UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL
PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE E DESENVOLVIMENTO NA REGIÃO CENTRO-OESTE

CAROLINA DI PIETRO FERNANDES

EFEITO DA SUPLEMENTAÇÃO DO ÓLEO DA SEMENTE DE UVA (*Vitis vinifera* L.) E DO ÓLEO DE SEMENTE DE LINHAÇA (*Linum usitatissimum*) EM CAMUNDONGO SWISS

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Dissertação apresentada ao Programa de Pós Graduação em Saúde e Desenvolvimento na Região Centro-Oeste, área de concentração Metabolismo e Nutrição, para obtenção do título de Mestre pela Universidade Federal de Mato Grosso do Sul (UFMS).

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RESUMO

Os óleos vegetais extraídos de sementes, grãos e frutas, são de suma importância à alimentação humana e ao setor industrial. Assim, os ácidos graxos mono e poli-insaturados, e as substâncias bioativas presentes possuem ações benéficas à saúde e são utilizadas para o desenvolvimento de produtos cosméticos, farmacêuticos e alimentícios. Desse modo, o óleo de semente de uva (G), o óleo de semente de linhaça (L) e a *Mistura* (GL), composta pelo óleo de semente de uva e óleo de linhaça em mesmo volume 1:1 (v/v), podem apresentar qualidade e estabilidade, além ter efeitos positivos em modelo experimental *in vivo*. Visto isso, avaliamos os óleos realizando análises físico-químicas, estabilidade oxidativa: Rancimat, análises térmicas como a termogravimetria/termogravimetria derivada (TG/DTG) e calorimetria exploratória diferencial (DSC), análises ópticas de absorção UV-Vis e matriz de emissão-excitação de fluorescência, carotenóides totais e cor, além de avaliações de ingestão, comportamento do peso, parametros bioquímicos e histológicos em ensaio experimental *in vivo*. O perfil de ácidos graxos apresentou maior fração de ácido linoléico (C18:2) em G (52,40 ± 1,34) e GL (42,53 ± 0,2) e maior presença de ácido alfa-linolênico (C18:3) em L (53,30 ± 0,03). Os índices de qualidade e identidade estão dentro das faixas de recomendação para todas as amostras analisadas, o valor de acidez foi maior em L (2,8 mg KOH/g) e indice de peróxido foi maior em G (6,5 $mEqO_2$/kg). Os valores dos índices de refração, iodo (Wijs), saponificação e densidade relativa, respaldam que os óleos são adequados para o consumo. As análises TG/DTG, DSC e rancimat comportaram-se de forma semelhante. As análises ópticas revelaram maior teor de carotenoides em L (13,67 ± 1,74) e GL (6,23 ± 0,54) em comparação à G (1,16 ± 0,15), pontuando congruência nos resultados da análise de cor. No ensaio *in vivo*, o grupo G, na dosagem de 2.000mg/kg/dia (G2) promoveu menor consumo ($p<0,05$), e o grupo GL, apresentou menor ganho de peso por grama de alimento consumido ($p<0,05$). Os grupos suplementados com G2 e GL apresentaram níveis mais elevados de HDL-c ($p<0,05$). O Grupo com suplementação de L na dosagem 2.000 mg/kg/dia (L2) apresentou o menor nível de colesterol total ($p<0,05$). Os grupos L2 e GL manifestaram os menores valores de MCP-1 e TNF-α ($p<0,05$). Além disso, as menores áreas de adipócitos foram notadas nos grupos suplementados com G, na dosagem de 1.000mg/kg/dia (G1) e GL. Nossos resultados sugerem que o óleo GL possui qualidade para consumo e pode influenciar nos perfis lipídicos, marcadores de inflamação e status antioxidante, sendo G2 e L2 também eficazes.

PALAVRAS CHAVES: Óleos vegetais; Ácidos Graxos; Antioxidante.

ABSTRACT

Vegetable oils extracted from seeds, grains and fruits are extremely important for human nutrition and the industrial sector. Thus, mono- and polyunsaturated fatty acids and the bioactive substances presented have beneficial effects on health and are used for the development of cosmetics, pharmaceutical products and foods. Thus, grape seed oil (G), linseed oil (L) and the Mixture (GL), composed of grape seed oil and linseed oil in the same volume 1:1 (v/v), can present quality and stability, in addition to having positive effects in an *in vivo* experimental model. Given this, we evaluate the oils, physical-chemical analyses, oxidative stability: Rancimat, thermal analyzes such as thermogravimetry/derivative thermogravimetry (TG/DTG) and differential scanning calorimetry (DSC), optical analyzes of UV-Vis absorption and emission matrix- fluorescence stimulation, total carotenoids and color, in addition to assessments of consumption, weight behavior, biochemical and histological parameters in an in vivo experimental test. The fatty acid profile showed a higher fraction of linoleic acid (C18:2) in G (52.40 ± 1.34) and GL (42.53 ± 0.2) and a higher fraction of alpha-linolenic acid (C18:3). in L (53.30 ± 0.03). The quality and identity indexes are within the recommended ranges for all samples tested, the acidity value was higher in L (2.8 mg KOH/g) and the peroxide index was higher in G (6.5 mEqO2/kg).). The values of the refractive indices, iodine (Wijs), saponification and relative density support that the oils are suitable for consumption. TG/DTG, DSC and rancimat analyzes behaved similarly. Optical analyzes revealed a higher carotenoid content in L (13.67 ± 1.74) and GL (6.23 ± 0.54) compared to G (1.16 ± 0.15), highlighting congruence in the analysis results by heart. In the in vivo test, group G, at a dosage of 2,000mg/kg/day (G2) promoted lower consumption ($p<0.05$), and group GL, showed lower weight gain per gram of food consumed ($p< 0.05$). The groups supplemented with G2 and GL had higher levels of HDL-c ($p<0.05$). The Group with L supplementation at a dosage of 2,000 mg/kg/day (L2) had lower total cholesterol levels ($p<0.05$). The L2 and GL groups had the lowest MCP-1 and TNF-α values ($p<0.05$). Furthermore, the smallest areas of adipocytes were noted in the groups supplemented with G, at a dosage of 1,000mg/kg/day (G1) and GL. Our results suggest that GL oil has quality for consumption and can influence lipid profiles, concentration markers and antioxidant status, with G2 and L2 also being effective.

KEYWORDS: Vegetable oils; Fatty acids; Antioxidant.

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1 INTRODUÇÃO

Desde o início das civilizações, os seres humanos utilizam os alimentos como forma de suprir as carências nutricionais diárias. Desse modo, a composição dos alimentos é dividida em macronutrientes, nos quais se enquadram os lipídeos, proteínas e carboidratos, e os micronutrientes definidos pelas vitaminas e minerais, que desempenham funções importantes para o organismo (Popkin; Reardon, 2018). As principais fontes de lipídios da dieta de origem animal são as gemas dos ovos, as carnes e seus derivados e as principais fontes de origem vegetal são os óleos vegetais comestíveis, obtidos através de frutos, sementes, castanhas e grãos (Silva Figueiredo *et al*., 2018; Zabot *et al*., 2022).

Os óleos vegetais extraídos de plantas estão presentes em 2/3 dos produtos alimentícios que fazem parte da alimentação humana (Yara-Varón *et al*., 2017). Sua presença nos métodos de preparo de alimentos e na incorporação de produtos alimentícios industrializados, propõem melhoras organolépticas e maior adesão do consumidor *(*Zabot *et al*., 2022). Geralmente os óleos vegetais são líquidos em temperatura ambiente (~ 25 $^{\circ}$C), por possuírem maior quantidade de ácidos graxos insaturados (Górska-Warsewicz *et al*., 2019). Assim, a qualidade e composição nutricional final de óleo vegetal depende de todas as etapas envolvidas durante sua extração (Redondo-Cuevas; Castellano; Raikos, 2017).

Ao visar uma alimentação saudável, há consenso sobre a preferência pelo consumo moderado de óleos vegetais em comparação às gorduras de origem animal, devido ao fato de que os óleos extraídos de sementes possuem atividade cardioprotetora e reduzem os índices lipídicos sanguíneos, independentemente do método de extração (Izar *et al*., 2021; TEH *et al*., 2019). Além disso, o Brasil está entre os dez maiores produtores de óleos vegetais no mundo, o que proporciona um contexto socioeconômico favorável à sua utilização e comercialização (Camelo; Fontgalland, 2021).

Consequentemente, tem-se conhecimento de que as sementes de uva são ricas em óleo e constituem um subproduto frequentemente descartado no processo de vinificação e produção de sucos (Lebaka *et al*., 2021; Tang *et al*., 2018). Essas sementes apresentam uma composição que varia de 6 à 20% de óleo, sendo este composto predominantemente por ácidos graxos insaturados, com destaque para o ácido graxo linoleico (C18:2) (65-75%), seguido pelo ácido oleico (C18:1) (20-40%), e apenas 10% de ácidos graxos saturados. Esses dados indicam o potencial de consumo e utilização das sementes de uva na alimentação humana (Fernandes *et al*., 2013; Lachman *et al*., 2015; Teh *et al*., 2019).

A agricultura de uvas da espécie *Vitis vinifera* L. possui tradição milenar, com impacto econômico principalmente nas regiões temperadas, como Itália, Espanha, norte da África e

Ásia ocidental (Migicovsky *et al*., 2017; Parihar; Sharma, 2021). A viticultura no Brasil, ocupa aproximadamente 78 mil hectares, com localização estabelecida desde a região sul até regiões próximas ao Equador. A produção do Brasil gera cerca de 1,5 milhões de toneladas de uvas por ano (De Oliveira *et al*., 2019), sendo destinadas ao processamento, como a elaboração de vinhos e sucos, comercialização do fruto e exportação (Górnaś *et al*., 2019).

Além disso, a composição de ácidos graxos do óleo de semente de uva, juntamente com a presença de substâncias bioativas, sugere uma ação anti-inflamatória ao atuar sobre as espécies reativas de oxigênio (ROS) e na redução de marcadores inflamatórios, como a secreção do fator de necrose tumoral alfa (TNF-α), interleucina 1 beta (IL-1β), interleucina 6 (IL-6), entre outras moléculas pró-inflamatórias (Cecchi *et al*., 2019; Han *et al*., 2019).

Outra semente reconhecida pela qualidade da composição do seu óleo é a semente de linhaça (*Linum usitatissimum* L.). Esta possui um alto conteúdo de ácidos graxos poli-insaturados (73%), destacando-se os ácidos alfa-linolênico (C18:3) (50-70%) e linoleico (C18:2) (14-20%), além da presença de ácidos graxos monoinsaturados, com ênfase no ácido oleico (C18:1) (18%), e apenas 9% de ácidos graxos saturados (Garavaglia *et al*., 2016). Além disso, esta semente contém lignanas, fitoestrógenos e fibras solúveis, que possuem aplicações terapêuticas (Bada *et al*., 2015; Lutterodt *et al*., 2011; Tamtaji *et al*., 2020).

A suplementação com óleo de semente de linhaça pode auxiliar no controle dos parâmetros lipídicos séricos, atuando na redução das concentrações de colesterol total (CT), lipoproteína de baixa densidade (LDL-c) e triglicerídeos séricos (TG) (Campos *et al*., 2019). Além disso, contribui para a supressão de danos teciduais com potencial inflamatório, decorrentes do estresse oxidativo, e para a diminuição de complicações cardiovasculares primárias, devido à concentração de ácido graxo α-linolênico (C18:3) (Ambikairajah; Walsh; Cherbuin, 2019; Kanikowska *et al*., 2022).

Ao comparar os tratamentos e prevenções terapêuticas baseadas em intervenções nutricionais com o alto custo e os efeitos adversos causados por medicamentos, torna-se primordial a inserção de alimentos protetores que atuem sobre o metabolismo, promovendo melhorias no estado de saúde-doença (Lyons; Kennedy; Roche, 2016). Assim, é necessário avaliar a biodisponibilidade de substâncias nutracêuticas presentes nos óleos de sementes, considerando os diferentes graus de maturação dos frutos, a qualidade do solo de plantio, o clima, a temperatura, entre outras influências naturais que impactam a composição nutricional das sementes e dos óleos extraídos delas (Kim *et al*., 2013; Martin *et al*., 2020).

Apesar do respaldo científico para o uso e comercialização dos óleos de semente de uva e de linhaça, não há registros na literatura sobre o uso combinado desses óleos.

Individualmente, esses óleos são reconhecidos por seus benefícios à saúde cardiovascular e efeitos anti-inflamatórios, devido às suas composições ricas em ácidos graxos poli-insaturados, antioxidantes e compostos bioativos. A combinação desses óleos pode resultar em um produto com perfis de ácidos graxos complementares, potencializando seus benefícios. Análises de qualidade, incluindo técnicas ópticas e estabilidade, são essenciais para avaliar a qualidade nutricional e o comportamento dessa mistura em comparação com os óleos individuais (Cao, 2019; Falade; Oboh; Okoh, 2017; Mirabelli *et al*., 2020; Pons *et al*., 2017).

Portanto, é necessário avaliar os óleos de semente de uva, de semente de linhaça e a mistura composta por ambos em proporção igual 1:1 (v/v) em relação às suas propriedades nutricionais, físicas e químicas. Além disso, é fundamental caracterizar e compreender o comportamento dos compostos presentes, suas vias de ação e os efeitos *in vivo*. Essa avaliação permitirá quantificar as melhores condições para a utilização desses óleos, promovendo o desenvolvimento econômico, nutritivo e tecnológico (Cao, 2019; Górska-Warsewicz *et al*., 2019; Mirabelli *et al*., 2020).

2 REVISÃO DE LITERATURA

2.1 Contexto da utilização de óleos vegetais

Óleos e gorduras vegetais extraídos de partes de plantas, como sementes, grãos e polpas de frutas, são compostos principalmente por triacilgliceróis, que consistem em três moléculas de ácidos graxos esterificadas com uma molécula de glicerol (Indelicato *et al*., 2017). Além disso, esses óleos contêm ácidos graxos livres, monoacilgliceróis e diacilgliceróis, bem como outros compostos menores, como esteróis, fosfolipídios, vitaminas lipossolúveis, pigmentos e minerais (Savva; Kafatos, 2016).

Os lipídeos, juntamente com proteínas e carboidratos, são macronutrientes que desempenham funções essenciais no organismo (Popkin; Reardon, 2018). Entre as formas de obtenção de lipídeos através da alimentação, os óleos vegetais se destacam pela maior presença de ácidos graxos monoinsaturados e poli-insaturados, caracterizando-se por serem líquidos à temperatura ambiente (Górska-Warsewicz *et al*., 2019).

Os óleos vegetais são substâncias hidrofóbicas amplamente utilizadas em diversos setores industriais, incluindo as indústrias química, farmacêutica, cosmética e alimentícia (Marcelino *et al*., 2022). A maioria dos óleos vegetais comestíveis derivados de sementes contém ácidos graxos monoinsaturados, que possuem apenas uma dupla ligação, sendo o ácido oleico (C18:1) o mais prevalente. Além disso, esses óleos contêm ácidos graxos poli-insaturados, que podem apresentar de duas a seis duplas ligações, sendo os principais o ácido linoleico (C18:2) e o ácido linolênico (C18:3). Em menor proporção, encontram-se os ácidos graxos saturados, que não possuem ligações duplas em suas moléculas, sendo os mais comuns o ácido palmítico (C16:0) e o ácido esteárico (C18:0) (Asefy *et al*., 2021; Parihar; Sharma, 2021).

A composição de ácidos graxos dos óleos vegetais é influenciada por diversos fatores, incluindo variações genéticas, manejo climático, condições do solo e período de plantio, além do tipo de processamento e grau de refino (Marcelino *et al*., 2022). Os compostos isolados de óleos vegetais, como fenóis, fitoesteróis, tocoferóis e carotenoides, podem ser utilizados como suplementos e ingredientes alimentares funcionais, com potencial para promover a saúde e aumentar a ingestão de componentes antioxidantes. Esses compostos são considerados seguros tanto para o ser humano, quanto para o meio ambiente (Falade; Oboh; Okoh, 2017; Pons *et al*., 2017).

Dessa maneira, os principais métodos de extração de um óleo vegetal são a extração por solvente e a prensagem a frio. A extração por solvente propõe maior economia e rendimento, sendo priorizada quando há baixo teor de lipídeos na matéria prima. Por este

método ocorre a separação por solvente orgânico (como o *n*-hexano), dando origem ao óleo vegetal refinado (Cravotto *et al*., 2022). O método de prensagem é utilizado quando a matéria-prima possui alto teor de lipídeos, permitindo a extração do óleo ao passar pela prensa. Esse método demanda mais tempo e uma maior quantidade de matéria-prima para produzir o óleo. No entanto, a vantagem é que o óleo não passa pelo processo de refinamento nem é submetido a outras substâncias químicas, apresentando-se como uma alternativa mais sustentável (Occhiuto *et al*., 2022).

O mercado de óleos comestíveis prensados a frio está em constante expansão devido a variedade de plantas e partes de plantas das quais é possível fazer a extração do óleo. Alguns destes óleos, são populares como o óleo de cânhamo, óleo de prímula e óleo de semente de linhaça. Estima-se que o cenário global de óleos prensados a frio atinjam valores monetários comercializados de US$ 36,40 bilhões até 2026, em comparação a US$ 24,62 bilhões em 2018, sendo atribuído a crescente busca pelos consumidores de alimentos que sejam ricos em compostos antioxidantes e bioativos (Roy *et al*., 2023).

Os óleos prensados a frio, em contraste com os óleos refinados, preservam uma maior quantidade de substâncias como compostos fenólicos, fitoesteróis, tocoferóis e carotenoides em sua composição (Durazzo; Fawzy Ramadan; Lucarini, 2022). Além disso, o método de prensagem a frio não requer insumos de alta energia nem a adição de produtos químicos, o que o torna um método sustentável. Por meio deste processo, é possível realizar a purificação por sedimentação, filtração e centrifugação (Grajzer *et al*., 2020).

Contudo, um fator que limita o amplo uso da prensagem a frio é a baixa eficiência do processo e a falta de padronização dos resultados dentro dos parâmetros estabelecidos. As características da matéria-prima são influenciadas pela variedade da subespécie, grau de maturação das sementes, condições climáticas, tratamentos agrotécnicos, condições de transporte e armazenamento, umidade dos grãos, limpeza e ausência de danos aos grãos, resultando em variações nas características físico-químicas do óleo extraído (Gaca *et al*., 2021; Prescha *et al*., 2014).

Somado a isso, a estabilidade oxidativa de óleos prensados a frio é menor do que os óleos provenientes de refino (Grajzer *et al*., 2020). Tal fato acontece, devido a remoção de produtos pró-oxidantes durante o refino (por exemplo, metais, corantes de clorofila e produtos de oxidação), que resultam em maior estabilidade oxidativa desses óleos (Gaca *et al*., 2021). O alto teor de compostos bioativos nos óleos prensados a frio demonstram ação como alimentos funcionais, pois possuem efeitos benéficos á saúde, além de suas propriedades nutricionais (Boskou, 2017).

