas, lipídios, proteínas, ácido desoxirribonucleico (DNA) e outras pequenas moléculas, resultando na interrupção da sinalização redox ocasionando disfunções celulares que podem culminar na morte celular (Luo et al., 2017).

Os compostos fenólicos podem auxiliar na remoção e/ou inibição de ER reduzindo o estresse oxidativo e consequentemente os danos a macromoléculas celulares e extracelulares através de diferentes mecanismos (Vuolo et al., 2018). A capacidade antioxidante dos compostos fenólicos está relacionada às suas propriedades de agentes redutores, uma vez que atuam como doadores de elétrons. Além disso, estes compostos têm a capacidade de quelar metais, em especial ferro e cobre, inibindo a formação de ER catalisadas por metais. Esta capacidade antioxidante dos compostos fenólicos está relacionada com o número de grupamentos hidroxila e sua posição em relação ao grupamento carboxila (Shahidi & Ambigaipalan, 2015; Tsimogiannis & Oreopoulou, 2019). Assim, o estresse oxidativo originado pelo excesso de ER, e a falta de antioxidantes enzimáticos e não-enzimáticos endógenos para prevenir ou remover estas espécies, pode ser amenizado através do consumo de compostos com potencial antioxidante oriundos da alimentação (Pisoschi & Pop, 2015).

Neste contexto, salientamos a importância dos compostos fenólicos presentes no bagaço de oliva, uma vez que são em sua maioria totalmente desperdiçados e estão associados a doenças como câncer, diabetes, doenças cardiovasculares, doenças neurodegenerativas, ambas relacionadas com o estresse oxidativo (Foscolou et al., 2018;

Gorzynik-Debicka et al., 2018). O hidroxitirosol (3,4-dihidroxifeniletanol, HT), um álcool fenólico presente no bagaço de oliva é capaz de exercer uma ampla gama de efeitos biológicos, cardioprotetores, anticarcinogênicos, antimicrobianos e neuroprotetores associados principalmente ao potencial antioxidante deste composto (Karkovic Markovic et al., 2019). O HT atua diretamente sobre o radical peroxil (ROO$^{\bullet}$) doando um átomo de hidrogênio, tornando esta ERO não reativa devido a presença da ligação intramolecular no radical fenoxil. A capacidade do HT remover radicais ROO$^{\bullet}$ é atribuída à presença de uma porção *o*-dihidroxifenil, como pode ser observado na Figura 1 (Karkovic Markovic et al., 2019).

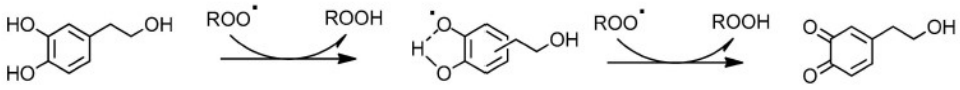

Figura 1 – Esquema do mecanismo de remoção do radical ROO• pelo HT (Karkovic Markovic et al., 2019).

Além disso, Calabriso et al. (2018) verificaram que o pré-tratamento de células endoteliais (HMEC-1) com HT (1 – 30 μmol L^{-1}) reduziu a produção mitocondrial do $^{\bullet}O^{-}_{2}$ e a peroxidação lipídica. Em contrapartida o pré-tratamneto com HT aumentou a atividade de SOD. Estes resultados sugerem que o HT apresenta capacidade antioxidante direcionada à mitocôndria (Calabriso et al., 2018).

O tirosol (2-(4-hidroxifenil)-etanol, Tir), assim como o HT também é um álcool fenólico e apresenta potencial antioxidante menor do que o HT. No entanto, o Tir é considerado um antioxidante eficaz devido à sua elavada estabilidade em condições críticas, resultando no acúmulo intracelular deste composto (Karkovic Markovic et al., 2019). Além de efeitos antioxidantes o Tir apresenta também efeitos anti-inflamatórios e neuroprotetores (Karkovic Markovic et al., 2019; Lee et al., 2018). Lee et al. (2018) verificaram que o Tir é eficaz na inibição do dano oxidativo de células musculares. O tratamento das células musculares (L6) com Tir (1, 30 e 100 μM) e H_2O_2 (0,5 mM) por 24 h resultou em efeito protetor do Tir contra o dano oxidativo induzido pelo H_2O_2. Este efeito protetor da presença de Tir inibiu a morte das células L6 em 22, 31 e 58 % nas concentrações de 1, 30 e 100 μM, respectivamente (Lee et al., 2018).

Os secoiridóides presentes no bagaço de oliva, como a oleuropeína (Ole) exercem potente capacidade antioxidante (Karkovic Markovic et al., 2019). A capacidade antioxidante da Ole é resultante da porção catecol (1,2-dihidroxibenzeno) presente em sua estrutura e é considerada a base de suas atividades anticarcinogênicas, cardioprotetoras, neuroprotetoras, gastroprotetoras, hepatoprotetoras, antidiabéticas, antiobesidade entre outras atividades (Karkovic Markovic et al., 2019). Shi et al. (2017) sugerem a Ole como um composto potencialmente útil para prevenção de hepatopatia. Segundo Shi et al. (2017), diferentes concentrações de Ole (7,4 μM, 14,8 μM e 29,6 μM) exerceram proteção das células humanas L-02 frente a morte celular induzida pelo H_2O_2 (100mM). Este efeito protetor da Ole foi associado ao aumento na expressão das enzimas antioxidantes endógenas SOD, CAT e GPx, de maneira dependente da concentração (Shi et al., 2017). Além disso, a Ole apresentou elevado potencial antioxidante (42,28 a 93,11 %) frente ao radical $^{\bullet}OH$ em concentrações que variaram de 20 a 2560 μM, através do ensaio *in vitro* da 2-desoxiribose (Shi et al., 2017).

A utilização de extratos oriundos do bagaço de oliva também tem sido alvo de interesse científico. Simonato et al. (2019) utilizaram bagaço de oliva como substituto da sêmola de trigo duro para melhorar as características tecnológicas e propriedades nutricionais de uma massa. A fortificação da massa com o bagaço de oliva (0, 5 e 10 g) resultou em aumento no conteúdo de compostos fenólicos totais e atividade antioxidante do produto. O conteúdo de compostos fenólicos totais da massa após cocção aumentou de 0,06 para 0,38 mg GAE g^{-1} e de 0,06 para 0,69 mg GAE g^{-1}, nas concentrações de 5 e 10 g de bagaço de oliva, respectivamente (Simonato et al., 2019). Segundo Simonato et al. (2019) as massas contendo o bagaço de oliva apresentaram um aumento de quatro vezes na capacidade antioxidante frente aos radicais ABTS e peroxil.

Bassani et al. (2016), verificaram que o extrato purificado de águas residuais oriundas do processamento do azeite de oliva, rico em HT, apresentou propriedades quimiopreventivas para células de câncer de cólon. As células humanas de câncer de cólon (HT-29) foram tratadas por 24 e 48 h com o extrato rico em HT (diluições de 1:50 até 1:1000). Independente da diluição do extrato e do tempo de exposição, o tratamento com o extrato rico em HT inibiu a proliferação, migração, invasão, adesão, surgimento de células de câncer de colo e liberação de células angiogênicas e citocinas pró-inflamatórias, caracterizando seu efeito quimiopreventivo (Bassani et al., 2016).

Além disso, o HT presente no óleo de oliva pode atuar como neuroprotetor através de sua capacidade antineuroinflamatória, associada a processos neurodegenerativos e

neurotóxicos (Zhang et al., 2020). O HT (25, 50 e 100 μM) foi capaz de reduzir a produção de mediadores pró-inflamatórios em células da micróglia (BV2) ativadas por lipopolissacarídeo (LPS), através da redução na expressão de fenótipos associados a via pró-inflamatória, sugerindo o efeito neuroprotetor deste composto (Zhang et al., 2020).

Diante do exposto, embora os compostos presentes no bagaço de oliva apresentem diferentes propriedades benéficas associadas à sua capacidade antioxidante tanto em sua forma isolada como em extratos purificados, é necessário conhecer os mecanismos de absorção e excreção, bem como a biodisponibilidade destes compostos quando ingeridos. Estas vias podem ser bastante complexas e necessitam de estudos aprofundados para compreender o metabolismo efeitos endógenos e exógenos destes compostos (Angeloni et al., 2017; Karkovic Markovic et al., 2019).

2.2.2. Propriedade anti-neuroinflamatória dos compostos fenólicos da oliva

Os compostos fenólicos apresentam diferentes propriedades benéficas à saúde, destacando sua capacidade antioxidante, anticâncer, antidiabética, antimicrobiana, hipolipidêmica, prevenção de doenças cardiovasculares e neuroproteção (Gulcin, 2020; Sylla et al., 2021). Com relação aos efeitos neuroprotetores, estes compostos têm sido amplamente estudados devido às suas características antineuroinflamatórias, uma vez que a neuroinflamação esta associada à diferentes doenças neurodegenerativas (Gallardo-fernández et al., 2019; Zhang et al., 2020; Zhao et al., 2021)

A prevalência de doenças neurodegenerativas tem aumentado de acordo com o aumento da expectativa de vida em diversos países (Hansson, 2021). A demência afeta cerca de 50 milhões de pessoas no mundo, e estima-se um aumento para 130 milhões até 2050. A doença de Alzheimer (DA) é a doença neurodegenerativa mais comum e é responsável por 60-70% dos casos de demência, seguida pela doença de Parkinson (DP) com uma prevalência global acima de 6 milhões (Hansson, 2021).

O sistema nervoso central (SNC) é constituído por uma complexa rede neuronal que é mantida e suportada por células gliais, representadas por oligodendrócitos, micróglia, células ependimárias, astrócitos e células de Schwann (Figura 2). As células gliais, especialmente microglias são consideradas as células imunes do SNC. A micróglia é uma célula chave no SNC, pois atua em resposta à processos neuroinflamatórios proporcionando a manutenção da homeostase cerebral (Virgin et al., 2019). O processo neuroinflamatório é considerado um dos principais mecanismos das doenças do sistema

nervoso central (SNC), que atua na contenção de agentes patogênicos ou em situações de comprometimento da fisiologia celular (Gallardo-fernández et al., 2019).

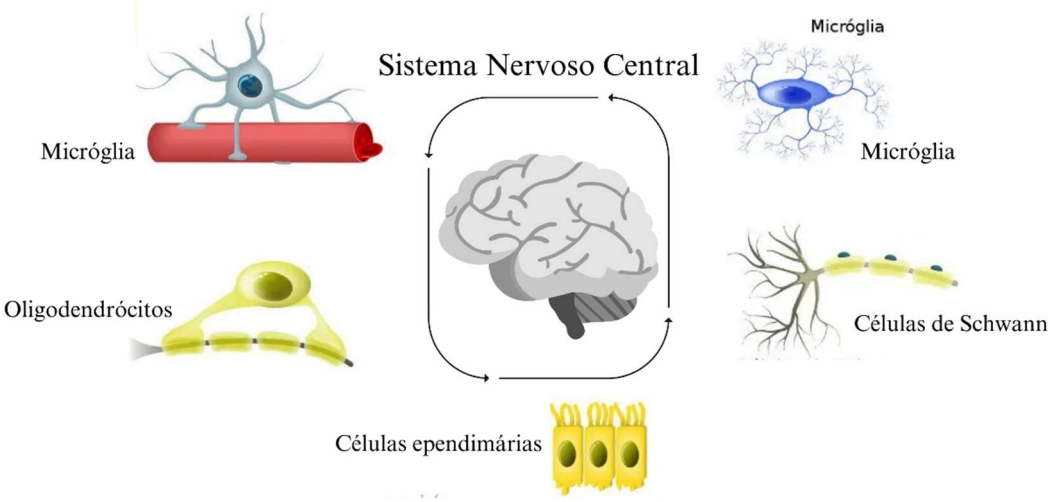

Figura 2 – Células gliais do SNC envolvidas no processo de neuroinflamação. Fonte: a autora.

A modulação do processo neuroinflamatório está associado a ativação das células da micróglia (Delgado, 2021). A ativação de células microgliais desencadeia uma resposta imune a lesões ou doenças do SNC, caracterizadas principalmente pelo aumento da produção de citocinas pró-inflamatórias (Cadoná et al., 2021). A produção de citocinas pró-inflamatórias pode levar a alterações dos fenótipos da micróglia com diferentes funções imunológicas. A ativação de células microgliais pode ativar características pró-inflamatórias/neurotóxicas ou anti-inflamatórias/neuroprotetoras, dependendo do ambiente cerebral (Sawikr et al., 2017).

A produção de citocinas e quimiocinas pró-inflamatórias induzida pela ativação de células da micróglia resulta em aumento do estresse oxidativo através da geração de EROs e ERNs (Souza et al., 2022). O estresse oxidativo tem sido associado a várias doenças, incluindo condições neuropsiquiátricas e neurodegenerativas, a maioria delas apresentando ativação imunológica crônica (Cadoná et al., 2021).

Nesse contexto, é importante desenvolver e buscar estratégias terapêuticas que possam modificar a polarização das células da micróglia ativadas, que, por sua vez, apresentam a via pró-inflamatória como principal resposta imune (Cadoná et al., 2021). Assim, os compostos fenólicos do bagaço de oliva podem ser uma alternativa capaz de modular o sistema imune no SNC. No entanto, a capacidade anti-neuroinflamatória e neuroprotetora dos compostos fenólicos do bagaço de oliva ainda são pouco

compreendidos (Zhang et al., 2020). As possíveis vias de atuação dos compostos fenólicos do óleo e bagaço de oliva no processo neuroinflamatório estão descritas na Figura 3.

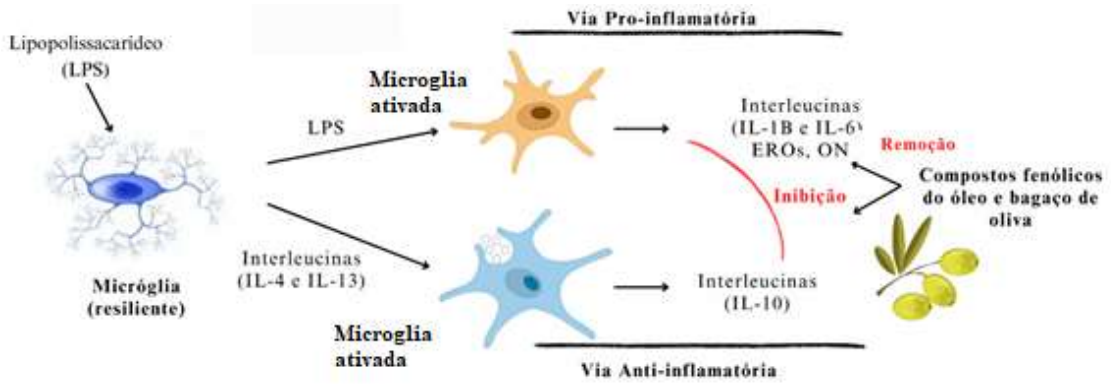

Figura 3 – Possíveis vias de atuação dos compostos fenólicos do óleo e bagaço de oliva no processo de neuroinflamação. Fonte: a autora.

O HT, presente no bagaço de oliva é capaz de suprimir a neuroinflamação induzida por lipopolissacarídeo (LPS) em células de micróglia (BV2) (Gallardo-fernández et al., 2019; Zhang et al., 2020). De acordo com Zhang et al. (2020), o HT reduziu a produção de mediadores pró-inflamatórios em células da micróglia (BV2). A análise fenotípica mostrou que o HT reduziu a expressão de CD86 (via pró-inflamatória) e aumentou a expressão de CD206 (via anti-inflamatória) (Zhang et al., 2020). Além disso, a administração de HT suprimiu a ativação da micróglia através da inibição da expressão do fator de necrose tumoral alfa (TNF-α) e de interleucina 1 beta (IL-1β) no hipocampo de camundongos submetidos ao estresse moderado imprevisível crônico (Zhao et al., 2021). Ole, um dos compostos fenólicos principais do bagaço de oliva, suprimiu o aumento induzido por LPS em células BV2 de mediadores pró-inflamatórios, como óxido nítrico, citocinas pró-inflamatórias e geração de ER (Park et al., 2017). Estes resultados demonstram a importância dos compostos fenólicos do bagaço de oliva na prevenção e combate de doenças neuroinflamatórias. Destacamos ainda, a necessidade de conhecer os efeitos dos compostos fenólicos presentes em uma matrix complexa, como o bagaço de oliva, e como estes compostos atuam sinergisticamente para promover ação antineuroinflamatória.

2.3 Biodisponibilidade dos compostos fenólicos do óleo e bagaço de oliva

Os compostos fenólicos presentes no bagaço e azeite de oliva são rapidamente metabolizados e absorvidos no trato gastrointestinal. Entretanto, o metabolismo destes compostos é dependente da concentração circulante e das enzimas envolvidas (Angeloni et al., 2017). Outro fator que contribui para a biodisponibilidade dos compostos fenólicos do bagaço de oliva é o tamanho de partícula. Neste contexto se destacam técnicas como o fracionamento granulométrico e o processo de micronização (Sefrin et al., 2021b). Ambas as técnicas visam a redução do tamanho de partícula, e contribuem também a obtenção de amostras homogêneas. O aumento da biodisponibilidade se dá através do aumento da área de contato com os compostos responsáveis pela biotransformação dos compostos fenólicos presentes no bagaço de oliva (Mushtaq et al., 2020).

O HT é um dos compostos presentes no bagaço de oliva que apresenta menor biodisponibilidade. A biodisponibilidade do HT é resultante da baixa concentração de HT circulante, devido ao metabolismo de fase I e fase II durante a primeira passagem no intestino e no fígado. Assim, a maior parte do HT circulante está associada a hidrólise de seus precursores (Ole e Ole aglicona) durante o processo de digestão gastrointestinal produzindo o HT como principal metabólito (Domínguez-Perles et al., 2017; López de las Hazas et al., 2018).

Um dos principais fatores envolvidos na baixa biodisponibilidade do HT, está relacionado ao seu caráter polar. Esta característica dificulta sua solubilidade em meios lipídicos e impede sua passagem através da membrana intestinal (Mateos et al., 2011). No entanto, se a concentração de HT circulante for elevada, parte pode ser absorvida e rapidamente incorporada ao plasma sanguíneo, atuando como antioxidante e cardioprotetor (Fernández-Ávila et al., 2015; Robles-Almazan et al., 2018).

O metabolismo de fase I do HT ocorre no interior dos enterócitos e em seguida no fígado. As enzimas envolvidas nesta fase são aldeído desidrogenases (ALDH) localizadas no citosol e presentes na parede intestinal (Angeloni et al., 2017; Rodríguez-Morató et al., 2016). O HT também pode ser encontrado no cérebro como um subproduto do metabolismo da dopamina e da tiramina (Figura 4), sendo essa a via de origem endógena deste composto (López de las Hazas et al., 2018). O processo de desaminação da dopamina mediado pela monoaminoxidase (MAO) resulta na produção de 3,4 dihidroxifenilacetaldeído (DOPAL). DOPAL é um produto instável e tóxico que é facilmente oxidado por ALDH à ácido 3,4-dihidroxifenilacético (DOPAC). Em menor quantidade, DOPAL é reduzido a HT por aldeído/aldose redutase (ALR) e a reação

inversa mediada pela enzima álcool desidrogenase (ADH) pode retornar à formação de DOPAL. DOPAC ainda pode ser convertido a HT por DOPAC redutases. Este mecanismo é responsável pela presença de HT circulante, bem como, a formação deste composto, que pode ainda atuar como antioxidante endógeno no cérebro (Karkovic Markovic et al., 2019).

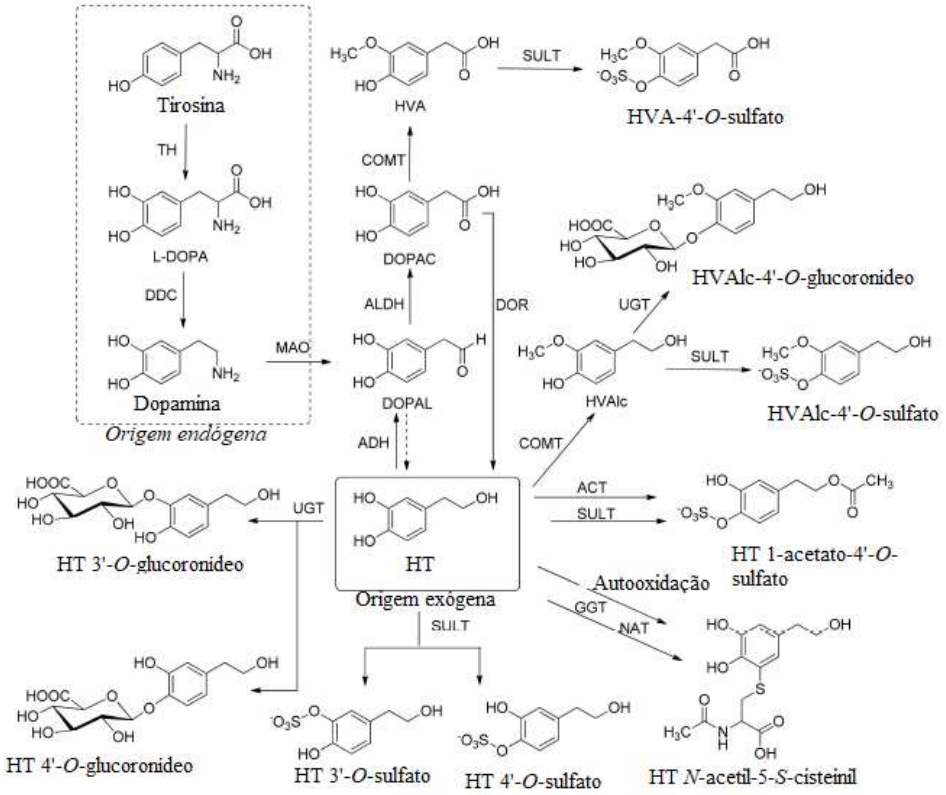

Figura 4 – Esquema das vias metabólicas endógenas e exógenas do hidroxitirosol (HT). Adaptado de (Karkovic Markovic et al., 2019). HVAlc: álcool homovanílico; HVA: ácido homovanílico; TH: titosina hidroxilase; DDC: dopa decarboxilase; MAO: monoaminoxidase; ALDH: aldeído dehidrogenase; ALR: aldeído/aldose redutase; ADH: álcool dehidrogenase; DOR: DOPAC redutase; COMT: catecol-*O*-metiltransferase; UGT: uridina 5'-difosfoglucoronosil transferase; SULT: sulfotransferase; ACT: *O*-acetiltransferase; GGT: glutamil transpeptidase; NAT: *N*-acetil transferase.

Nas reações de fase II (Figura 4) do HT as enzimas sulfotransferases (SULT), uridina 5'-difosfoglucuronosil transferases (UGT) e catecol-*O*-metiltransferases (COMT) são responsáveis pela formação dos principais metabólitos do HT, ácido homovanílico (HVA) e o álcool homovanílico (HVAlc). Outro metabólito resultante desta fase é o *N*-acetil-5-*S*-cisteinil HT formado a partir da autoxidação do HT em HT quinona. Em seguida, a reação com a glutationa (GSH) produz o conjugado que é clivado pelas enzimas

glutamil transpeptidases (GGT) e N-acetil transferases (NAT) formando sulfato HT. O sulfato HT, em suas diferentes formas, é o principal metabólito circulante no plasma após o metabolismo de fase I e II do HT (Karkovic Markovic et al., 2019; Robles-Almazan et al., 2018).

Assim como o HT, o Tir apresenta baixa biodisponibilidade e sua presença no plasma sanguíneo é resultante do metabolismo de secoiridóides como a Ole, que resultam na formação de Tir como seu matabólito (Boronat et al., 2019). A formação endógena de Tir (Figura 5) resulta do metabolismo oxidativo da tiramina, uma monoamina formada a partir da descarboxilação da tirosina. A porção amina da tiramina e removida pela MAO formando o 4-hidroxifenilacetaldeído (4-HPAL), que pode ser oxidado a 4-hidroxifenilacético (4-HPAA) pela ALDH formando um ácido ou reduzido a Tir por ALR gerando Tyr. Os metabólitos de maior abundância resultantes do metabolismo de fase II do Tir são o 4'-*O*-glucuronídeo e o 4'-*O*-sulfato (Figura 5) (Karkovic Markovic et al., 2019).

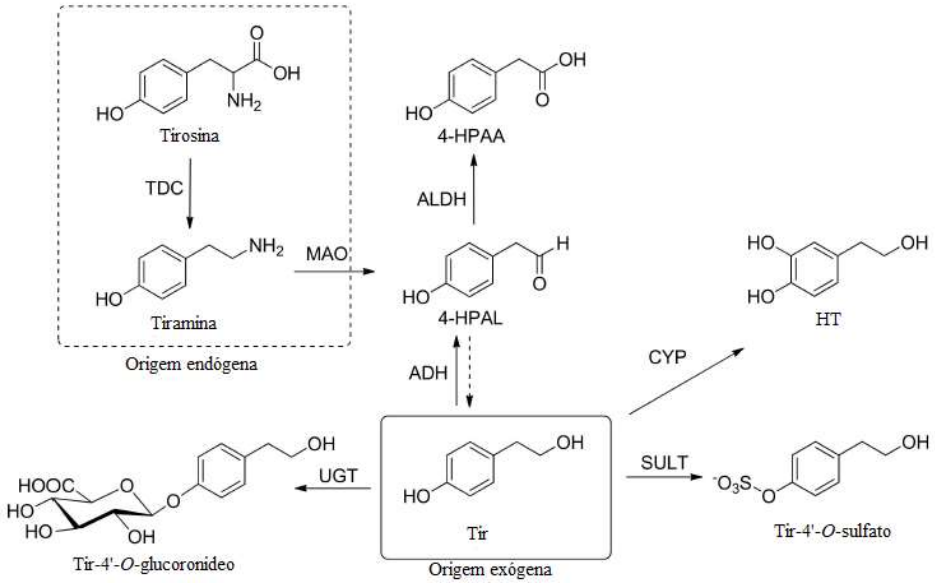

Figura 5 - Esquema das vias metabólicas endógenas e exógenas do tirosol (Tir). Adaptado de (Karkovic Markovic et al., 2019). HT: hidroxitirosol; 4-HPAA: ácido 4-hidroxifenilacético; 4-HPAL: 4-hidroxifenilacetaldeído; TDC: Tirosina descarboxilase; MAO: monoaminoxidase; ALDH: aldeído deshidrogenase; ALR: aldeído/aldose redutase; ADH: álcool deshidrogenase; CYP: citocromo P450; UGT: uridina 5'-difosfoglucoronosil transferase; SULT: sulfotransferase.

A Ole, bem como o Tir e HT é estável em nível gástrico durante o processo digestivo, o que resulta em maior disponibilidade de seu principal metabólito, o HT (Figura 6). A formação deste metabólito da Ole e sua distribuição e concentração são dependentes do pH do meio e do tempo de permanência da molécula no estômago (López de las Hazas et al., 2018). O processo de hidrólise cliva a ligação glicosídica da Ole liberando a porção aglicona (3,4-DHPA-EA) e uma molécula de glicose. A Ole aglicona (3,4-DHPA-EA) é hidrolisada formando HT e ácido elenólico que são posteriormente metabolizados. O catabolismo da Ole pela microbiota leva a formação de ácido fenilacético (PA) e seus derivados e, produtos fenilpropiônicos (PP) e derivados. Já no intestino grosso, Ole é degrada pela microbiota formando o HT que expressa sua atividade biológica frente as células do intestino grosso (Karkovic Markovic et al., 2019).

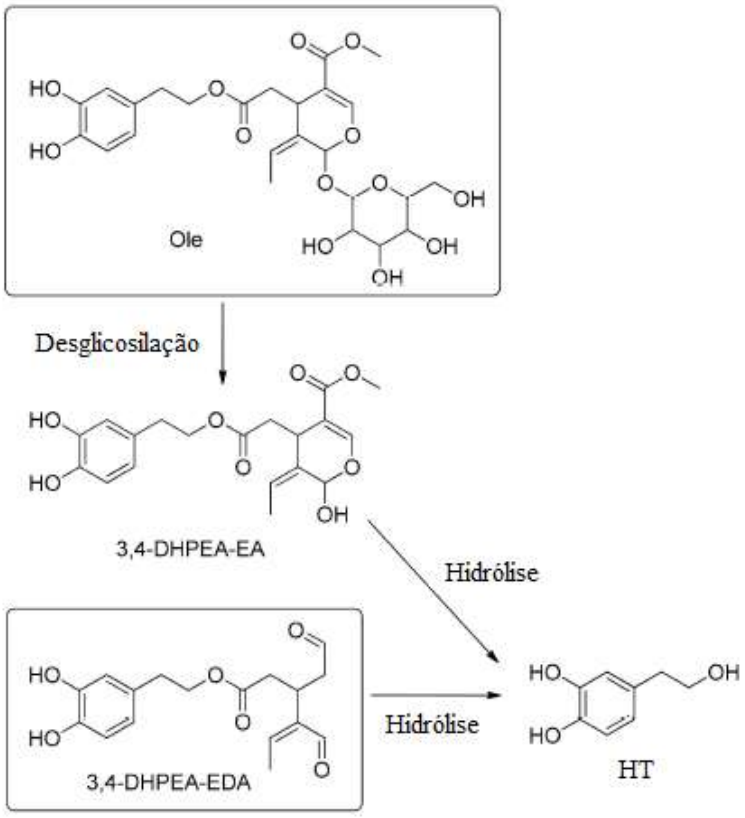

Figura 6 - Esquema das vias metabólicas da oleuropeína (Ole). Adaptado de (Karkovic Markovic et al., 2019). 3,4-DHPEA-EDA: oleaceína; 3,4-DHPEA-EA: oleuropeína aglicona monoaldeído.

Nos últimos anos, o interesse no comportamento da microbiota intestinal em presença de compostos fenólicos atraiu atenção, pois a maioria dos compostos fenólicos

não são absorvidos no intestino delgado intestino delgado e são expostos a microbiota no colo (López de las Hazas et al., 2018). A interação dos compostos fenólicos com a microbiota intestinal levou ao desenvolvimento de reações mútuas e formação de diferentes metabólitos. Desta forma, inicialmente os compostos fenólicos não digeridos são biotransformados pela microbiota intestinal através de reações de oxidação e desidroxilação, em seus principais metabólitos (Figura 7), resultando em aumento de sua biodisponibilidade (García-Villalba et al., 2014; López de las Hazas et al., 2018).

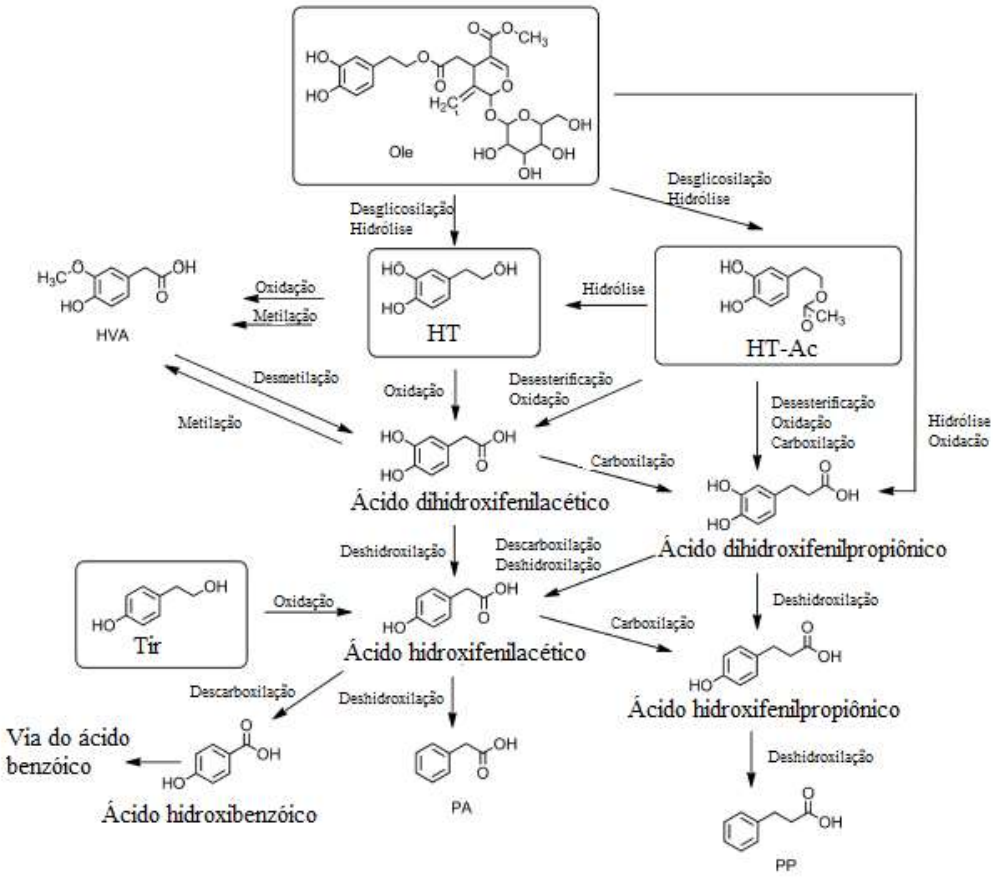

Figura 7 – Esquema de via colônica de biotransformação do hitroxitirosol (HT), tirosol (Tir), hitroxitirosol acetato (HT-Ac) e oleuropeína (Ole). Adaptado de (Karkovic Markovic et al., 2019). HVA: ácido homovanílico; PA: ácido fenilacético; PP: ácido fenilpropionico.

No intestino e no ceco, o HT é totalmente convertido em ácido fenilacético (PA) e seus derivados pela microbiota. No entanto, a microbiota pode converter o acetato de HT (HT-Ac) em derivados fenilpropiônicos. Ambos os metabólitos formados no colon podem ser absorvidos e biotransformados em metabólitos de fase II (Mosele et al., 2015).

Em contrapartida. Os compostos fenólicos podem auxiliar de maneira positiva na modulação da composição da microbiota intestinal, especialmente através da inibição do desenvolvimento de bactérias patogênicas e estímulo do desenvolvimento de bactérias benéficas, como as como as bifidobactérias, atuando como compostos com propriedades prebióticas (Karkovic Markovic et al., 2019; López de las Hazas et al., 2018; Mosele et al., 2015).

Diante deste contexto, o estudo dos diferentes efeitos benéficos à saúde oriundos dos compostos fenólicos do bagaço de oliva é de extrema relevância. Além disso, compreender como a biodisponibilidade destes compostos afetam estas propriedades auxilia na compreensão dos reais efeitos benéficos dos compostos fenólicos da oliva. Este estudo visa enfatizar o reaproveitamento de subprodutos de alto valor agregado, como o bagaço de oliva, provenientes da indústria de alimentos. Uma vez que o bagaço de oliva é rico em compostos fenólicos que apresentam inúmeras propriedades benéficas á saúde, dentre elas destacamos seu potencial antioxidante.

3. OBJETIVOS

3.1 Objetivo geral

Avaliar o efeito antioxidante dos compostos fenólicos biodisponíveis do bagaço de oliva.

3.2 Objetivos específicos

3.2.1. Realizar uma vasta revisão de literatura abordando as propriedades biológicas e a presença de diferentes contaminantes no óleo e bagaço de oliva;

3.2.2. Avaliar o efeito dos compostos fenólicos biodisponíveis do bagaço de oliva sobre a capacidade antioxidante *in vitro* em modelo *cell free*;

3.2.3. Avaliar a capacidade antioxidante dos compostos fenólicos biodisponíveis do bagaço de oliva em células da microglia (BV2);

3.2.4. Avaliar a atividade antioxidante dos compostos fenólicos biodisponíveis do bagaço de oliva em modelo de *Caenorhabditis elegans*.

4. METODOLOGIA

4.1 Amostras

As amostras de bagaço de oliva, cv. ‘Arbequina’, foram: (a) OPF - Fração de bagaço de oliva obtida por fracionamento úmido em peneira (partículas < 2 mm) e liofilizado, moído em moinho de facas comum e desengordurado e (b) OPM - Fração de bagaço de oliva obtida por fracionamento úmido em peneira e liofilizado, moído em moinho de facas comum, desengordurado e micronizado (partículas < 20 μm). As amostras de bagaço de oliva foram provenientes de uma empresa extratora de azeite de oliva, localizada na cidade de Formigueiro, RS, Brasil (29° 59′01 ″ S; 53° 21 ′ 50 ″ W).

4.2 Desenho experimental

Os experimentos de avaliação do potencial antioxidante *in vitro* e avaliação de capacidade antioxidante em células da micróglia (BV2) e em *C. elegans*, foram conduzidos em, no mínimo, triplicatas. As concentrações avaliadas nos experimentos *in vitro* e *in vivo* dos compostos fenólicos biodisponíveis do bagaço de oliva fracionado (OPF) e micronizado (OPM) variaram de 0,03 até 3 mg L^{-1}.

5. RESULTADOS

5.1 Phenolic compounds and contaminants in olive oil and pomace - A review of their biological and toxic effects

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Phenolic compounds and contaminants in olive oil and pomace – A narrative review of their biological and toxic effects

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ABBREVIATIONS LIST

AC – (+)-Acetoxypinoresinol
AFs – Aflatoxins
ALT – Alanine aminotransferase
AST – Aspartate aminotransferase
ATP – Adenosine triphosphate
Aβ – β-Amyloid
BaA – Benzo(a)anthracene
BaP – Benzo(a)pyrene
BbF – Benzo(B)fluoranthene
Chry – Chrysene
CVD – Cardiovascular diseases
DDT - 1,1'-(2,2,2-Trichloroethane-1,1-diyl)bis(4-chlorobenzene)
DNA – Deoxyribonucleic acid
ET-1 – Endothelin-1

EU – European union

FB – Fumonisins

GABA - ácido gama-aminobutírico

H_2O_2 – Hydrogen peroxide

HA – Homovanyl alcohol

HDL – High density lipoprotein

HIF-1 – Hypoxia-inducing factor

HT – Hydroxytyrosol

LDL – Low density lipoprotein

NO – Nitric oxide

Ole – Oleuropein

OO – Olive oil

OP – Olive pomace

OTA – Ochratoxin A

PA – Penitrem A

PAH – Polycyclic aromatic hydrocarbons

PC – Phenolic Compounds

RNS – Reactive nitrogen species

ROS – Reactive oxygen species

SOD – Superoxide dismutase

T2D – Type-2 diabetes mellitus

Tyr – Tyrosol

ZEA – Zearalenone

ABSTRACT - Olive oil is considered one of major component of the Mediterranean diet, with a consumption of 25-50 mL per capita/day. During olive oil production, a solid residue is generated, the olive pomace. Both olive oil and olive pomace are products rich in phenolic compounds and these bioactive compounds are most likely involved in their bioactivities. The most representative phenolic compounds in olive oil and olive pomace are oleuropein, tyrosol and hydroxytyrosol and they can act in the prevention and combat of several diseases, from cardiovascular diseases to cancers and neurodegenerative diseases. On the other hand, olives and their derivatives are constantly exposed to contaminants such as mycotoxin, pesticide residues and polycyclic aromatic hydrocarbons. Thus, phenolic compounds from olive oil and olive pomace may act by

reducing the toxicity of the contaminants present in these products. On the other hand, the contaminants in olive oil and pomace can also affect the beneficial properties attributed to these products. This review summarized current evidence regarding olive pomace and oil phenolic compounds content and their biological effects. In addition, we discussed the beneficial effects of phenolic compounds against toxic compounds present in olive products. Furthermore, we highlighted the importance of developing further studies to understand the interactions between bioactive and toxic compounds in olive oil and pomace -in order to state the actual health benefits of these products.

KEYWORDS – Hydroxytyrosol; Oleuropein; Pesticides; Mycotoxins; Polycyclic aromatic hydrocarbons

1. INTRODUCTION

The olive tree, *Olea europaea* L., is an evergreen tree cultivated for more than 7,000 years that can grow in sparsely fertile soils during periods of drought and can be found worldwide, especially in Mediterranean countries (Pang et al., 2021). Olive cultivation takes place in more than 40 countries and is one of the European Union's (EU) primary economic resources, contributing to approximately 70% of the world's olive oil (OO) production (Iannaccone et al., 2019). OO is considered the primary source of lipids in the Mediterranean diet, with a per capita consumption of 25-50 mL per day (Pang et al., 2021). The Mediterranean diet is associated with a reduced risk of developing chronic non-communicable diseases, such a cardiovascular diseases (CVD) and certain types of cancers. These benefits are linked to the lifestyle and diet adopted. The OO nutritional properties, associated with its bioactive compounds profile, can help to prevent and combat these different diseases (Pang et al., 2021).

During olives processing, liquid and solid residues rich in organic compounds are generated, which hinder degradation and can be harmful to the environment (Dini et al., 2020). The wastewater from mills and olive pomace (OP), composed of pits and fragments of bark and pulp, are the main by-products of olive processing. A ton of olives generates around 400 kg of olive pomace and 1,200 L of residual mill water (Tapia-Quirós et al., 2020). Estimates indicate that the seasonal production of OO generates, in a short period, almost 3,000,000 tons of OP per year worldwide (Nunes et al., 2016). Just like OO, these residues from the processing of the olives are also rich in bioactive compounds, which can be recovered and used in many food applications to improve the nutritional profile, such as, for example, the elaboration of functional foods (Tapia-Quirós et al., 2020). In addition to using OP to supplement animal feed and its biomass for energy production, OP can be used to extract residual oil. After the refining process, this residual oil has food quality and can be used in preparing food for human consumption (Chanioti and Tzia, 2017).

The main bioactive compounds in OO and OP are phenolic compounds (PC). The presence of these compounds contributes to the olive antioxidant, antidiabetic, anticancer, hypolipidemic, neuroprotective, cardioprotective, and antimicrobial properties (Tzekaki et al., 2021). Among the PC in the olive fruit, the secoiridoids compounds are the most representative, especially oleuropein (Ole) and its aglycone form, as well as the products resulting from its hydrolysis, tyrosol (Tyr) and hydroxytyrosol (HT). Tyr and HT

correspond to 30% of the total PC in olive fruit (Rafehi et al., 2012). In addition, the metabolism of these compounds forms the vanillic and homovanilic acids, which have bioactive properties similar to that presented by the original compounds (Reboredo-Rodríguez et al., 2018).

PC from OO and OP may act by reducing the toxicity of the contaminants present in these products, and their benefits against pesticides, mycotoxin and polycyclic aromatic hydrocarbons (PAHs) effects have already been described (Abdulrhman et al., 2021; Bertoz et al., 2021). PC from OO and OP prevented the cytotoxicity induced by Ochratoxin A (OTA), a food contaminant mycotoxin with severe nephrotoxic effects (Crupi et al., 2020). Furthermore, the interaction of the synthetic herbicide, metamitron, with the PC from OO and OP may have a beneficial effect on bone through modulation of osteoblastic physiology, resulting in reduced teratogenicity of this pesticide (Abdulrhman & El-aal, 2020).

This review summarizes current evidence regarding PC content in OO and OP and their biological effects, as well as, how these PC can prevent the toxicity of contaminats in olive crops.

2. METHODS

The PubMed (https://pubmed.ncbi.nlm.nih.gov), ScienceDirect (https://www.sciencedirect.com) and Scopus (https://www.scopus.com) databases were used to search articles by a combination of terms: olive, olive pomace, olive oil, AND phenolic compounds, secoiridoids, nutrition, phytochemicals, bioactive compounds, cardiovascular disease, neuroprotective effects, antidiabetic properties, anticancer action, hypolipidemic effects, antimicrobial action, oxidative stress, mycotoxins, pesticides, and aromatic polycyclic hydrocarbons. As this is not a systematic review, exclusion and inclusion criteria were not defined. However, articles up to March 1^{rst}, 2023 were considered and those providing relevant data for the discussion were included in the review.

3. OLIVE CULTIVATION

Olive cultivation is an essential agro-industrial activity for more than 40 countries, where it covers an area of around 10 million hectares. The EU countries, more precisely those of the Mediterranean region, are the leading producers of approximately 70% of the OO consumed in the world. Moreover, around 53% of world consumption of OO is

centralized in the EU, with Greece having the highest consumption per capita, approximately 12 kg per year (Iannaccone et al., 2019). The primary producer of table olives is Spain, followed by Egypt, Turkey, Algeria, Italy, Greece, and Portugal. However, its production is booming in other countries in South America, the Middle East, and Australia. The production of table olives in the 2017/18 harvest was close to 3 million tons (Perpetuini et al., 2020).

3.1 Olive fruit caracterization and processing

The olive tree (*Olea europaea* L.) is a characteristic of the Mediterranean basin. Olive cultivation has as its primary objective the extraction of OO or table olives to be consumed after undergoing a process to remove the bitterness caused by the presence of Ole (Perpetuini et al., 2020). Ole is a biologically active water-soluble compound with recognized biological effects. However, since it is responsible for the bitter taste of the fruits, it needs to be removed or degraded (Nunes et al., 2016). For this, there are different processes to improve the sensory characteristics and guarantee the safety of the consumer, such as an alkaline treatment or brine/salting, fermentation, or acidification (Perpetuini et al., 2020).

The main constituents of olives are water (from 60% to 75% of the total), lipids (from 10% to 25%) and reducing sugars (from 2% to 5%) (Nunes et al., 2020). Olives are also rich in PC (from 1% to 3%). However, as most of these compounds are polar, they have poor lipid solubility (Nunes et al., 2020). Thus, only 2% of the total value of PC present in olives migrates to the oil phase and is present in extra virgin OO. After extraction (Figure 1), OO goes through centrifugation processes, aiming at separating the oil, water and the OP. The filtration process, followed by decantation, is associated with a more stable final product and a longer shelf life, since it ensures the separation of the oil and possible solid impurities. However, the OO processing steps remove a large part of its PC (Pang et al., 2021). Despite this, OO is still widely recognized as a source of oleic acid, and in smaller percentages, contains other fatty acids, such as omega 6 and omega 3 (Pang et al., 2021).

The extraction of OO occurs exclusively by mechanical techniques, which consist of crushing the fruits and, consequently, releasing the oil contained in the vacuoles of the plant cells (Nunes et al., 2020). Mixing the olive paste will induce the release of oil and, subsequently, centrifugation or pressing will recover this liquid (Figure 1) (Nunes et al., 2020). Olive fruit variety, degree of maturity, conditions of cultivation, aling with the

efficiency of extraction equipment are some of the factors that influence OO performance and extraction capacity (Titouh et al., 2020). Thus, the main objective of olive groves is to maximize the yield of good quality oil, reducing losses in the by-products generated (Titouh et al., 2020). The milling and mixture processes are considered critical steps that cause important changes in the PC composition of the final OO (Titouh et al., 2020).

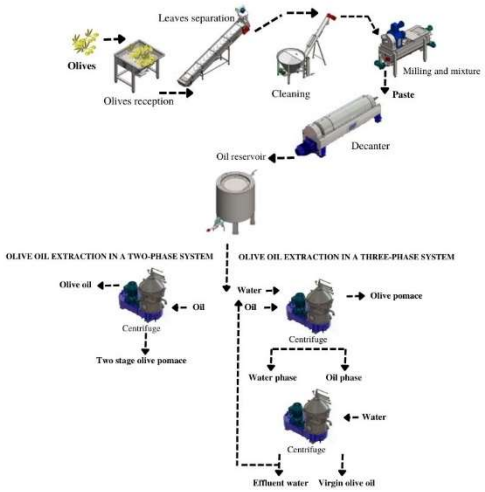

Fig. 1. Fig. 1 shows the stages of olive processing to obtain oil and olive pomace. Oil extraction and olive pomace separation can be carried out according to 2 methods, the two-phase system extraction method and the three-phase system extraction method. Both methods lead to obtaining oil and olive pomace.

The production of OO has undergone evolutionary changes, with the traditional discontinuous pressing process being replaced by continuous centrifugation, first in a three-phase system and later in a two-phase system (Figure 1) (Fernández-Bolaños et al., 2006). Aiming at a greater quantity of oil, in some of these processing steps, water is added during the pressing of the olives. Besides, according to the method used, a specific type of waste will be generated (Fernández-Bolaños et al., 2006). In the oldest method, discontinuous pressing, the olives, after being crushed, are spread on fiber disks stacked on top of each other and then pressed. To facilitate the separation of the oil from the different phases, little amount of water is added and this process produces the OP (Dermeche et al., 2013). The continuous three-phase process, still used in some OO-producing countries, consists of a decanter that will separate all phases by centrifugation

based on the difference in the density of the components of the olive paste (Dermeche et al., 2013). In the three-phase process, a large amount of warm water is added in the centrifugation step, reaching up to 50 liters for every 100 kg of paste, generating three fractions: OP, OO and effluent (Figure 1) (Fernández-Bolaños et al., 2006). The most significant disadvantage is the high production of effluents, representing a major environmental problem due to organic substances, such as reducing sugars and PC (Nunes et al., 2016).

This three-phase system is increasingly being replaced by the two-phase system, which has no added water, making it more sustainable (Dermeche et al., 2013). As the name says, the olive paste is separated into two phases, OO and OP, with high moisture content (Nunes et al., 2016). In this way, waste reduction occurs and generates a pomace rich in residual oil and bioactive compounds (Dermeche et al., 2013; Nunes et al., 2016).

Olive growing is considered an environmentally friendly activity, as large quantities of chemicals or fertilizers are not used and energy use is limited to gasoline instruments or electrical items (Nunes et al., 2016). Due to the sector's innovation, new plantations and investments, there has been a global intensification of OO production (Nunes et al., 2016). However, while OO production increases, the number of its by-products, such as OP, also increases, making these products targets of interest in terms of their potential beneficial properties for health (Ribeiro et al., 2021).

3.2 Olive pomace (OP)

OP is the heterogeneous material that remains after removing most of the oil from the olive paste, with a significant moisture and oil content (Berbel and Posadillo, 2018). For every 100 kg of olives, between 35 kg and 40 kg of pomace are generated, according to the technology used (Frankel et al., 2013). The composition of OP consists of olive pulp, bark, stone fragments, and water (Iannaccone et al., 2019).

The different OO extraction procedures result in differences in the quantity and composition of the olive pomace. For example, the pomace obtained by the two-phase extraction procedure has higher moisture content and lower oil content than the pomace obtained by the three-phase centrifugation procedure (Frankel et al., 2013; Peralbo-Molina et al., 2012).

The by-products from the OO oil industry, including OP, are characterized by the presence of phytotoxic components resulting from the degradation of PC by microorganisms (Berbel and Posadillo, 2018). Therefore, their direct release into the

environment is not recommended, as it is a pollutant waste (Berbel and Posadillo, 2018). OP and other solid residues have a strong odor and a pasty texture, making them difficult to handle and transport (Berbel and Posadillo, 2018). Because of these issues, the use of by-products is an alternative that has been growing as a positive solution for job creation, economic return, besides being environmentally friendly.

The exhausted OP generates electricity in biomass plants or domestic heating systems (Berbel & Posadillo, 2018). However, its use as a renewable fuel presents problems due to the emissions of particles of aromatic compounds (benzene, toluene, and phenol), sulfur, dioxins, and furans, considered harmful to the environment and human health (Berbel & Posadillo, 2018).

OP is also being used in animal feeding. However, due to the low available protein and high-fat content, it can reduce the total feed intake by animals (Berbel & Posadillo, 2018). Thus, it is considered a good supplement for animals, but it should be limited to 10% of the total diet (Berbel & Posadillo, 2018). Furthermore, besides reducing animal feeding costs, the addition of OP as a dietary supplement for ruminants positively affected the nutritional properties of milk, reducing the atherogenic and thrombogenic indexes and saturated fatty acids content (Berbel and Posadillo, 2018; Iannaccone et al., 2019).

Another possible use of exhausted OP is as a soil corrective to maintain the stability of soil aggregates, as it did not demonstrate any toxic effect on the plants' performance and induced an increase in potassium availability in the soil (Lanfranchi et al., 2016). As the olive tree requires a high amount of potassium, the use of the exhausted OP could reduce the amount of fertilizer and reduce the expenses in the management of the olive grove (Lanfranchi et al., 2016).

Since OP is formed by a complex mixture of organic and inorganic compounds, as well as, PC (Pavez et al., 2019), it has attracted interest in studies aimed at recovering these functional compounds, from their extraction to their application in the food industry (Berbel and Posadillo, 2018; Iannaccone et al., 2019; Nunes et al., 2020).

4. OLIVE PHENOLIC COMPOUNDS (PC)

The olive PC are involved in the beneficial properties attributed to the consumption of this product and its derivatives. The formation of these bioactive compounds is a result of environmental and climatic conditions during the cultivation of the olive tree, as these compounds are produced in the form of secondary metabolites for plant protection (Difonzo et al., 2021). Throughout the olive processing steps for oil

production, part of the PC remains in the lipid fraction (2%). However, most of the PC are present in the olive (98%) are hydrophilic and end up migrating to the OP, being eliminated along with this by-product (Ferreira et al., 2022).

As the OP contains oil, the highest concentration of PC e remains in the water-soluble fraction of the pomace, which makes this by-product an essential source of bioactive compounds with different properties beneficial to human health (Difonzo et al., 2021; Torić et al., 2020).

4.1 PC composition in OO

The main PC in OO have a lipophilic characteristic and can be found in free, bonded or esterified forms. The total PC content in OO ranges from 213 to 450 mg kg^{-1} and more than 30 different PC (Table 1) are reported (Rafehi et al., 2012). The phenolic acids in OO are represented by *p*-cumaric, ferulic, gallic, syringic, vanillic, and synaptic acids (Jimenez-Lopez et al., 2020). In addition, OO also contains flavonoid, lignans, hydroxy-isochromans, secoiridoids and phenolic alcohols(Jimenez-Lopez et al., 2020).

The main flavonoids found in OO are luteolin, apigenin, and its different derivative compounds, while the main lignans are represented by pinoresinol. In addition, the secoiridoids compounds are abundant in OO (Jimenez-Lopez et al., 2020), being the most common Ole, dimethyloleuropein, ligstroside, and their aglycone forms. Isochroman compounds are found at low concentrations in OO and are represented mainly by 1-phenyl-6,7-dihydroxy-isochroman and 1- (30Methoxy-40-hydroxy) phenyl-6,7-dihydroxy-isochroman (Gorzynik-Debicka et al., 2018). The main phenolic alcohols present in OO are Tyr and HT, present in small concentrations in fresh oil, but their concentration increases during storage by the hydrolysis of the secoiridoids that also originate HT and Tyr (Gorzynik-Debicka et al., 2018; Jimenez-Lopez et al., 2020).

Table 1 – Olive oil phenolic compounds.

	Compound	**Samples description**	**Reference**
Phenolic Acids	Gallic, caffeic, vanillic, ferulic and syringic acids	Virgin olive oil of *O. europaea* L. (Campania region, Italy)	(Cioffi et al., 2010)

	Vanillic acid	Extra-virgin olive oil*	Fernández-Arroyo et al., 2012)
	Ferulic and vanillic acids	Extra-virgin olive oil**	Presti et al., 2017)
	p-coumaric, vanillic, ferulic, benzoic and cinnamic acids	Extra-virgin olive oil ***	(Torić et al., 2020)
Secoiridoids	Oleuropein and ligstroside	Extra-virgin olive oil**	(Presti et al., 2017)
	Oleuropein Aglycon	Extra-virgin olive oil*	(Fernández-Arroyo et al., 2012)
	Methyl D-oleuropein aglycon, oleuropein aglycon, hydroxy D-oleuropein aglycon, 10-hydroxi oleuropein aglycon, decarboxymethyl oleuropein aglycon, ligstroside aglycon, decarboxymethyl ligstroside aglycon and hydroxy D-ligstroside aglycon	Extra-virgin olive oil****	(García-Villalba et al., 2010)
	Oleuropein, oleuropein aglycon and ligstroside aglycon	Virgin olive oil of *O. europaea* L. (Campania region, Italy)	(Cioffi et al., 2010)

	Oleacein	Extra-virgin olive oil ***	(Torić et al., 2020)
Phenolic alcohols	Hydroxytyrosol and tyrosol	Virgin olive oil of *O. europaea* L. (Campania region, Italy)	(Cioffi et al., 2010)
	Hydroxytyrosol and hydroxytyrosol acetate	Extra-virgin olive oil*	(Fernández-Arroyo et al., 2012)
	Tyrosol, hydroxytyrosol and hydroxytyrosol acetate	Extra-virgin olive oil****	(García-Villalba et al., 2010)
	Tyrosol and hydroxytyrosol	Extra-virgin olive oil ***	(Torić et al., 2020)
	Hydroxytyrosol, tyrosol and tyrosol acetate	Extra-virgin olive oil**	(Presti et al., 2017)
Flavonoids	Luteolin and apigenin	Extra-virgin olive oil*	(Fernández-Arroyo et al., 2012)
	Luteolin, methyl luteolin and apigenin	Extra-virgin olive oil**	(Presti et al., 2017)
	Apigenin and luteolin	Extra-virgin olive oil****	(García-Villalba et al., 2010)
	Apigenin	Extra-virgin olive oil ***	(Torić et al., 2020)
Lignans	Pinoresinol, acetoxypinoresinol and syringaresinol	Extra-virgin olive oil*	(Fernández-Arroyo et al., 2012;)

Pinoresinol, acetoxypinoresinol and syringaresinol	Extra-virgin olive oil****	(García-Villalba et al., 2010)
Pinoresinol	Extra-virgin olive oil**	(Presti et al., 2017)
Pinoresinol	Extra-virgin olive oil ***	(Torić et al., 2020)

*These samples correspond to two Hojiblanca variety olive oils produced in Málaga and Seville; seven Picual variety oils produced in Málaga, Jaén and Granada; one Cornezuelo variety oil produced in Granada; one Manzanilla variety oil produced in Seville; three Arbequina variety oils produced in Tarragona and Seville.

** These samples correspond to 32 Italian olive oil samples from Trapani geographical área (Trapani, Sicily) and 19 Spanish olive oil samples. The Italian samples were a blend of the Nocellara del Belice, Biancolilla and Cerasuola cultivars while the Spanish ones were monocultivar samples of Arbequina and Picual olives.