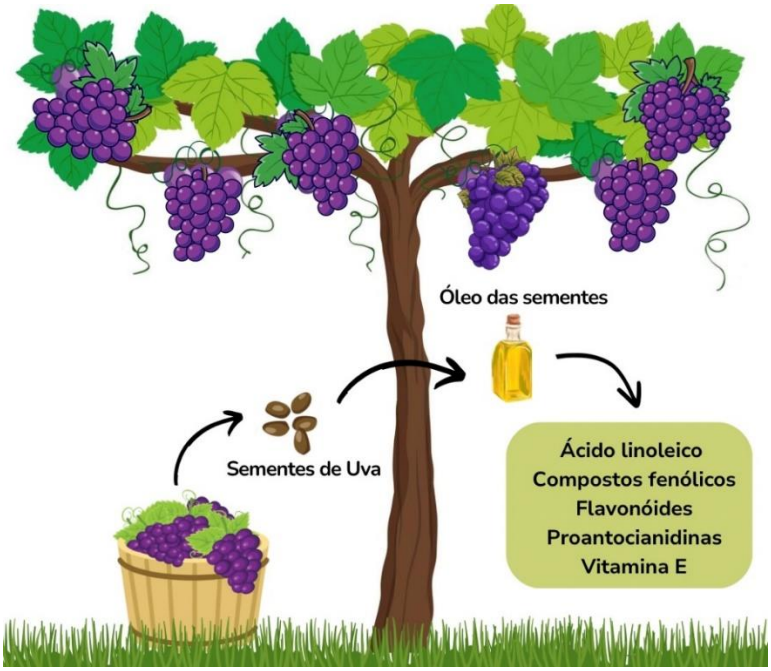
Somado a isso, grande parte das sementes que são descartadas tanto pela indústria, quanto pela população em geral, possuem alta quantidade de óleo. Sendo possível realizar a extração (por prensagem a frio) para o aproveitamento integral dos alimentos, promovendo a utilização pela indústria para elaboração de produtos alimentícios, cosméticos e farmacológicos à população (Karaman *et al*., 2015; Lau; Sabran; Shafie, 2021). Assim, essas sementes, juntamente com talos e cascas são classificados como resíduos alimentares e não são aproveitados, desprezando a presença de fitoquímicos com potenciais antimicrobianos e antioxidantes, que poderiam auxiliar no processo saúde-doença e para um consumo sustentável (Conrad *et al.*, 2018; Tang *et al*., 2021).

Desse modo, a utilização de óleos vegetais com propriedades antioxidantes, antitumorais e antidiabéticas tem sido amplamente aplicada à saúde humana (Brahmi *et al*., 2022; Periasamy; Athinarayanan; Alshatwi, 2016). Assim, a utilização de óleos ricos em substâncias bioativas visa agregar qualidade nutricional e aumentar o tempo de prateleira dos produtos, superando desafios técnicos como a baixa estabilidade desses compostos (Donsì; Ferrari, 2016).

2.2 Óleo de semente de uva

As uvas da espécie *Vitis vinifera* L., produzidas pela videira (*Vitis* sp.), são cultivadas tanto para a produção de vinhos quanto para o consumo in natura. A produção dessas uvas possui especificações de acordo com as subespécies dos frutos, como a presença ou ausência de sementes, diferentes colorações (avermelhada, preta e branca), além de variações nos tamanhos e formatos dos frutos (Arroyo-García *et al*., 2006). De igual modo, as propriedades nutricionais são singulares a cada subespécie. Além disso, outras influências de origem natural, como o grau de maturação dos frutos, a qualidade nutricional do solo de plantio e o clima, impactam diretamente na qualidade nutricional e na presença de compostos bioativos nas sementes de uva e no óleo extraído delas (Figura 1) (Kim *et al*., 2013; Martin *et al*., 2020).

Figura 1 - Óleo de semente de Uva, origem e componentes bioativos



Fonte: Autoria própria

As sementes das uvas possuem diversas substâncias bioativas, como polifenois, terpenoides, fitoesterois, entre outros compostos (Lee *et al*., 2016; Sławińska; Prochoń; Olas, 2023). Somado a isso, devido ao seu perfil químico, as sementes de uva, bem como óleo extraído delas apresenta potencial de aplicação na indústria farmacêutica, cosmética e alimentícia, sendo possível o desenvolvimento de suplementos alimentares e cosméticos (Cecchi *et al*., 2019; Ma; Zhang, 2017).

O óleo de semente de uva representa de 6-20% da composição da semente e possui constituintes lipofílicos, como alta quantidade de flavonoides, teor de fenóis totais 10 vezes maior do que na casca (Tang *et al*., 2018), vitamina E e fitoesterois, que proporcionam propriedades antioxidantes ao óleo (KARAMAN *et al*., 2015). Sendo assim, a composição do óleo extraído da semente de uva possui em média 90% de ácidos graxos insaturados, sendo os principais o ácido graxo linoleico (C18:2) (65–75%), ácido graxo oleico (C18:1) (20–40%) e cerca de 10% de ácidos graxos saturados (Lau; Sabran; Shafie, 2021; Tang *et al*., 2018).

O óleo de semente de uva extraído por meio da prensagem a frio tem a capacidade de preservar compostos que previnem o estresse oxidativo, como β-caroteno, γ-caroteno, fitoesteróis, tocoferóis e procianidinas, os quais indicam a atividade antioxidante do óleo. Assim, em comparação com as bagas e extratos alcoólicos, o óleo de semente de uva é capaz

de preservar as moléculas lipofílicas e biocompostos (Goufo; Singh; Cortez, 2020; Han *et al*., 2019).

Os principais grupos de substâncias bioativas de caráter lipídico presentes no óleo de semente de uva são os isômeros da vitamina E (até 50 mg por 100 g de óleo) e o teor de fitosterol, com destaque para os tocotrienóis. Essas substâncias demonstram notável atividade antioxidante e ação protetora para a saúde humana. No entanto, sua concentração é diretamente influenciada pelo grau de maturação das uvas e bagas, sendo que maiores concentrações de vitamina E são observadas em frutos mais maduros, e a presença de tocotrienóis aumenta com o crescimento das sementes (Górnaś *et al*., 2019).

Em relação aos tocotrienóis, os isômeros gama são os mais abundantes, atuando como constituintes antioxidantes que devem ser consumidos pela dieta, uma vez que os mamíferos não possuem produção endógena desses compostos. Além disso, sabe-se que todas as formas de tocoferol são suscetíveis à degradação por exposição à luz e ao ar, o que representa um obstáculo tanto para os processos de extração do óleo quanto para sua quantificação por meio de análises (Dos Santos Freitas *et al*., 2008).

Assim, a presença de ácido gálico, catequina, epicatequina, procianidinas e proantocianidinas presentes nas sementes e no óleo de semente de uva promovem função antioxidante. O mecanismo biológico antioxidante está associado à remoção de radicais livres, como o radical hidroxila e quelação de metais, que influenciam na redução de ROS e inibem a oxidação lipídica (Rodríguez *et al*., 2022).

As substâncias antioxidantes presentes no óleo de semente de uva atuam na modulação da expressão gênica anti-inflamatória, como nas vias celulares de liberação de ácido araquidônico, produção de citocinas e atividade da óxido nítrico sintase (NO) (Zarev *et al*., 2023). Os tocotrienois, isômeros da vitamina E, podem diminuir a inflamação adiposa relacionada à obesidade em linhagens celulares (Zhao *et al*., 2015).

As condições inflamatórias crônicas afetam a resistência à insulina ao promoverem a degradação da proteína quinase ativada por mitógeno (AMPK), resultado à redução da captação de glicose pelas células musculares e adiposas e na liberação de moduladores pró-inflamatórios por macrófagos estimulados por LDL-c, estresse oxidativo e síntese de eicosanoides (Mattos *et al*., 2017; Pasini *et al*., 2019). Visto isso, estudos apresentam o efeito do óleo de semente de uva evitando a degradação da AMPK, devido à presença de tocotrienois, e diminuindo os níveis de LDL-c, atenuando o processo inflamatório (Bagherniya *et al*., 2022; Lai *et al*., 2014; Shinagawa *et al*., 2017; Vilahur *et al*., 2019).

O ácido graxo linoleico, abundante no óleo de semente de uva, possui ação neuroprotetora (Berahmand *et al*., 2020), e propõe efeito anti-inflamatório em células de mamíferos (KOLAR *et al*., 2019). O óleo de semente de uva também apresenta efeito hepatoprotetor, pela presença de tocotrienois, que reduzem a peroxidação lipídica e promovem maior integridade da membrana plasmática, levando à diminuição da inflamação, apoptose e fragmentação do ácido desoxirribonucleico (DNA) (Ismail; Moawed; Mohamed, 2015; Ismail; Salem; Eassawy, 2016).

O potencial cardioprotetor do óleo de semente de uva pode ser justificado pela redução *in vitro* da adesão plaquetária e pela melhora dos perfis lipídicos sanguíneos em animais (Di Pietro Fernandes *et al*., 2023) incluindo a redução dos níveis de colesterol total (CT) e LDL-c (Mattos *et al*., 2017; Pasini *et al*., 2019; Wall-Medrano *et al*., 2017; Wang; Liu; Wang, 2023). Além disso, o ácido linoleico, abundante no óleo de semente de uva, contribui para a saúde cardiovascular em experimentos animais, demonstrando potencial como suplemento alimentar (Garavaglia *et al*., 2016; Kolar *et al*., 2019; Teh *et al*., 2019). No entanto, o efeito do óleo de semente de uva no perfil lipídico humano ainda requer mais pesquisas.

O óleo de semente de uva desperta interesse alimentício, pois seu uso culinário a frio não afeta as propriedades sensoriais dos alimentos e apresenta um sabor discreto. O interesse da indústria alimentícia por óleos vegetais tem impulsionado o desenvolvimento de novos produtos com maior qualidade nutricional. Um exemplo disso são os produtos à base de carne e salsichas, nos quais o óleo de semente de uva é adicionado em substituição à gordura animal (Kim *et al*., 2020; Lee *et al*., 2016).

Tanto em animais quanto em humanos, o óleo de semente de uva apresenta atividade cicatrizante (Al-Warhi *et al*., 2022). Na indústria de cosméticos, este óleo é utilizado como ingrediente em produtos hidratantes para a pele, uma vez que a perda de ácido linoleico cutâneo está correlacionada à perda de água pela epiderme. Assim, a aplicação regular de cosméticos contendo óleo de semente de uva, isoladamente ou misturado com outros óleos vegetais, é recomendada para olheiras e alopecia androgenética (Cho *et al*., 2014).

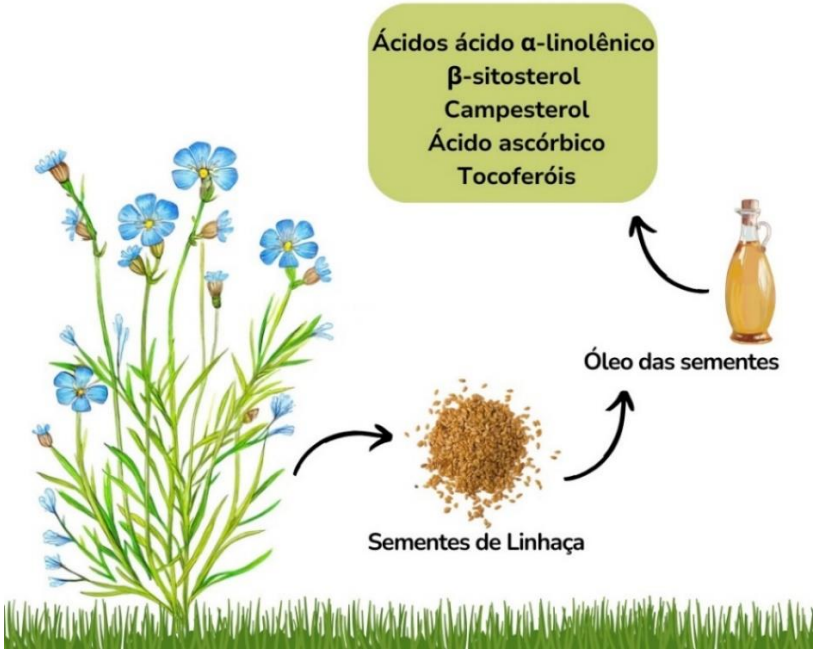
Além disso, o óleo de semente de uva mostra-se um competidor promissor para fontes fósseis de combustível devido à presença de ácidos graxos insaturados. A produção de biodiesel, incluindo o refino após a extração, é uma alternativa econômica e ambientalmente correta em regiões com altos volumes de produção de vinho (Hariram; Bose; Seralathan, 2019). Sugere-se o aproveitamento integral das uvas a partir do reaproveitamento das partes não comestíveis, visto que a alta quantidade de sementes descartadas em processos industriais

demonstra um elevado potencial de aplicação do óleo de semente de uva (Karaman *et al*., 2015).

2.3 Óleo de semente de linhaça

A semente de linhaça (*Linum usitatissimum* L.), pertencente à família Linaceae, é cultivada para o aproveitamento da fibra (linho), para o consumo integral da semente e para a extração do óleo da semente (Figura 2). O linho é uma das plantas mais antigas domesticadas, cujo manejo tinha como objetivo principal a confecção de roupas e objetos. Apesar da continuidade de seu cultivo, os métodos de cultivo e extração do óleo e das fibras foram aprimorados, e a morfologia da planta evoluiu. Assim, o linho utilizado para a extração do óleo é mais baixo, com cápsulas maiores e maior quantidade de ramos, em comparação àqueles destinados ao uso da fibra. Dessa forma, a adaptação das sementes contribuiu para seu amplo cultivo em regiões que variam de moderadamente frias a moderadamente quentes (Heller *et al*., 2015).

Figura 2 - Óleo de semente de linhaça, origem e componentes bioativos



Fonte: Autoria própria

Os maiores produtores de linhaça são Canadá, China, Índia, Estados Unidos e Rússia. A produção mundial de semente de linhaça varia entre 2.300.000 e 2.500.000 toneladas anuais, sendo o Canadá o principal produtor (Siva Kumar *et al*., 2017). A semente de linhaça

contém aproximadamente 35-45% de óleo, com mais de 60% de ácido alfa-linolênico, cerca de 20% de ácidos graxos monoinsaturados, principalmente ácido oleico, e apenas 9-10% de ácidos graxos saturados, destacando-se os ácidos palmítico (C16:0) e esteárico (C18:0) (Martinchik *et al*., 2012).

O teor de proteína nas sementes de linhaça varia de 20-30%, com destaque para o aminoácido lisina, que apresenta alta digestibilidade. O teor de fibra alimentar corresponde a 20% do peso das sementes inteiras de linhaça. A linhaça também possui uma alta concentração de lignanas (0,7-1,5% do peso seco da semente), com destaque ao secoisolariciresinol diglucosídeo (Martinchik *et al*., 2012). Estas, presentes na semente de linhaça exercem ação metabólica em parâmetros séricos sanguíneos, promovendo a redução do colesterol total (CT) e do LDL-c, além de possibilitar o aumento da lipoproteína de alta densidade (HDL-c). Dessa forma, juntamente com os fitoestrógenos e outras fibras solúveis, as lignanas possuem aplicação terapêutica (Bada *et al*., 2015).

Assim, o ácido alfa-linolênico, polifenóis e lignanas possuem propriedades fitoestrogênicas e promovem ações hipolipidêmicas e antiaterogênicas (Mirshekar; Dastar; Shams Shargh, 2021). As formas de ingestão incluem a semente de linhaça inteira, a semente de linhaça triturada, o óleo de linhaça e a farinha de linhaça parcialmente desengordurada (Parikh; Netticadan; Pierce, 2018). Além disso, vegetarianos e veganos frequentemente consomem o óleo de linhaça como alternativa ao óleo de peixe, devido ao seu rico conteúdo de ômega-3 (Rabail *et al*., 2021; Yadav *et al*., 2018).

O processamento das sementes, a temperatura e a duração do armazenamento influenciam diretamente na estabilidade do produto. A moagem ou esmagamento da semente de linhaça expõe o ácido alfa-linolênico a possíveis eventos oxidativos (Edel; Aliani; Pierce, 2015). Contudo, esse processo é necessário para tornar essas substâncias biodisponíveis, uma vez que o tegumento que reveste a semente impede a absorção de alfa-linolênico (Austria *et al*., 2008), sendo este mais biodisponível na forma de óleo (Edel; Aliani; Pierce, 2015) ou na forma moída (Austria *et al*., 2008). O armazenamento do óleo de linhaça em temperaturas mais baixas e sem exposição à luz, promove uma maior preservação do ácido alfa-linolênico contra possíveis degradações (Edel; Aliani; Pierce, 2015).

O papel anti-inflamatório do óleo de semente de linhaça ocorre pela presença de ácido α-linolênico e linoleico, estes competem pelas mesmas enzimas para sintetizar tanto o ácido araquidônico (C20:4), como os ácidos eicosapentaenoico (EPA) (C20:5) e ácido eicosatrienoico (ETA) (C20:3). Somado a isso, o ácido α-linolênico possui como derivado, o ácido docosaexaenoico (DHA) (C22:6), sendo uma das classes de lipídeos essenciais para a

síntese de eicosanoides juntamente com EPA (C20:5) e ácido araquidônico (C20:4). Os eicosanoides estão entre os principais mediadores e reguladores da inflamação por atuarem na ativação de prostaglandinas (PG), leucotrienos (LT), tromboxanos e outros derivados oxidados, produzidos pelas vias das enzimas cicloxigenase (COX) e lipoxigenase (LOX) (Borges *et al*., 2014).

A alta produção de eicosanoides possui correlação à distúrbios imunológicos, doenças cardiovasculares e inflamatórias. Assim, a ação anti-inflamatória e metabólica de ácidos graxos da família ω-3, como o ácido α-linolênico, EPA e DHA, são justificadas pela inibição competitiva à oxidação do ácido araquidônico pela COX para prostaglandinas e a conversão para leucotrienos (LT), pela via 5-lipoxigenase LOX (Borges *et al*., 2014).

Estudos *in vitro* indicam que os ácidos EPA e DHA contribuem para a inibição da produção de citocinas inflamatórias, como TNF-α, IL-1β e IL-6, entre outras (Cecchi *et al*., 2019; Rabail *et al*., 2021). A ação anti-inflamatória desses ácidos graxos, incluindo o ácido α-linolênico, pode ser explicada pelo fato de atuarem como precursores de mediadores lipídicos denominados resolvinas e protectinas, que inibem a migração transendotelial de neutrófilos, prevenindo a infiltração dessas células imunológicas no local da inflamação. Dessa forma, esses mediadores lipídicos podem atenuar a inflamação e limitar os danos causados aos tecidos (Vidar Hansen; Serhan, 2022; Yadav *et al*., 2018).

Os efeitos metabólicos do óleo de semente de linhaça em ratos espontaneamente hipertensos demonstraram uma diminuição da pressão arterial em condições agudas e crônicas (Udenigwe *et al*., 2012). O ácido alfa-linolênico presente no óleo de semente de linhaça desempenha um papel anti-inflamatório (Mali *et al*., 2019) e potencializa a ação antitumoral de drogas utilizadas na terapia oncológica. Dessa forma, o ácido alfa-linolênico atua sobre o receptor 2 do fator de crescimento epidérmico, potencializando os efeitos antitumorais (Calado *et al*., 2018; Mali *et al*., 2019).

Sabe-se que indivíduos mais velhos apresentam maiores concentrações de oxilipinas pró-inflamatórias, incluindo 5-HETE, 9,10,13-TriHOME e 9,12-13-TriHOME, que estão correlacionadas à inflamação crônica. Dessa forma, a suplementação dietética com óleo de semente de linhaça demonstrou corrigir o equilíbrio entre oxilipinas pró-inflamatórias e anti-inflamatórias, evidenciando um efeito benéfico sobre o envelhecimento (Caligiuri *et al*., 2014).