*** These samples correspond to three olive oil samples collected in Istrian Peninsula, Croatia: one from variety Bjelica produced by Oleum Maris d.o.o. (Vodnjan, Croatia) and two from variety Buža and Žižolera produced by family farm Matteo Beluci (Vodnjan, Croatia).

**** These samples correspond to eight olive oil samples acquired from a supermarket (Granada, Spain). Olive oil samples of three different olive fruit varieties so-called Picual, Hojiblanca and Arbequina and from different trademarks (Carbonell, Borges, Hojiblanca and Coosur).

4.2 PC composition in OP

Most of the PC in the olive fruits remain in the pomace after processing the oil. However, the PC in OO are also present in pomace in more significant quantities, making this by-product a product of high added value from the functional point of view (Mushtaq et al., 2020). In addition, OP has cellulose, hemicellulose, lignin, and pectins that represent nutritional interest due to its low digestibility and energy content (Simonato et al., 2019).

The OP presentes in its phenolic composition, both lipophilic and hydrophilic compounds. The pomace has moisture content close to 62%, which favors the migration of these compounds along the stages of OO processing (Simonato et al., 2019). Like OO, OP is rich in HT, Tyr, secoiridoids compounds and their derivatives, flavonoids, lignans, and phenolic acids (Table 2). The cinnamic, *p*-coumaric, caffeic, ferulic and vanillic phenolic acids most represent the phenolic acids in OP. The derivatives of Ole and the flavonoids such as rutin, apigenin, luteolin and quercetin are also present in the pomace (Difonzo et al., 2021; Mushtaq et al., 2020).

The richness of PC in OP makes this by-product extremely important for the food industry and the beneficial health properties associated with the consumption of these compounds. Among all the PC present in OO and OP, Ole, HT and Tyr stand out for their bioactivities, such as antioxidant, prevention of CVD, antidiabetic, anticancer, neuroprotective, and hypolipidemic effects (Jimenez-Lopez et al., 2020).

Table 2 – Olive pomace phenolic compounds.

	Compound	**Samples description**	**Reference**
Phenolic Acids	Vanillic, cinnamic, *p*-coumaric, caffeic, gallic, ferulic and protocatechuic acids	Olive pomace (different cultivars)*	(Peralbo-Molina et al., 2012)
	Vanillic, cinnamic, *p*-coumaric, caffeic and ferulic acids	Olive pomace (different cultivars)*	(Difonzo et al., 2021)
Phenolic Alcohols	Hydroxytyrosol	Olive pomace (different cultivars)*	(Chanioti & Tzia, 2017)
	Hydroxytyrosol glucoside, hydroxytyrosol, hydroxytyrosol diglucoside, hydroxytyrosol rhamnoside, tyrosol and tyrosol glucoside	Olive pomace (different cultivars)*	(Peralbo-Molina et al., 2012)

	Hydroxytyrosol and tyrosol	Olive pomace (different cultivars)*	(Difonzo et al., 2021)
Secoiridoids	Oleuropein	Olive pomace (different cultivars)*	(Chanioti & Tzia, 2017)
	Oleuropein	Olive pomace (different cultivars)*	(Difonzo et al., 2021)
	Oleuropein, 10-Hydroxy-oleuropein, oleuropein aglycone, oleuropein-aglycone mono-aldehyde, oleacein, oleocanthal and ligstroside aglycon	Olive pomace (different cultivars)*	(Peralbo-Molina et al., 2012)
Flavonoids	Luteolin-7-glucoside, luteolin, apigenin-7-*O*-glucoside, apigenin and rutin	Olive pomace (different cultivars)*	(Difonzo et al., 2021)
	Luteolin, apigenin and rutin	Olive pomace (different cultivars)*	(Chanioti & Tzia, 2017)
	Luteolin, luteolin-7-glucoside, rutin, apigenin, apigenin-7-*O*-glucoside, taxifolin, diosmetin and quercetin	Olive pomace (different cultivars)*	(Peralbo-Molina et al., 2012)
Lignans	Pinoresinol and acetoxypinoresinol	Olive pomace (different cultivars)*	(Difonzo et al., 2021)
	Hydroxypinoresinol, acetoxypinoresinol and pinoresinol	Olive pomace (different cultivars)*	(Peralbo-Molina et al., 2012)

* These olive pomace samples were obtained directly from the virgin olive oil production line.

5. BIOACTIVITIES OF OO AND OP

OO and OP have different beneficial properties for human health. The major bioactivities related to OO and OP include antioxidant (Ozkan et al., 2019; Gulcin, 2020),

anticancer (Calahorra et al., 2020; Memmola et al., 2022), antidiabetic (Marrano et al., 2021; Sylla et al., 2021), antimicrobial (Amini et al., 2017; Ribeiro et al., 2021), hypolipidemic (Farràs et al., 2015, 2019) CVD prevention (Katsiki et al., 2021; Widmer et al., 2013) and neuroprotective actions (Table 3) (Averna et al., 2018; Tzekaki et al., 2021). These properties are associated with the bioactive compounds present in these products, including PC. Besides the protective effects against some diseases described above, the putative protective potential of PC against Covid-19 has been recently discussed by our group (Augusti et al., 2020).

Table 3 – OO and OP biological properties - *in vitro* and *in vivo* studies.

Biological properties evaluated	Sample	Study model and analyzed concentrations	Results	References
ANTIOXIDANT EFFECT	*Virgin and refined virgin OO*	Virgin OO and refined virgin OO (50 mL daily) with differences in their PC concentration (161 mg kg^{-1} vs 14.67 mg kg^{-1}, respectivelly) were administered in 40 patients with stable coronary disease, over 3 weeks.	The consumption of virgin OO rich in PC (161 mg kg^{-1}) reduced lipids oxidation levels and increased antioxidant enzymes activities in the plasma of the patients.	(Fitó et al., 2005)
	OO	Administration of OO (20 mg kg^{-1} of PC daily), containing mostly HT, in adult male mice for 2 months.	Administration of OO protected against alcohol-induced oxidative stress.	(Carito et al., 2016)
	OP extracts	Effect of OP extracts (36 – 46 mg g^{-1} of PC) against radicals generated *in vitro*.	Arbequina OP extracts showed high *in vitro* antioxidant potential against	(Zhao et al., 2022)

			peroxil and DPPH radicals, as well as, ferric reducing antioxidant power.	
	OP extracts	Treatment of Caco-2 cells over 24 h withf OP extracts (1.5 mg mL^{-1} of PC).	OP extracts attenuated ROS generation induced by H_2O_2 in Caco-2 cells.	(Quero et al., 2022)
ANTIMICROBIAL EFFECT	*Virgin OO*	Subjects positive for *H. pylori* receiving virgin OO (60 g) for 2 periods of 14 days (with 1 month interval between periods).	The *H. pylori* eradication rate ranged from 27 to 40%, 72 hours after the last treatment.	(Castro et al., 2012)
	PC from OO and OP	*E. coli* wild-type were incubated with Tyr and Ole concentrations (7 and 10 mmol L^{-1}), for 1 hour at 37°C.	Tyr and Ole synergistically inhibited ATP synthase, the enzyme involved in the proliferation process of *E. coli.*	(Amini et al., 2017)
	Bioavailable PC from OP	Bioavailable PC from OP, obtained after the *in vitro* digestion process, were assessed for their antimicrobial and prebiotic potential, over 48h.	Bioavailable PC from OP combat the adhesion of pathogens such as *Bacillus cereus* (22.03%), *Listeria monocytogenes* (20.01%) and *Yersinia*	(Ribeiro et al., 2021)

			enterocolitica (3.51%) in the large intestine.	
ANTITUMORAL EFFECT	*OO phenolic extract*	Breast cancer cells (MCF-7) were treated with concentrations of OO extracts ranging from 0 to 1 mg L^{-1}, over 48h.	OO extracts reduced the MCF-7 cell proliferation.	(Reboredo-Rodríguez et al., 2018)
	Refined OO phenolicextracts	Exposure of human colon adenocarcinoma cells (SW480 and HT29) to refined OO extract (0.1%), over 24 h.	Refined OO rich in luteolin and Ole induced apoptosis and inhibited the cell proliferation.	(Fernández-Arroyo et al., 2012)
	OO phenolicextracts	Cervical (HeLa) and human colon (SW48) cancer cells were treated with different concentrations (0.02-0.25% v/v) of OO extract over 72h.	OO extracts inhibited cervical and human colon cancer cell proliferation.	(Torić et al., 2020)
	OP extracts	Human colon cancer cells (HCT-8) were treated with OP extracts (0.03 − 0.12 mg L^{-1}), over 12 h.	OP extracts have a cytotoxic effect for human colon cancer cells by inhibiting the expression of hypoxia inducible factor (HIF-1).	(Cells et al., 2021)
ANTIDIABETIC EFFECT	*PC from OO*	Endothelial like cells (ECV304) were exposed to different	PC from OO exhibited a protective effect on	(Storniolo et al., 2014)

	concentrations of PC from OO (10 – 120 µmol L^{-1}), over 48 h.	endothelial dysfunction by regulating NO and ET-1 intracellular levels and reducing the ROS induced by hyperglycemia and free fatty acids.	
OO	Daily consumption of OO (25 mL, equivalent to a concentration of 577 mg of PC kg^{-1}) by 8 overweight and 11 T2D patients, over 8 weeks.	OO improved the inflammatory status by reducing visfatin, adipokine responsible for triggering inflammatory processes.	(Santangelo et al., 2016)
PC from OO	Beta cells (1E) were exposed to PC extracted from OO (10 µmol L^{-1}) for 24h.	PC extracted from OO increased the beta cells proliferation, insulin biosynthesis and secretion of glucose-stimulated insulin.	(Marrano et al., 2021)
PC from OP	*In vitro* study with α-amylase treated (10 min.) with PC from OP (72.28 µg mL^{-1}).	PC from OP can inhibit the action of α-amylase, reducing the levels of sugars for absorption.	(Sylla et al., 2021)

CVD PREVENTION	*OO*	Administration of OO (30 mL daily), over 4 months in 82 patients with early atherosclerosis.	OO reduces the CVD incidence by improving the endothelial function.	(Widmer et al., 2013)
	PC extract from OP	Treatment of endothelial cells (EAhy926) with PC extract from OP (0.05 and 0.1 mg mL^{-1} of PC), over 24 h in human.	The PC extract from OP could offer protective effect in CVD by preventing endothelial disfunction.	(Palmieri et al., 2012)
	OO	33 Patients with hypercholesterolemia received OO (25 mL, 250 to 500 mg kg^{-1} of PC) per day for 3 weeks.	The OO consumption has beneficial effects on the HDL functionality.	(Farràs et al., 2015)
	OO	Daily consumption of OO (25 mL, 500 mg kg^{-1} of PC) by 33 hypercholesterolemic patients for 3 weeks	The OO consumption showed improvement in the HDL expression by regulating genes in mononuclear cells of patients with hypercholesterolemia.	(Farràs et al., 2019)
HYPOLIPIDEMIC EFFECT	*PC from OP oil*	Clinical study involving 72 volunteer patients, consumed OP oil (45 mL daily) over 4 weeks.	Consumption of OP oil resulted in an improved blood lipid profile	(González-Rámila et al., 2022)

			through the reduction of LDL.	
	Extra virgin OO	Male Wistar rats receinving extra virgin OO (1% of body weight) for 50 days.	OO reduced serum levels of total cholesterol, triglycerides and LDL, as well as increased serum HDL levels. In addition, virgin OO decreased lipid peroxidation and restored GSH levels in the liver.	(Kribeche, 2022)
NEUROPROTECTIVE EFFECT	*OO*	OO weekly consumption (1 L) by 447 healthy volunteers for over 6 years	OO consumption resulted in an improvement in cognitive function.	(Valls-Pedret et al., 2015)
	OO	Daily OO (0.7 g kg^{-1}, 680 mg kg^{-1} of oleocanthal) consumption by 5xFAD mouse model of AD, for 1 month.	OO consumption increased the effect of the drug donepezil. The combined consumption of OO and donepezil reduced the Aβ peptide load.	(Batarseh & Kaddoumi, 2018)
	OO	Daily administration of OO rich in PC (50 mL), for 80 individuals with mild cognitive	OO administration increases the neuroprotective protein BMI1 and	(Tzekaki et al., 2021)

	mpairment patients over 12 months.	reduced the levels of the Alzheimer disease-related biomarker p53	
PC from OP	Exposure of neuron-like cells to PC from OP (0.01 – 0.5 mg mL^{-1} of PC) over 24 h.	Neuron-like cells were protected by PC from OP from Ca2+-overloading and the pathological activation of calpain, key events in neurodegenerative disorders.	(Averna et al., 2018)

Abbreviations: Aβ - β-amyloid, AD - Alzheimer's disease, ATP - adenosine triphosphate, BMI1 - polycomb repressor complex 1, Caco-2 - intestinal cells, CVD - cardiovascular diseases, DPPH - 2,20 -Diphenyl-1-picrylhydrazyl, EAhy926 - human endothelial cells, ECV304 - endothelial cell line, ET-1 - endothelin-1, GSH - glutathione, H_2O_2 - hydrogen peroxide, HCT-8 - human colon cancer cells, HDL - high-density lipoprotein, HeLa - cervical cancer cell, HIF-1 - hypoxia-inducing factor, HT - hydroxytyrosol, LDL - low-density lipoprotein, MCF-7 - breast cancer cells, NO - nitric oxide, Ole - oleuropein, OO - Olive oil, PC - phenolic compounds, ROS - reactive oxigen species, SW48 - human colon cell, SW48 and HT29 - human colon adenocarcinoma cells and T2D - type-2 diabetes mellitus.

5.1 Antioxidant activity

PC fromOO and, especially Tyr, HT, and Ole, work to combat oxidative stress through their antioxidant properties (Ozkan et al., 2019). Oxidative stress results from an imbalance between oxidant and antioxidant substances. An increase in oxidant substances concentration in the bloodstream, especially reactive oxygen and nitrogen species (ROS and RNS), along with a deficiency in endogenous antioxidant defenses, leads to oxidative stress (Ozkan et al., 2019). This endogenous antioxidant defense deficiency can be

restored by ingesting compounds that can remove free radicals, ensuring redox balance and preventing damage to macromolecules, such as deoxyribonucleic acid (DNA), lipids and proteins (Georgakouli et al., 2016).

PC from OO and OP can act as exogenous antioxidants promoting the inactivation of free radicals by donating hydrogen atoms to these reactive species, forming inert species that do not have the initiate oxidative reactions capacity. Besides, due the presence of an unshared electron pair in the molecular structure, PC can act as metal ion chelating, especially copper and iron, agents that catalyze lipids oxidation (Gulcin, 2020).

PC content in OO oil may vary according to the type of processing adopted. The antioxidant effect of both virgin OO and refined virgin OO with differences in their PC concentration (161 mg kg^{-1} vs 14.67 mg kg^{-1}, respectivelly) on oxidative stress in 40 patients with stable coronary disease was evaluated. The consumption of 50 mL of virgin OO rich in PC per day, over three weeks, reduced lipids oxidation levels and increased antioxidant enzymes activities in the plasma of the evaluated patients (Fitó et al., 2005). The mechanisms by which OO rich in PC can exert its protective effect can be explained by the antioxidant potential of PC, keeping the oxidative stability of the LDL cholesterol abd avoiding fatty streak formation in vassels. Also, we can not rule out a combined protective effect of both PC and the monounsaturated fatty acids from OO. However, since the fatty acid composition and the content of other compounds with antioxidant activity were similar in the two OO, we may assume that PC plays an importante role in the observed effects (Fitó et al., 2005).

Administration of OO (20 mg kg^{-1} of PC daily), containing mostly HT, in adult male mice for two months, showed a protective potential against alcohol-induced oxidative stress (Carito et al., 2016). This protection of OO was associated to PC, which acted through the reduction of ROS and activation of endogenous antioxidants. Furthermore, OO supplementation resulted in the presence of HT metabolites (i.e., HT1 sulfate and HT2 sulfate), which demonstrates that this PC was absorbed in the gastrointestinal tract of alcoholic mice (Carito et al., 2016). Arbequina OP extracts (36 – 46 mg g^{-1} of PC) showed high *in vitro* antioxidant potential against peroxil and DPPH (2,20 -Diphenyl-1-picrylhydrazyl) radicals. In addition, OP extracts presented ferric reducing antioxidant power (Zhao et al., 2022). Moreover, OP extracts (1.5 mg mL^{-1} of PC) attenuated ROS generation induced by H_2O_2 in Caco-2 cells, after 24h of exposure. Therefore, PC from this extract could have a potential application in the management of gastrointestinal diseases related to oxidative stress (Quero et al., 2022). Micronization

process of OP increased the intestinal bioaccessibility of HT, Ole, luteolin and apigenin, as well as, increased the antioxidant capacity *in vitro*. Thus, micronization can be further exploited to improve the nutraceutical properties of OP by increasing the bioaccessibility and antioxidant capacity of PC (Sefrin et al., 2021a).

5.2 Antimicrobial activity

The PC present in OO and OP have also been associated with their antimicrobial action. OO showed bactericidal capacity *in vivo* against *Helicobacter pylori*, a pathogenic bacterium present in the gastric mucosa, and this effect was associated with its PC (Castro et al., 2012). Subjects positive for *H. pylori* receiving 60 g of virgin OO for 2 periods of 14 days (with 1 month interval between periods) showed *H. pylori* eradication rate from 27 to 40%, 72 hours after the last treatment (Castro et al., 2012). The high antimicrobial activity of the OO can be attributed in particular to Tyr and HT compounds, which have a structure similar to glutaraldehyde, a widely used synthetic biocide (Castro et al., 2012).

In addition, the *in vitro* antimicrobial action of PC present in OO and OP (Tyr, HT, and Ole) against *Escherichia coli* wild type demonstrated that Tyr and Ole (7 and 10 mM) synergistically inhibited adenosine triphosphate (ATP) synthase, the enzyme involved in the proliferation process of *E. coli.* (Amini et al., 2017). ATP synthase is the primary energy source in almost all organisms, from bacteria to humans and can be found on the surface of many types of cells, where it serves as a ligand-receptor and participates in cellular processes. The hydroxyl groups (OH) present in the PC from OO and OP act as ATP synthase inhibitors, resulting in the unblocking of the membrane of microorganisms and causing the extravasation of their cellular components and consequent reduction of celular proliferation (Amini et al., 2017).

Bioavailable PC from OP, obtained after the *in vitro* digestion process, can act as a prebiotic by inhibiting the adhesion of pathogens in the large intestine such as *Bacillus cereus* (22.03%), *Listeria monocytogenes* (20.01%) and *Yersinia enterocolitica* (3.51%) (Ribeiro et al., 2021). The mechanisms described above for PC (biocides and ATP synthase inhibitors) are also involved in this events (Amini et al., 2017; Castro et al., 2012; Ribeiro et al., 2021).

5.3 Antitumoral properties

The role of OO in cancer prevention is attributed to its PC and the mechanisms involved still need to be elucidated (Reboredo-Rodríguez et al., 2018). The

antiproliferative effect of OO phenolic extracts through breast cancer cells was described after MCF-7 cells have been treated with OO phenolic extract (0 - 1 mg L^{-1}), over 48 h. In this study, the secoiridoids compounds represented 83% of the total PC present in the OO phenolic extract and were associated with the antitumoral effect (Reboredo-Rodríguez et al., 2018). According to Reboredo-Rodríguez et al. (2018), it is proposed that not only the total amount of PC may exert beneficial effects on health, but also the presence of some "key" PC in low concentrations, such as apigenin and oleocanthal (Reboredo-Rodríguez et al., 2018). The OO PC act on hormone regulation by reducing estradiol. This effect demonstrates the OO anticancer role, since breast cancer is an estrogen-dependent neoplasm (at least in the early stages of carcinogenesis), being highly influenced by the levels of these hormones. However, these effects still remain inconclusive and few studies have been carried out with OO in the prevention and fight against breast cancer (Moral & Escrich, 2022).

In addition to antitumor capacity in breast cancer cells, refined OO phenolic extracts, rich in luteolin and Ole, were able to suppress cell growth of human colon adenocarcinoma cells (SW480 and HT29) (Fernández-Arroyo et al., 2012). The inhibition of human colon adenocarcinoma cell proliferation by the OO extract (0.1%), after 24 h, was accompanied by cells apoptosis. The metabolites associated with these results were hydroxylated luteolin and Ole in its aglycone form (Fernández-Arroyo et al., 2012). There are several hypotheses and mechanisms that may be involved in the pprevention of colon cancer by OO. One of the most relevant hypotheses is that the PC from OO hinder cell fixation, preventing integrin binding to various types of extracellular matrix proteins (Memmola et al., 2022). According to this hypothesis, PC from OO have anti-invasive effects, since integrins are crucial for cell invasion and migration, fundamental steps in the initiation, promotion and subsequent metastasis of cancer cells (Memmola et al., 2022).

OO (0.02 - 0.25% v/v) inhibited cervical cancer (HeLa) and human colon (SW48) cell proliferation, after 72h of treatment (Torić et al., 2020). However, these authors pointed out that in combination with anticancer drugs, the OO phenolic extract increased the HeLa and SW48 cancer cells metabolic activity, playing an adverse health effect by protecting tumor cells. Thus, these data imply the careful consumption of OO during treatment with chemotherapy in cancer patients (Torić et al., 2020).

OP extracts (0.03 − 0.12 mg L^{-1}) has a cytotoxic effect in human colon cancer cells (HCT-8), after 12h of exposure (Cells et al., 2021). The HCT-8 cells co-incubated

with the oxidant tBHP and OP extracts showed a decrease in ROS generation induced by tBHP. OP extract contains higher amounts of vanillic acid and luteolin-7-glucoside that inhibit cell proliferation, possibly by inhibiting the expression of hypoxia inducible factor (HIF-1). HIF-1 is a transcription factor regulation that increases glycolysis and drives tumor development under anoxic conditions. OP extracts act to inhibit this factor, resulting in reduced survival of cancer cells (Cells et al., 2021).

5.4 Antidiabetic properties

The antidiabetic properties attributed to PC from OO and OP consumption were not fully elucidated. However, the improvement of endothelial function is highlighted. The endothelium plays an essential role in regulating blood pressure by releasing vasodilators and vasoconstrictors, such as nitric oxide (NO) and endothelin-1 (ET-1). However, in type-2 diabetes mellitus (T2D), this mechanism is silenced, causing endothelial dysfunction, responsible for insulin resistance, hyperglycemia, and increased ROS generation (Storniolo et al., 2014). Exposure over 48 h of endothelial like cells (ECV304) to PC from OO (10 μM – 120 μM) resulted in a protective effect on endothelial dysfunction induced by hyperglycemia and free fatty acids (Storniolo et al., 2014). The main OO compound involved in this protection was HT, which acts on regulating NO and ET-1 intracellular levels, reducing the ROS (Storniolo et al., 2014). Accordingly, it is likely that the HT from OO are responsible for modulating NO production by controlling phospho-eNOS phosphorylation, which results in interruption of insulin signaling and endothelial dysfunction (Storniolo et al., 2014).

The daily consumption of OO (25 mL, equivalent to a concentration of 577 mg of PC kg^{-1}) by 8 overweight and 11 T2D patients, over 8 weeks, improved the inflammatory status, reducing serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and visfatin, adipokine responsible for triggering inflammatory processes in patients with T2D and overweight (Santangelo et al., 2016).

The initial development of T2D is associated with the loss of functional mass of beta cells, so the regeneration of these cells must be considered for the treatment of T2D (Marrano et al., 2021). Therefore, the effects of PC extracted from OO (10M) increased the beta cells proliferation (1E) after exposure (24 h). Furthermore PC from OO increased insulin biosynthesis and secretion of glucose-stimulated insulin (Marrano et al., 2021). Thus, the PC from OO, promote the health of beta cells, suggesting that these compounds

may act to improve insulin secretion and promote glycemic control in patients with T2D (Marrano et al., 2021).

Another hypothesis that may be associated with the PC from OO and OP anti-diabetes effect is the ability to inhibit enzymes responsible for hydrolyzing polysaccharides into monosaccharide units, existing in the cells that line the intestine (Sylla et al., 2021). *In vitro* studies support this hypothesis, where concentrations of PC (72.28 μg mL^{-1}) from OP can inhibit the action of α-amylase, reducing the levels of sugars for absorption, and reducing the need for insulin production (Sylla et al., 2021).

5.5 Hypolipidemic activity

The blood lipid profile is expressed by the presence of fats in the blood, such as cholesterol and triglycerides. Cholesterol is commonly classified into two types, high-density lipoprotein (HDL) and low-density lipoprotein (LDL), with higher levels of HDL bringing greater health benefits (Elsewedy et al., 2022). Hyperlipidemia is a disease characterized by the presence of extraordinary fats in the blood serum, particularly high levels of LDL along with low levels of HDL. The LDL accumulation in the blood results in oxidation by increasing levels of ROS or oxidative enzymes released by inflammatory cells. These oxidized lipids give rise to overexpression of adhesion and secretion of pro-inflammatory cytokines, that promote the generation of fatty streak cells laden with lipids, an component of atherosclerotic plaques (Kribeche, 2022). This way, OO and OP can play important roles on lipids concentration or their oxidative stability.

OO caused a reduction in serum levels of total cholesterol, triglycerides and LDL, accompanied by an HDL increase in male Wistar rats receinving extra virgin OO (1% of body weight) for 50 days (Kribeche, 2022). Moreover, virgin OO treatment decreased lipid peroxidation and restored GSH levels in the liver (Kribeche, 2022). This effect was most likelly mediated by PC antoxidant effects, by scavenging ROS and activateing endogenous antioxidant mechanisms (González-Rámila et al., 2022; Kribeche, 2022).

The daily consumption of OP oil (45 g) by 72 patients for 4 weeks resulted in decreased LDL, although blood pressure, peripheral artery tonometry, endothelial function and inflammation biomarkers remained unchanged(González-Rámila et al., 2022). Thus, we can not rule out that this lack of effects is related to the reduced levels of PC in this oil when compared to the crude OP.

5.6 Cardiovascular diseases prevention

CVD are multifactorial diseases associated with hyperlipidemia, inflammation, oxidative damage, endothelial disfunction, among other factors. Thus, OO consumption can attenuate several risk factors for CVD, such as blood pressure, dyslipidemia, endothelial dysfunction, thrombosis and carbohydrate metabolism (Katsiki et al., 2021). PC from OO and OP may represent an alternative for CVD prevention trough they antiinflamatory and antioxidant effects (Katsiki et al., 2021; Perrone et al., 2019).

The daily consumption (25 mL, 250 to 500 ppm of PC) of OO by 33 patients with hypercholesterolemia for 3 weeks has beneficial effects on the HDL functionality, since its action is more important than its circulating concentration (Farràs et al., 2015). Furthermore, HT from OO have been described as acting in hibiting the lipid oxidation by trapping peroxyl radicals. This way, a less proinflamatory and oxidized HDL could be more efficient in its pleotropic function (Crespo et al., 2020).

The OO daily consumption for 3 weeks (25 mL, 500 mg kg^{-1} of PC) showed improvement in the HDL expression by regulating genes in mononuclear cells of 33 patients with hypercholesterolemia (Farràs et al., 2019). However, the mechanisms involved in the expression of genes associated with these benefits are not yet understood (Farràs et al., 2019). Thus, it is possible that consuming OO rich in PC can improve the antiaterogenic capacity of HDL.

A diet rich in OO (30 mL per day) reduces the CVD incidence, over 4 months in 82 patients with early atherosclerosis by improving the endothelial function (Widmer et al., 2013). Along with the reduction of leukocytes, the improvement in endothelial function demonstrates an important benefic role of PC from OO, particularly Ole, Tyr and luteolin.

PC from OP (0.05 and 0.1 mg ml^{-1} of PC) showed protective effects against 24 h anoxic stress induced in human endothelial cells (EAhy926) (Palmieri et al., 2012). Anoxia modulates the expression of molecules associated with endothelial dysfunction and CVD. Thus, the treatment with PC extract from OP, particularly Ole and Tyr , could offer protective effect in CVD by preventing endothelial disfunction (Palmieri et al., 2012).

5.7 Neuroprotective activity

Neurodegenerative diseases are associated with oxidative damage, neuroinflammation, mitochondrial dysfunction, vascular impairment among other events.

In fact, vascular impairment mediates cognitive decline associated with aging, which becomes a risk factor for the development of dementia (Valls-Pedret et al., 2015). OO weekly consumption (1 L) by healthy volunteers (447), for over 6 years, resulted in an improvement in cognitive function (Valls-Pedret et al., 2015). The beneficial effect of an OO rich diet on cognition likely stems from the PC abundance in this product, which act as anti-inflammatory agents.

OO PC, especially secoiridoids, were linked to the attenuation of the β-amyloid (Aβ) pathology in Alzheimer's disease (AD). Daily OO (0.7 g kg^{-1}, 680 mg kg^{-1} of oleocanthal) consumption by rats, for a month, increased the effect of donepezil, (a medicament used in all stages of AD (Batarseh and Kaddoumi, 2018). The combined consumption of OO and donepezil reduced the Aβ peptide load. This reduction is associated with increased peptide elimination pathways through the blood-brain barrier and enzymatic degradation. In addition, OO attenuated the neuroinflammation process related to the Aβ peptide formation and toxicity responsible for AD development (Batarseh and Kaddoumi, 2018).

OO administration (50 ml per day) to 80 individuals over 64 years old, over 12 months, resulted in a reduction in oxidative stress and inflammatory responses, most likely mediated by PC from OO (Tzekaki et al., 2021). Besides, OO was able to modulate the immune system by affecting cytokine production and white blood cell activity (Gorzynik-Debicka et al., 2018; Tzekaki et al., 2021).

PC from OO and OP have the ability to counteract inflammation and oxidative stress that leads to myelin and neuron destruction. Therefore, these PC may be useful to delay the progression of demyelination and neurodegeneration (Giacometti et al., 2020). In this context, the main pathway of neuroprotection performed by the OO and OP PC is the morbidity reduction and delay in the progression of neurodegenerative diseases (Gorzynik-Debicka et al., 2018). In fact, neuron-like cells were protected by PC from OP treatment over 24 h (0.01 – 0.5 mg mL^{-1} of PC) from $Ca2^{+}$-overloading and the pathological activation of calpain, key events in neurodegenerative disorders (Averna et al., 2018). Regarding OP, chemical analysis demonstrated the presence of HT esters that are not present in olives, most likely synthesized in the OP by esterases that couple fatty acids and HT (Averna et al., 2018). Furthermore, HT esters exerted *in vitro* an important anti-inflammatory activity and appeared to be more bioavailable than HT itself, most likely due the increased lipophilicity resulting from a long fatty acid chain (Averna et al., 2018). Thus, it is possible that the increased lipophilicity of HT ester in OP could be

positive for its neuroprotective events, sinc lipophilic substances can easily cross brain blood barrier.

6. BIOAVAILABILITY OF DIETARY PC

All bioactivities associated to PC from OO and OP varies according to their bioavailability during digestion. During the digestive process stages, different enzymes act in the PC degradation, resulting in the bioavailable PC with biological potential (Speroni et al., 2019). Thus, gastric conditions are responsible for greater PC extraction from the food matrix, ensuring greater bioavailability without total loss of antioxidant capacity, although significant losses of some PCs occur. In addition, the intestinal microbiota action can contribute to the bioavailable phenolic metabolites formation with high antioxidant potential (Augusti et al., 2020). The OO and OP compounds with greater extraction during the digestive phases are Ole, HT, caffeic acid and luteolin (Speroni et al., 2019). Despite affecting the antioxidant capacity, the digestive process may also result in a prebiotic effect of undigested fractions of PC from OO and OP, stimulating the growth of beneficial intestinal microorganisms (Gavahian et al., 2019).

The process of PC uptake from OO is still not fully understood. However, it is known that PC go through the digestive stages, starting with the mouth and stomach, and proceeding to the small intestine and colon. Throughout these steps, structural changes occur through the action of enzymes and gut microbiota (Ditano-Vázquez et al., 2019). The digestive process contributes to the formation of PC derived from Ole, Tyr and HT, present in OO and OP, which have greater bioavailability. These bioavailable OO and OP PC can play different beneficial roles in human health (Ditano-Vázquez et al., 2019). In a dose-dependent manner, HT and Tyr from OO have the highest rate of intestinal absorption (40-60%). The secoiridoid compounds are stable in the mouth, but significant losses occur throughout the gastric, duodenal and colonic regions, through Ole degradation and formation of Tyr and HT, representing a bioavailability that varies between 7% and 34% (Ditano-Vázquez et al., 2019).

The PC bioavailability of OP is similar to OO since the most representative compounds of OP are also HT, Tyr, and Ole (Radić et al., 2020). The pre-treatment of human liver cells (HepG2) with the bioavailabe fraction of OP (41 μM) for 20 h was able to prevente cell damage and GSH depletion induced by 3 h exposure of cells to tert-butyl hydroperoxide (350 μM) (Radić et al., 2020). These results demonstrate that bioavailable

PC from OP can alter the redox state of the cell through multiple mechanisms, such as reducing potential, ROS elimination and GSH sinthesis (Radić et al., 2020).

The micronization of OP increases the intestinal bioaccessibility of their PC, after *in vitro* digestion. Micronized OP increased antioxidant capacity in the gastric phase and increased the HT, Ole and luteolin intestinal bioaccessibility when compared with OP that was only fractioned (Sefrin et al., 2021a). This reinforces the importance of developing adequate techniques to use OP for human consumption, targeting its nutraceutical properties by increasing the bioaccessibility and antioxidant capacity of its PC.

7. OLIVE CONTAMINANTS AND TOXYCOLOGICAL ASPECTS

Olive trees can be affected by various diseases caused by pests, fungi, and weeds. When they affect the olive trees, these diseases affect olive production due to precocious downfall (Arena et al., 2022). Therefore, the control of parasites and diseases helps to maintain high levels of OO production. However, residues of toxic compounds (added or generated in the plant) can migrate from the olive fruit and consequently contaminate the OO and OP (Arena et al., 2022). However, the presence of PC in OO and OP could protect from some toxic effects caused by the contaminants (Table 4).

Table 4 – Protective effect of PC from OO and OP on the toxic effects of pesticide residues, mycotoxins and PAH.

	Sample or PC	Contaminant	Experimental protocol	Results	Reference
Pesticide residues	OO	*Diazinon*	Daily supplementation of rats with OO (600 mg kg^{-1} bw) and diazinon (50 mg kg^{-1} bw) for 6 weeks.	OO attenuated the hepatorenal changes induced by diazinon.	(Al-Attar et al., 2017)
	Virgin OO	*Metamitron*	Daily administration of pregnant females rats with metamitron (33.94 - 67.88 mg kg^{-1} bw) and virgin OO (0.5 mL 200 g^{-1} bw).	Virgin OO reduced the metamitron teratogenic toxicity and improved the ossification process in rats pups.	(Abdulrhman & El-aal, 2020)
	OO	*Malathion*	Combined daily exposure of male rats with OO (400 mg kg^{-1} bw) and malathion (100 mg kg^{-1} bw), over 7 weeks.	OO prevented oxidative stress, nephrotoxicity and reprodutive toxicity induced by malathion.	(Al-asmari et al., 2022; Zeid et al., 2022)
	OO	*Imidacloprid*	Combined daily exposure of male albino rats to OO (10 mL kg^{-1} bw,	OO protected against imidacloprid mutagenicity.	(Mahmoud et al., 2020)

			for 2 weeks) and imidacloprid (22.5 mg kg^{-1} bw for 4, 8 and 12 weeks).		
Mycotoxyns	*Tyr and Ole*	*Alternariol*	Caco-2 intestinal cells exposed to alternariol (25 µmol L^{-1}) and PC from OO and OP (Tyr and Ole, 50 µmol L^{-1}) for 24h.	Tyr and Ole were able to reduce toxicity and ROS production in Caco-2 cells exposed to alternariol.	(Chiesi et al., 2015)
	HT	*Ochratoxin A*	Pre-incubation of kidney cells lines (1h) with HT (10 µmol L^{-1}), followed by exposure to ochratoxin A (2.5 to 25 µg mL^{-1}).	HT prevented the ochratoxin A cytotoxicity by attenuating the oxidative processes.	(Crupi et al., 2020)
	(+)-Acetoxypinoresinol	*Penitrem A*	Exposure of Swiss albino mouse to (+)-acetoxypinoresinol (30 µmol L^{-1}) and penitrem A (10 µmol L^{-1}), over 24 h.	(+)-Acetoxypinoresinol offers neuronal protection against the mycotoxin penitrem A.	(Qusa et al., 2020)
PAHs	OO	Benzo(a)pyrene	Treatment of liver microssomes of rats with 10 µL of OO and	OO normalized lipid peroxidation	(Devi et al., 2008)

		benzo(a)pyrene (25 μmol L^{-1}), over 1h.	and citotoxicity caused by benzo(a)pyrene.	
OO	Benzo(a)pyrene	Exposure of adult ApcMin mice with OO (300 mg kg^{-1} bw) and benzo(a)pyrene (25, 50, and 100 μg bw^{-1}) over 60.	Exposure to OO resulted in reduction of benzo(a)pyrene toxicity.	(Banks et al., 2016)

Abbreviations: ApcMin – adult mice, Caco-2 – intestinal cells, HT – hydroxytyrosol, Ole – oleuropein, OO – olive oil, OP – olive pomace, PAH - polycyclic aromatic hydrocarbons, PC – phenolic compounds, ROS – reactive oxigen species, Tyr – tyrosol.

7.1 Pesticide residues

Extra virgin OO (20 samples) from Milano (Italy) presented pesticides commonly used in olive cultivation, such as bromfenvinfos-methyl (<10 μg kg^{-1}), quinalphos (139 μg kg^{-1}), prothiofos (10 - 17 μg kg^{-1}) and phosalone (17 - 115 μg kg^{-1}) (Arena et al., 2022). The pesticides concentration of many samples was greater than the maximum residue level (MRL) of 10 μg kg^{-1} established by the European Union, this way representing a risk to human health (Arena et al., 2022).

OO from Iranian Market (28 domestic and 9 imported samples from Italy and Spain, all with glass packaging) presented residues of pretilachlor, heptachlor and 1,1'-(2,2,2-trichloroethane-1,1-diyl)bis(4-chlorobenzene) (DDT) in 29% of the analyzed OO samples (Razzaghi et al., 2018). Samples contaminated with pretilachlor comply with the maximum residue lavels (MRL) (50 ng g^{-1}) established by Iranian legislation for this pesticide in OO, while the use of the pesticide 2,4 DDT is forbiden in Iran (Razzaghi et al., 2018).

For the besto of our knowledge, countries such as Brazil, have no legislation for pesticides contente in OO. However, due to growing awareness of food safety and the widespread use of pesticides, the European Union has established MRL for pesticides in food. MRL are different for each pesticide and not all pesticides used in agriculture are

regulated and, in this case, an MRL value of 10 μg kg^{-1} must be assumed (Arena et al., 2022).

The toxicity of pesticides contaminants of OO and OP can be counteracted by PC administration. However, if this protection can occur still within in the food matrix remains to be elucidated. Daily supplementation of rats with OO (600 mg/kg bw) for 6 weeks, attenuated the hepatorenal changes induced by intoxication with diazinon (50 mg/kg bw) (Al-Attar et al., 2017). The protective effect of OO against hepatorenal changes induced by diazinon may be due to its flavonoids and phenolic acids and their antioxidant effects, which impair the activation of diazinon into the reactive form (Al-Attar et al., 2017).

The synthetic herbicide, metamitron (33.94 - 67.88 mg/kg bw) is toxic to the pregnant females rats and induces a delay in the skeleton fetal development after organogenesis (from the 6th to the 15th day of gestation). Virgin OO treatment (0.5 ml/200g b.w.) reduced the metamitron teratogenic toxicity effects and improved the ossification processo of rats pups, especially on the shortening of the last ribs of fetuses (Abdulrhman & El-aal, 2020). This beneficial effect on bones, was associated to PC of OO that may act through the modulation of osteoblastic physiology (Abdulrhman & El-aal, 2020).

Malathion is an effective and widely used organophosphate pesticide. This pesticide has a negative impact on the male reproductive system. The cell development and differentiation can be disrupted by the ROS generation, resulting in multiple effects on the male reproductive system (Zeid et al., 2022). In addition to its reproductive toxicity, malathion also exhibits nephrotoxicity (Al-asmari et al., 2022). Two parallel studies demonstrate that OO (400 mg/kg bw) prevented oxidative stress, nephrotoxicity and reprodutive toxicity induced by malathion (100 mg/kg bw) in male rats, after 7 weeks of combined exposure (Al-asmari et al., 2022; Zeid et al., 2022). It was verified that the reduction of the malathion oxidative estress and reproductive toxicity by OO was associated with the presence of Ole in the OO phenolic composition (Zeid et al., 2022). Oxidative stress and ROS scavenging by Ole increased the activity of SOD and CAT, which suggests that Ole can repair and maintain these enzymes, reducing oxidative stress associated with malathion toxicity (Zeid et al., 2022). The prevention of OO against oxidative stress and nephrotoxicity induced by malathion was associated with the HT presence in OO (Al-asmari et al., 2022). HT exhibit good interaction with the COX-2 enzyme that is significant in the pathophysiology of nephrotoxicity. The reduction of

COX-2 enzyme expression could be a vital technique in controlling nephron destruction (Al-asmari et al., 2022).

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-lideneamine] is a neonicotinoid insecticide, used for controlling insects and termites (Mahmoud et al., 2020). OO (10 ml/kg bw, for 2 weeks) presents protective effect against imidacloprid mutagenicity (22.5 mg/kg bw for 4, 8 and 12 weeks) in male albino rats (Mahmoud et al., 2020). OO protects against chromosomal damage due to the HT, Tyr, Ole and oleocanthal presence that act to ROS removal, before these species can reach the DNA and induce damage. In addition, these PC activate the defense mechanism by enzymatic induction or by stimulating mitochondrial biogenesis (Mahmoud et al., 2020).

7.2 Mycotoxins

Mycotoxins are secondary metabolites produced by filamentous fungi, such as fungi of the *Aspergillus*, *Penicillium*, and *Fusarium* strains. Filamentous fungi develop under variable temperature conditions (20 to 35 °C) humidity (20 to 60%) and under conditions of water stress. These factors favor the filamentous fungi development and the mycotoxin production during olive cultivation, leading to OO and OP contamination (Abdolmaleki et al., 2021; Khalil et al., 2019). Thus, the mycotoxin's presence in olive products represents a food safety problem since about 30% of the human diet is based on vegetable oil consumption (Abdolmaleki et al., 2021).

OO and OP contamination with aflatoxins (AFs) produced by the fungi *Aspergillus flavus*, *Aspergillus nomius* and *Aspergillus parasiticus* is a global foodsafety concern (Waqas et al., 2021). The AFB1 subclass of AFs is recognized as the most toxic and carcinogenic, categorized as a group 1 carcinogen by the International Agency for Research on Cancer. AFB_1 elevated concentrations were identified in 20 samples of OO from markets, superstores, and farmers from central cities of Punjab, Pakistan (Waqas et al., 2021). According to this study, OO AFB_1 and total AF occurrence were 8.51 and 12.78 μg kg^{-1}, respectively. These results demonstrate that OO samples have AFB_1 levels greater than the proposed limit set by the European Commission regulation (2 μg kg^{-1}) (Waqas et al., 2021).

The alternariol mycotoxin, produced by *Alternaria sp*. fungi, is common in OO (Chiesi et al., 2015). Virgin OO (20 samples) from shops in Fujian Province (southeast china) presented alternariol contamination (Lin et al., 2022). More than half of the products (60%) were positive for alternariol, with four samples detected in appreciable

concentrations (12.5 - 13.7 μg kg^{-1}). Alternariol exhibits mutagenic, cytotoxic, genotoxic, and carcinogenic effects, indicating that more extensive monitoring research is needed aimed at the food safety of these products (Lin et al., 2022).

Studies associating PC from OO and OP with a mitigation of mycotoxin toxicity have been the subject of scientific interest (Chiesi et al., 2015; Crupi et al., 2020). The PC HT and Ole (50 μM), present in OO and OP, were able to reduce toxicity and ROS production in Caco-2 intestinal cells exposed to alternariol (25 μM) for 24 h (Chiesi et al., 2015). The HT can act as a scavenger of peroxyl radicals near the membrane surface, while Ole acts as a scavenger of chain-propagating lipid hydroxyl radicals within membranes (Chiesi et al., 2015).

Ochratoxin A (OTA) is a food contaminant mycotoxin and has severe nephrotoxic effects (Crupi et al., 2020). Exposure of kidney cells lines to OTA (2.5 to 25 μg mL^{-1}) reduced cell survival and induced stress oxidative (Crupi et al., 2020). The kidney cell pre-incubation (1h) with HT (10 μM) prevented the OTA cytotoxicity (Crupi et al., 2020) by attenuating the oxidative processes (Crupi et al., 2020). This way, PC from OO and OP can contribute to reducing the development of kidney failure, associated with OTA exposure (Crupi et al., 2020).

One of the OO lignins, the (+)-acetoxypinoresinol (AC, 5 to 30 μM) offers neuronal protection against the mycotoxin Penitrem A (PA, 10 μM) in a Swiss albino mouse, after 24 h (Qusa et al., 2020). PA acts on calcium-dependent channels in the brain, causing motor system dysfunction, including tremors and seizures. Furthermore, PA has the ability to spontaneously increase the release of endogenous glutamate, γ-aminobutyric acid (GABA), and produce ROS in primary cerebellar granule neurons (Qusa et al., 2020). AC treatment proved to be protective of sciatic nerve against the PA toxicity (Qusa et al., 2020). Thus OO and OP lignans may play a promising role as tracks in controlling peripheral nerve injury caused by the micotoxin tremogenic PA (Qusa et al., 2020).

7.3 Polycyclic aromatic hydrocarbons residues (PAH)

PAH are environmental contaminants produced by incomplete combustion processes or organic matter pyrolysis (Bertoz et al., 2021). PAH are released through forest fires, volcanic eruptions, burning of fuels, tires, polypropylene or polystyrene, vehicle exhaust emissions, smoking, cooking processes, and waste incineration (Bertoz et al., 2021; Elaridi et al., 2020). PAH have two or more fused aromatic rings in their structure, making them poorly soluble in water and highly soluble in non-polar solvents

and edible oils, favoring OO and OP contamination (Bertoz et al., 2021; Rascón et al., 2018).

The primary sources of OO contamination by PHA are environmental pollution by dust deposition on the olive skin, olives falling on the ground during harvest, and storage in mills (Rascón et al., 2018). OO and OP ingestion is an important route of PAH exposure. However, only 16 PAH are usually monitored in food and the environment (Ekner et al., 2022). OO samples (16 samples, included extra virgin OO, ecological extra virgin OO and refined OO) presented total PAH levels of 30.1 μg kg^{-1}, which characterizes a high risk of exposure to these toxic compounds (Ekner et al., 2022).

Benzo(a)pyrene (BaP), benzo(a)anthracene (BaA), benzo(b)fluoranthene (BbF), and chrysene (Chry) are PAH with carcinogenic potential reported by the European Regulation (Cotugno et al., 2021). OO samples obtained from Syrian markets showed average levels of 15 PAHs of 63.7 μg kg^{-1} (Krajian and Odeh, 2018). Of 38 OO samples, 9 had BaP concentrations higher than those stipulated by the European Regulation (2 μg kg^{-1}). In addition, 2 samples exceeded the MRL (10 μg kg^{-1}) for the sum of PAHs (BaP, BaA, BbF and Chry) (Krajian and Odeh, 2018). According to these authors, the OO used for consumption in Syrian present a risk to the population, and there is a need to develop specific national legislation for the PAH levels in OO (Krajian and Odeh, 2018).

Lebanon presented high pollution levels, which resulted in the generation of PAH. OO samples (112 samples) obtained in Lebanon markets showed high PAH levels (Elaridi et al., 2020). Only one of the 112 OO samples analyzed, showed levels higher than those established by the European Regulation for BaP (11.9 μg kg^{-1}), posing an increased carcinogenic risk (Elaridi et al., 2020). This same OO sample showed a total of 26.7 μg kg^{-1} for four PAH (BaP, BaA, BbF, and Chry), which also exceeds the MRL established by the European Regulation (10 μg kg^{-1}) (Elaridi et al., 2020). These authors emphasize the need to develop appropriate processing steps to reduce the content of these carcinogenic compounds in OO (Elaridi et al., 2020).

Despite the PAH carcinogenicity, the harmful health effects associated with the consumption of OO and OP contaminated with PAH are still poorly understood. BaP presents a high carcinogenic risk induced by oxidative stress-mediated by the enzyme BaP hydroxylase (Devi et al., 2008). Treatment of liver microssomes of rats with 10 μL of OO normalized lipid peroxidation and citotoxicity caused by BaP (25 μM). Authors suggest that PC from OO can act by inhibiting BaP hidroxilase and effectively remove BaP metabolites, contributing to the protective effects of OO (Devi et al., 2008).

Ingestion of food contaminated with PAH contributes to the development of gastrointestinal carcinogenesis (Banks et al., 2016). However, exposure of adult ApcMin mice OO (300 mg kg^{-1} bw) over 60 days resulted in reduction of BaP (25, 50, and 100 µg bw^{-1}) toxicity. PC from OO promoted a rapid BaP detoxification, decreasing the concentrations of its organic metabolites and reducing the extent of DNA damage in the colon and liver (Banks et al., 2016).

Although there are relevant data regarding the presence of pesticide, mycotoxins and PHAs residues in OO, the number of publications is very low, indicating the need of more studies evaluating the content of these contaminats in OO available for consumers. Moreover, no studies evaluating the presence of these contaminants in OP were found until this moment. However, since these substances contamine olive fruits, their presence must also be expected in OP. Thus, quantification of contaminants in OP is extremely important to guarantee the safe utilization of this by-product and its biological properties (Arena et al., 2022; Razzaghi et al., 2018).

PC widely found in OO and OP were able to minimize negative effects of pesticides, mycotoxins PHAs. However, there is still a gap to be filled regarding studies using OO and OP in the evaluation of protective effects agains these food contaminats and most reported studies tested isolated PC instead samples of olive products. Despite the protective effect of isolated PC is not always the same observed in the food matrix, they may suitable to drive future studies with OO and OP samples.

Since PC from OO were able to counteract the toxicity of these contaminats *in vitro* and *in vivo*, we can hypothesize that PC could interact with then even whithin the food matrix, before consumption of OO and OP. This way, besides presenting toxicity for human health itself, toxic agents could also impair the benefits of some foods, as described for *Cabernet Sauvignon* wine contaminated with the mycotoxin Ocratoxin A (Schmidt et al., 2020). This way, it is possible that contaminants in OO and OP can affect the previously reported beneficial biological properties attributed to its PC. The effect of this possible interaction remains to be confirmed in further studies from our group.

8. FINAL CONSIDERATIONS

The importance of this review lays in to offer useful information regarding the bioactivities of OO and OP, such as antioxidant, antimicrobial, antitumoral, hypolipidemic, antidiabetes potential, along with protective effects on CVD and neurodegenerative disorders. The main PC associated to such bioactivities are Ole, Tyr

and HT and the extension of their effect depends on their biavailability after digestion processes.

On the other hand, OO and, most likely OP, may also present toxic compounds, such as mycotoxins, PAHs and pesticides. As few reports about the levels of these contaminats in OO and mainly in OP are available until this moment, studies on this regard should be carried out ia a near future.

PC may counteract the toxicity caused by contaminats from OO and OP, although most available studies use isolated PC instead samples of OO and OP. Thus, the data of this review may drive future experiments in order to fill this gap.

Another gap observed in the presente review is the small number of studies about contaminantion and bioactivities of OP. Since OP is an importante by product from OO production and presentes high levels of PC, it could be more explored in order to determine its use in human nutrition.

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11. AUTHOR CONTRIBUTIONS

Conceptualization and design, Schmidt L. e Augusti P.R.; Funding acquisition Moreira J.C.F. and Augusti P.R.; Investigation and data analysis, Schmidt L.; Writing–original draft, Schmidt L.; Writing–review and editing, Schmidt L., Moreira J.C.F., Augusti P.R. and Prestes O.D. All authors have read and agreed to the published version of the manuscript.

12. AUTHOR DECLARATIONS

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

13. REFERENCES

Abdolmaleki, K., Khedri, S., Alizadeh, L., Javanmardi, F., Oliveira, C. A. F., & Mousavi Khaneghah, A. (2021). The mycotoxins in edible oils: An overview of prevalence, concentration, toxicity, detection and decontamination techniques. Trends in Food Science and Technology, 115, 500–511. https://doi.org/10.1016/j.tifs.2021.06.057

Abdulrhman, S. M., & El-aal, R. A. H. A. (2020). Developmental toxicity of metamitron and the protective effect of olive oil on pregnant female rats and their offspring. Current Science International, 9, 594–606. https://doi.org/10.36632/csi/2020.9.4.52

Al-asmari, K. M., Altayb, H. N., Al-attar, A. M., Qahl, S. H., Al-thobaiti, S. A., & Abu, I. M. (2022). Saudi Journal of Biological Sciences Arabica coffee and olive oils mitigate malathion-induced nephrotoxicity in rat : In silico , immunohistochemical and biochemical evaluation. Saudi Journal of Biological Sciences, 29, 1–20. https://doi. org/10.1016/j.sjbs.2022.103307

Al-Attar, A. M., Elnaggar, M. H. R., & Almalki, E. A. (2017). Protective effect of some plant oils on diazinon induced hepatorenal toxicity in male rats. Saudi Journal of Biological Sciences, 24, 1162–1171. https://doi.org/10.1016/j.sjbs.2016.10.013

Amini, A., Liu, M., & Ahmad, Z. (2017). Understanding the link between antimicrobial properties of dietary olive phenolics and bacterial ATP synthase. International Journal of Biological Macromolecules, 101, 153–164. https://doi.org/10.1016/j. ijbiomac.2017.03.087

Arena, A., Zoccali, M., Mondello, L., & Tranchida, P. Q. (2022). A method for the determination of 70 pesticides in extra virgin olive oil based on a limited - volume solvent extraction step prior to comprehensive two - dimensional gas chromatography - tandem mass spectrometry. Analytical and Bioanalytical Chemistry, 1, 1–22. https://doi.org/10.1007/s00216-022-04494-6

Augusti, P. R., Conterato, G. M. M., Denardin, C. C., Prazeres, I. D., & Teresa, A. (2020). Bioactivity, bioavailability, and gut microbiota transformations of dietary phenolic compounds: Implications for COVID-19. Journal of Nutritional Biochemistry, 97, 1–19. https://doi.org/10.1016/j.jnutbio.2021.108787Averna, M., Casazza, A. A., Martines, A., Pedrazzi, M., Franchi, A., Tullio, R. De, Perego, P., Melloni, E., Averna, M., Casazza, A. A., Martines, A., & Pedrazzi, M. (2018). Cell protection from Ca -overloading by bioactive molecules extracted from olive pomace. Natural Product Research, 6419, 1–7. https://doi.org/10.1080/ 14786419.2017.1422181

Banks, L. D., Amoah, P., Niaz, M. S., Washington, M. K., Adunyah, S. E., & Ramesh, A. (2016). Olive oil prevents benzo(a)pyrene [B(a)P]-induced colon carcinogenesis through altered B(a)P metabolism and decreased oxidative damage in ApcMin mouse model. Journal of Nutritional Biochemistry, 28, 37–50. https://doi.org/10.1016/j.jnutbio.2015.09.023

Batarseh, Y. S., & Kaddoumi, A. (2018). Oleocanthal-rich extra-virgin olive oil enhances donepezil effect by reducing amyloid-β load and related toxicity in a mouse model of Alzheimer's disease. Journal of Nutritional Biochemistry, 55, 113–123. https://doi.org/10.1016/j.jnutbio.2017.12.006

Berbel, J., & Posadillo, A. (2018). Review and analysis of alternatives for the valorisation of agro-industrial olive oil by-products. Sustainability (Switzerland), 10, 1–9. https://doi.org/10.3390/su10010237

Bertoz, V., Purcaro, G., Conchione, C., & Moret, S. (2021). A review on the occurrence and analytical determination of pahs in olive oils. Foods, 10, 1–26. https://doi.org/10.3390/foods10020324

Calahorra, J., Martínez-Lara, E., Granadino-Rold´an, J. M., Martí, J. M., Ca˜nuelo, A., Blanco, S., Oliver, F. J., & Siles, E. (2020). Crosstalk between hydroxytyrosol, a major olive oil phenol, and HIF-1 in MCF-7 breast cancer cells. Scientific Reports, 10, 1–15. https://doi.org/10.1038/s41598-020-63417-6

Carito, V., Ceccanti, M., Vincenzo, C., Natella, F., Bello, C., Coccurello, R., Mancinelli, R., & Fiore, M. (2016). Olive Polyphenols effects in a mouse model of chronic ethanol addiction Valentina. Nutrition, 1, 1–22. https://doi.org/10.1016/j.nut.2016.08.014

Castro, M., Romero, C., De Castro, A., Vargas, J., Medina, E., Mill´an, R., & Brenes, M. (2012). Assessment of Helicobacter pylori eradication by virgin olive oil. Helicobacter, 17, 305–311. https://doi.org/10.1111/j.1523-5378.2012.00949.x

Cells, C. H. C. T., Centrone, M., Agostino, M. D., Difonzo, G., Bruno, A., De Mise, A., Di Ranieri, M., Montemurro, C., Valenti, G., Poiana, M., Caponio, F., & Tamma, G. (2021). Antioxidant efficacy of olive by-product extracts in human colon HCT8 cells. Foods, 10, 1–12. https://doi.org/10.3390/foods10010011

Chanioti, S., & Tzia, C. (2017). Optimization of ultrasound-assisted extraction of oil from olive pomace using response surface technology: Oil recovery, unsaponifiable matter, total phenol content and antioxidant activity. LWT - Food Science and Technology, 79, 178–189. https://doi.org/10.1016/j.lwt.2017.01.029

Chiesi, C., Fernandez-Blanco, C., Cossignani, L., Font, G., & Ruiz, M. J. (2015). Alternariol-induced cytotoxicity in Caco-2 cells. Protective effect of the phenolic fraction from virgin olive oil. Toxicon, 93, 103–111. https://doi.org/10.1016/j. toxicon.2014.11.230