O consumo de óleo de linhaça na dieta foi capaz de reduzir a presença de *Proteobacteria* e *Porphyromonadaceae* no microbioma intestinal, demonstrando um impacto positivo na doença hepática alcoólica, visto que alterações em bactérias específicas

no microbioma indicam uma melhora na saúde intestinal (Zhang et al., 2017). Além disso, o óleo de linhaça mostrou-se benéfico na redução da gastroenterite (Hanif Palla; Gilani, 2015; Power et al., 2016), atuando nos receptores muscarínicos intestinais e canais de potássio (Pulkrabek *et al*., 2017).

O óleo de semente de linhaça, proveniente de variedades como Bekoji, Belaye-96, Berene, Chilalo, Ci-1525, Ci-1652, Kassa-2 e Tole, apresenta variações de 55,7% a 60,1% de ácidos linolênicos (C18:3), 13,3% a 16,6% de ácido linoleico (C18:2), 15,9% a 20,3% de ácido oleico (C18:1) e 4,9% a 8,1% de ácido palmítico (C16:0) (Deme *et al*., 2021). Essas variações indicam que diferentes espécies de sementes, bem como as características relacionadas ao cultivo, influenciam diretamente as propriedades nutricionais dos óleos. A cor dessas sementes também é determinada pela quantidade de pigmentos presentes no revestimento externo (Lutterodt *et al*., 2011)

O óleo de linhaça também é obtido a partir da prensagem a frio das sementes, sendo o óleo vegetal encontrado na natureza com maior concentração de ácido graxo alfa-linolênico. Este, é considerado essencial para o consumo humano, pois não pode ser sintetizado e deve ser obtido pela alimentação (Al Juhaimi *et al*., 2017). Apesar de conter quantidades elevadas de ácido graxo alfa-linolênico, este óleo possui baixa estabilidade ao calor, não sendo indicado para a cocção de alimentos ou processos industriais com temperaturas elevadas (Joshi; Hegde; Zanwar, 2022).

Devido a isso, confere-se ao óleo propriedades protetoras à saúde, especialmente quanto às doenças cardiovasculares, por auxiliar na prevenção da aterioscleroses, diminuindo a produção de células espumosas e mediadores inflamatórios, tais como IL-1β, IL-6, IL-10, TNF-α (Pulkrabek *et al*., 2017). Assim, o óleo de semente de linhaça assume grande importância na nutrição humana devido ao seu potencial de redução do CT, LDL-c e triglicerídeos, por meio de sua ação antioxidante, sendo efetiva na redução do estresse oxidativo (Garavaglia *et al*., 2016).

O óleo de linhaça possui efeitos variáveis sobre marcadores inflamatórios, como as interleucinas IL-2, IL-4, IL-6, IL-10, TNF-α, interferon-gama (IFN-γ) E proteína C reativa, devido a supressão da produção de radicais de oxigênio pelos glóbulos brancos (Mahmudiono *et al*., 2022). O óleo de semente de linhaça também diminui a molécula 1 de adesão celular vascular solúvel e apresenta atenuação da agregação plaquetária, demonstrando possível ação contra a aterioscleroses (Rubilar *et al*., 2010; Yang *et al*., 2023).

O óleo de semente de linhaça apresenta resultados promissores para a saúde cardiovascular, além de auxiliar no tratamento de câncer e na saúde gastrointestinal (Parikh *et*

al., 2023). Além do ácido graxo alfa-linolênico, o óleo de semente de linhaça contém mono, di e tri-terpenos, como β-Sesquifelandreno, campesterol, ácido hexadecanoico, esqualeno e β-Sitosterol, bem como ácido ascórbico e gama-tocoferol. Esses compostos possuem funções antioxidantes importantes que auxiliam no diabetes mellitus tipo II (DM2), reduzindo a inflamação crônica de baixo grau e promovendo maior estabilidade glicêmica em pacientes diabéticos (Zhu *et al*., 2020). Além disso, o óleo de semente de linhaça promove proteção cardiovascular, antitumoral e anti-inflamatória (Tang *et al*., 2021; Zhu *et al*., 2020).

2.4 Ação dos ácidos graxos no organismo

Os óleos vegetais, após serem metabolizados, podem ser armazenados como reserva de energia, influenciam a composição lipídica sanguínea, compõem os fosfolipídios das membranas celulares e apresentam sinalização específica para interações hormonais (Calder, 2015). Dessa forma, a utilização dos lipídeos pelo organismo é influenciada pela quantidade e perfil de ácidos graxos ingeridos pela dieta, bem como pela produção endógena, que possui caráter e predisposição genética (Rezende *et al*., 2021).

Os óleos vegetais comestíveis possuem grande potencial nutricional, além de atributos sensoriais de cor, sabor e aroma, com importância na indústria agrícola e tecnológica (Campos *et al*., 2019). As substâncias fenólicas e bioativas (como as vitaminas e derivados de vitaminas), são denominadas de antioxidantes vegetais, e agem no organismo como compostos quimiopreventivos capazes de auxiliar no tratamento de doenças induzidas por radicais livres, incluindo câncer, diabetes e doenças neurodegenerativas (Ali *et al*., 2020). O mecanismo de defesa antioxidante dos compostos fenólicos e vitaminas, envolve atividade de sequestrador de radicais livres e proteção contra peroxidação lipídica, somado a quelação de metais tóxicos (Miller, 2020).

Assim, os ácidos graxos oriundos da alimentação podem contribuir a maior formação de HDL-c auxiliando a troca do colesterol por triglicérides. Desse modo, possibilitam maior transporte reverso para o fígado, reduzindo a formação de LDL-c a partir da lipoproteína de muito baixa densidade (VLDL-c), que carrega os triglicerídeos para os tecidos, como fígado e os músculos (Cinque *et al*., 2023). Os óleos vegetais comestíveis possuem intensa atividade biológica, por possuírem compostos antioxidantes, com funções metabólicas, como ação de veículo de absorção de vitaminas lipossolúveis (A, D, E e K) e participação na produção de hormônios como cortisol, estrógeno, progesterona, testosterona *(*Mirabelli *et al*., 2020).

As vitaminas lipossolúveis e os compostos antioxidantes, como os polifenóis, possuem ação metabólica e hepática, auxiliando como cofatores de vias endógenas que reduzem o

estresse oxidativo e a inflamação por melhora dos marcadores hepáticos e citocinas inflamatórias como IL-6, TNF-α e IL-1β (Dias *et al*., 2022; Laffin *et al*., 2023).

Estudos sugerem que a dieta mediterrânea pode auxiliar na redução da esteatose hepática não alcoólica e em doenças crônicas não transmissíveis, promovendo a prevenção de doenças cardiovasculares e metabólicas, como diabetes tipo 2, doenças inflamatórias e câncer (Calder, 2015; Mirabelli *et al*., 2020). Essa dieta é caracterizada por baixos níveis de bebidas açucaradas, açúcares refinados e carne vermelha, além do maior consumo de ácidos graxos mono e poli-insaturados em relação aos ácidos graxos saturados, quando comparada à dieta ocidental (Rad; Mousavi; Chiti, 2023; Venn, 2020).

Em relação aos ácidos graxos saturados, o ácido mirístico (C14:0) é encontrado na maioria das gorduras animais e em óleos vegetais, sua ação endógena pode contribuir ao aumento do CT plasmático e LDL-c, devido as ligações simples da molécula (Saraswathi *et al*., 2022). O mesmo ocorre com o ácido palmítico (C16:0), amplamente encontrado em óleos vegetais, principalmente nos óleos de sementes, que também pode atuar como precursor dos ácidos graxos naturais saturados e insaturados de cadeia mais longa (Ghasemi; Golabadi; Piadeh, 2021; Park *et al*., 2019).

Somado a isso, o ácido esteárico (C18:0) é menos prevalente no perfil de ácidos graxos de óleos de sementes em comparação com o ácido palmítico (C16:0), embora também esteja presente em óleos vegetais derivados de polpas de frutas. A ingestão de ácido esteárico pode contribuir para a redução dos níveis de colesterol total, uma vez que é utilizado na síntese de fosfolipídios de membrana ou convertido em ácido oleico no fígado *(*Afarani *et al*., 2023).

O ácido oleico (C18:1) é o principal ácido monoinsaturado presente em óleos vegetais (Martín-Reyes *et al*., 2023). Devido à sua ampla distribuição nos alimentos, está presente tanto na alimentação animal quanto na humana. O consumo de ácidos monoinsaturados, como os derivados do ácido oleico, pode estar associado à melhora do perfil lipídico, incluindo a diminuição dos níveis de LDL-c e o aumento de HDL-c. Além disso, o ácido oleico (C18:1) desempenha um papel na redução da adrenoleucodistrofia (ALD), uma condição que afeta o cérebro e as glândulas adrenais (Dobrzyńska; Przysławski, 2020).

Assim em relação os ácidos graxos poli-insaturados, o ácido linoleico (C18:2) é a fração majoritária na maioria dos perfis de ácidos graxos de óleos vegetais oriundos de sementes e oleaginosas. Anteriormente, o ácido linoleico (C18:2) era considerado apenas precursor do ácido araquidônico (C20:4) no organismo humano, contudo, atualmente

evidencia-se benefícios de sua ingestão, como efeito hipocolesterolêmico e redução dos níveis de lipoproteínas, como a LDL-c (Martin *et al*., 2020).

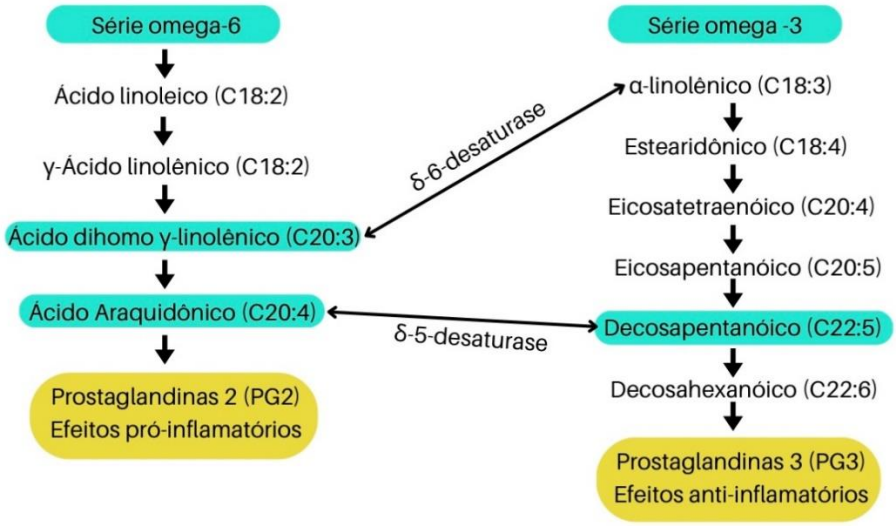
O ácido araquidônico (C20:4) é um precursor de prostaglandinas, prostaciclinas e eicosanoides das séries 2 e 4, como PGE2, tromboxano A2 (TXA2) e leucotrieno B4 (LTB4). Os eicosanoides, especialmente o PGE2, são responsáveis por induzir o aumento da permeabilidade vascular, vasodilatação, hiperemia e hiperalgesia. O TXA2, por sua vez, promove a síntese de citocinas inflamatórias, como IL-1β e TNF-α, por fagócitos mononucleares. No entanto, o ácido docosa-hexaenoico (DHA) atua inibindo a síntese de eicosanoides a partir do ômega 6, competindo com a oxidação do ácido araquidônico pela ciclooxigenase (COX) para a produção de prostaglandinas e sua conversão em leucotrienos pela via da 5-lipoxigenase, reduzindo assim a liberação de ácido araquidônico na membrana celular (Lutz; Cornett, 2013).

Os ácidos graxos poli-insaturados da família ômega-6 são amplamente encontrados em alimentos como óleos de milho e girassol, carnes, ovos e leite, e desempenham um papel crucial em várias vias metabólicas essenciais para o crescimento e desenvolvimento do organismo. Os benefícios desses ácidos graxos estão diretamente relacionados à quantidade adequada ingerida. No entanto, o consumo excessivo de ácidos graxos ômega-6, especialmente proveniente de produtos industrializados típicos da dieta ocidental, pode promover um desequilíbrio nos processos inflamatórios (Poli; Agostoni; Visioli, 2023).

O ácido α-Linolênico (C18:3) também é um ácido graxo poli-insaturado, e possui ações metabólicas de manutenção da pressão arterial e diminuição da agregação plaquetária, pela redução dos níveis de CT, triglicérideos e LDL-c, somado a diminuição de citocinas pró inflamatórias devido ao fato do ácido alfa-linolênico atuar como mensageiro e regulador da expressão gênica (Calder, 2015; Salman; Salman; Yildiz, 2022; Yang *et al*., 2018).

A conversão de ácido graxo linoleico (18:2) em ácido araquidônico (20:4) e de ácido graxo α-linolênico (18:3) em ácidos eicosapentaenoicos (20:3) no organismo humano ocorre pelas mesmas enzimas. Portanto, a maior presença desses ácidos graxos no organismo está relacionada à disponibilidade de substrato (Figura 3) (Beyer *et al*., 2023). O ácido graxo linoleico (18:2) atua como substrato para a síntese de ácido γ-linolênico (18:3) e ácido dihomo-γ-linolênico (20:3), que são incorporados aos fosfolipídios das membranas celulares de maneira semelhante (Beyer *et al*., 2023; Walker *et al*., 2015).

Figura 3 - Conversão metabólica dos ácidos graxos



Fonte: Autoria própria

A substituição de parte dos ácidos graxos saturados da dieta, por ácidos graxos insaturados, pontua os óleos vegetais como alternativa às gorduras animais, para manutenção do estado de saúde e prevenção de doenças crônicas não transmissíveis (Fernandes *et al*., 2013). Os óleos vegetais extraídos de sementes, independente do método de extração, melhoram a atividade cardioprotetora e atuam diminuindo as frações lipídicas sanguíneas, por auxiliarem como coadjuvante de reações enzimáticas anti-inflamatórias e possuírem perfil de ácidos graxos benéficos ao organismo quando comparado as gorduras de origem animal (Izar *et al*., 2021; Teh *et al*., 2019).

Considerando os aspectos fisiológicos, o colesterol atua como um constituinte essencial das membranas celulares, promovendo fluidez e ativação de enzimas de membrana, além de ser um precursor dos ácidos biliares, hormônios esteroides e participar na ativação da vitamina D (Marcelino *et al*., 2019). Dessa forma, uma dieta inadequada, rica em ácidos graxos saturados e açúcares refinados, aliada ao sedentarismo, pode levar à diminuição dos níveis de HDL-c, o que é justificado pelo estado inflamatório crônico de baixo grau (Kontush, 2020).

Dessa forma, as alterações estruturais da molécula de HDL-c ocorrem devido à diminuição do colesterol éster, juntamente com o aumento do colesterol livre, triglicerídeos e ácidos graxos livres. Um dos possíveis mecanismos para a diminuição da HDL-c é a redução da síntese de apolipoproteína A-I e o aumento da produção sérica de amiloide A (SAA) no fígado. A SAA, ao se ligar ao HDL-c, desloca a apolipoproteína A-I (Popeijus *et al*., 2021).

Além disso, a ação enzimática da fosfolipase A2 secretora (sPLA2) e da lipase das células endoteliais, induzidas por citocinas, também reduz a estabilidade e o metabolismo do HDL-c (Di Pietro Fernandes *et al*., 2023).

Os ácidos graxos mono e poli-insaturados adquiridos pela alimentação desempenham um papel crucial na responsividade celular e tecidual, regulando vias de sinalização antioxidantes e modulando processos inflamatórios (Oppedisano *et al*., 2020; Sakai *et al*., 2017). Dessa forma, eles exercem uma função essencial na saúde humana, incluindo a prevenção terapêutica de diversas doenças (Djuricic; Calder, 2021). As propriedades físico-químicas, a caracterização e o comportamento desses compostos influenciam as vias de ação no organismo, resultando em efeitos distintos sobre o metabolismo celular (Silva Figueiredo *et al*., 2018; Torres Silva *et al*., 2020).

As análises físico-químicas ressaltam a qualidade do óleo, visto que as etapas de extração influenciam na estabilidade oxidativa e qualidade nutricional dos óleos vegetais (Castelo-Branco *et al*., 2016). Além disso, valores de acidez, peróxidos, entre outros, complementam e comprovam a composição dos óleos vegetais, estabelecendo adequação ao consumo humano (Redondo-Cuevas *et al*., 2018). Assim, por meio da aferição da integridade de compostos e da qualidade físico-química evita-se a ingestão de ácidos graxos oxidados/degradados, que podem causar uma série de complicações no organismo, como danos celulares, modificações na composição lipídica de tecidos, lesões hepáticas e cardíacas, pela incorporação desses ácidos oxidados nas lipoproteínas, que aumentam o estresse oxidativo e desenvolvimento aterosclerótico (Grootveld, 2022).

2.5 Índices de qualidade e identidade de óleos vegetais

A qualidade final de um óleo depende de todas as etapas envolvidas em seu processamento. Quando sementes e grãos são armazenados em condições inadequadas, pode ocorrer o aquecimento exacerbado da matéria-prima, aumento da umidade e acidez, além de alterações no sabor, aroma e rendimento, devido às modificações químicas que podem ser quantificadas em análises após o processo de extração dos óleos vegetais (Menegazzo; Petenuci; Fonseca, 2014). Portanto, são estabelecidos parâmetros de qualidade e identidade por meio de análises físico-químicas, que propõem faixas de resultados adequados ao consumo humano. Resultados fora desse padrão são reconhecidos como óleo oxidado ou adulterado (Dodoo *et al*., 2022).

A oxidação ocorre devido à decomposição em hidroperóxidos, peróxidos e aldeídos de baixo peso molecular, resultando na formação de radicais livres. A presença de calor, metais

pesados, luz ultravioleta ou substâncias de nitrogênio presentes no óleo induz esses radicais a interagirem com o oxigênio, iniciando uma reação em cadeia que gera radicais peróxidos, hidroperóxidos e radicais alquil. Esses radicais podem reagir entre si, formando produtos de oxidação, como ácidos e longas cadeias de hidrocarbonetos. Juntamente com aldeídos e cetonas, esses compostos constituem os produtos não reativos resultantes da oxidação (Multari *et al*., 2019).

Considerando que o oxigênio da molécula se combina com ácidos graxos insaturados para produzir hidroperóxidos e radicais livres, os óleos vegetais com maior quantidade de ácidos graxos poli-insaturados são mais predispostos à oxidação (Kochhar; Henry, 2009). No entanto, para que a oxidação do óleo ocorra efetivamente, são necessárias temperaturas acima de 100 ºC, além de indicadores oxidativos, como componentes químicos oxidados, metais de transição (ferro ou cobre) ou enzimas (lipoxigenases) (Scherer; Siddiq, 2019).

Assim, o controle da oxidação lipídica é alvo da indústria alimentícia, visto que promove formação de odores (ranço), produtos tóxicos ao organismo e perda de valor nutricional (Rhee; Stubbs, 1978). A avaliação dos óleos vegetais é realizada por métodos analíticos que expressam a integridade da matéria prima. As análises físico-químicas, juntamente com outras técnicas como a cromatografia gasosa são utilizadas para conhecer o comportamento e conteúdo dos ácidos graxos que compõe estes óleos (JIANG; QIAN, 2023).

O índice de acidez é um parâmetro químico utilizado para avaliar a qualidade dos óleos vegetais, sendo definido como a massa (em mg) de hidróxido de potássio (KOH) necessário para neutralizar os ácidos graxos livres presentes em 1,0 grama de óleo. A acidez decorre da hidrólise parcial dos glicerídeos e mostra-se uma variável relacionada à natureza da matéria-prima, como por exemplo, processamento e conservação das sementes (MIKOŁAJCZAK et al., 2022). De acordo com o *Codex alimentarius* (1999), os parâmetros de acidez devem estar dentro da recomendação para óleos prensados a frio de < 4,0 mg KOH/g, sendo o resultado condizentes a quantidade de ácidos graxos livres presentes em um grama de óleo ou gordura (ENDO, 2018).

O índice de peróxido é um dos métodos mais utilizados para medir o estado de oxidação de óleos vegetais (MIKOŁAJCZAK et al., 2022), visto que os peróxidos são os primeiros produtos da oxidação de um óleo vegetal, assim, é um indicador relacionado à presença ou ausência de hidroperóxidos, formados nas etapas iniciais da auto-oxidação lipídica (ENDO, 2018). Desse modo, o limite preconizado para óleos virgens prensados a frio é de <15 $mEqO_2$/kg para indicar ausência de deterioração (Codex Alimentarius, 1999).

O índice de refração está relacionado à viscosidade apresentada pelo óleo vegetal, sendo que valores mais altos indicam um produto já deteriorado ou em processo de deterioração (ENDO, 2018). Os óleos vegetais apresentam diferentes índices de refração conforme sua natureza, variando na intensidade e velocidade com que os raios luminosos os atravessam quando medidos pelo refratômetro. Esse índice aumenta com o comprimento da cadeia carbônica e o grau de insaturação dos ácidos graxos constituintes, sendo essencial para determinar o nível de conservação e qualidade do óleo (JIANG; QIAN, 2023). O óleo de semente de uva e semente de linhaça apresentam uma recomendação específica pelo *Codex Alimentarius* (1999) de 1,467-1,477 (40°C), para óleo prensado a frio de semente de uva e 1,472-1,487 (40 °C) para óleo prensado a frio de semente de linhaça.

Além disso, o índice de iodo é utilizado para a determinação quantitativa das insaturações presentes nos óleos vegetais. Substâncias com cadeias insaturadas de carbono permitem a adição de halogênios, sendo assim, utiliza-se o número de centigramas de iodo absorvido por grama da amostra para mensurar a capacidade de polimerização sob aquecimento e a tendência à oxidação. Dessa forma, quanto maior a quantidade de insaturações, maior a predisposição do óleo vegetal à oxidação (AREMU; IBRAHIM; ANDREW, 2017). O índice de iodo previstos pelo *Codex Alimentarius* (1999ª) para óleo de semente de uva prensado a frio é de 128-150g I_2/100 g, e para óleo de semente de linhaça prensado a frio é de 170-211g I_2/100 g.

O índice de saponificação é um critério para a identificação de óleos vegetais, no qual é medido a quantidade de KOH necessária para saponificar o conteúdo lipídico de uma amostra. A reação de saponificação é realizada em soluções alcalinas, onde a reação ocorre levando à conversão de um éster em sal e álcool (ENDO, 2018). O índice de saponificação está relacionado aos ácidos graxos de menor peso molecular, de modo que quanto menor o peso molecular, maior os valores para este índice (Aoac, 2019). De acordo com *Codex Alimentarius* (1999a) os limites preconizados são de 188-194 mgKOH/g para óleo de semente de uva, e 185-197 mgKOH/g para o óleo de semente de linhaça, indicando segurança para consumo (MIKOŁAJCZAK et al., 2022).

Por fim, a densidade relativa, medida pelo método de picnômetro, indica a razão entre a massa da amostra e a massa da água a 25 ºC. Dessa forma, quanto menor a densidade de um óleo vegetal, menor é o peso molecular dos ácidos graxos e maior é a presença de insaturações (PISESKUL et al., 2023). Da mesma maneira, densidades que fogem do padrão podem indicar adulteração do óleo ou a presença de contaminantes. De acordo com o *Codex*

Alimentarius (1999a) para óleo de semente de linhaça possui valor proposto de 0,925- 0,935 mg/mL, já o óleo de semente de uva possui recomendação de 0,920- 0,926 mg/mL.

Junto à isso, ainda são utilizados métodos para mapear as propriedades do óleo, avaliar sua suscetibilidade à oxidação, como oxidação/polimerização térmica e características físicas e químicas, sendo o conjunto de resultados necessário para compor a adequação para o consumo do óleo (KOCH et al., 2023).

2.6 Estabilidade e qualidade de óleos vegetais

O método Rancimat é utilizado para determinar a estabilidade oxidativa dos óleos vegetais, e pode ser aplicado tanto em óleos vegetais destinados ao consumo humano, como em cosméticos enriquecidos com óleos (REDONDO-CUEVAS et al., 2018). O método baseia-se mudanças na condutividade da amostra exposta a temperaturas de 100 ºC e sob condições de fluxo de ar constante (SCHERER; SIDDIQ, 2019).

A alteração na condutividade acontece para a produção de ácidos orgânicos voláteis e iônicos, Assim, o período de indução (PI) é o período em horas em que ocorrem alterações químicas nos óleos vegetais, sendo uma indicação direta da oxidação durante o aquecimento (REDONDO-CUEVAS et al., 2018; YANG et al., 2018). Os óleos vegetais são suscetíveis a eventos oxidativos devido ao seu alto teor de ácidos graxos insaturados, sendo os ácidos graxos poli-insaturados os mais suscetíveis a serem perdidos durante a primeira etapa de aquecimento (MUEED et al., 2022).

Desse modo quanto maior o PI, maior a estabilidade oxidativa da amostra de óleo (REDONDO-CUEVAS et al., 2018). O perfil de ácidos graxos e compostos antioxidantes naturais presentes no óleo podem influenciar diretamente no PI, contudo, não há consenso na literatura sobre a adição de compostos antioxidantes e a melhora da estabilidade oxidativa dos óleos vegetais pelo aumento do PI (CHEN et al., 2019; PANTOJA et al., 2018).

Assim, sugere-se que o óleo de semente de linhaça e de semente de uva não devem ser utilizados em altas temperaturas devido à perda da composição nutricional, sendo melhor utilizado como suplementação em cápsulas ou adicionado a preparações frias (KOCHHAR; HENRY, 2009; LEITE et al., 2012).

A análise Rancimat também prevê a resistência do óleo para iniciar o processo oxidativo, caracterizado pelas reações dos radicais livres, por isso os resultados deste teste correlacionam-se com medidas de oxidação avançada por metodologias termoanalíticas, como a análise de Termogravimetria/Termogravimetria Derivada (TG/DTG) e a Calorimetria

exploratória diferencial (DSC), proporcionando resultados mais completos (REDONDO-CUEVAS et al., 2018).

As proporções de ácidos graxos saturados e insaturados nos óleos vegetais são influenciadas pela variedade de espécies, condições climáticas, qualidade do solo de plantio e armazenamento dos frutos e sementes pós-colheita. Alimentos industrializados processados ou ultraprocessados que contêm ácidos graxos estão frequentemente sujeitos a tratamento térmico durante o processamento, estocagem e preparação (RAOF et al., 2019; RIMEZ et al., 2008).

Assim, a estabilidade térmica dos óleos vegetais depende de sua estrutura química, sendo os óleos com maior quantidade de ácidos graxos saturados mais estáveis do que os com maior quantidade de ácidos graxos insaturados (REZENDE et al., 2021). Desse modo, o conhecimento da estabilidade térmica desses óleos propõe maior controle da qualidade e utilização para o desenvolvimento de produtos (SONG; SUI; JIANG, 2023). Junto a isso, as análises térmicas ainda possibilitam a aplicação para medidas de propriedades físicas, estudo das reações químicas e determinação da composição dos óleos vegetais (CHANDRAN et al., 2017).

Na TG, as decomposições ocorrem pela diminuição da massa da amostra, em relação ao aumento da temperatura, viabilizando a quantificação da perda de massa pela saída de compostos voláteis e decomposição de ácidos graxos. Os primeiros ácidos graxos a se deteriorarem são os poli-insaturados, seguido pelos monoinsaturados, e por fim, a decomposição dos ácidos graxos saturados (MISUTSU et al., 2015). Visto isso, a curva DTG possui a função de identificar em quantas etapas ocorre uma decomposição térmica ou uma degradação termo-oxidativa, sendo complementar a análise dos resultados da TG (VEGA-LIZAMA et al., 2015).

A técnica DSC é capaz de verificar alterações físicas em função do aumento ou diminuição da temperatura, pelos picos de fusão e cristalização, devido a transição térmica nas amostras. Esta análise permite avaliar a estabilidade em temperaturas extremas conforme os picos de temperatura inicial e final, sendo de suma importância para o conhecimento do comportamento térmico dos ácidos graxos e substâncias antioxidantes presentes no óleo, tais resultados são relevantes à indústria, alimentícia, farmacêutica e cosmética, priorizando o desenvolvimento seguro de novos produtos (CUVELIER; LACOSTE; COURTOIS, 2012; MISUTSU et al., 2015).

Somado a isso, as técnicas ópticas também são utilizadas para o maior conhecimento da matéria prima, como a qualidade do óleo, possível presença de impurezas e estabilidade

oxidativa, identificando a presença de compostos fenólicos e pigmentos (carotenoides e clorofilas). A fluorescência e absorbância, comprovam através de ondas captadas de emissão-excitação a presença destes compostos (DE OLIVEIRA et al., 2019).

O monitoramento da presença de tocotrienois e pigmentos, ocorre pela absorção e emissão de energia em determinados comprimentos de onda, assim é permitido a identificação e quantificação de compostos orgânicos nas amostras, sendo possível monitorar o avanço oxidativo dos óleos vegetais (MARCELINO et al., 2019; SILVA FIGUEIREDO et al., 2018).

A espectroscopia de absorção de luz ultravioleta-visível (UV-Vis) se baseia na medida da absorção de radiação eletromagnética nas regiões visível e ultravioleta por espécies químicas (moléculas e íons) desse modo, determina-se a presença de compostos como carotenoides, clorofilas e tocoferóis sendo possível mensurar através dos picos de absorção os produtos de degradação e oxidação lipídica, como peróxidos. Estes resultados são expressos em comprimento de onda (em nanômetros (nm)) pela absorbância, e são estabelecidos limites dos feixes proporcionais à concentração de cada espécie química determinada (MISUTSU et al., 2015).

Dessa forma, a espectroscopia de fluorescência utiliza o mecanismo de fotoluminescência, no qual a radiação eletromagnética é emitida por moléculas excitadas, resultando na mudança de orbitais energéticos dos elétrons conforme a absorção de fótons de luz. Esta análise é mais sensível que a absorbância e permite quantificar substâncias orgânicas e inorgânicas na amostra (CUVELIER; LACOSTE; COURTOIS, 2012), complementando a avaliação da qualidade e estabilidade dos óleos vegetais.

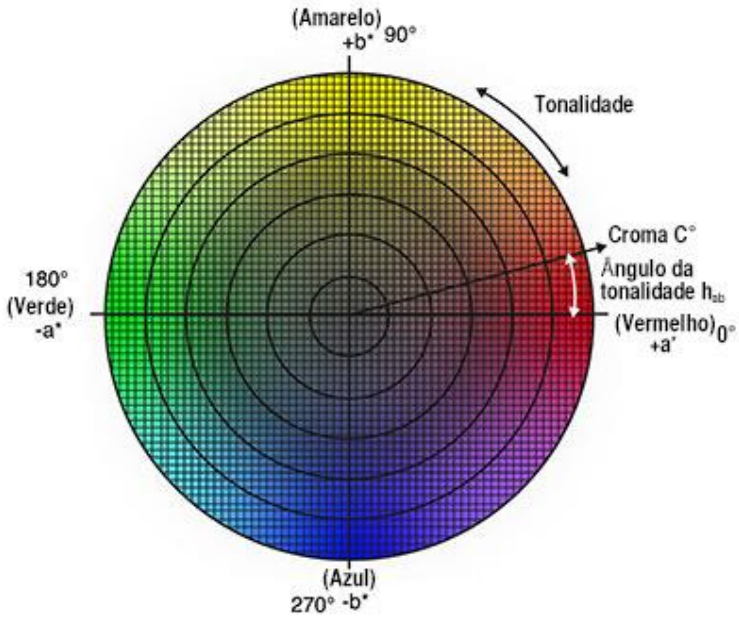
2.7 Coloração e composição de carotenoides de óleos vegetais

A coloração é um parâmetro de qualidade dos óleos vegetais, pois representa a estabilidade na produção, a aceitação de compra e a presença de pigmentos com ação metabólica e antioxidante no organismo. A definição de cor pode ser descrita como a capacidade da amostra de absorver e refletir luz, sendo um parâmetro subjetivo que sofre influência de fatores externos, como luz, tamanho e local de visualização do material (CHEN; SUN, 2023).

Os parâmetros colorimétricos são sistemas criados para mensurar as cores de maneira mais precisa por meio de três eixos: luminosidade (L^*) e cromaticidade (a^* e b^*). A L^* é o parâmetro da faixa de cor classificada de 0 a 100, onde 0 indica uma luminosidade mais escura e 100 indica uma luminosidade mais brilhante, onde é possível identificar as alterações visuais da incidência de luz (CHEN; SUN, 2023).

As coordenadas de cromaticidade indicam a direção das cores, onde *a** varia no eixo das abscissas e tem faixa de coloração do vermelho (+*a**) ao verde (-*a**), e *b**, no eixo das ordenadas e tem faixa de coloração do amarelo (+*b**) ao azul (-*b**). A saturação da cor (*C**), corresponde à distância do eixo da luminosidade, onde quanto mais próximo ao centro mais escuro é o alimento. Além disso, o parâmetro da tonalidade (*hue*), indica o grau de coloração por meio de uma escala que varia de 0 a 360º que se inicia no eixo +*a** e se movimenta no sentido anti-horário, indicando o ângulo da tonalidade (Figura 4) (CHEN; SUN, 2023).

Figura 4 - Espaço de Cor CIE *L**, *C**, *Hue*



Fonte: Commission Internationale de l'Eclairage (CIE), 1913

Durante a colheita, armazenamento, transporte e processamento, os frutos passam por alterações bioquímicas que variam de acordo com os diferentes graus de maturação, clima, composição nutricional do solo de plantio e temperatura. Além disso, outras influências, como o método de extração, impactam diretamente na qualidade nutricional das sementes e dos óleos extraídos delas, afetando os pigmentos naturais responsáveis pela coloração (Martin *et al*., 2020).

Os pigmentos naturais encontrados nas sementes como carotenoides e clorofilas, permanecem após o processamento devido sua solubilidade em óleo (Etzbach *et al*., 2018; Ojeda-Amador *et al*., 2018). Os carotenos são os principais grupos de pigmentos e conferem

ao óleo coloração na faixa amarela a vermelha, exemplos deste grupo são o β-caroteno, β-criptoxantina, α-caroteno, licopeno e luteína (Etzbach *et al*., 2018).

A presença de β-caroteno auxilia na redução dos estágios iniciais do estresse oxidativo e na inativação dos radicais peroxila, atuando como um antioxidante natural (Siriamornpun; Kaewseejan, 2017). De forma geral, os seres humanos não conseguem sintetizar esses pigmentos, sendo necessária sua obtenção por meio da alimentação (Arathi *et al*., 2015). Devido às suas propriedades antioxidantes, o consumo de flavonoides, carotenoides, clorofilas e outros pigmentos presentes nos alimentos tem sido associado à prevenção de doenças como câncer, diabetes e doenças crônicas não transmissíveis (Arathi *et al*., 2015; Siriamornpun; Kaewseejan, 2017).

Os compostos fenólicos presentes nos pigmentos, como carotenoides e clorofilas, protegem os óleos vegetais contra a ação de radicais livres envolvidos na peroxidação lipídica e possuem ação direta em reações oxidativas, impedindo a ação de agentes pró-oxidantes (Zeb, 2020). Os agentes oxidantes ou pró-oxidantes podem ser substâncias endógenas ou exógenas com atuação intracelular, capazes de oxidar moléculas-alvo, como ROS, que inibem sistemas antioxidantes (Milani *et al*., 2017).

Dentre as moléculas exógenas que compõem os agentes oxidantes, podemos citar medicamentos, oxigênio, ozônio, nitrato de potássio, perborato de sódio, peróxidos, halogênios e poluentes ambientais. Já as moléculas endógenas incluem enzimas da cadeia respiratória, inflamações, peroxissomos e fagócitos. Além disso, metais de transição como ferro e cobre, provenientes de fontes dietéticas, são utilizados como doadores de elétrons e auxiliam na formação de alguns radicais livres, potencializando seus efeitos no organismo (Jomova *et al*., 2023; Milani *et al*., 2017; Zeb, 2020),

O estresse oxidativo pode danificar células e tecidos, tornando essencial o consumo de uma variedade de alimentos ricos em micronutrientes e substâncias bioativas que atuam como coenzimas e substratos para enzimas antioxidantes, como superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GPX), peroxirredoxinas (PRX) e tiorredoxinas (Trx). Além disso, a glutationa (GSH), peroxirredoxinas, tiorredoxinas e proteínas transportadoras de íons de metais de transição também demonstram atividade antioxidante. O ácido ascórbico, α-tocoferol e seus derivados, juntamente com carotenoides, seus derivados e polifenóis, são eficientes nesse mecanismo de ação, atuando como sequestradores de elétrons e participando indiretamente das reações enzimáticas (Jomova *et al*., 2023; Rana *et al*., 2022).

Durante a peroxidação lipídica, ocorrem oxidações sucessivas em lipídeos insaturados, iniciando pela oxidação dos ácidos graxos poli-insaturados e, em seguida, dos

monoinsaturados, resultando na formação de radicais de ácidos graxos e hidroperóxidos de lipídeos. A retirada sucessiva de hidrogênio do agrupamento metil dos ácidos graxos gera um radical que se liga ao oxigênio, formando radicais peróxidos (Cuffaro; Digiacomo; Macchia, 2023; Rana *et al*., 2022).

Os hidroperóxidos e peróxidos lipídicos alteram a estrutura, propriedades e função das membranas celulares, tornando-as instáveis e mais suscetíveis ao rompimento. Consequentemente, os produtos do estresse oxidativo, como os aldeídos reativos, incluindo o malondialdeído (MDA), podem causar danos à estrutura do DNA e das proteínas, resultando em processos mutagênicos (Arathi *et al*., 2015; Cuffaro; Digiacomo; Macchia, 2023).

Visto isso, a presença destas substâncias antioxidantes em óleos vegetais, como o β-caroteno, mostra-se benéfico à saúde por sua interação às espécies reativas de oxigênio e radicais livres (Speranza *et al*., 2016). Os carotenoides e clorofilas são avaliados pela classificação dos pigmentos “a” para coloração azul-esverdeada, e “b” para coloração amarelo-esverdeada (Kochhar; Henry, 2009).