Cioffi, G., Pesca, M. S., De Caprariis, P., Braca, A., Severino, L., & De Tommasi, N. (2010). Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. Food Chemistry, 121, 105–111. https://doi.org/ 10.1016/j.foodchem.2009.12.013

Crespo, C., Carmen, L., & Alberto, D. (2020). Olive oil consumption and its repercussions on lipid metabolism. Nutrition Reviews, 1, 1–17. https://doi.org/10.1093/nutrit/ nuaa014

Crupi, R., Palma, E., Siracusa, R., Fusco, R., Gugliandolo, E., Cordaro, M., Impellizzeri, D., De Caro, C., Calzetta, L., Cuzzocrea, S., & Di Paola, R. (2020). Protective effect of hydroxytyrosol against oxidative stress induced by the ochratoxin in kidney cells: In vitro and in vivo study. Frontiers in Veterinary Science, 7, 1–13. https://doi.org/10.3389/fvets.2020.00136

Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., & Michaud, P. (2013). Olive mill wastes: Biochemical characterizations and valorization strategies. Process Biochemistry, 48, 1532–1552. https://doi.org/10.1016/j.procbio.2013.07.010

Devi, K. P., Kiruthiga, P. V., Pandian, S. K., Archunan, G., & Arun, S. (2008). Olive oil protects rat liver microsomes against benzo(a)pyrene-induced oxidative damages: An in vitro study. Molecular Nutrition & Food Research, 52, 95–102. https://doi.org/ 10.1002/mnfr.200800047

Difonzo, G., Troilo, M., Squeo, G., Pasqualone, A., & Caponio, F. (2021). Functional compounds from olive pomace to obtain high-added value foods – a review. Journal of the Science of Food and Agriculture, 101, 15–26. https://doi.org/10.1002/ jsfa.10478

Dini, I., Graziani, G., Fedele, F. L., Sicari, A., Vinale, F., Castaldo, L., & Ritieni, A. (2020). An environmentally friendly practice used in olive cultivation capable of increasing commercial interest in waste products from oil processing. Antioxidants, 9, 1–13. https://doi.org/10.3390/antiox9060466

Ditano-V´azquez, P., Torres-Pe˜na, J. D., Galeano-Valle, F., P´erez-Caballero, A. I., Demelo- Rodríguez, P., Lopez-Miranda, J., Katsiki, N., Delgado-Lista, J., & Alvarez-Sala- Walther, L. A. (2019). The fluid aspect of the mediterranean diet in the prevention and management of cardiovascular disease and diabetes: The role of polyphenol content

in moderate consumption of wine and olive oil. Nutrients, 11, 1–28. https://doi.org/10.3390/nu11112833

Ekner, H., Dreij, K., & Sadiktsis, I. (2022). Determination of polycyclic aromatic hydrocarbons in commercial olive oils by HPLC/GC/MS – occurrence, composition and sources. Food Control, 132, 1–11. https://doi.org/10.1016/j. foodcont.2021.108528

Elaridi, J., Fakhro, M., Yamani, O., Dimassi, H., Othman, H., & Attieh, Z. (2020). GC–MS analysis of polycyclic aromatic hydrocarbons in bottled olive oil marketed in Lebanon. Toxicological Research, 36, 211–220. https://doi.org/10.1007/s43188-019-00015-3

Elsewedy, H. S., Shehata, T. M., Almostafa, M. M., & Soliman, W. E. (2022). Hypolipidemic activity of olive oil-based nanostructured lipid carrier containing atorvastatin. Nanomaterials, 1, 1–18. https://doi.org/10.3390/nano12132160

Farr`as, M., Arranz, S., Carri´on, S., Subirana, I., Mu˜noz-Aguayo, D., Blanchart, G., Kool, M., Sol`a, R., Motilva, M. J., Escol`a-Gil, J. C., Rubi´o, L., Fern´andez-Castillejo, S., Pedret, A., Estruch, R., Covas, M. I., Fit´o, M., Hern´aez, A., & Casta˜ner, O. (2019). A functional virgin olive oil enriched with olive oil and thyme phenolic compounds improves the expression of cholesterol efflux-related genes: A randomized, crossover, controlled trial. Nutrients, 11, 1–10. https://doi.org/10.3390/ nu11081732

Farr`as, M., Casta˜ner, O., Martín-Pel´aez, S., Hern´aez, A., Schr¨oder, H., Subirana, I., Mu˜noz-Aguayo, D., Gaixas, S., Torre, R. de la, Farr´e, M., Rubi´o, L., Díaz, O., Fern´andez-Castillejo, S., Sol`a, R., Motilva, M. J., & Fit´o Colomer, M. (2015). Complementary phenol-enriched olive oil improves HDL characteristics in hypercholesterolemic subjects. A randomized, double-blind, crossover, controlled trial. The VOHF study. Molecular Nutrition & Food Research, 59, 1758–1770. https://doi.org/10.1002/mnfr.201500030

Fern´andez-Arroyo, S., G´omez-Martínez, A., Rocamora-Reverte, L., Quirantes-Pin´e, R., Segura-Carretero, A., Fern´andez-Guti´errez, A., & Ferragut, J. A. (2012). Application of nanoLC-ESI-TOF-MS for the metabolomic analysis of phenolic compounds from extra-virgin olive oil in treated colon-cancer cells. Journal of Pharmaceutical and Biomedical Analysis, 63, 128–134. https://doi.org/10.1016/j.jpba.2012.01.033

Fern´andez-Bola˜nos, J., Rodríguez, G., Rodríguez, R., Guill´en, R., & Jim´enez, A. (2006). Extraction of interesting organic compounds from olive oil waste. Grasas y Aceites, 57, 95–106. https://doi.org/10.3989/gya.2006.v57.i1.25

Ferreira, V., Cristina, A., Moura, G. L., Castilhos, J. De, Gemelli, T., Hoffmann, F., Ziegler, V., Ferreira, C. D., Moura, G. L., Castilhos, J. De, Gemelli, T., & Hoffmann, J. F. (2022). Antimicrobial and cytotoxic activity to human colon adenocarcinoma cell lines (HT-29) potential of olive oil extraction residue. Natural Product Research, 36, 4486–4491. https://doi.org/10.1080/ 14786419.2021.1986708

Fit´o, M., Cladellas, M., De La Torre, R., Martí, J., Alc´antara, M., Pujadas-Bastardes, M., Marrugat, J., Bruguera, J., L´opez-Sabater, M. C., Vila, J., Covas, M. I., Schr¨oder, H., Weinbrenner, T., Mu˜noz, D., Farr´e, M., Menoyo, E., Mir´o-Casas, E., Closas, N., De La Torre-Boronat, C., … L´opez, M. C. (2005). Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: A randomized, crossover, controlled, clinical trial. Atherosclerosis, 181, 149–158. https://doi.org/10.1016/j. atherosclerosis.2004.12.036

Frankel, E., Bakhouche, A., Lozano-S´anchez, J., Segura-Carretero, A., & Fern´andez-Guti´errez, A. (2013). Literature review on production process to obtain extra virgin olive oil enriched in bioactive compounds. Potential use of byproducts as alternative sources of polyphenols. Journal of Agricultural and Food Chemistry, 61, 5179–5188. https://doi.org/10.1021/jf400806z García-Villalba, R., Larrosa, M., Possemiers, S., Tom´as-Barber´an, F. A., & Espín, J. C. (2014). Bioavailability of phenolics from an oleuropein-rich olive (Olea europaea) leaf extract and its acute effect on plasma antioxidant status: Comparison between pre- and postmenopausal women. European Journal of Nutrition, 53, 1015–1027. https://doi.org/10.1007/s00394-013-0604-9

Gavahian, M., Mousavi Khaneghah, A., Lorenzo, J. M., Munekata, P. E. S., Garcia-Mantrana, I., Collado, M. C., Mel´endez-Martínez, A. J., & Barba, F. J. (2019). Health benefits of olive oil and its components: Impacts on gut microbiota antioxidante activities, and prevention of noncommunicable diseases. Trends in Food Science and Technology, 88, 220–227. https://doi.org/10.1016/j.tifs.2019.03.008

Georgakouli, K., Mpesios, A., Kouretas, D., Petrotos, K., Mitsagga, C., Giavasis, I., & Jamurtas, A. Z. (2016). The effects of an olive fruit polyphenol-enriched yogurt on body composition, blood redox status, physiological and metabolic parameters and yogurt microflora. Nutrients, 8, 1–14. https://doi.org/10.3390/nu8060344

Giacometti, J., Muhvi´c, D., Grubi´c-Kezele, T., Nikoli´c, M., ˇSoi´c-Vrani´c, T., & Bajek, S. (2020). Olive leaf polyphenols (Olps) stimulate glut4 expression and translocation in the skeletal muscle of diabetic rats. International Journal of Molecular Sciences, 21, 1–31. https://doi.org/10.3390/ijms21238981

Gonz´alez-R´amila, S., Sarri´a, B., Seguido, M. A., García-Cordero, J., Mateos, R., & Bravo, L. (2022). Olive pomace oil can improve blood lipid profile: A randomized, blind, crossover, controlled clinical trial in healthy and at-risk volunteers. European Journal of Nutrition, 1, 1–15. https://doi.org/10.1007/s00394-022-03001-y Gorzynik-Debicka, M., Przychodzen, P., Cappello, F., Kuban-Jankowska, A., Gammazza, A. M., Knap, N., Wozniak, M., & Gorska-Ponikowska, M. (2018). Potential health benefits of olive oil and plant polyphenols. International Journal of Molecular Sciences, 19, 547–560. https://doi.org/10.3390/ijms19030686

Gulcin, ˙I. (2020). Antioxidants and antioxidant methods: An updated overview. Archives of Toxicology, 94, 1–65. https://doi.org/10.1007/s00204-020-02689-3

Iannaccone, M., Ianni, A., Ramazzotti, S., Grotta, L., Marone, E., Cichelli, A., & Martino, G. (2019). Whole blood transcriptome analysis reveals positive effects of dried olive pomace-supplemented diet on inflammation and cholesterol in laying hens. Animals, 9, 1–19. https://doi.org/10.3390/ani9070427

Jimenez-Lopez, C., Carpena, M., Lourenço-Lopes, C., Gallardo-Gomez, M., Lorenzo, J. M., Barba, F. J., Prieto, M. A., & Simal-Gandara, J. (2020). Bioactive compounds and quality of extra virgin olive oil. Foods, 9, 1–31. https://doi.org/10.3390/ foods9081014

Katsiki, N., P´erez-martínez, P., Lopez-miranda, J., & Lopez-miranda, J. (2021). Olive oil intake and cardiovascular disease prevention: " seek and you shall find". Current Cardiology Reports, 23, 2–6. https://doi.org/10.1007/s11886-021-01496-1

Khalil, A. M. A., Hashem, A. H., & Abdelaziz, A. M. (2019). Occurrence of toxigenic Penicillium polonicum in retail green table olives from the Saudi Arabia market. Biocatalysis and Agricultural Biotechnology, 21, 1–7. https://doi.org/10.1016/j. bcab.2019.101314

Krajian, H., & Odeh, A. (2018). Levels of 15 + 1 EU priority polycyclic aromatic hydrocarbons in different edible oils available in the Syrian market. Polycyclic Aromatic Compounds, 38, 369–378. https://doi.org/10.1080/ 10406638.2016.1220958

Kribeche, A. (2022). The beneficial effects of virgin olive oil against oxidative stress induced by hypercholesterolemia in rats. Acta Scientiarum, 44, 1–9. https://doi.org/ 10.4025/actascihealthsci.v44i1.58558

Lanfranchi, M., Giannetto, C., & De Pascale, A. (2016). Economic analysis and energy valorization of by-products of the olive oil process: "valdemone DOP" extra virgin olive oil. Renewable and Sustainable Energy Reviews, 57, 1227–1236. https://doi.org/ 10.1016/j.rser.2015.12.196

Lin, H., Ni, L., Chen, H., & Xu, W. (2022). Analytica Chimica Acta A simple and versatile strategy for sensitive SIDA-UHPLC-MS/MS analysis of Alternaria toxins in olive oil. Analytica Chimica Acta, 1232, 1–8. https://doi.org/10.1016/j.aca.2022.340451

Mahmoud, A. S., Amein, K. A., Yahia, D., & Sharkawy, A. A. (2020). Mutagenic effect of imidacloprid insecticide : The ameliorative effect of pre and post exposure to olive oil. Journal of Food Biochemistry, 1, 1–8. https://doi.org/10.1111/jfbc.13221

Marrano, N., Spagnuolo, R., Biondi, G., Cignarelli, A., Perrini, S., Vincenti, L., Laviola, L., Giorgino, F., & Natalicchio, A. (2021). Effects of extra virgin olive oil polyphenols on beta-cell function and survival. Plants, 10, 1–12. https://doi.org/10.3390/plants10020286

Memmola, R., Petrillo, A., Lorenzo, S. Di, Altuna, S. C., Habeeb, B. S., Soggiu, A., Bonizzi, L., Garrone, O., & Ghidini, M. (2022). Correlation between olive oil intake and gut microbiota in colorectal cancer prevention. Nutrients, 14, 1–9. https://doi.org/10.3390/nu14183749

Moral, R., & Escrich, E. (2022). Influence of olive oil and its components on breast cancer : Molecular mechanisms. Molecules, 27, 1–38. https://doi.org/10.3390/molecules27020477

Mushtaq, A., Hanif, M. A., Ayub, M. A., Bhatti, I. A., & Romdhane, M. (2020). Olive. Medicinal Plants of South Asia, 40, 541–555. https://doi.org/10.1016/b978-0-08-102659-5.00040-9

Nunes, M. A., Pimentel, F. B., Costa, A. S. G., Alves, R. C., & Oliveira, M. B. P. P. (2016). Olive by-products for functional and food applications: Challenging opportunities to face environmental constraints. Innovative Food Science and Emerging Technologies, 35, 139–148. https://doi.org/10.1016/j.ifset.2016.04.016

Nunes, M. A., Reszczy´nski, F., P´ascoa, R. N. M. J., Costa, A. S. G., Alves, R. C., & Oliveira, M. B. P. P. (2020). Influence of olive pomace blending on antioxidant activity: Additive, synergistic, and antagonistic effects. Molecules, 26, 1–11. https://doi.org/10.3390/molecules26010169

Ozkan, H., Koksal, N., Dorum, B. A., Kocael, F., Ozarda, Y., Bozyigit, C., Dogan, P., Guney Varal, I., & Bagci, O. (2019). New-generation fish oil and olive oil lipid for prevention of oxidative damage in preterm infants: Single center clinical trial at university hospital in Turkey. Pediatrics International, 61, 388–392. https://doi.org/10.1111/ped.13798

Palmieri, D., Aliakbarian, B., Alberto, A., Ferrari, N., Spinella, G., Pane, B., Cafueri, G., Perego, P., & Palombo, D. (2012). Effects of polyphenol extract from olive pomace on anoxia-induced endothelial dysfunction. Microvascular Research, 83, 281–289. https://doi.org/10.1016/j.mvr.2012.02.010

Pang, K. L., Lumintang, J. N., & Chin, K. Y. (2021). Thyroid-modulating activities of olive and its polyphenols: A systematic review. Nutrients, 13, 1–14. https://doi.org/ 10.3390/nu13020529

Pavez, I. C., Lozano-S´anchez, J., Borr´as-Linares, I., Nu˜nez, H., Robert, P., & Segura-Carretero, A. (2019). Obtaining an extract rich in phenolic compounds from olive pomace by pressurized liquid extraction. Molecules, 24, 1–17. https://doi.org/ 10.3390/molecules24173108 Peralbo-Molina, ´A., Priego-Capote, F., & Luque De Castro, M. D. (2012). Tentative identification of phenolic compounds in olive pomace extracts using liquid chromatography-tandem mass spectrometry with a quadrupole-quadrupole-time-of- flight mass detector. Journal of Agricultural and Food Chemistry, 60, 11542–11550. https://doi.org/10.1021/jf302896m

Perpetuini, G., Prete, R., Garcia-gonzalez, N., Alam, M. K., & Corsetti, A. (2020). Table olives more than a fermented food. Foods, 9, 1–16. https://doi.org/10.3390/ foods9020178

Perrone, M. A., Gualtieri, P., Gratteri, S., Ali, W., Sergi, D., Muscoli, S., Cammarano, A., Bernardini, S., Renzo, L. Di, & Romeo, F. (2019). Effects of postprandial hydroxytyrosol and derivates on oxidation of LDL, cardiometabolic state and gene expression: A nutrigenomic approach for cardiovascular prevention. Journal of Cardiovascular Medicine, 20, 419–426. https://doi.org/10.2459/ JCM.0000000000000816

Quero, J., Ballesteros, L. F., Ferreira-santos, P., Velderrain-rodriguez, G. R., Rocha, C. M. R., Pereira, R. N., Teixeira, J. A., & Martin-belloso, O. (2022). Unveiling the antioxidant therapeutic functionality of sustainable olive pomace active ingredients. Antioxidants, 11, 1–22. https://doi.org/10.3390/antiox11050828

Qusa, M. H., Abdelwahed, K. S., Meyer, S. A., & El Sayed, K. A. (2020). Olive oil lignan (+)-Acetoxypinoresinol peripheral motor and neuronal protection against the tremorgenic mycotoxin penitrem A toxicity via STAT1 pathway. ACS Chemical Neuroscience, 11, 3575–3589. https://doi.org/10.1021/acschemneuro.0c00458

Radi´c, K., Vinkovi´c Vrˇcek, I., Paviˇci´c, I., & ˇCepo, D. V. (2020). Cellular antioxidant activity of olive pomace extracts: Impact of gastrointestinal digestion and cyclodextrin encapsulation. Molecules, 25, 1–15. https://doi.org/10.3390/ molecules25215027

Rafehi, H., Ververis, K., & Karagiannis, T. C. (2012). Mechanisms of action of phenolic compounds in olive. Journal of Dietary Supplements, 9, 96–109. https://doi.org/ 10.3109/19390211.2012.682644

Rasc´on, A. J., Azzouz, A., & Ballesteros, E. (2018). Multiresidue determination of polycyclic aromatic hydrocarbons in edible oils by liquid-liquid extraction–solid- phase extraction–gas chromatography–mass spectrometry. Food Control, 94, 268–275. https://doi.org/10.1016/j.foodcont.2018.07.015

Razzaghi, N., Ziarati, P., Rastegar, H., Shoeibi, S., Amirahmadi, M., Conti, G. O., Ferrante, M., Fakhri, Y., & Mousavi Khaneghah, A. (2018). The concentration and probabilistic health risk assessment of pesticide residues in commercially available olive oils in Iran. Food and Chemical Toxicology, 120, 32–40. https://doi.org/ 10.1016/j.fct.2018.07.002 Reboredo-Rodríguez, P., Gonz´alez-Barreiro, C., Cancho-Grande, B., Forbes- Hern´andez, T. Y., Gasparrini, M., Afrin, S., Cianciosi, D., Carrasco-Pancorbo, A., Simal-G´andara, J., Giampieri, F., & Battino, M. (2018). Characterization of phenolic extracts from Brava extra virgin olive oils and their cytotoxic effects on MCF-7 breast cancer cells. Food and Chemical Toxicology, 119, 1–49. https://doi.org/10.1016/j. fct.2018.05.026

Ribeiro, T. B., Costa, C. M., Bonif´acio - Lopes, T., Silva, S., Veiga, M., Monforte, A. R., Nunes, J., Vicente, A. A., & Pintado, M. (2021). Prebiotic effects of olive pomace powders in the gut: In vitro evaluation of the inhibition of adhesion of pathogens, prebiotic and antioxidant effects. Food Hydrocolloids, 112, 1–14. https://doi.org/ 10.1016/j.foodhyd.2020.106312

Santangelo, C., Filesi, C., Varì, R., Scazzocchio, B., Filardi, T., Fogliano, V., D'Archivio, M., Giovannini, C., Lenzi, A., Morano, S., & Masella, R. (2016). Consumption of extra-virgin olive oil rich in phenolic compounds improves metabolic control in patients with type 2 diabetes mellitus: A possible involvement of reduced levels of circulating visfatin. Journal of Endocrinological Investigation, 39, 1295–1301. https://doi.org/10.1007/s40618-016-0506-9

Schmidt, L., Heck, N. de V., Ferreira, I., G¨oethel, G., Somacal, S., Emanuelli, T., Rodrigues, E., Garcia, S. C., Welke, J. E., & Augusti, P. R. (2020). Ochratoxin A presence in Cabernet Sauvignon wine changes antioxidant activity in vitro and oxidative stress

markers in vivo. Food Additives & Contaminants Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 1, 1–10. https://doi.org/10.1080/19440049.2020.1802067

Simonato, B., Trevisan, S., Tolve, R., Favati, F., & Pasini, G. (2019). Pasta fortification with olive pomace: Effects on the technological characteristics and nutritional properties. LWT - Food Science and Technology, 114, 1–6. https://doi.org/10.1016/j.lwt.2019.108368

Speroni, C. S., Stiebe, J., Guerra, D. R., Beutinger Bender, A. B., Ballus, C. A., dos Santos, D. R., Dal Pont Morisso, F., da Silva, L. P., & Emanuelli, T. (2019). Micronization and granulometric fractionation improve polyphenol content and antioxidant capacity of olive pomace. Industrial Crops and Products, 137, 347–355. https://doi.org/10.1016/j.indcrop.2019.05.005

Storniolo, C. E., Rosell´o-Catafau, J., Pint´o, X., Mitjavila, M. T., & Moreno, J. J. (2014). Polyphenol fraction of extra virgin olive oil protects against endothelial dysfunction induced by high glucose and free fatty acids through modulation of nitric oxide and endothelin-1. Redox Biology, 2, 971–977. https://doi.org/10.1016/j. redox.2014.07.001

Sylla, N., Bouyahya, A., Taha, D., Dakka, N., & Elhajji, H. (2021). Biocatalysis and Agricultural Biotechnology Study of the antioxidant and antidiabetic activity in vitro of free and encapsulated phenolic compounds of olive pomace. Biocatalysis and Agricultural Biotechnology, 36, 1–9. https://doi.org/10.1016/j.bcab.2021.102126

Tapia-Quir´os, P., Montenegro-Landívar, M. F., Reig, M., Vecino, X., Alvarino, T., Cortina, J. L., Saurina, J., & Granados, M. (2020). Olive mill and winery wastes as viable sources of bioactive compounds: A study on polyphenols recovery. Antioxidants, 9, 1–15. https://doi.org/10.3390/antiox9111074

Titouh, K., Mazari, A., & Aït Meziane, M. Z. (2020). Contribution to improvement of the traditional extraction of olive oil by pressure from whole and stoned olives by addition of a co-adjuvant (talc). Oilseeds & fats Crops and Lipids, 27, 1–7. https://doi.org/10.1051/ocl/2020017

Tori´c, J., Brozovic, A., Lonˇcar, M. B., Brala, C. J., Markovi´c, A. K., Benˇci´c, D., & Barbari´c, M. (2020). Biological activity of phenolic compounds in extra virgin olive oils through their phenolic profile and their combination with anticancer drugs observed in human cervical carcinoma and colon adenocarcinoma cells. Antioxidants, 9, 1–15. https://doi.org/10.3390/antiox9050453

Tzekaki, E. E., Papaspyropoulos, A., Tsolaki, M., Lazarou, E., Kozori, M., & Pantazaki, A. A. (2021). Restoration of BMI1 levels after the administration of early harvest extra virgin olive oil as a therapeutic strategy against Alzheimer's disease. Experimental Gerontology, 144, 1–10. https://doi.org/10.1016/j.exger.2020.111178

Valls-Pedret, C., Sala-Vila, A., Serra-Mir, M., Corella, D., De La Torre, R., Martínez-Gonz´alez, M.´A., Martínez-Lapiscina, E. H., Fit´o, M., P´erez-Heras, A., Salas-Salvad´o, J., Estruch, R., & Ros, E. (2015). Mediterranean diet and age-related cognitive decline: A randomized clinical trial. JAMA Internal Medicine, 175, 1094–1103. https://doi.org/10.1001/jamainternmed.2015.1668

Waqas, M., Iqbal, S. Z., Abdull Razis, A. F., Pervaiz, W., Ahmad, T., Usman, S., Ali, N. B., & Asi, M. R. (2021). Occurrence of aflatoxins in edible vegetable seeds and oil samples available in pakistani retail markets and estimation of dietary intake in consumers. International Journal of Environmental Research and Public Health, 18, 1–12. https://doi.org/10.3390/ijerph18158015

Widmer, R. J., Freund, M. A., Flammer, A. J., Sexton, J., Lennon, R., Romani, A., Mulinacci, N., Vinceri, F. F., Lerman, L. O., & Lerman, A. (2013). Beneficial effects of polyphenol-rich olive oil in patients with early atherosclerosis. European Journal of Nutrition, 52, 1223–1231. https://doi.org/10.1007/s00394-012-0433-2

Zeid, I. M. A., Al-asmari, K. M., Altayb, H. N., Al-attar, A. M., & Qahl, S. H. (2022). Predominance of antioxidants in some edible plant oils in ameliorating oxidative stress and testicular toxicity induced by malathion. Life, 12, 1–10. https://doi.org/10.3390/life12030350

Zhao, H., Avena-bustillos, R. J., & Wang, S. C. (2022). Extraction-, purification and in vitro antioxidant activity evaluation of phenolic compounds in California olive pomace. Foods, 11, 1–17. https://doi.org/10.3390/foods11020174

5.2 Bioavailable Phenolic Compounds from Olive Pomace Present Anti-Neuroinflammatory Potential on Microglia Cells

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Abstract: The neuroinflammatory process is considered one of the main characteristics of central nervous system diseases, where a pro-inflammatory response results in oxidative stress through the generation of reactive oxygen and nitrogen species (ROS and RNS). Olive (*Olea europaea L.*) pomace is a by-product of olive oil production that is rich in phenolic compounds (PCs), known for their antioxidant and anti-inflammatory properties. This work looked at the antioxidant and anti-neuroinflammatory effects of the bioavailable PC from olive pomace in cell-free models and microglia cells. The bioavailable PC of olive pomace was obtained through the process of *in vitro* gastrointestinal digestion of fractionated olive pomace (OPF, particles size < 2 mm) and micronized olive pomace (OPM, particles size < 20 μm). The profile of the PC that is

present in the bioavailable fraction as well as its *in vitro* antioxidant capacity were determined. The anti-neuroinflammatory capacity of the bioavailable PC from olive pomace (0.03–3 mg L^{-1}) was evaluated in BV-2 cells activated by lipopolysaccharide (LPS) for 24 h. The total bioavailable PC concentration and antioxidant activity against peroxyl radical were higher in the OPM than those observed in the OPF sample. The activation of BV-2 cells by LPS resulted in increased levels of ROS and nitric oxide (NO). The bioavailable PCs from both OPF and OPM, at their lowest concentrations, were able to reduce the ROS generation in activated BV-2 cells. In contrast, the highest PC concentration of OPF and OPM was able to reduce the NO levels in activated microglial cells. Our results demonstrate that bioavailable PCs from olive pomace can act as anti-neuroinflammatory agents in vitro, independent of particle size. Moreover, studies approaching ways to increase the bioavailability of PCs from olive pomace, as well as any possible toxic effects, are needed before a final statement on its nutritional use is made.

Keywords: neuroinflammation; oxygen reactive species; nitric oxide; gastrointestinal digestion; BV-2 cells

1. Introduction

Neuronal diseases associated with neurodegeneration and neuroinflammation are reported as a public health problem worldwide [1]. Alzheimer's disease is the most common neurodegenerative disease and accounts for 60 to 70% of all dementia cases, followed by Parkinson's disease [2,3]. It is estimated that approximately 50 million individuals worldwide are afflicted by dementia, a figure that is projected to rise to 130 million by 2050 [3].

The neuroinflammation process is considered one of the main mechanisms of the central nervous system (CNS) for dealing with pathogenic agents or situations of impairment in cellular physiology, and it is associated with the activation of microglia cells, which are considered the immune cells of the CNS [4,5]. The activation of microglial cells initiates an immune response to injuries or diseases in the central nervous system, characterized by an increased production of pro-inflammatory cytokines [1,5].

The production of pro-inflammatory cytokines and chemokines induced by activated microglia cells results in an increase in oxidative stress through the generation of reactive oxygen and nitrogen species (ROS and RNS) [6,7]. Oxidative stress has been associated with several diseases, including neuropsychiatric and neurodegenerative

conditions, most of which present chronic immune activation [7,8]. In fact, the inflammatory inducers phytohemagglutinin (PHA) and lipopolysaccharide (LPS) are capable of inducing ROS and RNS generation along with cell inflammation in murine macrophages and microglia cells, respectively [1,6].

In this context, it is important to seek therapeutic strategies through the anti-neuroinflammatory capacity that can modify the polarization of activated microglia cells, in which the pro-inflammatory pathway is their main immune response [1]. Thus, natural compounds capable of modulating the immune response of activated microglia cells and/or reducing oxidative stress seem to be a promising alternative [1].

PCs are widely produced by plants, such as berries, nuts, and olives, and are known to present antioxidant and anti-inflammatory activities, especially in models associated with the CNS [2,5]. Besides regular sources, PCs can be obtained from waste and by-products from the food industry, which has been a sustainable and great alternative for obtaining these compounds [6].