Os compostos carotenoides são lipossolúveis e estão presentes na composição de óleos vegetais (Mezzomo; Ferreira, 2016). Contudo, o óleo de semente de uva, mesmo com menores concentrações de carotenoides, contém polifenóis e vitamina E, que melhoram a cicatrização e reduzem a inflamação sistêmica (Vitale *et al*., 2022). Comparado a outros óleos vegetais, o óleo de semente de uva possui a maior quantidade de isômeros de vitamina E, como o tocotrienol, que apresenta propriedades antioxidantes, anti-inflamatórias e antitumorais, destacando-se pela sua atividade antioxidante (Lu *et al*., 2022; Magalingam *et al*., 2022; Shinagawa *et al*., 2017).

De forma semelhante, os flavonoides, ácidos fenólicos e tocoferóis são produtos insaponificáveis isolados dos óleos de semente de uva e linhaça, que demonstram propriedades anti-inflamatórias (Poljšak; Kočevar Glavač, 2022). Além disso, a fácil degradação dos carotenoides devido à exposição à luz, oxigênio, evaporação do solvente durante a análise e técnicas de extração do óleo das sementes pode alterar sua quantificação (Shinagawa *et al*., 2017; Speranza *et al*., 2016). As variações nos resultados de carotenoides são influenciadas por características genéticas de cada subespécie vegetal, condições ambientais, grau de maturação e métodos de extração utilizados para a fabricação do óleo (Marcelino *et al*., 2019).

Considerando as informações apresentadas nos subtópicos anteriores, a revisão de literatura demonstra um potencial terapêutico tanto do óleo de semente de uva quanto do óleo de semente de linhaça, devido ao perfil de ácidos graxos e presença de compostos bioativos, com destaque à saúde cardiovascular e efeitos anti-inflamatórios. A sinergia entre esses óleos, quando combinados em uma *Mistura* 1:1 (v/v), pode potencializar seus efeitos benéficos. No presente estudo, verificamos por meio de técnicas analíticas as características físico-químicas, térmicas, ópticas, presença de compostos bioativos e estabilidade oxidativa dessa *Mistura* em comparação com os óleos individuais, juntamente ao modelo experimental *in vivo* utilizando a suplementação dos óleos isolados e a *Mistura* 1:1 (v/v).

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3 OBJETIVOS

3.1 Objetivo geral

Avaliar o efeito do consumo do óleo da semente de uva, óleo de semente de linhaça e a *Mistura*, composta pelo óleo de semente de uva e óleo de linhaça em mesmo volume 1:1 (v/v), em camundongos *Swiss*, bem como avaliar a sua qualidade e estabilidade oxidativa.

3.2 Objetivos específicos

- Determinar o perfil de ácidos graxos, índices de qualidade e identidade do óleo da semente de uva, óleo da semente de linhaça e *Mistura* 1:1 (v/v);

- Caracterizar os óleos por meio de técnicas ópticas, assim como avaliar sua estabilidade oxidativa e térmica;

- Avaliar a capacidade antioxidante, pela presença de compostos carotenoides e coloração destes óleos;

- Verificar o impacto do consumo desses óleos no peso corporal, ingestão alimentar, parâmetros séricos (colesterol total e frações, triacilglicerídeos e glicose) e nas adipocinas de animais;

- Avaliar parâmetros morfológicos e alterações histológicas nos animais.

4 MATERIAIS E METODOS

4.1 Matéria prima

Neste estudo, os óleos vegetais prensados a frio de sementes de Vitis vinifera L. e Linum usitatissimum L. foram adquiridos de fornecedores conceituados da Indústria Pazze Alimen tos™, Panambi (RS), Brasil. Estes óleos foram obtidos pela técnica de prensagem a frio, garantindo baixos índices de acidez e peróxido, verificados pelo fabricante. Esse processo preservou a pureza e uniformidade dos óleos, fundamentais para a integridade desta pesquisa. Para desenvolver uma mistura consistente de óleos, a mistura meticulosa de volumes iguais de óleo de semente de uva e óleo de linhaça dourada foi conduzida utilizando balões volumétricos para estabelecer uma proporção precisa de 1:1 (v/v). Foi empregada agitação delicada para evitar a introdução de bolhas de ar que pode comprometer a estabilidade da mistura. Posteriormente, a mistura de óleos foi armazenada em recipiente hermético e estéril, protegido da exposição solar direta e mantido em temperatura estável para preservar sua qualidade e inibir a deterioração oxidativa.

Neste estudo foi utilizado azeite virgem extra da marca Andorinha Portugal™ Ferreira do Alentejo, Portugal, para o grupo de controlo suplementado com azeite, com a sua identidade e detalhes de qualidade especificados no rótulo.

4.2 Perfil de Ácidos Graxos

Os ácidos graxos foram esterificados de acordo com metodologia adaptada de Maya e Rodriguez-Amaya (1993). Os ésteres metílicos de ácidos graxos foram analisados por cromatografia gasosa (GC 2010, Shimadzu, Japão) para obter seus picos individuais. No equipamento utilizou-se um detector de ionização de chama (FID) e uma coluna capilar (BPX-70, diâmetro interno de 0,25 mm, 30 m de comprimento e filme de 0,25 mm de espessura). Com temperatura de injetor e detector de 250 °C. A temperatura inicial da coluna foi mantida a 80 °C por 3 minutos e então aumentada a 10 °C/min até atingir 140 °C, seguida de um aumento para 240 °C a 5 °C/min por 5 minutos. Picos individuais de FAME (ésteres metílicos de ácidos graxos) foram identificados comparando os tempos relativos de retenção com o padrão FAME (Supelco C22, 99% puro). O cálculo dos teores de ácidos graxos foi realizado integrando-se as áreas dos picos (porcentagem de área), e os resultados são expressos em g de ácidos graxos/g de óleo extraído.

Junto a isso, foram utilizados o índice de aterogenicidade apresentado na Equação 1 e o índice de trombogenicidade na Equação 2 (Ulbricht; Southgate, 1991).

$$\text{Índice de aterogenicidade} = \frac{[(C12{:}0+(4\times C14{:}0)+C16{:}0)]}{(\sum MUFA + \sum\omega 6 + \sum\omega 3)} \qquad (1)$$

$$\text{Índice de trombogenicidade} = \frac{(C14{:}0 + C16{:}0 + C18{:}0)}{[(0.5 \times \sum MUFA\ )+(0.5 \times \sum\omega 6)+(3 \times \sum\omega 3)+(\omega 3/\omega 6)]} \qquad (2)$$

Onde MUFA é a soma dos ácidos graxos monoinsaturados dos óleos estudados.

4.3 Análises de qualidade e identidade dos óleos vegetais

As análises de identidade e qualidade dos óleos vegetais foram feitas de acordo com a metodologia apresentada por (Läubli; Bruttel, 1986).

4.3.1 Índice de acidez

O índice de acidez foi determinado pela adição de solução éter-álcool (1:1) neutralizado e fenolftaleína como indicador de mudança de coloração aos óleos. Como titulante foi utilizado hidróxido de potássio (KOH) 0,1N até o aparecimento da coloração rosa. Os resultados foram expressos em mgKOH/g e g de ácido oleico/100g óleo.

4.3.2 Índice de peróxido

O índice de peróxido foi realizado com 5,0 mL solução ácido acético-clorofórmio (3:2), 0,1 mL de solução saturada de iodeto de potássio e 0,1 mL de solução de amido solúvel 1% como indicador de alteração de coloração adicionados aos óleos, com posterior repouso ao abrigo de luz. A titulação foi realizada com solução de tiossulfato de sódio a 0,01N. Os resultados foram expressos em $mEqO_2/kg$.

4.3.3 Índice de refração

O índice de refração dos óleos foi obtido com o auxílio de refratômetro de Abbé (RL3, Tecnal, Brasil) calibrado com água destilada, com o índice de refração de 1,3330, em 27 °C, com temperatura corrigida para 40 °C.

4.3.4 Índice de iodo

O índice de iodo foi obtido a partir de tetracloreto de carbono e solução de Wijs adicionados aos óleos, e tiossulfato de sódio padronizado como titulante até a mudança de coloração de preto para rosa. Os resultados foram expressos em $gI_2/100g$.

4.3.5 Índice de saponificação

O índice de saponificação foi determinado pela adição de solução alcóolica de hidróxido de potássio (KOH) a 4% às amostras, sendo refluxadas durante 1 hora. Em seguida adicionou-se fenolftaleína como indicador para mudanças das colorações e titulação com ácido clorídrico (HCl) 0,5 N. Os resultados foram expressos em mgKOH/g.

4.3.6 Densidade relativa a 25 °C/25 °C

A densidade relativa foi realizada de acordo com o Método do Picnômetro, previamente tarado em estufa a 105 °C. Sendo adicionado água resfriada a 20-23 °C e colocado em banho com temperatura constante (25 ± 0,1 °C). Após 30 minutos, foi ajustado o nível de água e pesado em balança analítica. Os mesmos procedimentos foram realizados com os óleos. Os resultados foram expressos em mg/mL.

4.4 Análises de estabilidade oxidativa e térmica dos óleos vegetais

4.4.1 Estabilidade oxidativa: Rancimat

A estabilidade oxidativa foi obtida através do período de indução (PI) resultado do teste de Rancimat, segundo o método EN 14112, utilizando o equipamento Rancimat (893 Professional Biodiesel Rancimat, Metrohm, Brasil). As análises foram realizadas com 3,0 g do óleo sem diluição a 110 °C e analisada sob fluxo de ar constante de 10 L/h, que passou pelas amostras, seguido por um recipiente contendo 50 mL de água deionizada no qual a condutividade gerada pelos produtos voláteis durante a degradação dos óleos vegetais foram medidos em função do tempo (Läubli; Bruttel, 1986).

4.4.2 Termogravimetria e Termogravimetria Derivada (TGA/DTG)

Para as curvas TGA/DTG, utilizamos 4,0 mg de cada óleo em um sistema TGA Q50 (TA Instruments, Eden Prairie, MN, EUA, EUA) sob atmosfera de nitrogênio, com fluxo de 60 mL/min no forno e taxa de aquecimento de 10 ◦C/min em temperaturas variando entre ambiente e 700 ◦C, com cadinhos de platina usados como suporte (Meireles *et al*., 2022).

4.4.3 Calorimetria Diferencial de Varredura (DSC)

Para avaliar os processos de cristalização e fusão dos óleos, foram realizadas curvas DSC com massa de amostra em torno de 3 mg, em um sistema de análise térmica DSC Q20 da TA Instruments – EUA, equipado com um sistema de refrigeração de duplo estágio RCS 90. As curvas de resfriamento e aquecimento foram programadas em ciclos. Inicialmente a temperatura foi equilibrada a 60 °C, seguido de isoterma de 10 minutos e rampa de resfriamento até -60 °C, a razão de 5 °C/min completando o ciclo 1. Na sequência a temperatura foi equilibrada a -60 °C, seguido de isoterma por 10 minutos e rampa de aquecimento a 5 °C/min até temperatura final de 60 °C completando o ciclo 2. O tempo total de análise: próximo de 70 minutos.

4.5 Análises ópticas

4.5.1 Absorção UV-Vis e matriz de fluorescência de emissão-excitação.

As amostras foram diluídas em grau de espectroscópio hexano (Sigma-Aldrich > 99%), nas concentrações de 1,0; 5,0 e 90 g/dm^3. Os espectros de absorção foram obtidos utilizando-se um espectrômetro UV-vis Lambda 265 (PerkinElmer®) coletado entre 200 e 600 nm, sendo utilizada uma cubeta de quartzo com 10 mm de comprimento óptico e 3,5 mL de capacidade.

Os mapas de fluorescência foram medidos em espectrômetro de fluorescência FS-2 (Scinco®), para as amostras na concentração de 90 g/dm^3. A excitação é variada em uma faixa de 270 a 450 nm com delta de 5nm. E a emissão das amostras foi medida entre 290 e 750 nm com resolução de 1 nm.

4.6 Coloração

A coloração foi determinada em espectrofotômetro portátil da marca Konica Minolta® modelo CM-2300d. Os resultados foram expressos em L*, a* e b* conforme o espaço de cor L* a* b* empregando a escala CIE *L*a*b***, onde o L* representa a luminosidade, variando entre 0 (sem luminosidade ou preto) e 100 (branco), o a* representa as cores que vão do vermelho (+a*) ao verde (-a*) e o b* representa as faixas de cores que vão do amarelo (+b*) ao azul (-b*). A partir dos resultados foram obtidos os índices do ângulo *Hue* representados pela Equação 3, onde foi possível definir a tonalidade em graus e Croma (*C**) na Equação 4, no qual indica-se a saturação da cor das amostras.

$$Hue = b * a * \qquad (3)$$

Onde o valor de Hue é a tonalidade de cor definida em graus, b* indica a cromaticidade no eixo variando do amarelo/azul e a* mostra a cromaticidade no eixo variando do vermelho/verde (Rodriguez-Amaya, 1999; Minolta Corporation, 1994).

$$C* = a*2 + b*2 \qquad (4)$$

Onde o valor de C* (croma) indica a saturação da cor, b* indica a cromaticidade no eixo variando do amarelo/azul e a* mostra a cromaticidade no eixo variando do vermelho/verde (Minolta Corporation, 1994).

4.7 Determinação de carotenoides

Amostras de óleo de semente de uva, linhaça e *Mistura* foram inicialmente filtradas a vácuo e em ambiente protegido da luz, para retirada de possíveis impurezas. Foram realizados diversos ensaios para definir a concentração do óleo em éter de petróleo P.A. (Dinâmica) a fim de obter absorbâncias entre 0,2 e 0,8. Alíquotas de óleo foram diluídos em éter de petróleo e o volume ajustado com éter de petróleo em balão volumétrico (10 mL). As etapas subsequentes foram realizadas de acordo com a metodologia descrita por (Rodriguez-Amaya, 1999). Todas as etapas foram realizadas protegidas da luz com papel alumínio, evitando a fotodegradação dos carotenoides. Depois de preparado os extratos foram realizados leituras de cada extrato em cubeta de quartzo em espectrofotômetro UV-Visível (Biochrom Libra S60PC) em comprimento de onda de 450 nm. O éter de petróleo P.A. foi utilizado como branco. O teor de carotenoides foi calculado através da Equação 5, com valor da absortividade do β-caroteno de 2592, o resultado foi expresso em µg/100g de óleo.

$$Carotenoides\left(\mu g/g\right) = \frac{A \times V \times 10^4}{E^{1\%}_{1cm} \times m} \qquad (5)$$

Onde A é a absorbância no pico máximo de absorção, V é o volume final da amostra (mL), m é a massa da amostra (g) e $E^{1\%}_{1cm}$ é o coeficiente de extinção (β-caroteno = 2592 em éter de petróleo).

4.8 Experimento In vivo

4.8.1 Animais

Desenvolvemos este estudo de acordo com os preceitos éticos da Lei n. 11.794, de 8 de outubro de 2008, do Decreto n. 6.899, de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (Concea) e aprovadas pela Comissão de Ética no Uso de Animais (CEUA) da UFMS (n. 1339/2022). Foram utilizados 126 camundongos da linhagem Swiss Webster (Mus musculus), machos adultos, com 12 semanas de idade e peso médio de 36 g. Foram fornecidos pelo Biotério Central da Universidade Federal de Mato Grosso do Sul. A aclimatação período ocorreu 7 dias antes do início do período experimental. Durante esse período, os animais foram mantidos em ambiente com temperatura controlada (22 ± 1 °C), sob ciclo claro/escuro de 12 horas, com acesso *ad libitum* a comida e água, com quatro a cinco animais por gaiola.

4.8.2 Delineamento experimental

Os Este estudo incluiu dois grupos de controle padrão: um suplementado com água destilada (C) e outro suplementado com azeite (O). Além disso, houve cinco grupos experimentais (L1, L2, G1, G2, GL). O desenho experimental, conforme representado na Figura 1, inspirou-se em estudos anteriores de Torres *et al*. (2020), Marcelino *et al*. (2022), e Silva *et al*. (2023). Esses estudos utilizaram a administração por gavagem de óleo de Caryocar brasiliense Cambess, óleo de Mauritia flexuosa e óleo de Acrocomia aculeata em camundongos Swiss nas dosagens correspondentes de 1.000 mg/kg/dia e 2.000 mg/kg/dia, demonstrando eficácia em seus resultados. Após o período de aclimatação, foram administradas dosagens diárias durante 11 semanas (Tabela 1 e Figura 1).

Tabela 1 - Determinação dos grupos experimentais, suplementação e dosagem

Grupos	Suplementação	Dosagem (mg/kg/animal)
Controle (C)	Água destilada	1000
Azeite de oliva (O)	Azeite de oliva virgem extra	1000
Óleo de semente de linhaça (L1)	Óleo de semente de linhaça dourada	1000
Óleo de semente de linhaça (L2)	Óleo de semente de linhaça dourada	2000
Óleo de semente de uva (G1)	Óleo de semente de uva	1000
Óleo de semente de uva (G2)	Óleo de semente de uva	2000

Mistura (GL)*	Óleo de semente de uva + Óleo de semente de linhaça dourada 1:1 (v/v)*	2000

Figura 5 - Fluxograma dos grupos experimentais após 7 dias de adaptação.

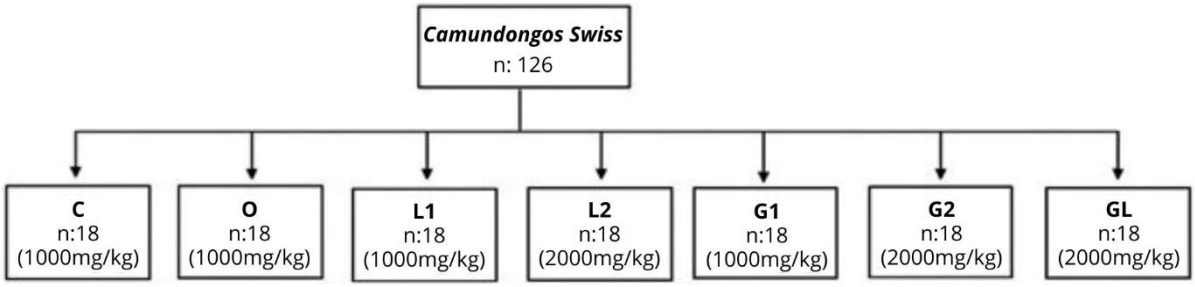

Figura 1. C: grupo controle (água destilada na dose de 1000 mg/kg/animal); O: Grupo azeite virgem extra (1000 mg/kg/animal); L1: Grupo óleo de linhaça (1000 mg/kg/animal); L2: Grupo óleo de linhaça dourada (2000 mg/kg/animal); G1: Grupo óleo de semente de uva (1000 mg/kg/animal); G2: Grupo óleo de semente de uva (2000 mg/kg/animal); GL: Óleo de mistura (2000 mg/kg/animal).

4.8.3 Ingestão alimentar e ganho de peso.

Todos os animais receberam dieta padrão normocalórica comercial (Nuvital®), sendo suplementados via *gavagem* durante 11 semanas, sendo as doses foram reajustadas semanalmente de acordo coma evolução do peso dos animais. As avaliações do consumo alimentar e ganho de peso foram realizadas semanalmente durante 11 semanas de experimento, sendo utilizado o cálculo de coeficiente de eficácia alimentar (CEA) apresentado na Equação 6, para determinar quanto um grama de ração ingerida promove em aumento de peso corporal (Nery *et al*., 2011).

$$\mathrm{CEA} = (\mathrm{PF} - \mathrm{PI}) \div \mathrm{TA} \qquad (6)$$

Onde, PF é o peso corporal final em gramas, o PI é equivalente ao peso corporal inicial em gramas e o TA representa a quantidade total de alimento ingerido em gramas (Nery *et al*., 2011).

Para mensurar o ganho de peso dos animais foi utilizado o cálculo do coeficiente de ganho de peso por consumo calórico (CGPCC), realizado com o objetivo de obter a capacidade do animal em converter energia alimentar consumida em peso corporal (Equação 7).

$$\text{CGPCC} = (PF - PI) \div \text{Kcal ingerida} \qquad (7)$$

Onde, FW representa o peso corporal final em gramas, WI é equivalente ao peso corporal inicial em gramas e kcal ingested é o valor calórico da dieta ingerida (Nery *et al*., 2011).

A composição da ração normocalórica comercial (Nuvital®), foi apresentada na Tabela 2, na qual está descrita os ingredientes, percentual de macronutrientes (carboidratos, proteínas e lipídeos) e a quantidade de caloria por grama de ração.

Tabela 2 - Composição da ração normocalórica comercial (g/kg de ração)

Ingredientes (g/kg)	Nuvital®
Amido	725.67
Caseína (≥82% proteína)	40.00
DL-metionina	100.00
Óleo de soja	40.00
Celulose	100.00
Mix de Minerais**	35.00
Mix de Vitaminas**	10.00
L-cistina	1.80
Bitartarato de colina	2.50
Tertbutil hidroquinona	0.008
Energy (cal/kg)	4.360,00
Carboidratos (%)	75.75%
Proteínas (%)	16.00%
Lípidos (%)	8.25%
Calorias/g dieta	4.36

Mix de Minerais e vitaminas de acordo com as recomendações do fabricante.**

4.8.4 Eutanásia

A eutanásia foi realizada com os animais em jejum por seis horas, com água *ad libium*. A anestesia dos animais ocorreu com dose letal de isoflurano com posterior exsanguinação pela via cava inferior para os a avaliação dos parâmetros séricos. Foram retirados os 5 sítios de gordura (epididimal, mesentérica, omental, retroperitoneal e perirrenal) e o fígado, foram

pesados em balança eletrônica semianalítica (Bel Diagnóstica®) e os valores expressos em miligramas (mg), sendo o tecido epididimal e fígado armazenados em formal a 10% para análise histológica.

O índice de adiposidade (%) foi calculado através fórmula adaptada de Taylor e Phillips (1996) (Equação (8)).

$$\text{Índice de adiposidade (\%)} = \frac{(\text{Soma dos sítios de gordura}) \times 100}{Peso\ final} \quad (8)$$

4.8.5 Parâmetros séricos

Após a coleta de sangue pela veia cava posterior, o sangue foi transferido para tubo com gel separador e centrifugado a 5.000 rpm por 10 minutos em centrífuga refrigerada (hettich, universal 320) para obter o soro. Para os parâmetros séricos foram determinados colesterol total (CT), lipoproteína de alta densidade (HDL-c), triglicerídeos (TG) e glicemia em jejum (GL) utilizando-se o método enzimático-colorimétrico e medições por espectrofotometria (Hagen; Hagen, 1962).

Foram apresentados os cálculos de lipoproteína de muito baixa densidade (VLDL-c) (Equação (9)), lipoproteína de baixa densidade (LDL-c) (Equação 10)), e colesterol não-HDL (não-HDL-c) (Equação (11)).

$$\text{Valor de VLDL} = \text{Valores de Triglicerídeos}/5 \quad (9)$$

$$\text{LDL–c} = \text{Colesterol Total} - (\text{HDL–c} + \text{VLDL–c}) \quad (10)$$

$$\text{Colesterol não–HDL} = \text{Colesterol Total} - \text{HDL–c} \quad (11)$$

4.8.6 Citocinas

As concentrações de adipocinas IL-6, monocyte chemoattractant protein-1 (MCP-1), TNF-α, inibidor do ativador de plasminogênio-1 (PAI-1) total, insulina, leptina e resistina foram mensuradas elo kit comercial MAD-KMAG-71K da Merck-Sigma Aldrich São Paulo-Brasil, leitura da placa no Luminex by Merck-Sigma Aldrich São Paulo-Brasil sendo utilizado o software MAGPIX da Merck-Sigma Aldrich São Paulo-Brasil, e valores de concentração foram obtidos em μg/mL do soro do sangue coletado após a eutanásia dos animais e centrifugação em tubo com gel separador. O soro foi submetido ao Vortex por 30 segundos e levado a centrífuga (6000 rpm por 10 minutos). Em seguida 10 μL do soro de cada animal foram acondicionados em uma placa com 96 poços juntamente com 10 μL de solução Assay

buffer e 25 µL de solução contendo sete adipocinas. Foram também preparados os parâmetros branco, padrão e controle conforme instruções (Miliplex MAP kit, USA). Após realizada a leitura no limine pelo software MAGPIX™ e obtidos os valores de concentração em pg/mL.

4.8.7 Histologia do tecido adiposo epididimal e fígado

Para a análise histológica, o fígado e o tecido adiposo epididimal foram armazenados em potes coletores com solução de formol a 10%. A análise histológica do fígado foi realizada por patologista e as análises do efeito da suplementação nos hepatócitos foram realizadas utilizando o sistema de (Kleiner *et al*., 2005). A análise da área dos adipócitos do tecido adiposo epididimal foi realizada de acordo com metodologia descrita por *Pereira et al*., (2012).

4.9 Análise estatística

As análises foram realizadas utilizando os software's Jandel Sigma Stat, versão 3.5 (Systat software, Incs., San Jose, CA, EUA) e Sigma Plot, versão 12.5 (Systat Software Inc., San Jose, CA, EUA) e apresentados a média ± desvio padrão (DP). Os grupos foram comparados utilizando-se ANOVA on ranks seguido do pós-teste de Tukey, com diferenças consideradas significativas quando $p<0.05$. Kruskal-Wallis seguido de Dunns, com diferenças consideradas significativas quando $p<0.05$. Os dados histológicos foram descritos em frequências absolutas (n) e relativas (%), O teste do qui-quadrado foi utilizado para avaliar a associação na análise histológica, seguido da correção de Bonferroni, pelo programa estatístico Bioestat 5.0. O nível de significância considerado foi de $p<0.05$.

CONCLUSÃO

Tendo em vista os resultados apresentados, o ácido graxo poliinsaturado predominante no óleo de semente de uva e no óleo *Mistura* foi o ácido linoleico (C18:2), enquanto no óleo de linhaça prevalece o ácido alfa-linolênico (C18:3). Em todos os óleos, o ácido oleico (C18:1) foi o ácido graxo monoinsaturado majoritário e o principal ácido graxo saturado foi o ácido palmítico (C16:0). Os índices físico-químicos dos óleos estudados estão dentro das faixas recomendadas, indicando ausência de oxidação e adequação para o consumo. A estabilidade oxidativa e as análises térmicas (TGA/DTA e DSC) comportaram-se de forma semelhante, e as análises ópticas apresentam variações por causa da presença de pigmentos (carotenoides e clorofilas) com resultados condizentes aos achados nas análises de carotenoides totais e coloração. O óleo de semente de uva na dosagem de 2.000 mg/kg/dia, promoveu menor consumo alimentar, e o óleo *Mistura* apresenta menor ganho de peso por grama de ração consumida e consumo calórico total. Ambos óleos indicam níveis mais elevados de lipoproteína HDL-c, enquanto o óleo de linhaça na dosagem de 2.000 mg/kg/dia proporciona níveis reduzidos de colesterol total. Além disso, o óleo de semente de uva, linhaça e o óleo *Mistura* indicam ação mais atenuada das citocinas pró-inflamatórias (MCP-1 e TNF-α), destacando-se a ação anti-inflamatória da suplementação, de mesma forma que o óleo de semente de uva na dosagem de 1000 mg/kg/dia e o óleo *Mistura* demonstram as menores áreas de adipócitos, avaliadas na análise histológica.

Em perspectivas futuras, deve-se haver mais estudos experimentais e clínicos sobre a utilização dos óleos de semente de uva, linhaça e possíveis misturas, uma vez que os resultados apontam propriedades funcionais no modelo de estudo apresentado, com expectativa de potencial utilização nas indústrias alimentícia, farmacêutica e cosmética.

REFERÊNCIAS

AFARANI, O. R. et al. Altering palmitic acid and stearic acid ratios in the diet of early-lactation Holsteins under heat stress: Feed intake, digestibility, feeding behavior, milk yield and composition, and plasma metabolites. **Journal of Dairy Science**, v. 106, n. 9, p. 6171–6184, set. 2023.

AL JUHAIMI, F. et al. Bioactive Properties, Fatty Acid Composition and Mineral Contents of Grape Seed and Oils. **South African Journal of Enology & Viticulture**, v. 38, n. 2, out. 2017.

ALI, A. A. et al. Application of Biosensors for Detection of Pathogenic Food Bacteria: A Review. **Biosensors**, v. 10, n. 6, p. 58, 30 maio 2020.

AL-WARHI, T. et al. Antioxidant and Wound Healing Potential of Vitis vinifera Seeds Supported by Phytochemical Characterization and Docking Studies. **Antioxidants**, v. 11, n. 5, p. 881, 29 abr. 2022.

AMBIKAIRAJAH, A.; WALSH, E.; CHERBUIN, N. Lipid profile differences during menopause: a review with meta-analysis. **Menopause**, v. 26, n. 11, p. 1327–1333, nov. 2019.

ARATHI, B. P. et al. Metabolomics of carotenoids: The challenges and prospects – A review. **Trends in Food Science & Technology**, v. 45, n. 1, p. 105–117, set. 2015.

AREMU, M. O.; IBRAHIM, H.; ANDREW, C. Comparative Studies on the Lipid Composition of Blood Plum () Pulp and Seed Oils. **The Open Biochemistry Journal**, v. 11, n. 1, p. 94–104, 28 out. 2017.

ARROYO-GARCÍA, R. et al. Multiple origins of cultivated grapevine (*Vitis vinifera* L. ssp. *sativa*) based on chloroplast DNA polymorphisms. **Molecular Ecology**, v. 15, n. 12, p. 3707–3714, out. 2006.

ASEFY, Z. et al. Unsaturated fatty acids as a co-therapeutic agents in cancer treatment. **Molecular Biology Reports**, v. 48, n. 3, p. 2909–2916, mar. 2021.

AUSTRIA, J. A. et al. Bioavailability of Alpha-Linolenic Acid in Subjects after Ingestion of Three Different Forms of Flaxseed. **Journal of the American College of Nutrition**, v. 27, n. 2, p. 214–221, abr. 2008.

BADA, J. C. et al. Characterization of grape seed oil from wines with protected denomination of origin (PDO) from Spain. **Grasas y Aceites**, v. 66, n. 3, p. e085, 30 set. 2015.

BAGHERNIYA, M. et al. The effects of phytochemicals and herbal bio-active compounds on tumour necrosis factor-α in overweight and obese individuals: a clinical review. **Inflammopharmacology**, v. 30, n. 1, p. 91–110, fev. 2022.

BERAHMAND, F. et al. Grape Seed Oil as a Natural Therapy in Male Rats with Alzheimer's Diseases. **Advanced Pharmaceutical Bulletin**, v. 10, n. 3, p. 430–436, 11 maio 2020.

BEYER, M. P. et al. Potential Clinical Applications of Pro-Resolving Lipids Mediators from Docosahexaenoic Acid. **Nutrients**, v. 15, n. 15, p. 3317, 26 jul. 2023.

BORGES, M. C. et al. Ácidos graxos poli-insaturados ômega-3 e lúpus eritematoso sistêmico: o que sabemos? **Revista Brasileira de Reumatologia**, v. 54, n. 6, p. 459–466, nov. 2014.

BOSKOU, D. Edible Cold Pressed Oils and Their Biologically Active Components. **Journal of Experimental Food Chemistry**, v. 03, n. 01, 2017.

BRAHMI, F. et al. Optimization of the conditions for ultrasound-assisted extraction of phenolic compounds from Opuntia ficus-indica [L.] Mill. flowers and comparison with conventional procedures. **Industrial Crops and Products**, v. 184, p. 114977, set. 2022.

CALADO, A. et al. The Effect of Flaxseed in Breast Cancer: A Literature Review. **Frontiers in Nutrition**, v. 5, p. 4, 7 fev. 2018.

CALDER, P. C. Functional Roles of Fatty Acids and Their Effects on Human Health. **Journal of Parenteral and Enteral Nutrition**, v. 39, n. 1S, set. 2015.

CALIGIURI, S. P. B. et al. Elevated levels of pro-inflammatory oxylipins in older subjects are normalized by flaxseed consumption. **Experimental Gerontology**, v. 59, p. 51–57, nov. 2014.

CAMELO, S. M. B.; FONTGALLAND, I. L. Análise da produção e comercialização de óleo vegetal no Brasil: o estudo de caso da empresa Cargill. **E-Acadêmica**, v. 2, n. 2, p. e062229, 14 ago. 2021.

CAMPOS, J. R. et al. Linseed Essential Oil – Source of Lipids as Active Ingredients for Pharmaceuticals and Nutraceuticals. **Current Medicinal Chemistry**, v. 26, n. 24, p. 4537–4558, 11 out. 2019.

CAO, Y. Adipocyte and lipid metabolism in cancer drug resistance. **Journal of Clinical Investigation**, v. 129, n. 8, p. 3006–3017, 2 jul. 2019.

CASTELO-BRANCO, V. N. et al. Antioxidant capacity is a surrogate measure of the quality and stability of vegetable oils. **European Journal of Lipid Science and Technology**, v. 118, n. 2, p. 224–235, fev. 2016.

CECCHI, L. et al. In depth study of phenolic profile and PTP-1B inhibitory power of cold-pressed grape seed oils of different varieties. **Food Chemistry**, v. 271, p. 380–387, jan. 2019.

CHANDRAN, J. et al. Oxidative stability, thermal stability and acceptability of coconut oil flavored with essential oils from black pepper and ginger. **Journal of Food Science and Technology**, v. 54, n. 1, p. 144–152, jan. 2017.

CHEN, J. et al. The characterization of soybean germ oil and the antioxidative activity of its phytosterols. **RSC Advances**, v. 9, n. 68, p. 40109–40117, 2019.

CHEN, X.; SUN, S. Color Reversion of Refined Vegetable Oils: A Review. **Molecules**, v. 28, n. 13, p. 5177, 3 jul. 2023.

CHO, Y. H. et al. Effect of Pumpkin Seed Oil on Hair Growth in Men with Androgenetic Alopecia: A Randomized, Double-Blind, Placebo-Controlled Trial. **Evidence-Based Complementary and Alternative Medicine**, v. 2014, p. 1–7, 2014.

CINQUE, F. et al. Nutritional and Lifestyle Therapy for NAFLD in People with HIV. **Nutrients**, v. 15, n. 8, p. 1990, 20 abr. 2023.

CODEX ALIMENTARIUS (FAO/WHO). Codex Standard for Named Vegetable Oils, CODEX STAN 210 (Amended 2003). Codex Alimentarius, Roma, Itália, 2003.

CONRAD, Z. et al. Relationship between food waste, diet quality, and environmental sustainability. **PLOS ONE**, v. 13, n. 4, p. e0195405, 18 abr. 2018.

CRAVOTTO, C. et al. Towards Substitution of Hexane as Extraction Solvent of Food Products and Ingredients with No Regrets. **Foods**, v. 11, n. 21, p. 3412, 28 out. 2022.

CUFFARO, D.; DIGIACOMO, M.; MACCHIA, M. Dietary Bioactive Compounds: Implications for Oxidative Stress and Inflammation. **Nutrients**, v. 15, n. 23, p. 4966, 30 nov. 2023.

CUVELIER, M.-E.; LACOSTE, F.; COURTOIS, F. Application of a DSC model for the evaluation of TPC in thermo-oxidized oils. **Food Control**, v. 28, n. 2, p. 441–444, dez. 2012.

DE OLIVEIRA, J. B. et al. Chemical characteristics of grapes cv. Syrah (*Vitis vinifera* L.) grown in the tropical semiarid region of Brazil (Pernambuco state): influence of rootstock and harvest season. **Journal of the Science of Food and Agriculture**, v. 99, n. 11, p. 5050–5063, 30 ago. 2019.

DEME, T. et al. Fatty Acid Profile, Total Phenolic Content, and Antioxidant Activity of Niger Seed (Guizotia abyssinica) and Linseed (Linum usitatissimum). **Frontiers in Nutrition**, v. 8, p. 674882, 2 ago. 2021.

DI PIETRO FERNANDES, C. et al. Nutraceutical Potential of Grape (Vitis vinifera L.) Seed Oil in Oxidative Stress, Inflammation, Obesity and Metabolic Alterations. **Molecules**, v. 28, n. 23, p. 7811, 28 nov. 2023.

DIAS, B. V. et al. EPA/DHA and linseed oil have different effects on liver and adipose tissue in rats fed with a high-fat diet. **Prostaglandins & Other Lipid Mediators**, v. 159, p. 106622, abr. 2022.

DJURICIC, I.; CALDER, P. C. Beneficial Outcomes of Omega-6 and Omega-3 Polyunsaturated Fatty Acids on Human Health: An Update for 2021. **Nutrients**, v. 13, n. 7, p. 2421, 15 jul. 2021.

DOBRZYŃSKA, M.; PRZYSŁAWSKI, J. The effect of camelina oil (α-linolenic acid) and canola oil (oleic acid) on lipid profile, blood pressure, and anthropometric parameters in postmenopausal women. **Archives of Medical Science**, 31 mar. 2020.

DODOO, D. et al. Postmarketing Surveillance for the Photosensitised Oxidation of Vegetable Oils in the Marketplace. **Journal of Oleo Science**, v. 71, n. 6, p. 795–811, 2022.

DONSÌ, F.; FERRARI, G. Essential oil nanoemulsions as antimicrobial agents in food. **Journal of Biotechnology**, v. 233, p. 106–120, set. 2016.

DOS SANTOS FREITAS, L. et al. Pressurized liquid extraction of vitamin E from Brazilian grape seed oil. **Journal of Chromatography A**, v. 1200, n. 1, p. 80–83, jul. 2008.

DURAZZO, A.; FAWZY RAMADAN, M.; LUCARINI, M. Editorial: Cold Pressed Oils: A Green Source of Specialty Oils. **Frontiers in Nutrition**, v. 8, p. 836651, 11 fev. 2022.

EDEL, A. L.; ALIANI, M.; PIERCE, G. N. Stability of bioactives in flaxseed and flaxseed-fortified foods. **Food Research International**, v. 77, p. 140–155, nov. 2015.

ENDO, Y. Analytical Methods to Evaluate the Quality of Edible Fats and Oils: The JOCS Standard Methods for Analysis of Fats, Oils and Related Materials (2013) and Advanced Methods. **Journal of Oleo Science**, v. 67, n. 1, p. 1–10, 2018.

ETZBACH, L. et al. Characterization of carotenoid profiles in goldenberry (Physalis peruviana L.) fruits at various ripening stages and in different plant tissues by HPLC-DAD-APCI-MS. **Food Chemistry**, v. 245, p. 508–517, abr. 2018.