Olive (*Olea europaea L.*) pomace is a by-product of olive oil production, consisting of pulp, peel, and olive pits and representing about 80% (v/v) of the total amount of processed olives. Olive pomace has a considerable amount of PCs (0.4 to 2.4%) [9]. Due to their hydrophilic characteristics, 98% of the PCs present in the olive migrates to the pomace fraction during olive oil extraction [10]. Additionally, PCs from olive fruits, such as hydroxytyrosol and oleuropein, present antioxidant and anti-inflammatory effects in microglia cells activated by lipopolysaccharide (LPS), a known model for inflammation [11–13].

There is an abundance of PCs in the olive pomace, which makes it extremely promising for the food industry, and processes that increase the bioavailability of these compounds are extremely important [14]. Particle size reduction processes, such as micronization, are capable of increasing the intestinal bioavailability of olive pomace PCs after *in vitro* digestion [9]. However, throughout the digestive process, structural changes occur in PCs due to the action of enzymes and intestinal microbiota [15]. The process of digestion of olive pomace contributes to the formation of PCs derived from oleuropein, tyrosol, and hydroxytyrosol, which have a greater bioavailability [9]. Depending on the dose, hydroxytyrosol and tyrosol have the highest intestinal absorption rates (40–60%). Secoiridoid compounds are stable in the mouth, but significant losses occur in the gastric, duodenal, and intestinal regions due to the degradation of oleuropein and the formation of tyrosol and hydroxytyrosol [9,14].

Therefore, olive pomace PCs are promising compounds for the food industry and have different biological properties, such as antioxidant and anti-neuroinflammatory potential [14]. Thus, it is important to evaluate the beneficial potential of the bioavailable fraction of PC in foods before digestion processes. Based on these considerations, the aims of this work were to evaluate the antioxidant activity in cell-free models and the anti-neuroinflammatory capacity of bioavailable PCs from olive pomace on activated microglia cells.

2. Material and Methods

2.1. Olive Pomace Samples

Samples of olive pomace, cv. 'Arbequina', were obtained from an olive oil extractor company located in the city of Formigueiro, RS, Brazil (29◦5900100 S; 53◦2105000 W) and were processed according to the methodology described by [16]. The OP without physical modification was freeze-dried in a freeze dryer (LS 3000, Terroni Equipments Cientifiques, São Paulo, Brazil) and crushed in a knife mill (MA 630, Marconi®, São Paulo, Brazil). The raw OP was subjected to granulometry fractionation with a 2 mm sieve and centrifuged (1774× g for 10 min). The sediment was collected, lyophilized in a lyophilizer (LS 3000, Terroni Equipments Scientific, São Paulo, Brazil), crushed in a food mill knife (MA 630, Marconi®, SP, Brazil), and degreased with n-hexane. The fraction obtained was called OPF. Subsequently, the OPF was micronized in a planetary ball mill (PM 100, Retsch Co., Haan, Germany), using a 250 mL container with six stainless steel spheres (30 mm in diameter each). The grinding time was optimized for 15 g of sample ground at 300 rpm min^{-1} for 5 h, with a 2 min pause every 10 min of grinding. After micronization, the sample was named OPM. In this way, the raw OP sample resulted in two samples with different particle sizes: (a) OPF-Olive pomace obtained via moist fractionation in a strainer (particles < 2 mm) and lyophilized, milled in a common knife mill, and degreased; and (b) OPM-Olive pomace obtained as described above and micronized into particles < 20 µm.

2.2. Gastrointestinal Digestion of Olive Pomace to Obtain Bioavailable PCs

OPF and OPM olive pomace samples were submitted to the gastrointestinal digestion process following three sequential steps (Figure 1): (1) mouth digestion, (2) gastric digestion (stomach), and (3) duodenal digestion (small intestine), according to the *in vitro* international digestion protocol [17]. The mouth digestion step was simulated

using artificial saliva (pH = 7.5) incubated at 37 °C for 2 min under constant agitation. At the end of the mouth digestion step, the pH of the digested sample was adjusted to pH 3.0 by the addition of an artificial gastric solution containing electrolytes (K^+, Na^+, Cl^-, $H_2PO_4^-$, HCO_3^-, CO_3^{2-}, Mg_4^+, NH_4^+, and Ca_2^+). Then, the digested sample was submitted to gastric digestion via incubation (37 °C for 2 h under agitation) and the addition of pepsin (2000 U mL^{-1}). Subsequently, the pH of the gastric digested sample was adjusted to 7.0 using 1 M NaOH and submitted to intestinal digestion through the addition of pancreatin (100 U mL^{-1}) and bile salts (10 mM). The final digested sample was placed into dialysis membranes (12000 Da, Sigma Aldrich, São Paulo, Brazil) immersed in 200 mL of phosphate buffer (24.96 mM, pH 7.2) and incubated at 37 °C for 2 h under sporadic agitation. At the end of intestinal digestion, two fractions, IN and OUT, were collected. The IN fraction was the one retained inside the dialysis membrane (corresponding to the digested fraction that remains in the small and large intestines), whereas the OUT fraction was the fraction able to be dialyzed, representing the bioavailable PCs from the OPF and OPM olive pomace samples. The bioavailable PCs from olive pomace were subjected to the evaluation of antioxidant and anti-neuroinflammatory ability in a cell-free system and microglial cells, respectively.

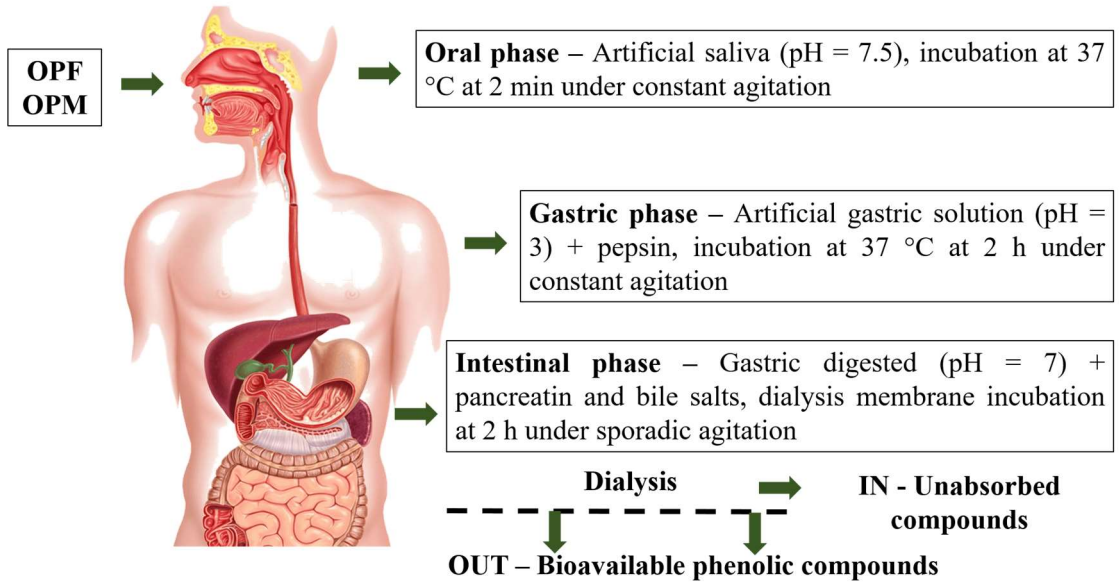

Figure 1. Olive pomace gastrointestinal digestion process steps. OPF—fractionated olive pomace (<2 mm) and OPM—fractionated and micronized olive pomace (<20 µm).

2.3. The Profile of Bioavailable PCs from Olive Pomace

According to [16], the bioavailable PCs in the OPF and OPM samples were extracted using an acidified acetone solution (0.35% formic acid, v/v; 7 mL). The phenolic extracts obtained were identified and quantified using an ultra-high-performance liquid chromatograph (Nexera XR, Shimadzu, Kioto, Japan) coupled to a triple-quadrupole mass spectrometer (UHPLC-MS/MS), according to [16]. Samples were injected (10 µL) in a C18 analytical column (4.6 mm × 150 mm, 1.8 µm particle size; Agilent Technologies, Santa Clara, CA, USA) at 35 °C. The mobile phase was HPLC-grade water with 0.5% acetic acid (eluent A) and acetonitrile (eluent B) at 0.2 mL min^{-1}, and the chromatographic separation was performed in reverse-phase mode. Analytical curves were constructed using commercial standards of verbascoside (Chromadex, Los Angeles, C18, CO, USA), protocatechuic acid, 3-hydroxytyrosol, 4-hydroxybenzoic acid, tyrosol, caffeic acid, vanillic acid, homovanillic acid, p-coumaric acid, ferulic acid, oleuropein, luteolin, and apigenin (Sigma-Aldrich, St. Louis, MO, USA). PCs were quantified using authentic reference standards except for hydroxytyrosol-glycoside, which was quantified as an equivalent of hydroxytyrosol, and oleuropein aglycone, which was quantified as an equivalent of oleuropein [16]. The experiments were performed by setting the concentration of bioavailable PCs in OPF and OPM samples at 0.03, 0.15, 0.30, 1.5, and 3 mg L^{-1}.

2.4. In Vitro Antioxidant Capacity of Bioavailable PCs from Olive Pomace

The bioavailable PCs from OPF and OPM samples were analyzed for their antioxidant potential against hydroxyl radical ($^{\bullet}OH$) generation, ability to protect against glutathione (GSH) oxidation, and ability to protect against the peroxyl ($ROO^{\bullet}$) radical using the methods described below [18–20].

2.4.1. Hydroxyl Radical Generation

The ability of a sample to remove the $^{\bullet}OH$ radical generated via the Fenton reaction is evaluated in the deoxyribose assay [19]. The bioavailable PCs from olive pomace (0.03, 0.15, 0.30, 1.50, and 3 mg L^{-1}) were incubated for 1 h at 37 °C in test tubes containing potassium phosphate buffer (TFK, 50 mM), ethylendiaminetetraacetic acid (EDTA, 1 mM), iron chloride ($FeCl_3$, 1 mM), water, ascorbic acid (2 mM), deoxyribose (60 mM), and hydrogen peroxide (H_2O_2, 10 mM). To make pink or orange compounds, we added 1 mL of thiobarbituric acid (TBA) and 1 mL of hydrochloric acid (HCl, 25%) to each tube. Then, we put the tubes in a water bath at 100 °C for 15 min. The reduction

in the intensity of the pink or orange color caused by the samples was measured using a spectrophotometer at 532 nm, and the results were expressed as a percentage of •OH radical generation [19].

2.4.2. GSH Protection Capacity

The GSH assay was used to see if bioavailable PCs from olive pomace could prevent GSH oxidation and the reduction in sulfhydryl groups caused by H_2O_2 [18]. The samples were incubated (0.03, 0.15, 0.30, 1.50, and 3 mg L^{-1}) for 1 h in test tubes containing TFK (1 mM), water, H_2O_2 (5 mM), and GSH (6 mM). At the end of the incubation, the measurement of the reduction in the sulfhydryl groups from GSH was carried out using 5,5 dithio bis-(2-nitrobenzoic acid) (DTNB). The results were expressed in GSH content (nmol GSH mL^{-1} of DTNB) [18].

2.4.3. Protective Capacity against the Radical ROO•

The Oxygen Radical Absorbance Capacity (ORAC) method measures the scavenging capacity of a compound against the peroxyl radical, generated through 2,2-azobis-2- methylpropanoamidine (AAPH) at 37 °C. In 96-well plates, 25 µL of bioavailable PCs from olive pomace, previously diluted (100 times) in potassium phosphate buffer (75 mM) and 150 µL of fluorescein working solution (81 nM), was added. The plate was incubated for 10 min at 37 °C, for the last 3 min under constant agitation. After the incubation period, 25 µL of the AAPH solution was added (152 mM). Monitoring of fluorescence decay was followed in a fluorescence reader (Enspire 2300, Multimode Plate Reader, Perkin Elmer, Waltham, MA, USA) at 37 °C for 90 min. Excitation and emission wavelengths of 485 nm and 528 nm, respectively, were used. Results were expressed as µmol of Trolox equivalents per mL of sample [20].

2.5. Evaluation of the Anti-Neuroinflammatory Capacity of Bioavailable PCs from Olive Pomace in Microglial Cells

2.5.1. Cell Culture and Treatments

The microglial cell line BV-2 (ATCC®CRL-2467TM) was purchased from the Cell Bank of Rio de Janeiro (the "Banco de Células do Rio de Janeiro", BCRJ, Rio de Janeiro, RJ, Brazil). The cells were cultured in RPMI 1640 cell culture medium (Sigma-Aldrich, #R8758, São Paulo, SP, Brazil). The cells were kept in a CO_2 incubator under ideal conditions for cell culture (5% CO_2 at 37 °C). The culture medium was

supplemented with 10 mM of HEPES, 10% of fetal bovine serum (FBS) (Sigma-Aldrich, #F2442, São Paulo, SP, Brazil), and 1% penicillin (100 U mL^{-1})/streptomycin (100 mg mL^{-1}) (Sigma-Aldrich, #P4333, São Paulo, SP, Brazil). Treatments were conducted on BV-2 cells after 6–8 passages [6].

BV-2 cells were seeded in 96-well plates (density of 2.5 × 105 cells mL^{-1} per well) and treated with a concentration-response curve of bioavailable PCs from OPF and OPM samples (0.03, 0.15, 0.30, 1.50, and 3 mg L^{-1}) for 24 h to evaluate the effect of the treatments through cell proliferation and analysis of NO production. Hydrogen peroxide (H_2O_2) (100 µM) was used as a positive control for cell death induction [21].

In a second moment, BV-2 cells were treated with 1 µg mL^{-1} after exposure to LPS, and activated cells were treated with the bioavailable PCs from OPF and OPM samples (0.03, 0.15, 0.30, and 1.50 mg L^{-1}) for an additional 24 h to verify the ability of the samples to reduce the inflammatory response. The anti-neuroinflammatory capacity was evaluated through the determination of cell viability and the measurement of levels of ROS and RNS [1].

2.5.2. Cellular Viability Determination

The viability of BV-2 microglial cells inactivated or activated with LPS and exposed to the samples of this study was determined using MTT bromide (3-(4,5-Dimethyl-2-thiazolyl2,5-diphenyl-2H-tet-razonium) reagent (Sigma-Aldrich-M2128; St. Louis, MO, USA). After the incubation period with the bioavailable PCs from the OPF and OPM samples, the treatments were removed, and the cells were resuspended in phosphate buffer (PBS) 1×, adding 20 µL of 5 mg mL^{-1} MTT. After incubation at 37 °C for 2 h, the intracellular formazan crystals were solubilized using demethylsulfoxide (DMSO). Absorbance was determined at 570 nm using a plate reader (Biochrom® Anthos 2010, London, UK) [22].

2.5.3. Measurement of NO Production

NO levels were determined through an indirect method based on the use of the Greiss reagent (1% sulfanilamide + 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride) [23]. After activation by LPS and treatment with the bioavailable PCs from OPF and OPM samples, the cell supernatant was added to the Greiss reagent and analyzed. The Greiss reagent can detect organic nitrite (NO metabolite). The sulfanilamide in this reagent interacts with the nitrite in the sample, forming diazonium

salts that turn pink or purple when in contact with N-(naphthyl 1-dihydrochloride) ethylenediamine. The staining intensity is directly proportional to the NO production. Absorbance was measured at 540 nm using a plate reader (Biochrom®Anthos 2010, London, UK) [23].

2.5.4. Measurement of ROS Levels

Total ROS levels in LPS-inactivated and -activated cells after treatment with the bioavailable PCs from OPF and OPM samples were determined using DCFH-DA (20 Dichlorodihydrofluorescein 20 diacetate) (Sigma Aldrich-D6883; Sao Paulo, SP, Brazil) [24]. DCFH-DA is metabolized by intracellular enzymes, forming dichlorodihydrofluorescein (DCFH). ROS can reduce DCFH into dichlorofluorescein (DCF). DCF emits fluorescence that can be measured at 525 nm excitation and 488 nm emission. The fluorescence intensity was measured using a plate reader (Spectra Max i3, Molecular Devices, San Jose, CA, USA) [24].

2.6. Statistical Analyses

All experiments were repeated at least three times. The results were expressed as the mean ± standard error of the mean (SEM). All data analyses were performed using Graph Pad Prism Software version 5.0 (La Jolla, CA, USA), and differences were considered significant when $p < 0.05$, evaluated by one-way analysis of variance (ANOVA) followed by the Tukey test.

To investigate the association between HPLC-MS assessment of PCs and the antineuroinflamatory effect, chemometric analyses such as principal component analysis (PCA) and cluster analysis (CA) were carried out. The CA was performed for treatments using the Distance, Cluster, and Trace procedures, using the average Euclidean distance as a dissimilarity measure and Ward as a clustering method. PCA was performed using the PRINQUAL, PRINCOMP, and FACTOR procedures (Khattree and Naik 2000). MANOVA was performed on the SAS® System for Windows™ version 9.4 (SAS Institute Inc., Cary, NC, USA) at a 5% significance level.

3. Results

3.1. The Profile of Bioavailable PC in OPF and OPM Samples

The total concentration of the bioavailable PCs in the OPM sample was higher than that observed in the OPF sample (Table 1, $p < 0.05$). The main groups of PCs present

in olive pomace are flavonols, secoiridoids, phenolic acids, phenolic alcohols, and lignins. The phenolic alcohols found in the bioavailable fractions were hydroxytyrosol-glucoside, hydroxytyrosol, and tyrosol, with hydroxytyrosol-glucoside being the most representative in both samples. OPM showed higher concentrations of hydroxytyrosol-glucoside when compared with OPF (Table 1, $p < 0.05$). This same profile was observed for secoiridoid compounds, represented by oleuropein aglycone, 4-hydroxybenzoic acid, and chlorogenic acid, where OPM presented a higher content than OPF (Table 1, $p < 0.05$).

Table 1. Bioavailable phenolic composition (mg L^{-1}) of fractionated (OPF) and micronized (OPM) olive pomace.

Phenolic compound	**OPF**	**OPM**
Hydroxytyrosol	0.10 ± 0.02^{a}	0.18 ± 0.04^{a}
Hydroxytyrosol -glycoside	4.53 ± 0.08^{b}	8.07 ± 0.60^{a}
4-Hydroxybenzoic Acid	0.008 ± 0.002^{b}	0.015 ± 0.00^{a}
Tyrosol	1.15 ± 0.07^{a}	1.56 ± 0.31^{a}
Chlorogenic Acid	< LOQ	0.01 ± 0.00^{a}
Vanillic Acid	0.13 ± 0.04^{a}	0.12 ± 0.05^{a}
Oleuropein aglycone	4.65 ± 0.07^{b}	5.95 ± 0.12^{a}
p-Coumaric Acid	0.063 ± 0.01^{a}	0.093 ± 0.01^{a}
Oleuropein	< LOQ	< LOQ
Total PC content	10.65 ± 0.04^{b}	16.02 ± 0.35^{a}

Results are presented as mean ± standard error of mean (SEM), and different letters indicate significant differences ($p < 0.05$) via one-way ANOVA and Tukey test. LOQ: limit of quantification.

3.2. In Vitro Cell-Free Antioxidant Capacity of the Bioavailable PCs from OPF and OPM Samples

The bioavailable PCs from olive pomace did not show antioxidant potential ($p \geq 0.05$) against the $^{\bullet}OH$ radical and H_2O_2 regardless of the concentration evaluated and the particle size of the olive pomace. However, the bioavailable PCs from olive pomace

showed antioxidant capacity against the $ROO^{\bullet}$ radical, and this effect was higher for the OPM sample when compared with OPF (4.30 μM Trolox mL^{-1} vs. 3.31 μM Trolox mL^{-1}, $p < 0.05$).

3.3. Anti-Neuroinflammatory Capacity of the Bioavailable PCs from Olive Pomace in Microglial Cells

As expected, H_2O_2 decreased the cellular viability and increased NO production when compared with the negative control. The OPF sample, at the highest evaluated concentration (3 mg L^{-1}), reduced per se the cell viability of BV-2 cells by 83.82% (Figure 2A, $p < 0.05$). This behavior was not observed for the OPM sample (Figure 2B, $p > 0.05$). Additionally, all the OPM evaluated concentrations were shown to be safe, since none of the concentrations caused cellular mortality (Figure 2B). Also, none of the olive pomace samples induced increased NO levels compared with the negative control by itself (Figure 2C, D). Thus, the concentration of 3 mg L^{-1} was not evaluated for the anti-neuroinflammatory capacity of olive pomace samples in BV-2 cells activated by LPS.

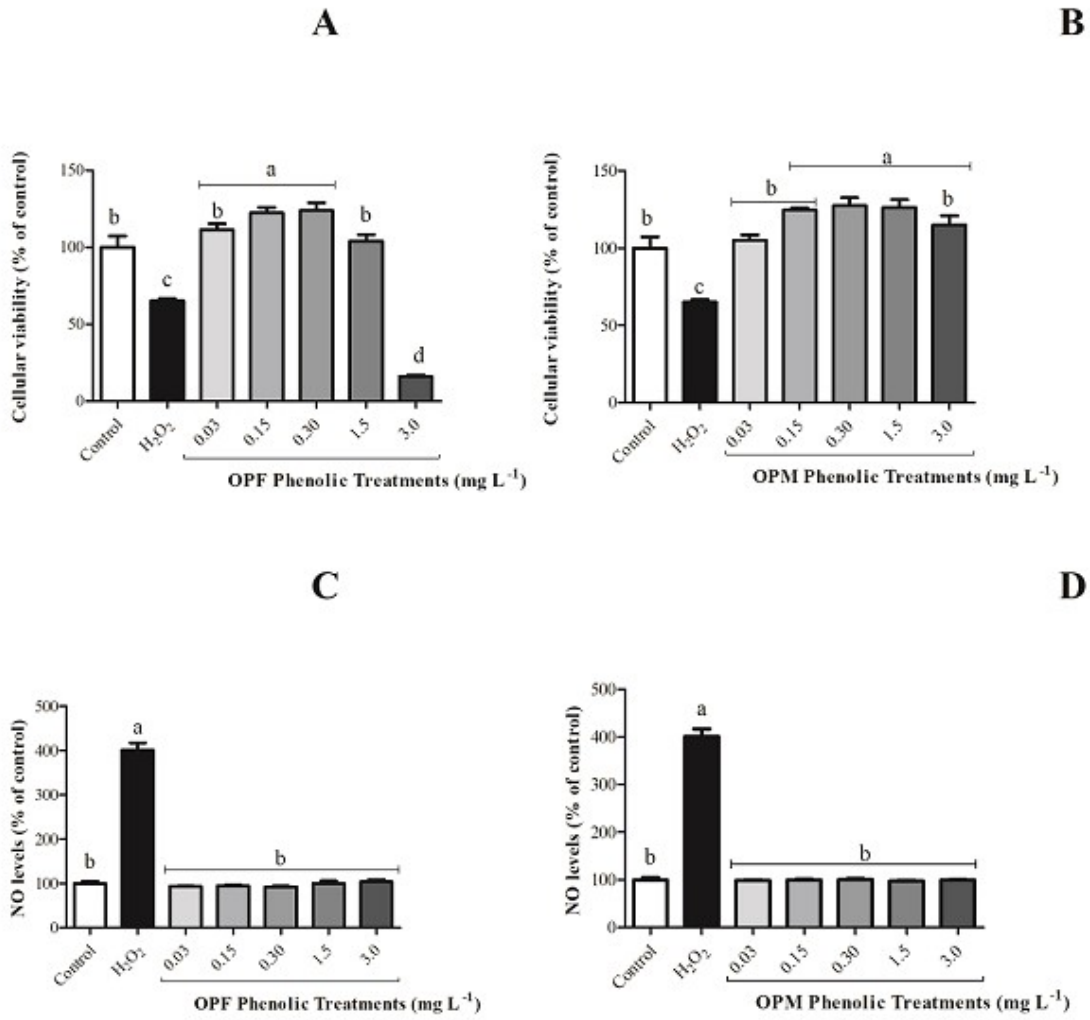

Figure 2. *Per se* effect of bioavailable PCs from OPF and OPM samples (0.03, 0.15, 0.30, 1.5, and 3 mg L^{-1}) on cell viability (A, B) and on NO levels (C, D) in BV-2 microglial cells after 24 h of treatment. Results are shown in percentages and presented as mean ± standard error of mean (SEM), and different letters indicate significant differences between groups. Statistical analysis was carried out via one-way ANOVA followed by Tukey post hoc. Results with $p < 0.05$ were considered significant.

The activation of microglial cells by LPS did not affect cell viability although it resulted in an increase in ROS and NO levels when compared with the control group (Figure 3, $p < 0.05$). The OPF sample was able to significantly attenuate ROS generation in activated microglial cells only at its lowest concentration (0.03 mg L^{-1}), while the OPM sample presented a protective effect of reducing ROS levels at both 0.03 and 0.15 mg L^{-1} (Figure 3A, B $p < 0.05$). In contrast, only the highest concentration of PCs (1.5 mg L^{-1})

from both OPF and OPM samples was able to significantly attenuate (approximately 25%) the increase in NO levels induced by LPS in BV-2 cells ($p < 0.05$, Figure 3C, D).

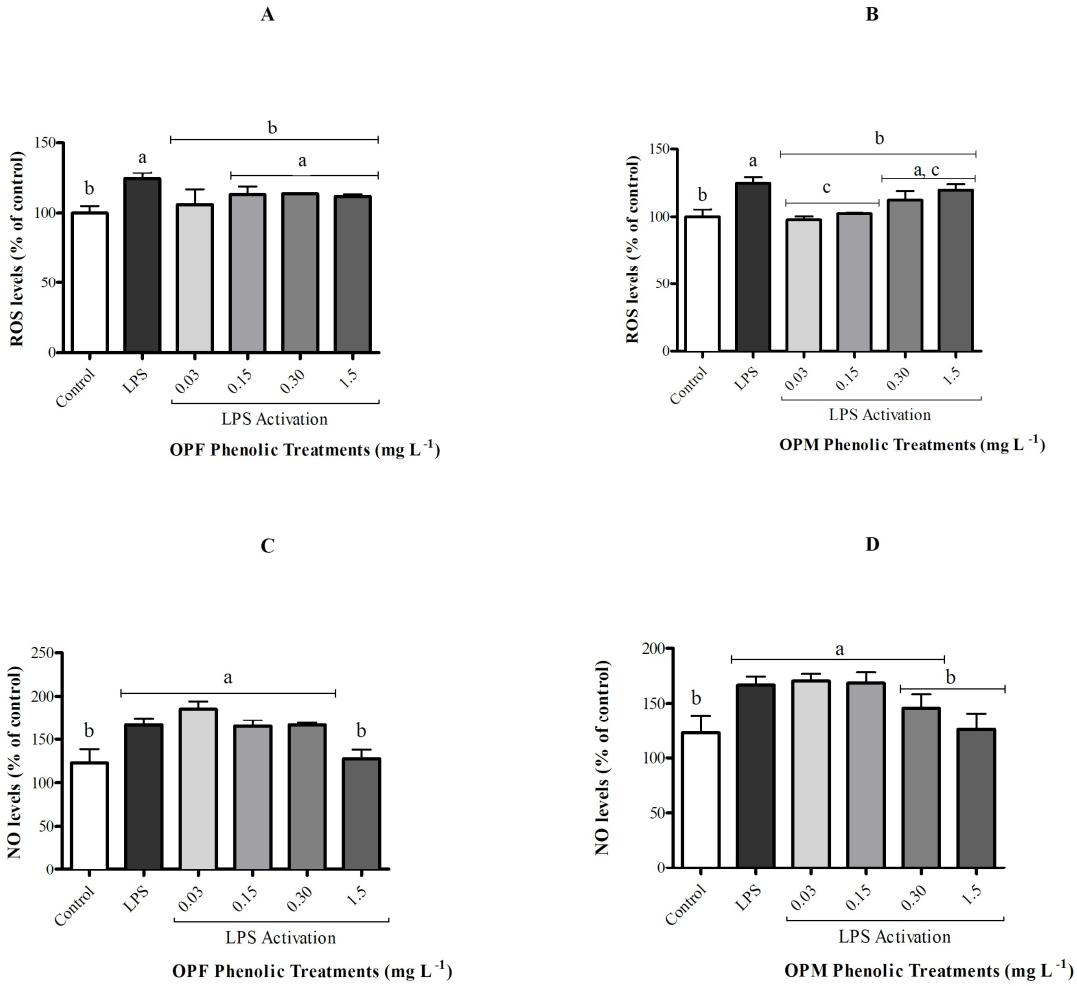

Figure 3. Effect of bioavailable PCs from the OPF and OPM samples (0.03, 0.15, 0.30, and 1.5 mg L^{-1}) on the ROS (A,B) and NO levels (C,D) on LPS-activated (1 µg mL^{-1}) microglial cells after 24h of treatment. Results are shown in percentages and presented as mean ± standard error of mean (SEM), and different letters indicate significant differences between groups. Statistical analysis was carried out via one-way ANOVA followed by Tukey post hoc. Results with $p < 0.05$ were considered significant.

3.4. Chemometric Analyses to Identify the Bioactive PCs of Olive Pomace

The relationship between PCs from olive pomace and the anti-neuroinflammatory effect was investigated using MANOVA. MANOVA using the Wilks, Pillai, Hotelling–

Lawley, and Roy tests revealed that when all variables were analyzed together, there was a significant interaction effect (types of processing × inclusion levels).