FALADE, A.; OBOH, G.; OKOH, A. Potential Health Implications of the Consumption of Thermally-Oxidized Cooking Oils – a Review. **Polish Journal of Food and Nutrition Sciences**, v. 67, n. 2, p. 95–105, 30 jun. 2017.

FERNANDES, L. et al. Seed oils of ten traditional Portuguese grape varieties with interesting chemical and antioxidant properties. **Food Research International**, v. 50, n. 1, p. 161–166, jan. 2013.

GACA, A. et al. Changes in Volatile Compound Profiles in Cold-Pressed Oils Obtained from Various Seeds during Accelerated Storage. **Molecules**, v. 26, n. 2, p. 285, 8 jan. 2021.

GARAVAGLIA, J. et al. Grape Seed Oil Compounds: Biological and Chemical Actions for Health. **Nutrition and Metabolic Insights**, v. 9, p. NMI.S32910, jan. 2016.

GHASEMI, E.; GOLABADI, D.; PIADEH, A. Effect of supplementing palmitic acid and altering the dietary ratio of *n* -6: *n* -3 fatty acids in low-fibre diets on production responses of dairy cows. **British Journal of Nutrition**, v. 126, n. 3, p. 355–365, 14 ago. 2021.

GÓRNAŚ, P. et al. Diversity of oil yield, fatty acids, tocopherols, tocotrienols, and sterols in the seeds of 19 interspecific grapes crosses. **Journal of the Science of Food and Agriculture**, v. 99, n. 5, p. 2078–2087, 30 mar. 2019.

GÓRSKA-WARSEWICZ, H. et al. Butter, Margarine, Vegetable Oils, and Olive Oil in the Average Polish Diet. **Nutrients**, v. 11, n. 12, p. 2935, 3 dez. 2019.

GOUFO, P.; SINGH, R. K.; CORTEZ, I. A Reference List of Phenolic Compounds (Including Stilbenes) in Grapevine (Vitis vinifera L.) Roots, Woods, Canes, Stems, and Leaves. **Antioxidants**, v. 9, n. 5, p. 398, 8 maio 2020.

GRAJZER, M. et al. Characteristics and Antioxidant Potential of Cold-Pressed Oils—Possible Strategies to Improve Oil Stability. **Foods**, v. 9, n. 11, p. 1630, 8 nov. 2020.

GROOTVELD, M. Evidence-Based Challenges to the Continued Recommendation and Use of Peroxidatively-Susceptible Polyunsaturated Fatty Acid-Rich Culinary Oils for High-Temperature Frying Practises: Experimental Revelations Focused on Toxic Aldehydic Lipid Oxidation Products. **Frontiers in Nutrition**, v. 8, p. 711640, 5 jan. 2022.

HAN, H. et al. Grape Seed Procyanidins Attenuates Cisplatin-induced Human Embryonic Renal Cell Cytotoxicity by Modulating Heme Oxygenase-1 in Vitro. **Cell Biochemistry and Biophysics**, v. 77, n. 4, p. 367–377, dez. 2019.

HANIF PALLA, A.; GILANI, A.-H. Dual effectiveness of Flaxseed in constipation and diarrhea: Possible mechanism. **Journal of Ethnopharmacology**, v. 169, p. 60–68, jul. 2015.

HARIRAM, V.; BOSE, A.; SERALATHAN, S. Dataset on optimized biodiesel production from seeds of Vitis vinifera using ANN, RSM and ANFIS. **Data in Brief**, v. 25, p. 104298, ago. 2019.

HELLER, K. et al. A comparative study between Europe and China in crop management of two types of flax: linseed and fibre flax. **Industrial Crops and Products**, v. 68, p. 24–31, jun. 2015.

INDELICATO, S. et al. Triacylglycerols in edible oils: Determination, characterization, quantitation, chemometric approach and evaluation of adulterations. **Journal of Chromatography A**, v. 1515, p. 1–16, set. 2017.

ISMAIL, A. F. M.; MOAWED, F. S. M.; MOHAMED, M. A. Protective mechanism of grape seed oil on carbon tetrachloride-induced brain damage in γ-irradiated rats. **Journal of Photochemistry and Photobiology B: Biology**, v. 153, p. 317–323, dez. 2015.

ISMAIL, A. F. M.; SALEM, A. A. M.; EASSAWY, M. M. T. Hepatoprotective effect of grape seed oil against carbon tetrachloride induced oxidative stress in liver of γ-irradiated rat. **Journal of Photochemistry and Photobiology B: Biology**, v. 160, p. 1–10, jul. 2016.

IZAR, M. C. D. O. et al. Posicionamento sobre o Consumo de Gorduras e Saúde Cardiovascular – 2021. **Arquivos Brasileiros de Cardiologia**, v. 116, n. 1, p. 160–212, 27 jan. 2021.

JIANG, Y.; QIAN, H.-Y. Transcription factors: key regulatory targets of vascular smooth muscle cell in atherosclerosis. **Molecular Medicine**, v. 29, n. 1, p. 2, 5 jan. 2023.

JOMOVA, K. et al. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. **Archives of Toxicology**, v. 97, n. 10, p. 2499–2574, out. 2023.

JOSHI, A.; HEGDE, M.; ZANWAR, A. Flaxseed oil and palm olein blend to improve omega-6: omega-3 ratio. **Journal of Food Science and Technology**, v. 59, n. 2, p. 498–509, fev. 2022.

KANIKOWSKA, D. et al. Effect of Flaxseed (Linum usitatissimum L.) Supplementation on Vascular Endothelial Cell Morphology and Function in Patients with Dyslipidaemia—A Preliminary Observation. **Nutrients**, v. 14, n. 14, p. 2879, 13 jul. 2022.

KARAMAN, S. et al. Recovery Potential of Cold Press Byproducts Obtained from the Edible Oil Industry: Physicochemical, Bioactive, and Antimicrobial Properties. **Journal of Agricultural and Food Chemistry**, v. 63, n. 8, p. 2305–2313, 4 mar. 2015.

KIM, T.-K. et al. Effects of replacing pork fat with grape seed oil and gelatine/alginate for meat emulsions. **Meat Science**, v. 163, p. 108079, maio 2020.

KIM, W.-K. et al. Monocyte Chemoattractant Protein-1 Deficiency Attenuates Oxidative Stress and Protects against Ovariectomy-Induced Chronic Inflammation in Mice. **PLoS ONE**, v. 8, n. 8, p. e72108, 19 ago. 2013.

KLEINER, D. E. et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. **Hepatology**, v. 41, n. 6, p. 1313–1321, jun. 2005.

KOCH, E. et al. Beyond Autoxidation and Lipoxygenases: Fatty Acid Oxidation Products in Plant Oils. **Journal of Agricultural and Food Chemistry**, v. 71, n. 35, p. 13092–13106, 6 set. 2023.

KOCHHAR, S. P.; HENRY, C. J. K. Oxidative stability and shelf-life evaluation of selected culinary oils. **International Journal of Food Sciences and Nutrition**, v. 60, n. sup7, p. 289–296, jan. 2009.

KOLAR, M. J. et al. Linoleic acid esters of hydroxy linoleic acids are anti-inflammatory lipids found in plants and mammals. **Journal of Biological Chemistry**, v. 294, n. 27, p. 10698–10707, jul. 2019.

KONTUSH, A. HDL and Reverse Remnant-Cholesterol Transport (RRT): Relevance to Cardiovascular Disease. **Trends in Molecular Medicine**, v. 26, n. 12, p. 1086–1100, dez. 2020.

LACHMAN, J. et al. Evaluation of oil content and fatty acid composition in the seed of grapevine varieties. **LWT - Food Science and Technology**, v. 63, n. 1, p. 620–625, set. 2015.

LAFFIN, L. J. et al. Comparative Effects of Low-Dose Rosuvastatin, Placebo, and Dietary Supplements on Lipids and Inflammatory Biomarkers. **Journal of the American College of Cardiology**, v. 81, n. 1, p. 1–12, jan. 2023.

LAI, X. et al. The protective effects and genetic pathways of thorn grape seeds oil against high glucose-induced apoptosis in pancreatic β-cells. **BMC Complementary and Alternative Medicine**, v. 14, n. 1, p. 10, dez. 2014.

LAU, K. Q.; SABRAN, M. R.; SHAFIE, S. R. Utilization of Vegetable and Fruit By-products as Functional Ingredient and Food. **Frontiers in Nutrition**, v. 8, p. 661693, 15 jun. 2021.

LÄUBLI, M. W.; BRUTTEL, P. A. Determination of the oxidative stability of fats and oils: Comparison between the active oxygen method (AOCS Cd 12-57) and the rancimat method. **Journal of the American Oil Chemists' Society**, v. 63, n. 6, p. 792–795, jun. 1986.

LEBAKA, V. R. et al. Nutritional Composition and Bioactive Compounds in Three Different Parts of Mango Fruit. **International Journal of Environmental Research and Public Health**, v. 18, n. 2, p. 741, 16 jan. 2021.

LEE, J.-H. et al. Antiviral effects of black raspberry (*Rubus coreanus*) seed extract and its polyphenolic compounds on norovirus surrogates. **Bioscience, Biotechnology, and Biochemistry**, v. 80, n. 6, p. 1196–1204, 2 jun. 2016.

LEITE, C. D. F. C. et al. Effects of flaxseed on rat milk creamatocrit and its contribution to offspring body growth. **Jornal de Pediatria**, v. 88, n. 1, p. 74–8, 15 fev. 2012.

LU, Y. et al. Tocotrienol-Rich Fractions Offer Potential to Suppress Pulmonary Fibrosis Progression. **International Journal of Molecular Sciences**, v. 23, n. 22, p. 14331, 18 nov. 2022.

LUTTERODT, H. et al. Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. **Food Chemistry**, v. 128, n. 2, p. 391–399, set. 2011.

LUTZ, C. S.; CORNETT, A. L. Regulation of genes in the arachidonic acid metabolic pathway by RNA processing and RNA -mediated mechanisms. **WIREs RNA**, v. 4, n. 5, p. 593–605, set. 2013.

LYONS, C.; KENNEDY, E.; ROCHE, H. Metabolic Inflammation-Differential Modulation by Dietary Constituents. **Nutrients**, v. 8, n. 5, p. 247, 27 abr. 2016.

MA, Z.; ZHANG, H. Phytochemical Constituents, Health Benefits, and Industrial Applications of Grape Seeds: A Mini-Review. **Antioxidants**, v. 6, n. 3, p. 71, 15 set. 2017.

MAGALINGAM, K. B. et al. Tocotrienol-Rich Fraction and Levodopa Regulate Proteins Involved in Parkinson's Disease-Associated Pathways in Differentiated Neuroblastoma Cells: Insights from Quantitative Proteomic Analysis. **Nutrients**, v. 14, n. 21, p. 4632, 3 nov. 2022.

MAHMUDIONO, T. et al. The effect of flaxseed oil consumtion on blood pressure among patients with metabolic syndrome and related disorders: A systematic review and meta-analysis of randomized clinical trials. **Phytotherapy Research**, v. 36, n. 10, p. 3766–3773, out. 2022.

MALI, A. V. et al. Anticancer and antimetastatic potential of enterolactone: Clinical, preclinical and mechanistic perspectives. **European Journal of Pharmacology**, v. 852, p. 107–124, jun. 2019.

MARCELINO, G. et al. Characterization and oxidative stability of oils and bioactive compounds of the fruits of *Byrsonima cydoniifolia* A. Juss. at different ripening stages. **Journal of the Science of Food and Agriculture**, v. 99, n. 6, p. 2855–2864, abr. 2019.

MARCELINO, G. et al. Characterization of Buriti (Mauritia flexuosa) Pulp Oil and the Effect of Its Supplementation in an In Vivo Experimental Model. **Nutrients**, v. 14, n. 12, p. 2547, 19 jun. 2022.

MARTIN, M. E. et al. Grape (Vitis vinifera L.) Seed Oil: A Functional Food from the Winemaking Industry. **Foods**, v. 9, n. 10, p. 1360, 25 set. 2020.

MARTINCHIK, A. N. et al. [Nutritional value and functional properties of flaxseed]. **Voprosy Pitaniia**, v. 81, n. 3, p. 4–10, 2012.

MARTÍN-REYES, F. et al. Oleic acid regulates the circadian rhythm of adipose tissue in obesity. **Pharmacological Research**, v. 187, p. 106579, jan. 2023.

MATTOS, G. N. et al. Grape by-product extracts against microbial proliferation and lipid oxidation: a review. **Journal of the Science of Food and Agriculture**, v. 97, n. 4, p. 1055–1064, mar. 2017.

MEIRELES, B. R. L. D. A. et al. Catole coconut (Syagrus cearensis) oil: physicochemical characterization and thermo-oxidative stability by TG/DTG/DTA and Rancimat. **Journal of Thermal Analysis and Calorimetry**, v. 147, n. 5, p. 3591–3598, mar. 2022.

MENEGAZZO, M. L.; PETENUCI, M. E.; FONSECA, G. G. Production and characterization of crude and refined oils obtained from the co-products of Nile tilapia and hybrid sorubim processing. **Food Chemistry**, v. 157, p. 100–104, ago. 2014.

MEZZOMO, N.; FERREIRA, S. R. S. Carotenoids Functionality, Sources, and Processing by Supercritical Technology: A Review. **Journal of Chemistry**, v. 2016, p. 1–16, 2016.

MIGICOVSKY, Z. et al. Patterns of genomic and phenomic diversity in wine and table grapes. **Horticulture Research**, v. 4, n. 1, p. 17035, dez. 2017.

MIKOŁAJCZAK, N. et al. Efficacy of canolol and guaiacol in the protection of cold-pressed oils being a dietary source linoleic acid against oxidative deterioration. **Food Chemistry**, v. 393, p. 133390, nov. 2022.

MILANI, A. et al. Carotenoids: biochemistry, pharmacology and treatment. **British Journal of Pharmacology**, v. 174, n. 11, p. 1290–1324, jun. 2017.

MILLER, E. F. Nutrition Management Strategies for Nonalcoholic Fatty Liver Disease: Treatment and Prevention. **Clinical Liver Disease**, v. 15, n. 4, p. 144–148, abr. 2020.

MIRABELLI, M. et al. Mediterranean Diet Nutrients to Turn the Tide against Insulin Resistance and Related Diseases. **Nutrients**, v. 12, n. 4, p. 1066, 12 abr. 2020.

MIRSHEKAR, R.; DASTAR, B.; SHAMS SHARGH, M. Supplementing flaxseed oil for long periods improved carcass quality and breast fatty acid profile in Japanese quail. **animal**, v. 15, n. 2, p. 100104, fev. 2021.

MISUTSU, M. Y. et al. Thermoanalytical Methods in Verifying the Quality of Biodiesel. Em: BIERNAT, K. (Ed.). **Biofuels - Status and Perspective**. [s.l.] InTech, 2015.

MUEED, A. et al. Flaxseed Bioactive Compounds: Chemical Composition, Functional Properties, Food Applications and Health Benefits-Related Gut Microbes. **Foods**, v. 11, n. 20, p. 3307, 21 out. 2022.

MULTARI, S. et al. Changes in the volatile profile, fatty acid composition and other markers of lipid oxidation of six different vegetable oils during short-term deep-frying. **Food Research International**, v. 122, p. 318–329, ago. 2019.

NERY, C. D. S. et al. Medidas murinométricas e eficiência alimentar em ratos provenientes de ninhadas reduzidas na lactação e submetidos ou não ao exercício de natação. **Revista Brasileira de Medicina do Esporte**, v. 17, n. 1, p. 49–55, fev. 2011.

OCCHIUTO, C. et al. Comparative Evaluation of the Nutrients, Phytochemicals, and Antioxidant Activity of Two Hempseed Oils and Their Byproducts after Cold Pressing. **Molecules**, v. 27, n. 11, p. 3431, 26 maio 2022.

OJEDA-AMADOR, R. M. et al. Characterization of virgin walnut oils and their residual cakes produced from different varieties. **Food Research International**, v. 108, p. 396–404, jun. 2018.

OPPEDISANO, F. et al. The Anti-Inflammatory and Antioxidant Properties of n-3 PUFAs: Their Role in Cardiovascular Protection. **Biomedicines**, v. 8, n. 9, p. 306, 25 ago. 2020.

PANTOJA, S. et al. High-Quality Biodiesel Production from Buriti (Mauritia flexuosa) Oil Soapstock. **Molecules**, v. 24, n. 1, p. 94, 28 dez. 2018.

PARIHAR, S.; SHARMA, D. A Breif Overview on Vitis vinifera. **Scholars Academic Journal of Pharmacy**, v. 10, n. 12, p. 231–239, 22 dez. 2021.

PARIKH, H. et al. Assessing diatom distribution in Cambay Basin, Western Arabian Sea: impacts of oil spillage and chemical variables. **Environmental Monitoring and Assessment**, v. 195, n. 8, p. 993, ago. 2023.

PARIKH, M.; NETTICADAN, T.; PIERCE, G. N. Flaxseed: its bioactive components and their cardiovascular benefits. **American Journal of Physiology-Heart and Circulatory Physiology**, v. 314, n. 2, p. H146–H159, 1 fev. 2018.

PARK, K.-H. et al. Palmitic Acid-Enriched Diet Induces Hepatic Steatosis and Injury in Adult Zebrafish. **Zebrafish**, v. 16, n. 6, p. 497–504, 1 dez. 2019.

PASINI, F. et al. Recovery of Oligomeric Proanthocyanidins and Other Phenolic Compounds with Established Bioactivity from Grape Seed By-Products. **Molecules**, v. 24, n. 4, p. 677, 14 fev. 2019.

PEREIRA, S. S. et al. Differences in adipose tissue inflammation and oxidative status in C57BL/6 and ApoE−/− mice fed high fat diet. **Animal Science Journal**, v. 83, n. 7, p. 549–555, jul. 2012.

PERIASAMY, V. S.; ATHINARAYANAN, J.; ALSHATWI, A. A. Anticancer activity of an ultrasonic nanoemulsion formulation of Nigella sativa L. essential oil on human breast cancer cells. **Ultrasonics Sonochemistry**, v. 31, p. 449–455, jul. 2016.

PISESKUL, J. et al. Optimization of Enzyme-Assisted Mechanical Extraction Process of Hodgsonia heteroclita Oilseeds and Physical, Chemical, and Nutritional Properties of the Oils. **Foods**, v. 12, n. 2, p. 292, 8 jan. 2023.

POLI, A.; AGOSTONI, C.; VISIOLI, F. Dietary Fatty Acids and Inflammation: Focus on the n-6 Series. **International Journal of Molecular Sciences**, v. 24, n. 5, p. 4567, 26 fev. 2023.

POLJŠAK, N.; KOČEVAR GLAVAČ, N. Vegetable Butters and Oils as Therapeutically and Cosmetically Active Ingredients for Dermal Use: A Review of Clinical Studies. **Frontiers in Pharmacology**, v. 13, p. 868461, 25 abr. 2022.

PONS, Z. et al. Chronic administration of grape-seed polyphenols attenuates the development of hypertension and improves other cardiometabolic risk factors associated with the metabolic syndrome in cafeteria diet-fed rats. **British Journal of Nutrition**, v. 117, n. 2, p. 200–208, 28 jan. 2017.