The CA of bioavailable PCs revealed the formation of two distinct groups, which were able to explain 84.4% of the total data variation (Figure 4A). One of the groups includes chlorogenic acid, hydroxytyrosol, tyrosol, oleuropein aglycone, hydroxytyrosolglucoside, nitric oxide, vanillic acid, *p*-coumaric acid, and 4-hydroxybenzoic acid. This grouping suggests that the NO levels were first influenced by vanillic, p-coumaric, and 4-hydroxybenzoic acids.

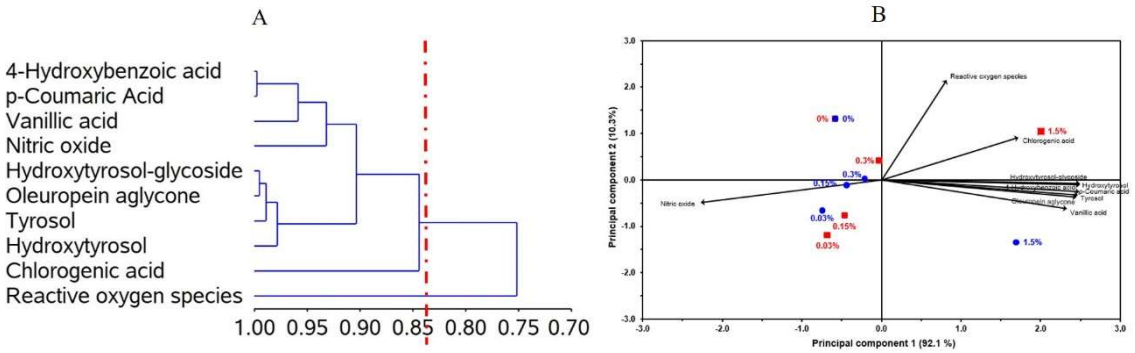

Figure 4. Dendrogram of polyphenols, anti-inflammatory, and antioxidant activities (ordinate axis) in relation to the coefficient of determination (r^2; abcissa axis) using the correlation matrix as similarity measure and principal component as clustering method (A). Bidimensional biplot from olive pomace (OP) under different processing types (OPF = fractioned, OPM = micronized) and inclusion levels (0.03, 0.15, 0.30, and 1.50 mg L^{-1}) versus polyphenols, anti-inflammatory, and antioxidant activities (loadings) in relation to the principal components (B). (A) = 84.4% and (B) = 92.1%.

The PCA was used as an exploratory analysis to verify whether the distinct types of olive pomace processing and their different concentrations affected the anti-neuroinflammatory capacity of bioavailable PCs from olive pomace (Figure 4B). A two-dimensional biplot of different processing types and different concentrations vs. PC, NO, and ROS levels presented a proportion of explained variance of 92.1% of the data. According to the PCA (Figure 4B), we can see that, for both samples, lower concentrations (0.03, 0.15, and 0.30 mg L^{-1}) were associated with a high antioxidant potential (reduced ROS levels) and a low anti-neuroinflammatory effect (higher NO levels). On the other hand, at higher concentrations (1.5 mg L^{-1}) of bioavailable PCs from olive pomace, lower NO levels were observed, suggesting an anti-neuroinflammatory effect, while less potential for reducing ROS levels was observed.

3.5. Distribution of Bioavailable PC Classes from OPF and OPM

The ability of OPF and OPM to reduce NO and ROS levels may likely be associated with the PCs presented in the samples. The distribution of different classes of bioavailable PCs in OPF and OPM olive pomace is reported in Figure 5. The OPF presents in its composition 15% phenolic acids (4-hydroxybenzoic, vanillic, and p-coumaric acids), 55% phenolic alcohols (hydroxytyrosol, hydroxytyrosol–glycoside, and tyrosol), and 30% secoiridoid compounds (oleuropein aglycone). On the other hand, OPM presented a different distribution, where only 5% of the bioavailable PCs were represented by phenolic acids (4-hydroxybenzoic, vanillic, p-coumaric, and chlorogenic acids). Phenolic alcohols and secoiridoid compounds were in greater proportions in the OPM sample, at 60% and 35%, respectively.

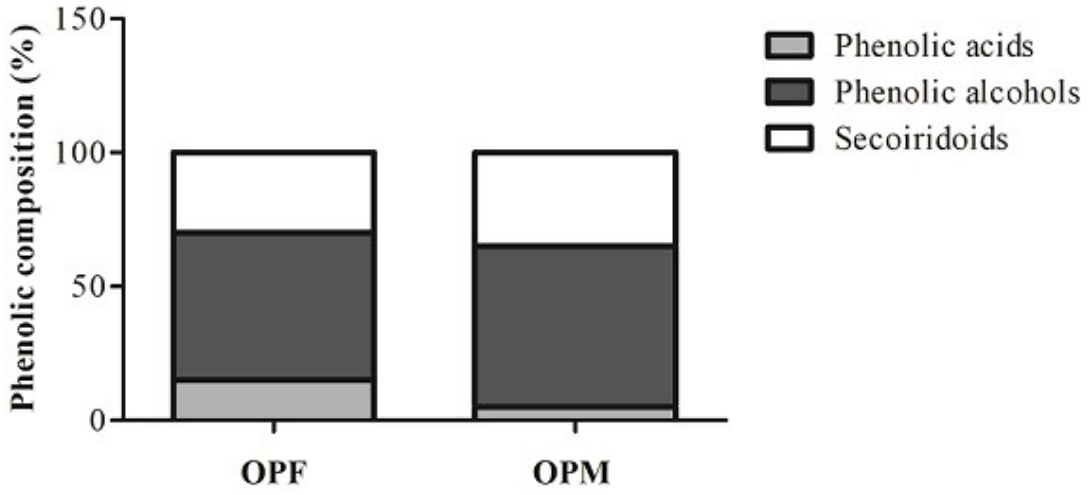

Figure 5. Changes in the distribution of bioavailable phenolic compound classes from fractionated (OPF) and micronized (OPM) olive pomace.

4. Discussion

Most of the PCs present in olive pomace are rapidly metabolized and absorbed in the gastrointestinal tract [14,25]. The *in vitro* gastrointestinal digestion process of the OPF and OPM samples resulted in two fractions, the IN and OUT fractions. The IN fraction is retained on the dialysis membrane and corresponds to the digested fraction that remains in the colon for subsequent colonic fermentation. The OUT fraction is the one that permeates the dialysis membrane and corresponds to the bioavailable fraction of the digested sample [26]. In this study, our interest was focused on the OUT fractions of the

OPF and OPM samples, which correspond to the bioavailable PCs that perform different protective functions in the human body [27].

The amount of the bioavailable PCs in the OPM sample was higher than that observed in the OPF sample, and this result may be associated with the particle size of the samples, where the smallest particle size (OPM) results in a greater contact surface with the enzymes involved in the gastrointestinal digestion process, resulting in a greater extraction of PCs [16]. These results corroborate those presented by other researchers, where hydroxytyrosol, hydroxytyrosol glycoside, tyrosol, apigenin, and p-coumaric acid were identified in the bioavailable fraction of olive pomace after the *in vitro* digestion process [16,26,28]. Oleuropein is the most representative secoiridoid compound in olive pomace, followed by phenolic alcohols, tyrosol, and hydroxytyrosol. A large variety of phenolic acids are present in olive pomace, especially vanillic, caffeic, p-coumaric, ferulic, and gallic acids. Pinoresinol and its derivatives are the most common lignins in olive pomace, while rutin, apigenin, and luteolin are the most representative flavonol compounds [14,29]. In agreement with the results above, the bioavailable PCs from OPM had a higher antioxidant capacity against peroxyl radical than the ones from OPF. This characteristic of the bioavailable PCs from olive pomace can be helpful in the mediation of oxidative and neuroinflammatory processes [6,14,26].

In the present study, the bioavailable PCs of olive pomace acted as neuroprotectors by attenuating neuroinflammatory processes. The digestive process is important in defining PC biological properties, as it causes significant changes in the PC profile in foods [14]. Throughout the digestion stages, PCs may undergo changes in their structures, due to changes in pH. These alterations result in an increased reactivity and, consequently, a possible increase in the bioactivity of the PCs present in the food matrix [28,30]. In fact, of the total ingested PCs, it is estimated that only 10%, at the end of the digestive process, remain bioavailable to play their protective role in the body [14].

The solubility characteristic of a PC is what determines its permeability across the blood–brain barrier (BBB), where less polar compounds (i.e., O-methylated derivatives) are more permeable when compared with more highly polar PCs (i.e., sulfated and glucuronidated derivatives) [31]. This peculiarity of the PCs in olive oil and olive pomace makes them compounds with potential for the treatment of neuroinflammation [32]. The ability to cross the BBB has already been described for PCs from several sources [33–35]. Moreover, the bioavailability of PCs as epigallocatechin gallate, epicatechin, and anthocyanins after oral administration in the brain is low (less than 1 nmol g^{-1}). However,

even at low concentrations, they play an important role in neuroprotection through the regulation of pro-inflammatory genes [31].

In fact, the OPM sample presented a higher amount of bioavailable PCs and antioxidant in vitro activity than the OPF. However, all evaluated concentrations were correct based on their phenolic composition, so both OPF and OPM contain the same amount of PCs. Nonetheless, OPM exhibits a distinct distribution of PC subclasses in comparison with OPF. Thus, the present study shows that the different proportions of bioavailable PCs in the OPF and OPM samples may directly affect their potential to reduce ROS and NO levels, since PCs may act synergistically [14]. That could explain the lack of differences among OPF and OPM on ROS and NO levels in cells, as revealed via the PCA. Moreover, the PCA also revealed that higher concentrations of bioavailable PCs from olive pomace are needed for the anti-neuroinflammatory potential when compared with those required for the antioxidant effects in activated microglia cells, independently of the particle size evaluated. We suggest that this fact may be associated with oxidative stress induced by neuroinflammation, which, as it is an event that involves different factors, results in the need for higher concentrations of PCs. However, regarding the antioxidant effect, we highlight that lower concentrations of PCs can be beneficial, since we demonstrated for the first time in our study that higher concentrations of PCs from olive pomace can induce cytotoxicity, which may be the result of a possible pro-oxidant effect at higher concentrations.

The reactivity of PCs to ROS depends on their structures, which may imply different complementary mechanisms where PCs act simultaneously [36]. Thus, we cannot rule out that the observed effects may be a result of the proportion and distribution of PCs in each analyzed sample rather than the amount of a specific compound. Accordingly, the combination of PCs in olive oil (equimolar proportion of oleuropein, tyrosol, and pcoumaric acid) was more efficient in delaying neuronal death and combating oxidative stress and neuroinflammation associated with neurodegenerative diseases [36].

The CA suggests that NO levels were first influenced by vanillic, *p*-coumaric, and 4-hydroxybenzoic acids. In fact, the OPF sample had a higher phenolic acids content than the OPM. Accordingly, p-coumaric acid (80 mg kg^{-1} bw) is able to inhibit the generation of pro-inflammatory mediators induced by LPS, especially through its antioxidant action and ability to inhibit cytokine production [37]. This phenolic acid (75 mg kg^{-1} bw) also reduced the expression of pro-inflammatory cytokines (IL-1β and TNFα), protecting against depression and memory loss in a mouse model of depression induced by

costicosterone [38]. Similarly, vanillic acid (80 mg kg^{-1} bw) can reduce oxidative stress induced by β-amyloid peptide (Aβ1-42), which demonstrates its neuroprotective role [39]. Furthermore, chlorogenic acid (4 mg kg^{-1} bw) was able to inhibit the pro-inflammatory pathway in BV-2 cells of male C57BL/6 mice activated by LPS and promote their polarization to the anti-neuroinflammatory pathway [40].

On the other hand, OPM presented a higher proportion of phenolic alcohols and secoiridoid compounds than OPF. Oleuropein and its main metabolite, hydroxytyrosol, are the main PCs in olive oil and pomace and have been widely studied, especially because of their easy administration through food [32]. Isolated hydroxytyrosol and oleuropeín presented antioxidant and anti-inflammatory effects in microglial cells activated by LPS [12,32] or α-synuclein [11]. One of the main PCs from olive pomace, hydroxytyrosol (50–200 mg kg^{-1} bw), alleviates oxidative stress and neuroinflammation, as well as enhances hippocampal neurotrophic signaling in a depression model in mice [36].

PCs from oil and olive pomace are recognized for their antioxidant and neuroprotective properties but, in most cases, they are studied individually. One study has shown that whole extra-virgin olive oil, which is rich in oleuropein, tyrosol, and hydroxytyrosol, was able to reduce the production of inflammatory mediators and suppress the secretion of pro-inflammatory cytokines in BV-2 microglial cells activated by LPS [34]. Furthermore, the Mediterranean diet, characterized by a high consumption of olive oil, highlights the importance of olive PCs and the different health benefits that are linked to the consumption of these compounds [1]. Although studies on the beneficial potential of PCs from olive pomace are still scarce, our study is a pioneer in describing some of the benefits of this by-product. Thus, we highlight the importance of further studies aimed at guaranteeing the biological properties of the PCs present and the food safety of olive pomace as a diet enricher, in addition to studies on possible food applications.

Currently, olive pomace is considered a problem for the olive oil industry, as there is no specific purpose for this by-product. Although olive pomace can be used in animal feed, soil fertilization, and energy generation, these uses still do not meet the demands of industries [12]. Furthermore, the use of olive pomace can increase the sustainability of the olive oil industry and, in turn, lead to relevant economic benefits [12]. In this study, we showed the promising role of PCs from olive pomace in the prevention and mediation

of oxidative and neuroinflammatory processes in microglia cells. Thus, this bioactivity may improve the range of olive pomace applications.

Moreover, we reported for the first time that the OPF sample at the highest evaluated concentration (3 mg L^{-1}) reduced the cell viability of BV-2 cells per se. These results indicate that high concentrations of OPF may be toxic to microglial cells. We can propose that this toxic effect of the higher concentration of OPF was possibly due to the lower concentrations of the compounds hydroxytyrosol-glycoside, 4-hydroxybenzoic acid, and oleuropein aglycone, which act synergistically with the others. This same toxic potential was not observed for OPM, where the concentrations of hydroxytyrosol-glycoside, 4-hydroxybenzoic acid, and oleuropein aglycone were higher and may have acted synergistically with the other compounds, not resulting in a toxic potential for microglial cells. Therefore, we highlight that it is always important to consider the distribution of PCs in different concentrations in a complex matrix, such as food.

5. Conclusions

This study shows, for the first time, that bioavailable PCs from olive pomace present antioxidant potential in cell-free systems and anti-neuroinflammatory potential in microglia cells. The antioxidant and the anti-neuroinflammatory effects are most likely associated with the distribution of bioavailable PCs in olive pomace samples and their synergistic action. We demonstrated that bioavailable PCs from olive pomace are a promising option for combating diseases associated with neuroinflammation, regardless of particle size. However, studies on its possible toxic effects and safe concentrations are essential for its nutritional application. We highlight the latter as one of the main gaps to be filled to understand the actual health benefits of the PCs present in olive pomace. Olive pomace is considered a problem for the olive oil production industry, as there is no specific purpose for this by-product. In this study, we showed the promising role of PCs from olive pomace in the prevention and mediation of oxidative and neuroinflammatory processes, highlighting their possible food application. Furthermore, the use of olive pomace can increase the sustainability of the olive oil industry and, in turn, lead to relevant economic benefits.

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Writing-Original draft, L.S.; Writing-Review and editing, L.S., J.C.F.M., P.R.A., R.d.O.M., A.K.M., T.E. and M.A.Z.A. All authors have read and agreed to the published version of the manuscript.

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References

1. Angeloni, C.; Malaguti, M.; Barbalace, M.C.; Hrelia, S. Bioactivity of Olive Oil Phenols in Neuroprotection. Int. J. Mol. Sci. 2017, 18, 2230. [CrossRef] [PubMed]

2. Augusti, P.R.; Conterato, G.M.; Denardin, C.C.; Prazeres, I.D.; Serra, A.T.; Bronze, M.R.; Emanuelli, T. Bioactivity, bioavailability, and gut microbiota transformations of dietary phenolic compounds: Implications for COVID-19. J. Nutr. Biochem. 2020, 97, 108787. [CrossRef] [PubMed]

3. Berk, M.; Kapczinski, F.; Andreazza, A.; Dean, O.; Giorlando, F.; Maes, M.; Yücel, M.; Gama, C.; Dodd, S.; Dean, B.; et al. Pathways underlying neuroprogression in bipolar disorder: Focus on inflammation, oxidative stress and neurotrophic factors. Neurosci. Biobehav. Rev. 2011, 35, 804–817. [CrossRef] [PubMed]

4. Boligon, A.A.; Sagrillo, M.R.; Machado, L.F.; Filho, O.d.S.; Machado, M.M.; da Cruz, I.B.M.; Athayde, M.L. Protective Effects of Extracts and Flavonoids Isolated from Scutia buxifolia Reissek against Chromosome Damage in Human Lymphocytes Exposed to Hydrogen Peroxide. Molecules 2012, 17, 5757–5769. [CrossRef] [PubMed]

5. Brodkorb, A.; Egger, L.; Alminger, M.; Alvito, P.; Assunção, R.; Ballance, S.; Bohn, T.; Bourlieu-Lacanal, C.; Boutrou, R.; Carrière, F.; et al. INFOGEST static in vitro

simulation of gastrointestinal food digestion. Nat. Protoc. 2019, 14, 991–1014. [CrossRef]
6. Cadoná, F.C.; de Souza, D.V.; Fontana, T.; Bodenstein, D.F.; Ramos, A.P.; Sagrillo, M.R.; Salvador, M.; Mota, K.; Davidson, C.B.; Ribeiro, E.E.; et al. Açaí (Euterpe oleracea Mart.) as a Potential Anti-neuroinflammatory Agent: NLRP3 Priming and Activating Signal Pathway Modulation. Mol. Neurobiol. 2021, 58, 4460–4476. [CrossRef]
7. Choi, W.-S.; Shin, P.-G.; Lee, J.-H.; Kim, G.-D. The regulatory effect of veratric acid on NO production in LPS-stimulated RAW264.7 macrophage cells. Cell. Immunol. 2012, 280, 164–170. [CrossRef]
8. Cong, L.; Lei, M.-Y.; Liu, Z.-Q.; Liu, Z.-F.; Ma, Z.; Liu, K.; Li, J.; Deng, Y.; Liu, W.; Xu, B. Resveratrol attenuates manganese-induced oxidative stress and neuroinflammation through SIRT1 signaling in mice. Food Chem. Toxicol. 2021, 153, 112283. [CrossRef]
9. Costa, F.; Barbisan, F.; Assmann, C.E.; Duarte, A.; Henrique, M.; Thiago, M.; Medeiros, M.M.; Duarte, F.; Beatrice, I.; Costa, F.; et al. Influence of Val16Ala-SOD2 polymorphism on sperm quality parameters. Hum. Fertil. 2017, 21, 212–219. [CrossRef]
10. Delgado, A.; Cholevas, C.; Theoharides, T.C. Neuroinflammation in Alzheimer's disease and beneficial action of luteolin. BioFactors 2021, 47, 207–217. [CrossRef]
11. Ellman, G.L. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 1959, 82, 70–77. [CrossRef] [PubMed]
12. Foti, P.; Pino, A.; Romeo, F.V.; Vaccalluzzo, A.; Caggia, C.; Randazzo, C.L. Olive Pomace and Pâté Olive Cake as Suitable Ingredients for Food and Feed. Microorganisms 2022, 10, 237. [CrossRef] [PubMed]
13. Gallardo-Fernández, M.; Hornedo-Ortega, R.; Alonso-Bellido, I.M.; Rodríguez-Gómez, J.A.; Troncoso, A.M.; García-Parrilla, M.C.; Venero, J.L.; Espinosa-Oliva, A.M.; de Pablos, R.M. Hydroxytyrosol Decreases LPS- and α-Synuclein-Induced Microglial Activation In Vitro. Antioxidants 2019, 9, 36. [CrossRef] [PubMed]
14. Halliwell, B.; Gutteridge, J.M.; Aruoma, O.I. The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem. 1987, 165, 215–219. [CrossRef] [PubMed]
15. Hansson, O. Biomarkers for neurodegenerative diseases. Nat. Med. 2021, 27, 954–963. [CrossRef] [PubMed]
16. Hornedo-Ortega, R.; Cerezo, A.B.; de Pablos, R.M.; Krisa, S.; Richard, T.; García-Parrilla, M.C.; Troncoso, A.M. Phenolic Compounds Characteristic of the Mediterranean

Diet in Mitigating Microglia-Mediated Neuroinflammation. Front. Cell. Neurosci. 2018, 12, 373. [CrossRef]
17. Huang, D.; Ou, B.; Prior, R.L. The Chemistry behind Antioxidant Capacity Assays. J. Agric. Food Chem. 2005, 53, 1841–1856. [CrossRef]
18. Ketnawa, S.; Reginio, F.C., Jr.; Thuengtung, S.; Ogawa, Y. Changes in bioactive compounds and antioxidant activity of plant-based foods by gastrointestinal digestion: A review. Crit. Rev. Food Sci. Nutr. 2022, 62, 4684–4705. [CrossRef]
19. Kujawska, M.; Jodynis-Liebert, J. Polyphenols in Parkinson's Disease: A Systematic Review of In Vivo Studies. Nutrients 2018, 10, 642. [CrossRef]
20. Leri, M.; Vasarri, M.; Carnemolla, F.; Oriente, F.; Cabaro, S.; Stio, M.; Degl'innocenti, D.; Stefani, M.; Bucciantini, M. EVOO Polyphenols Exert Anti-Inflammatory Effects on the Microglia Cell through TREM2 Signaling Pathway. Pharmaceuticals 2023, 16, 933. [CrossRef]
21. Li, Y.; Li, M.; Wang, L.; Li, Z. Effect of particle size on the release behavior and functional properties of wheat bran phenolic compounds during in vitro gastrointestinal digestion. Food Chem. 2022, 367, 130751. [CrossRef] [PubMed]
22. Mushtaq, A.; Hanif, M.A.; Ayub, M.A.; Bhatti, I.A.; Romdhane, M. Olive. Med. Plants South Asia 2020, 40, 541–555. [CrossRef]
23. Park, J.; Min, J.; Chae, U.; Yeop, J.; Song, K.; Lee, H.; Jun, H.; Lee, S.; Lee, D. Anti-in fl ammatory effect of oleuropein on microglia through regulation of Drp1-dependent mitochondrial fi ssion. J. Neuroimmunol. 2017, 306, 46–52. [CrossRef]
24. Reboredo-rodr, P.; Gonz, C.; Mart, E.; Rial-otero, R.; Cancho-grande, B. Applicability of an In-Vitro Digestion Model to Assess the Bioaccessibility of Phenolic Compounds from Olive-Related Products. Molecules 2021, 26, 6667. [CrossRef]
25. Ribeiro, B.; Bonif, T.; Silva, S.; Veiga, M.; Monforte, A.R. Food Hydrocolloids Prebiotic effects of olive pomace powders in the gut: In vitro evaluation of the inhibition of adhesion of pathogens, prebiotic and antioxidant effects. Food Hydrocoll. 2021, 112, 106312. [CrossRef]
26. Sánchez-Martínez, J.D.; Garcia, A.R.; Alvarez-Rivera, G.; Valdés, A.; Brito, M.A.; Cifuentes, A. In Vitro Study of the Blood–Brain Barrier Transport of Natural Compounds Recovered from Agrifood By-Products and Microalgae. Int. J. Mol. Sci. 2023, 24, 533. [CrossRef]
27. Monteiro, C.S.; Bortolazzo, P.C.; Bonini, C.A.A.; Dluzniewski, L.T.; da Silva, D.T.; Baranzelli, J.; Smaniotto, F.A.; Ballus, C.A.; Lozano-Sánchez, J.; Somacal, S.; et al.

Effect of micronization on olive pomace biotransformation in the static model of colonic fermentation. Food Chem. 2023, 418, 135921. [CrossRef]
28. Schmidt, L.; Damian, O.; Rossini, P. Phenolic compounds and contaminants in olive oil and pomace—A narrative review of their biological and toxic effects. Food Biosci. 2023, 53, 102626. [CrossRef]
29. Sefrin, C.; Rigo, D.; Betine, A.; Bender, B.; Stiebe, J.; Augusto, C.; Picolli, L.; Lozano-s, J.; Emanuelli, T. Micronization increases the bioaccessibility of polyphenols from granulometrically separated olive pomace fractions. Food Chem. 2021, 344, 128689. [CrossRef]
30. Simon, A.; Darcsi, A.; Kéry, Á.; Riethmüller, E. Blood-brain barrier permeability study of ginger constituents. J. Pharm. Biomed. Anal. 2020, 177, 112820. [CrossRef]
31. Simonato, B.; Trevisan, S.; Tolve, R.; Favati, F.; Pasini, G. Pasta fortification with olive pomace: Effects on the technological characteristics and nutritional properties. LWT 2019, 114, 108368. [CrossRef]
32. Singh, S.S.; Rai, S.N.; Birla, H.; Zahra, W.; Rathore, A.S.; Singh, S.P. NF-κB-Mediated Neuroinflammation in Parkinson's Disease and Potential Therapeutic Effect of Polyphenols. Neurotox. Res. 2020, 37, 491–507. [CrossRef] [PubMed]
33. de Souza, D.V.; Pappis, L.; Bandeira, T.T.; Sangoi, G.G.; Fontana, T.; Rissi, V.B.; Sagrillo, M.R.; Duarte, M.M.; Duarte, T.; Bodenstein, D.F.; et al. Açaí (Euterpe oleracea Mart.) presents anti-neuroinflammatory capacity in LPS-activated microglia cells. Nutr. Neurosci. 2022, 25, 1188–1199. [CrossRef] [PubMed]
34. Taticchi, A.; Urbani, S.; Albi, E.; Servili, M.; Codini, M.; Traina, G.; Balloni, S.; Patria, F.F.; Perioli, L.; Beccari, T.; et al. In Vitro Anti-Inflammatory Effects of Phenolic Compounds from Moraiolo Virgin Olive Oil (MVOO) in Brain Cells via Regulating the TLR4/NLRP3 Axis. Molecules 2019, 24, 4523. [CrossRef] [PubMed]
35. Yu, X.D.; Zhang, D.; Xiao, C.L.; Zhou, Y.; Li, X.; Wang, L.; He, Z.; Reilly, J.; Xiao, Z.Y.; Shu, X. P-Coumaric Acid Reverses Depression-Like Behavior and Memory Deficit Via Inhibiting AGE-RAGE-Mediated Neuroinflammation. Cells 2022, 11, 1594. [CrossRef]
36. Zhao, Y.-T.; Zhang, L.; Yin, H.; Shen, L.; Zheng, W.; Zhang, K.; Zeng, J.; Hu, C.; Liu, Y. Hydroxytyrosol alleviates oxidative stress and neuroinflammation and enhances hippocampal neurotrophic signaling to improve stress-induced depressive behaviors in mice. Food Funct. 2021, 12, 5478–5487. [CrossRef]

37. Zhang, L.; Zhang, J.; Jiang, X.; Yang, L.; Zhang, Q.; Wang, B.; Cui, L.; Wang, X. Hydroxytyrosol Inhibits LPS-Induced Neuroinflammatory Responses via Suppression of TLR-4-Mediated NF-κB P65 Activation and ERK Signaling Pathway. Neuroscience 2020, 426, 189–200. [CrossRef]
38. Zhu, T.; Wang, L.; Wang, L.-P.; Wan, Q. Therapeutic targets of neuroprotection and neurorestoration in ischemic stroke: Applications for natural compounds from medicinal herbs. Biomed. Pharmacother. 2022, 148, 112719. [CrossRef]
39. Khaneghah, A.M.; Martins, L.M.; von Hertwig, A.M.; Bertoldo, R.; Sant'Ana, A.S. Deoxynivalenol and its masked forms: Characteristics, incidence, control and fate during wheat and wheat based products processing—A review. Trends Food Sci. Technol. 2018, 71, 13–24. [CrossRef]
40. Xiong, S.; Su, X.; Kang, Y.; Si, J.; Wang, L.; Li, X.; Ma, K. Effect and mechanism of chlorogenic acid on cognitive dysfunction in mice by lipopolysaccharide-induced neuroinflammation. Front. Immunol. 2023, 14, 1178188. [CrossRef]

5.3 Avaliação da atividade antioxidante dos compostos fenólicos biodisponíveis do bagaço de oliva em *Caenorhabditis elegans*

Manuscrito formatado para submissão em periódico a ser definido.

6. DISCUSSÃO GERAL

O bagaço de oliva, um subproduto da produção do óleo de oliva, é rico em diferentes compostos fenólicos, o que torna este produto de alto valor agregado. No entanto, não existe uma aplicação deste subproduto que possa suprir a demanda total da indústria beneficiadore de oliva. Atualmente, o bagaço de oliva é utilizado em parte para alimentação animal, algumas aplicações como adubação de solo e geração de energia (Berbel and Posadillo, 2018; Iannaccone et al., 2019). Embora estas atividades sejam importantes, a riqueza em composição fenólica do bagaço de oliva é interesse de estudos em várias áreas, buscando a utilização deste sub-produto, de maneira segura, para desempenhar diferentes efeitos benéficos à saúde humana.

Os compostos fenólicos mais representativos no óleo e bagaço de oliva são a oleuropeína, o tirosol e o hidroxitirosol. Estes compostos podem contribuir, através de diferentes vias, para a prevenção e controle de doenças, desde doenças cardiovasculares ao câncer e doenças neurodegenerativas (Jimenez-Lopez et al., 2020). Os principais compostos fenólicos associados as diferentes bioatividades do óleo e bagaço de oliva são Ole, Tir e HT, e a extensão de sua ação depende de sua biodisponibilidade após os processos digestivos (Difonzo et al., 2021).

A constante exposição das olivas à contaminantes como micotoxinas, resíduos de pesticidas e hidrocarbonetos aromáticos policíclicos destaca a importância de compreender como os compostos fenólicos do óleo e bagaço de oliva atuam na toxicidade desses contaminantes nesses produtos (Arena et al., 2022). Ainda existem poucos relatos sobre os níveis desses contaminantes em óleo e principalmente em bagaço de oliva. Assim, enfatizamos a necessidade de estudos a esse respeito. Além disso, é de extrema importância, a criação de uma legislação específica para o monitoramento dos níveis destes contaminantes em óleo e bagaço de oliva, visando garantir a segurança destes produtos.

Neste contexto, os compostos fenólicos podem neutralizar a toxicidade causada por contaminantes nos derivados da oliva, embora a maioria dos estudos disponíveis atualmente, utilize compostos fenólicos isolados, em vez de amostras de óleo e bagaço de oliva. Portanto, os dados apresentados podem estimular estudos futuros, visando preencher esta lacuna. Outra lacuna que identificamos ao longo de nossas pesquisas, é a necessidade de mais estudos sobre a contaminação e bioatividade do bagaço de oliva. Contudo, é de extrema importância realizar mais estudos para entender as interações entre compostos fenólicos e tóxicos no óleo e bagaço de oliva para determinar os reais

benefícios à saúde desses produtos, e a possível aplicação do bagaço de oliva na alimentação humana.

Buscando contribuir com os estudos a cerca da bioatividade do bagaço de oliva, desenvolvemos estudos para avaliar a capacidade antioxidante em *C. elegans* e em células da micróglia dos compostos fenólicos biodisponíveis do bagaço de oliva. O bagaço de oliva foi submetido a dois tipos de fracionamento, o primeiro foi apenas um fracionamento (tamanho de partícula < 2 mm), amostra OPF. O segundo foi um fracionamento seguido de micronização (tamanho de partícula < 2 μm), amostra OPM. Estas amostras foram submetidas ao processo de digestão gastrointestinal *in vitro*, visando obter os compostos fenólicos biodisponíveis das amostras de bagaço de oliva.

O tamanho de partícula influenciou na concentração e perfil final de compostos fenólicos biodisponíveis das amostras OPF e OPM. A amostra OPM apresentou concentração de compostos fenólicos biodisponíveis superior a amostra OPF ($p \leq 0.05$). Este comportamento está associado ao tamanho de partícula, a amostra OPM possuí menor tamanho de partícula e maior superfície de contato com as enzimas do processo digestivo *in vitro*, o que resulta em maior concentração de compostos fenólicos biuodisponíveis (Sant et al., 2023).

Os compostos fenólicos identificados e quantificados nas amostras biodisponíveis do OPF e OPM foram hidroxitirosol-glicosídeo, tirosol e hidroxitirosol, oleuropeína e oleuropeína aglicona, ácidos 4-hidroxibenzóico e fenólicos clorogênicos. A concentração de todos os compostos fenólicos analisados foi superior na amostra biodisponível do OPM, quando comparada à OPF ($p \leq 0,05$). Esses resultados corroboram com os apresentados por outros pesquisadores, onde hidroxitirosol, hidroxitirosol-glicosídeo, tirosol, apigenina e ácido p-cumárico foram identificados na fração biodisponível do bagaço de oliva, após o processo de digestão *in vitro* (Reboredo-Rodríguez et al., 2021; Ribeiro et al., 2021; Sant et al., 2023).

Os compostos fenólicos biodisponíveis do bagaço de oliva apresentaram capacidade antioxidante *in vitro* contra o radical peroxil $ROO^{\bullet}$, sendo esse efeito maior nos compostos fenólicos biodisponíveis na amostra OPM quando comparado ao potencial dos compostos fenólicos biodisponíveis do OPF. Estas características antioxidantes e do perfil fenólico das amostras biodisponíveis de bagaço de oliva podem contribuir com a prevenção e atuar na mediação de processos associados ao estresse oxidativo (Sant et al., 2023).

A característica lipofílica dos compostos fenólicos é o que determina sua permeabilidade através da barreira hematoencefálica (BHE), onde compostos menos polares (isto é, derivados O-metilados) são mais permeáveis quando comparados a compostos fenólicos altamente polares (isto é, sulfatados e derivados glicuronidados) (Singh et al., 2020). Neste contexto, avaliamos o possível potencial anti-neuroinflamatório dos compostos fenólicos biodisponíveis do bagaço de oliva em células da microglia (BV2) ativadas por LPS (1 μg mL^{-1}). Em paralelo avaliamos o potencial antioxidantes do compostos fenólicos biodisponíveis do bagaço de oliva frente ao dano oxidativo induzido po H_2O_2 (300 mM) em *C. elegans*.

Os resultados associados ao efeito *per se* dos compostos fenólicos biodisponíveis das amostras de bagaço de oliva, indicam que concentrações fenólicas de 3 mg L^{-1} de OPF podem ser tóxicas para células microgliais, pois ocasionaram redução de viabilidade celular. Desta forma, essa concentração não foi avaliada quanto a sua capacidade anti-neuroinflamatória em células BV-2 ativadas por LPS.

Os compostos fenólicos biodisponíveis das amostras OPF e OPM não afetaram a viabilidade celular das células BV2 ativadas por LPS, independente da concentração avaliada. Os compostos fenólicos biodisponíveis da amostra OPF atenuaram a geração de EROs nas células BV2 ativadas, apenas em sua menor concentração (0,03 mg L^{-1}), enquanto a amostra OPM apresentou efeito protetor nas concentrações de 0,03 e 0,15 mg L^{-1}. Por outro lado, apenas a maior concentração de compostos fenólicos biodisponíveis (1,5 mg L^{-1}) das amostras de OPF e OPM foi capaz de atenuar, em aproximadamente 25%, o aumento nos níveis de NO induzido por LPS nas células BV2 ativadas. Desta maneira, este é o primeiro estudo que relata o efeito antioxidante e possível potencial anti-neuroinflamatória de compostos fenólicos biodisponíveis do bagaço de oliva. A biodisponibilidade dos compostos fenólicos no cérebro é extremamente importante na neuroproteção por meio da regulação de genes pró-inflamatórios (Singh et al., 2020). No entanto, não há informações sobre a biodisponibilidade dos compostos fenólicos do bagaço de oliva no cérebro.

Em contrapartida, os compostos fenólicos biodisponíveis do OPF apresentaram toxicidade para o *C. elegans* nas concentrações de 1,5 e 3 mg L^{-1}, o que foi evidenciado pela redução da sobrevivência dos nematoides quando comparados ao grupo controle não tratado. Este comportamento foi acompanhado de aumento na geração de ERs. No entanto, os compostos fenólicos biodisponíveis do OPM não afetaram a sobrevivência

dos nematoides. Estes efeitos podem estar associados ao diferente perfil fenólico das amostras, resultado dos diferentes tamanhos de partícula.

As maiores concentrações de compostos fenólicos biodisponíveis avaliadas tanto para OPF quanto para OPM foram tóxicas para o *C. elegans*. De acordo com Santhi et al. (2019), altas concentrações (2,4 mg L^{-1}) de compostos fenólicos são letais para nematóides na fase juvenil. No entanto, em menores concentrações os compostos fenólicos biodisponíveis do OPF e OPM (0,03, 0,15 e 0,30 mg L^{-1}) atenuaram a morte e a geração de ER induzida pelo H_2O_2 nos nematoides após exposição aguda. Os compostos fenólicos biodisponíveis do OPF protegeram a SOD da oxidação e reduziram a peroxidação lipídica nas maiores concentrações (0,15 e 0,30 mg L^{-1}). Em contrapartida, os compostos fenólicos biodisponíveis do OPM apresentaram este comportamento nas menores concentrações (0,03 e 0,15 mg L^{-1}). Além disso, concentrações de compostos fenólicos biodisponíveis menores para OPF (0,03 mg L^{-1}), e maiores para OPM (0,30 mg L^{-1}) aumentaram os níveis de peroxidação lipídica, o que indica possível dano oxidativo.

Os resultados obtidos neste estudo demonstram a importância de se estudor sub-produtos como o bagaço de oliva, que apresenta elevado valor agregado devido a sua composição fenólica. Neste estudo, demonstramnos que os compostos fenólicos biodisponíveis do bagaço de oliva, em concentrações inferiores a 0,30 mg L^{-1} podem atuar como antioxidantes tanto em modelo de células da micróglia quanto em modelo de *C. elegans*. Além disso, o tamanho de partícula influenciou diretamente na extração dos compostos fenólicos ao longo da digestão *in vitro*, onde par OPM a concentração fenólica total foi superior ao OPF. Por fim, destacamos que esta tese segue os princípios de sustentabilidade através da utilização de um sub-produto da indústria de beneficiamento do óleo de oliva.

7. CONSIDERAÇÕES FINAIS

Os resultados obtidos no presente trabalho nos permitem concluir que:

- O bagaço de oliva apresenta uma variedade de compostos fenólicos, responsáveis por inúmeras bioatividades. Entretanto, o óleo de oliva (e possivelmente o bagao de oliva também) podem estar contaminados com substancias tóxicas utilizadas durante o cultivo da oliveira (micotoxinas, pesticidas, etc.). Entretanto, essa interação entre os compostos bioativos e tóxicos necessita ser avaliada em um futuro próximo.
- Os compostos fenólicos obtidos após digestão gastrointestinal do bagaço de oliva apresentaram potencial antioxidante em células da micróglia;
- Os compostos fenólicos obtidos após digestão gastrointestinal do bagaço de oliva atenuaram o estresse oxidativo causado por H_2O_2 *in vivo* em modelo de *C. elegans*.

Diante dos resultados relatados no artigo de revisão e estudos tanto *in vitro* quanto *in vivo*, destacamos a importância dos compostos fenólicos do bagaço de oliva. Em nosso trabalho, demonstramos o potencial antioxidante dos compostos biodisponíveis do bagaço de oliva mesmo após o processo de digestão. Estas informações destacam a importância de estudos a cerca das bioatividades e possíveis aplicações do bagaço de oliva na indústria de alimentos, visando garantir sua segurança e propor possíveis utilizações na alimentação humana.

8. PERSPECTIVAS FUTURAS

Este estudo apresenta como perspectivas futuras propor uma possível aplicação alimentícia do bagaço de oliva, buscando o aproveitamento de sua riqueza em compostos fenólicos. Em contra partida, ainda são necessários maiores estudos para compreender como os compostos fenólicos atuam, e como o tipo de processamento pode afetar as bioatividades destes compostos. Outro lacuna a ser preenchida é a avaliação da interação dos compostos fenólicos do bagaço de oliva com diferentes contaminantes, comuns do cultivo da azeitona, como micotoxinas, pesticidas e hidrocarbonetos policiclicos aromáticos.

9. REFERÊNCIAS

Altindiş, M., Aslan, F. G., Uzuner, H., Ünal, H., Köroğlu, M., Kulaç, S., & Karadenizli, A. (2020). Comparison of antiviral effect of olive leaf extract and propolis with acyclovir on herpes simplex virus type 1. Mikrobiyoloji Bulteni, 54, 79–94. https://doi.org/10.5578/mb.69019.

Angeloni, C., Malaguti, M., Barbalace, M. C., & Hrelia, S. (2017). Bioactivity of olive oil phenols in neuroprotection. International Journal of Molecular Sciences, 18, 1–27. https://doi.org/10.3390/ijms18112230.

Arena, A., Zoccali, M., Mondello, L., & Tranchida, P. Q. (2022). A method for the determination of 70 pesticides in extra virgin olive oil based on a limited - volume solvent extraction step prior to comprehensive two - dimensional gas chromatography - tandem mass spectrometry. Analytical and Bioanalytical Chemistry, 1, 1-8. https://doi.org/10.1007/s00216-022-04494-6.

Bassani, B., Rossi, T., De Stefano, D., Pizzichini, D., Corradino, P., Macrì, N., Noonan, D. M., Albini, A., & Bruno, A. (2016). Potential chemopreventive activities of a polyphenol rich purified extract from olive mill wastewater on colon cancer cells. Journal of Functional Foods, 27, 236–248. https://doi.org/10.1016/j.jff.2016.09.009.

Berbel, J., & Posadillo, A. (2018). Review and analysis of alternatives for the valorisation of agro-industrial olive oil by-products. Sustainability (Switzerland), 10, 1–9. https://doi.org/10.3390/su10010237.

Borges, T. H., Pereira, J. A., Cabrera-Vique, C., Lara, L., Oliveira, A. F., & Seiquer, I. (2017). Characterization of Arbequina virgin olive oils produced in different regions of Brazil and Spain: Physicochemical properties, oxidative stability and fatty acid profile. Food Chemistry, 215, 454–462. https://doi.org/10.1016/j.foodchem.2016.07.162.

Boronat, A., Mateus, J., Soldevila-Domenech, N., Guerra, M., Rodríguez-Morató, J., Varon, C., Muñoz, D., Barbosa, F., Morales, J. C., Gaedigk, A., Langohr, K., Covas, M. I., Pérez-Mañá, C., Fitó, M., Tyndale, R. F., & de la Torre, R. (2019). Cardiovascular benefits of tyrosol and its endogenous conversion into hydroxytyrosol in humans. A randomized, controlled trial. Free Radical Biology and Medicine, 143, 471–481. https://doi.org/10.1016/j.freeradbiomed.2019.08.032.

Calabriso, N., Gnoni, A., Stanca, E., Cavallo, A., Damiano, F., Siculella, L., & Carluccio, M. A. (2018). Hydroxytyrosol ameliorates endothelial function under inflammatory conditions by preventing mitochondrial dysfunction. Oxidative Medicine and Cellular Longevity, 2018, 1-14. https://doi.org/10.1155/2018/9086947.

Delgado, A. (2021). Neuroinflammation in Alzheimer ' s disease and beneficial action of luteolin. Biofactors, 2020, 207–217. https://doi.org/10.1002/biof.1714.

Difonzo, G., Troilo, M., Squeo, G., Pasqualone, A., & Caponio, F. (2021). Functional compounds from olive pomace to obtain high-added value foods – a review. Journal of the Science of Food and Agriculture, 101, 15–26. https://doi.org/10.1002/jsfa.10478.

Domínguez-Perles, R., Auñón, D., Ferreres, F., & Gil-Izquierdo, A. (2017). Gender differences in plasma and urine metabolites from Sprague–Dawley rats after oral administration of normal and high doses of hydroxytyrosol, hydroxytyrosol acetate, and DOPAC. European Journal of Nutrition, 56, 215–224. https://doi.org/10.1007/s00394-015-1071-2.

Dumitrescu, L., Popescu-Olaru, I., Cozma, L., Tulbă, D., Hinescu, M. E., Ceafalan, L. C., Gherghiceanu, M., & Popescu, B. O. (2018). Oxidative stress and the microbiota-gut-brain axis. Oxidative Medicine and Cellular Longevity, 2018, 1-12. https://doi.org/10.1155/2018/2406594.

Fernández-Ávila, C., Montes, R., Castellote, A. I., Chisaguano, A. M., Fitó, M., Covas, M. I., Muñoz-Aguallo, D., Nyyssönen, K., Zunft, H. J., & López-Sabater, M. C. (2015). Fast determination of virgin olive oil phenolic metabolites in human high-density lipoproteins. Biomedical Chromatography, 29, 1035–1041. https://doi.org/10.1002/bmc.3389.

Foscolou, A., Critselis, E., & Panagiotakos, D. (2018). Olive oil consumption and human health: A narrative review. Maturitas, 118, 60–66. https://doi.org/10.1016/j.maturitas.2018.10.013.

Gallardo-fernández, M., Hornedo-ortega, R., Alonso-bellido, I. M., & José, A. (2019). Hydroxytyrosol Decreases LPS- and α -Synuclein- Induced Microglial Activation In Vitro. Antioxidants, 9, 1–18. https://doi.org/10.3390/antiox9010036.

García-Villalba, R., Larrosa, M., Possemiers, S., Tomás-Barberán, F. A., & Espín, J. C. (2014). Bioavailability of phenolics from an oleuropein-rich olive (Olea europaea) leaf extract and its acute effect on plasma antioxidant status: Comparison between pre- and postmenopausal women. European Journal of Nutrition, 53, 1015–1027. https://doi.org/10.1007/s00394-013-0604-9.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: an updated overview. Archives of Toxicology, 94, 651-715. https://doi.org/10.1007/s00204-020-02689-3.

Hansson, O. (2021). Biomarkers for neurodegenerative diseases. Nature Medicine, 27, 954–963. https://doi.org/10.1038/s41591-021-01382-x.4

Jimenez-Lopez, C., Carpena, M., Lourenço-Lopes, C., Gallardo-Gomez, M., M. Lorenzo, J., Barba, F. J., Prieto, M. A., & Simal-Gandara, J. (2020). Bioactive compounds and quality of extra virgin olive oil. Foods, 9, 1-31. https://doi.org/10.3390/foods9081014.

Joseph, S. V., Edirisinghe, I., & Burton-Freeman, B. M. (2016). Fruit Polyphenols: A Review of Anti-inflammatory Effects in Humans. Critical Reviews in Food Science and Nutrition, 56, 419–444. https://doi.org/10.1080/10408398.2013.767221.

Karkovic Markovic, A., Toric, J., Barbaric, M., & Jakobušic Brala, C. (2019). Potential Effects on Human Health. Molecules, 24, 1-39. https://doi.org/10.3390/molecules24102001.

Lanfranchi, M., Giannetto, C., & De Pascale, A. (2016). Economic analysis and energy valorization of by-products of the olive oil process: "valdemone DOP" extra virgin olive oil. Renewable and Sustainable Energy Reviews, 57, 1227–1236. https://doi.org/10.1016/j.rser.2015.12.196.

Lee, K. M., Hur, J., Lee, Y., Yoon, B. R., & Choi, S. Y. (2018). Protective effects of tyrosol against oxidative damage in L6 muscle cells. Food Science and Technology Research, 24, 943–947. https://doi.org/10.3136/fstr.24.943.

López de las Hazas, M. C., Godinho-Pereira, J., Macià, A., Almeida, A. F., Ventura, M. R., Motilva, M. J., & Santos, C. N. (2018). Brain uptake of hydroxytyrosol and its main circulating metabolites: Protective potential in neuronal cells. Journal of Functional Foods, 46, 110–117. https://doi.org/10.1016/j.jff.2018.04.028.

Luo, H., Chiang, H. H., Louw, M., Susanto, A., & Chen, D. (2017). Nutrient Sensing and the Oxidative Stress Response. Trends in Endocrinology and Metabolism, 28, 449–460. https://doi.org/10.1016/j.tem.2017.02.008.

Martín-García, B., Pimentel-Moral, S., Gómez-Caravaca, A. M., Arráez-Román, D., & Segura-Carretero, A. (2020). Box-Behnken experimental design for a green extraction method of phenolic compounds from olive leaves. Industrial Crops and Products, 154, 1-10. https://doi.org/10.1016/j.indcrop.2020.112741.

Mateos, R., Pereira-Caro, G., Saha, S., Cert, R., Redondo-Horcajo, M., Bravo, L., & Kroon, P. A. (2011). Acetylation of hydroxytyrosol enhances its transport across differentiated Caco-2 cell monolayers. Food Chemistry, 125, 865–872. https://doi.org/10.1016/j.foodchem.2010.09.054.

Mosele, J. I., Macià, A., & Motilva, M. J. (2015). Metabolic and microbial modulation of the large intestine ecosystem by non-absorbed diet phenolic compounds: A review. Molecules, 20, 17429–17468. https://doi.org/10.3390/molecules200917429.

Mushtaq, A., Hanif, M. A., Ayub, M. A., Bhatti, I. A., & Romdhane, M. (2020). Olive. Medicinal Plants of South Asia, 1, 541–555. https://doi.org/10.1016/b978-0-08-102659-5.00040-9.

Park, J., Min, J., Chae, U., Yeop, J., Song, K., Lee, H., Jun, H., Lee, S., & Lee, D. (2017). Anti-in fl ammatory effect of oleuropein on microglia through regulation of Drp1-dependent mitochondrial fi ssion. Journal of Neuroimmunology, 306, 46–52. https://doi.org/10.1016/j.jneuroim.2017.02.019.

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. European Journal of Medicinal Chemistry, 97, 55–74. https://doi.org/10.1016/j.ejmech.2015.04.040.

Reboredo-Rodríguez, P., González-Barreiro, C., Cancho-Grande, B., Forbes-Hernández, T. Y., Gasparrini, M., Afrin, S., Cianciosi, D., Carrasco-Pancorbo, A., Simal-Gándara, J., Giampieri, F., & Battino, M. (2018). Characterization of phenolic extracts from Brava extra virgin olive oils and their cytotoxic effects on MCF-7 breast cancer cells. Food and Chemical Toxicology, 119, 73-85. https://doi.org/10.1016/j.fct.2018.05.026.

Ribeiro, T. B., Costa, C. M., Bonifácio - Lopes, T., Silva, S., Veiga, M., Monforte, A. R., Nunes, J., Vicente, A. A., & Pintado, M. (2021). Prebiotic effects of olive pomace powders in the gut: In vitro evaluation of the inhibition of adhesion of pathogens, prebiotic and antioxidant effects. Food Hydrocolloids, 112, 1-14. https://doi.org/10.1016/j.foodhyd.2020.106312.

Robles-Almazan, M., Pulido-Moran, M., Moreno-Fernandez, J., Ramirez-Tortosa, C., Rodriguez-Garcia, C., Quiles, J. L., & Ramirez-Tortosa, Mc. (2018). Hydroxytyrosol: Bioavailability, toxicity, and clinical applications. Food Research International, 105, 654–667. https://doi.org/10.1016/j.foodres.2017.11.053.

Rodríguez-Morató, J., Boronat, A., Kotronoulas, A., Pujadas, M., Pastor, A., Olesti, E., Pérez-Mañá, C., Khymenets, O., Fitó, M., Farré, M., & de la Torre, R. (2016). Metabolic disposition and biological significance of simple phenols of dietary origin: hydroxytyrosol and tyrosol. Drug Metabolism Reviews, 48, 218–236. https://doi.org/10.1080/03602532.2016.1179754

Romero-García, J. M., Niño, L., Martínez-Patiño, C., Álvarez, C., Castro, E., & Negro, M. J. (2014). Biorefinery based on olive biomass. State of the art and future trends. Bioresource Technology, 159, 421–432. https://doi.org/10.1016/j.biortech.2014.03.062.

Salehi, B., Vlaisavljevic, S., Oluwaseun, C., Bunmi, J., Kregiel, D., Antolak, H., Pawlikowska, E., Uprety, Y., Mileski, K. S., Prasad, H., Shari, J., Das, G., Kumar, J., Kumar, A., Segura-carretero, A., & Contreras, M. (2019). Plants of the genus Vitis : Phenolic compounds , anticancer properties and clinical relevance. Trends in Food Science & Technology, 91, 362–379. https://doi.org/10.1016/j.tifs.2019.07.042

Monteiro, A., Colpo, P., Araujo, C., Bonini, A., Tamires, L., Trivisiol, D., Baranzelli, J., Aline, F., Augusto, C., Lozano-s, J., Somacal, S., & Emanuelli, T. (2023). Effect of micronization on olive pomace biotransformation in the static model of colonic fermentation. Food Chemistry, 418, 1-8. https://doi.org/10.1016/j.foodchem.2023.135921.

Santhi, V. S., Salame, L., Muklada, H., Azaizeh, H., Haj-Zaroubi, M., Awwad, S., Landau, S. Y., & Glazer, I. (2019). Toxicity of phenolic compounds to entomopathogenic nematodes: A case study with Heterorhabditis bacteriophora exposed to lentisk (Pistacia lentiscus) extracts and their chemical components. Journal of Invertebrate Pathology, 160, 43–53. https://doi.org/10.1016/j.jip.2018.12.003.

Sawikr, Y., Yarla, N. S., & Peluso, I. (2017). Neuroinflammation in Alzheimer's Disease: The Preventive and Therapeutic Potential of Polyphenolic Nutraceuticals. Stress and Inflammation in Disorders, 108, 33-57. https://doi.org/10.1016/bs.apcsb.2017.02.001,

Sefrin, C., Rigo, D., Betine, A., Bender, B., Stiebe, J., Augusto, C., Picolli, L., Lozano-s, J., & Emanuelli, T. (2021b). Micronization increases the bioaccessibility of polyphenols from granulometrically separated olive pomace fractions. Food Chemistry, 344, 1-10. https://doi.org/10.1016/j.foodchem.2020.128689.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects - A review. Journal of Functional Foods, 18, 820–897. https://doi.org/10.1016/j.jff.2015.06.018.

Shi, C., Chen, X., Liu, Z., Meng, R., Zhao, X., Liu, Z., & Guo, N. (2017). Oleuropein protects L-02 cells against H2O2-induced oxidative stress by increasing SOD1, GPx1 and CAT expression. Biomedicine and Pharmacotherapy, 85, 740–748. https://doi.org/10.1016/j.biopha.2016.11.092.

Simonato, B., Trevisan, S., Tolve, R., Favati, F., & Pasini, G. (2019). Pasta fortification with olive pomace: Effects on the technological characteristics and nutritional properties. Lwt, 114, 1-6. https://doi.org/10.1016/j.lwt.2019.108368.

Singh, S. Sen, Rai, S. N., Birla, H., Zahra, W., & Rathore, A. S. (2020). NF- κ B-Mediated Neuroinflammation in Parkinson's Disease and Potential Therapeutic Effect of Polyphenols. Neurotoxicity Research, 37, 491–507. https://doi.org/10.1007/s12640-019-00147-2.

Souza, D. V. De, Pappis, L., Bandeira, T. T., Sangoi, G., Fontana, T., Rissi, V. B., Sagrillo, M. R., Duarte, M. M., Duarte, T., Bodenstein, D. F., Andreazza, C., Beatrice, I., Ribeiro, E. E., Antoniazzi, A., Ourique, A. F., Machado, A. K., Sangoi, G., Fontana, T., Rissi, V. B., … Machado, A. K. (2022). Açaí (Euterpe oleracea Mart .) presents anti-neuroinflammatory capacity in LPS-activated microglia cells. Nutritional Neuroscience, 25, 1188-1199. https://doi.org/10.1080/1028415X.2020.1842044.

Speroni, C. S., Stiebe, J., Guerra, D. R., Beutinger Bender, A. B., Ballus, C. A., dos Santos, D. R., Dal Pont Morisso, F., da Silva, L. P., & Emanuelli, T. (2019). Micronization and granulometric fractionation improve polyphenol content and antioxidant capacity of olive pomace. Industrial Crops and Products, 137, 347–355. https://doi.org/10.1016/j.indcrop.2019.05.005.

Sylla, N., Bouyahya, A., Taha, D., Dakka, N., & Elhajji, H. (2021). Biocatalysis and Agricultural Biotechnology Study of the antioxidant and antidiabetic activity in vitro of free and encapsulated phenolic compounds of olive pomace. Biocatalysis and Agricultural Biotechnology, 36, 1-9. https://doi.org/10.1016/j.bcab.2021.102126.

Virgin, M., Oil, O., Taticchi, A., Urbani, S., Albi, E., Servili, M., Codini, M., Traina, G., Balloni, S., Patria, F. F., Perioli, L., Beccari, T., & Conte, C. (2019). In Vitro Anti-Inflammatory Effects of Phenolic. Molecules, 24, 1–17. https://doi.org/10.3390/molecules24244523.

Vuolo, M. M., Lima, V. S., & Maróstica Junior, M. R. (2018). Phenolic Compounds: Structure, Classification, and Antioxidant Power. Bioactive Compounds: Health Benefits and Potential Applications, 1, 33-50. https://doi.org/10.1016/B978-0-12-814774-0.00002-5.

Yun-Tao Zhao, Zhang, L., Yin, H., Shen, L., Zheng, W., Zhang, K., Zeng, J., Hu, C., & Liu, Y. (2021). Hydroxytyrosol alleviates oxidative stress, neuroinflammation and enhances hippocampal neurotropic signaling to improve stress-induced depressive behaviors in mice. Function and Funtion, 1, 1–24. https://doi.org/10.1039/D1FO00210D.

Zhang, L., Zhang, J., Jiang, X., Yang, L., Zhang, Q., Wang, B., & Wang, X. (2020). N EUROSCIENCE Hydroxytyrosol Inhibits LPS-Induced Neuroinflammatory Responses via Suppression of TLR-4-Mediated NF- j B P65 Activation and ERK Signaling Pathway. Neuroscience, 426, 189–200. https://doi.org/10.1016/j.neuroscience.2019.12.005.