POPEIJUS, H. E. et al. Potential Contribution of Short Chain Fatty Acids to Hepatic Apolipoprotein A-I Production. **International Journal of Molecular Sciences**, v. 22, n. 11, p. 5986, 1 jun. 2021.

POPKIN, B. M.; REARDON, T. Obesity and the food system transformation in LATIN AMERICA. **Obesity Reviews**, v. 19, n. 8, p. 1028–1064, ago. 2018.

POWER, K. A. et al. Dietary flaxseed modulates the colonic microenvironment in healthy C57Bl/6 male mice which may alter susceptibility to gut-associated diseases. **The Journal of Nutritional Biochemistry**, v. 28, p. 61–69, fev. 2016.

PRESCHA, A. et al. The Antioxidant Activity and Oxidative Stability of Cold-Pressed Oils. **Journal of the American Oil Chemists' Society**, v. 91, n. 8, p. 1291–1301, ago. 2014.

PULKRABEK, M. et al. Flaxseed- and Buckwheat-Supplemented Diets Altered *Enterobacteriaceae* Diversity and Prevalence in the Cecum and Feces of Obese Mice. **Journal of Dietary Supplements**, v. 14, n. 6, p. 667–678, 2 nov. 2017.

RABAIL, R. et al. An Intricate Review on Nutritional and Analytical Profiling of Coconut, Flaxseed, Olive, and Sunflower Oil Blends. **Molecules**, v. 26, n. 23, p. 7187, 26 nov. 2021.

RAD, Z. A.; MOUSAVI, S. N.; CHITI, H. A low-carb diet increases fecal short-chain fatty acids in feces of obese women following a weight-loss program: randomized feeding trial. **Scientific Reports**, v. 13, n. 1, p. 18146, 24 out. 2023.

RANA, A. et al. Health benefits of polyphenols: A concise review. **Journal of Food Biochemistry**, v. 46, n. 10, out. 2022.

RAOF, N. A. et al. Effect of molecular structure on oxidative degradation of ester based transformer oil. **Tribology International**, v. 140, p. 105852, dez. 2019.

REDONDO-CUEVAS, L. et al. Revealing the relationship between vegetable oil composition and oxidative stability: A multifactorial approach. **Journal of Food Composition and Analysis**, v. 66, p. 221–229, mar. 2018.

REDONDO-CUEVAS, L.; CASTELLANO, G.; RAIKOS, V. Natural antioxidants from herbs and spices improve the oxidative stability and frying performance of vegetable oils.

International Journal of Food Science & Technology, v. 52, n. 11, p. 2422–2428, nov. 2017.

REZENDE, D. A. D. C. S. et al. Effect of the essential oils of *Satureja montana* L., *Myristica fragrans* H. and *Cymbopogon flexuosus* S. on mycotoxin-producing *Aspergillus flavus* and *Aspergillus ochraceus* antifungal properties of essential oils. **FEMS Microbiology Letters**, v. 368, n. 19, p. fnab137, 26 out. 2021.

RHEE, K. S.; STUBBS, A. C. Oxidative Deterioration in Vegetable Oils: Health-Food Oils Versus Conventional Oils. **Journal of Food Protection**, v. 41, n. 6, p. 443–446, jun. 1978.

RIMEZ, B. et al. The thermal degradation of poly(vinyl acetate) and poly(ethylene-co-vinyl acetate), Part I: Experimental study of the degradation mechanism. **Polymer Degradation and Stability**, v. 93, n. 4, p. 800–810, abr. 2008.

RODRÍGUEZ, L. et al. A Comprehensive Literature Review on Cardioprotective Effects of Bioactive Compounds Present in Fruits of Aristotelia chilensis Stuntz (Maqui). **Molecules**, v. 27, n. 19, p. 6147, 20 set. 2022.

RODRIGUEZ-AMAYA, D. B. Changes in carotenoids during processing and storage of foods. **Archivos Latinoamericanos De Nutricion**, v. 49, n. 3 Suppl 1, p. 38S-47S, set. 1999.

ROY, S. et al. Recent Advances in the Carotenoids Added to Food Packaging Films: A Review. **Foods**, v. 12, n. 21, p. 4011, 2 nov. 2023.

RUBILAR, M. et al. FLAXSEED AS A SOURCE OF FUNCTIONAL INGREDIENTS. **Journal of soil science and plant nutrition**, v. 10, n. 3, jul. 2010.

SAKAI, C. et al. Fish oil omega-3 polyunsaturated fatty acids attenuate oxidative stress-induced DNA damage in vascular endothelial cells. **PLOS ONE**, v. 12, n. 11, p. e0187934, 9 nov. 2017.

SALMAN, H. B.; SALMAN, M. A.; YILDIZ, E. A. The effect of omega-3 fatty acid supplementation on weight loss and cognitive function in overweight or obese individuals on weight-loss diet. **Nutrición Hospitalaria**, 2022.

SARASWATHI, V. et al. Myristic Acid Supplementation Aggravates High Fat Diet-Induced Adipose Inflammation and Systemic Insulin Resistance in Mice. **Biomolecules**, v. 12, n. 6, p. 739, 24 maio 2022.

SAVVA, S. C.; KAFATOS, A. Vegetable Oils: Dietary Importance. Em: **Encyclopedia of Food and Health**. [s.l.] Elsevier, 2016. p. 365–372.

SCHERER, R.; SIDDIQ, F. The relation between students' socioeconomic status and ICT literacy: Findings from a meta-analysis. **Computers & Education**, v. 138, p. 13–32, set. 2019.

SHINAGAWA, F. B. et al. Chemical composition of cold pressed Brazilian grape seed oil. **Food Science and Technology**, v. 38, n. 1, p. 164–171, 19 out. 2017.

SILVA FIGUEIREDO, P. et al. An Overview of Novel Dietary Supplements and Food Ingredients in Patients with Metabolic Syndrome and Non-Alcoholic Fatty Liver Disease. **Molecules**, v. 23, n. 4, p. 877, 11 abr. 2018.

SILVA, L. P. R. D. et al. Bocaiuva (Acrocomia aculeata) nut oil: composition and metabolic impact in an experimental study. **Food Science and Technology**, v. 43, p. e43522, 2023.

SIRIAMORNPUN, S.; KAEWSEEJAN, N. Quality, bioactive compounds and antioxidant capacity of selected climacteric fruits with relation to their maturity. **Scientia Horticulturae**, v. 221, p. 33–42, jul. 2017.

SIVA KUMAR, S. et al. Effect of flaxseed oil and flour on sensory, physicochemical and fatty acid profile of the fruit yoghurt. **Journal of Food Science and Technology**, v. 54, n. 2, p. 368–378, fev. 2017.

SŁAWIŃSKA, N.; PROCHOŃ, K.; OLAS, B. A Review on Berry Seeds—A Special Emphasis on Their Chemical Content and Health-Promoting Properties. **Nutrients**, v. 15, n. 6, p. 1422, 15 mar. 2023.

SONG, X.; SUI, X.; JIANG, L. Protection Function and Mechanism of Rosemary (Rosmarinus officinalis L.) Extract on the Thermal Oxidative Stability of Vegetable Oils. **Foods**, v. 12, n. 11, p. 2177, 28 maio 2023.

SPERANZA, P. et al. Amazonian Buriti oil: chemical characterization and antioxidant potential. **Grasas y Aceites**, v. 67, n. 2, p. e135, 30 jun. 2016.

TAMTAJI, O. R. et al. Effects of flaxseed oil supplementation on biomarkers of inflammation and oxidative stress in patients with metabolic syndrome and related disorders: A systematic review and meta-analysis of randomized controlled trials. **Clinical Nutrition ESPEN**, v. 40, p. 27–33, dez. 2020.

TANG, G.-Y. et al. Potential of Grape Wastes as a Natural Source of Bioactive Compounds. **Molecules**, v. 23, n. 10, p. 2598, 11 out. 2018.

TANG, Z. et al. Flaxseed oil: Extraction, health benefits and products. **Quality Assurance and Safety of Crops & Foods**, v. 13, n. 1, p. 1–19, 2 jan. 2021.

TAYLOR, B. A.; PHILLIPS, S. J. Detection of Obesity QTLs on Mouse Chromosomes 1 and 7 by Selective DNA Pooling. **Genomics**, v. 34, n. 3, p. 389–398, jun. 1996.

TEH, H. E. et al. Hypocholesterolemic Effects of Expeller-Pressed and Solvent-Extracted Fruit Seed Oils and Defatted Pomegranate Seed Meals. **Journal of Agricultural and Food Chemistry**, v. 67, n. 22, p. 6150–6159, 5 jun. 2019.

TORRES SILVA, G. et al. Caryocar brasiliense Cambess. Pulp Oil Supplementation Reduces Total Cholesterol, LDL-c, and Non-HDL-c in Animals. **Molecules**, v. 25, n. 19, p. 4530, 3 out. 2020.

UDENIGWE, C. C. et al. Low molecular weight flaxseed protein-derived arginine-containing peptides reduced blood pressure of spontaneously hypertensive rats faster than amino acid form of arginine and native flaxseed protein. **Food Chemistry**, v. 132, n. 1, p. 468–475, maio 2012.

ULBRICHT, T. L. V.; SOUTHGATE, D. A. T. Coronary heart disease: seven dietary factors. **The Lancet**, v. 338, n. 8773, p. 985–992, out. 1991.

VEGA-LIZAMA, T. et al. Thermogravimetric analysis as a rapid and simple method to determine the degradation degree of soy biodiesel. **Fuel**, v. 156, p. 158–162, set. 2015.

VENN, B. J. Macronutrients and Human Health for the 21st Century. **Nutrients**, v. 12, n. 8, p. 2363, 7 ago. 2020.

VIDAR HANSEN, T.; SERHAN, C. N. Protectins: Their biosynthesis, metabolism and structure-functions. **Biochemical Pharmacology**, v. 206, p. 115330, dez. 2022.

VILAHUR, G. et al. Phytosterols and Inflammation. **Current Medicinal Chemistry**, v. 26, n. 37, p. 6724–6734, 17 dez. 2019.

VITALE, S. et al. Phytochemistry and Biological Activity of Medicinal Plants in Wound Healing: An Overview of Current Research. **Molecules**, v. 27, n. 11, p. 3566, 1 jun. 2022.

WALKER, E. B. et al. Polyethylene glycol-mediated fusion of herpes simplex type 1 virions with the plasma membrane of cells that support endocytic entry. **Virology Journal**, v. 12, n. 1, p. 190, dez. 2015.

WALL-MEDRANO, A. et al. Lipidomic and Antioxidant Response to Grape Seed, Corn and Coconut Oils in Healthy Wistar Rats. **Nutrients**, v. 9, n. 1, p. 82, 20 jan. 2017.

WANG, Y.; LIU, J.; WANG, Y. Role of TNF-α-induced m6A RNA methylation in diseases: a comprehensive review. **Frontiers in Cell and Developmental Biology**, v. 11, p. 1166308, 24 jul. 2023.

YADAV, R. K. et al. Modulation of oxidative stress response by flaxseed oil: Role of lipid peroxidation and underlying mechanisms. **Prostaglandins & Other Lipid Mediators**, v. 135, p. 21–26, mar. 2018.

YANG, K.-M. et al. Quality Characterization and Oxidative Stability of Camellia Seed Oils Produced with Different Roasting Temperatures. **Journal of Oleo Science**, v. 67, n. 4, p. 389–396, 2018.

YANG, Z. et al. Application evaluation of linseed oil as a substitute for fish oil in the commercial diet for juvenile Portunus trituberculatus. **Aquaculture Reports**, v. 29, p. 101504, abr. 2023.

YARA-VARÓN, E. et al. Vegetable Oils as Alternative Solvents for Green Oleo-Extraction, Purification and Formulation of Food and Natural Products. **Molecules**, v. 22, n. 9, p. 1474, 5 set. 2017.

ZABOT, G. L. et al. Encapsulation of Bioactive Compounds for Food and Agricultural Applications. **Polymers**, v. 14, n. 19, p. 4194, 6 out. 2022.

ZAREV, Y. et al. Exploring Phytochemical Composition and In Vivo Anti-Inflammatory Potential of Grape Seed Oil from an Alternative Source after Traditional Fermentation Processes: Implications for Phytotherapy. **Plants**, v. 12, n. 15, p. 2795, 28 jul. 2023.

ZEB, A. Concept, mechanism, and applications of phenolic antioxidants in foods. **Journal of Food Biochemistry**, v. 44, n. 9, set. 2020.

ZHANG, X. et al. Flaxseed oil ameliorates alcoholic liver disease via anti-inflammation and modulating gut microbiota in mice. **Lipids in Health and Disease**, v. 16, n. 1, p. 44, dez. 2017.

ZHAO, L. et al. Muscadine grape seed oil as a novel source of tocotrienols to reduce adipogenesis and adipocyte inflammation. **Food & Function**, v. 6, n. 7, p. 2293–2302, 2015.

ZHU, L. et al. Dietary flaxseed oil rich in omega-3 suppresses severity of type 2 diabetes mellitus via anti-inflammation and modulating gut microbiota in rats. **Lipids in Health and Disease**, v. 19, n. 1, p. 20, dez. 2020.

ANEXO

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Review

Nutraceutical Potential of Grape (*Vitis vinifera* L.) Seed Oil in Oxidative Stress, Inflammation, Obesity and Metabolic Alterations

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Abstract: *Vitis vinifera* L. (grapevine) is a perennial plant of the Vitaceae family that is widely used to produce grapes and wines. Grape seed oil is rich in fatty acids such as linoleic acid (65–75%), vitamin E (50 mg), and phytosterols in addition to phenolic compounds, such as catechins (414 mg), epicatechins (130.4 mg), and gallic acid (77 µg), shows promise as a nutritional compound and is outstanding as a therapeutic substance with active properties for health, detected mainly by in vitro studies, as well as some in vivo studies. The benefits of consuming this oil include modulating the expression of antioxidant enzymes, anti-atherosclerotic and anti-inflammatory effects, and protection against oxidative cell damage and some types of cancer. However, experimental findings confirm that therapeutic functions remain scarce; thus, more studies are needed to determine the mechanisms of action involved in the indicated therapeutic qualities.

Keywords: plant oil; linoleic acid; phenolics; resveratrol; therapeutic potential; oxidative stress; inflammation; obesity

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1. Introduction

The Vitaceae family has about 15 genera and 800 species that are present in temperate regions such as Europe, most of North America, western Asia, southern South America, and the extreme north and south of Africa [1]. The species *Vitis vinifera* L. is produced by the vine (*Vitis* sp.) and is cultivated to produce wine and the fresh consumption of fruit in Europe. This vine of the Vitaceae family has the grape as fruit, which has been cultivated for thousands of years by different civilizations [2].

The commercialization and cultivation of grapes have been going on for more than two millennia in Western Europe and more than four millennia in the Eastern Mediterranean, with genetic alterations resulting from spontaneous reproduction in addition to the definition of methods for the genetic preservation of those most accepted and preferred by the local population. Thus, the grape varieties produced in Western Europe are currently

the basis of the global wine industry, the main planting varieties being Cabernet Sauvignon, Merlot, Grenache, Sauvignon Blanc, Tempranillo, Chardonnay, Syrah, Pinot Noir, Airen, and Trebbiano Toscano [3].

Grape production has many specifications regarding the existing subspecies of this fruit, such as the presence or absence of seeds, different colors (reddish, black, and white), as well as fruit size and shape [4]. Therefore, there are beneficial nutritional properties present in parts of the plant, such as the root, stem, leaf, seed, and pulp, that can be used by the food industry to contribute to the health–disease process in different ways [5–7].

Various nutritional constituents have also been found in grapes (*V. vinifera* L.). The nutritional content of grapes includes proteins, lipids, carbohydrates, minerals, and vitamins. Each part of the vines or any other grape-based product contains different nutrients. Grape seeds (*Vitis vinifera* L.) have in their nutritional composition lipids (10.45–16.73 g), proteins (8.7–9.8 g), carbohydrates (18.2–19.8 g), and fiber (40.2–43.7 g) per 100 g [1,8,9].

The composition of *V. vinifera* seeds comprises 90% unsaturated fatty acids, with the major ones being linoleic fatty acid (65–75%) and oleic fatty acid (20–40%), and 10% saturated fatty acids [8,9], flavonoids (59.7 g/100 g), catechins (414 mg/100 g), procyanidins (2.5 g/100 g), phenolic compounds (73 µg/100 g) and tannins (14 mg CE/100 g); thus, they have antioxidant activity (1390.6 mMT/100 g) [10–14].

Grape seeds have shown antioxidant, antifungal, anti-inflammatory, and anti-obesity effects, as well as anticholinergic cicatrizing action [15]. Grape seed oil has the capacity to preserve important compounds to prevent oxidative stress, such as ascorbic acid, β-carotene, phytosterols, and tocopherols due to the presence of procyanidins in the plant's stems and leaves; procyanidins in the seeds also indicate the antioxidant activity of the oil, according to Goufo, Singh and Cortez (2020) [16].

Grape seeds have health benefits, as scientific studies have demonstrated different biological and medicinal characteristics [15,16]. However, few studies have reported on their therapeutic potential for metabolic dysfunctions through experimental models of obesity, oxidative stress, or inflammation. Thus, our review investigated the nutritional value and bioactive compounds of *V. vinifera* seeds, in addition to ethnobotanical knowledge and possible metabolic, antioxidant, and anti-inflammatory applications.

2. Contextual Relationship of *Vitis vinifera* L.

Growing predominantly in regions with temperate and subtropical climates in the Northern Hemisphere [17], the European vine *V. vinifera* is considered the most relevant species and the most planted worldwide to meet the market demand for fine wines, sparkling wines and fresh fruit [18]. The vine (from the Latin *viere*, to fix) is a shrubby climber whose growth is controlled by pruning for grape quantity and quality [19].

Generally, grapes have a bunch of axes, juice, pulp, peel, and seeds [7]. The seeds represent about 10–12% of the solid residue left by the vinification process [20], and studies have shown that processing protocols can affect the content of seeds [21,22]. However, phenols and other components of grape seeds can still be used as nutraceuticals or can prevent diseases, which promotes the full utilization of grapes by reusing the non-edible parts [23].

Nutritional Phytochemical Composition of Vitis vinifera L. Seed Oil

Grape (*V. vinifera*) seeds contain approximately 17% lipids (10.45–16.73 g/100 g), 10% proteins (8.7–9.8 g/100 g), 20% carbohydrates (18.2–19.8 g/100 g) and between 40 and 44% food fiber (40.2–43.7 g/100 g) (Table 1) [8,9].

Table 1. Nutrients and fiber in *Vitis vinifera* L. seeds (g/100 g; minimum and maximum values) [8,9,24].

Component	Quantity per 100 g
Carbohydrates (g)	18.2–19.8
Proteins (g)	8.7–9.8
Lipids (g)	10.6–16.7
Monounsaturated fatty acids (g)	8.8–22.1
Polyunsaturated fatty acids (g)	67.2–78.2
Saturated fatty acids (g)	7.0–12.8
Fiber (g)	40.2–43.7
Energy (Kcal/100 g)	216.8–237.4

Grape pomace is a solid organic by-product consisting of grape skin, stem fractions, pulp, and seed, which are discarded in the manufacture of wines or juices and can either be fermented or not [25]. The pomace powder presents amounts of moisture (8.9 ± 0.08), ash (24.97 ± 2.4), carbohydrates (6.13 ± 0.01), total fiber (2.16 ± 0.01), fat (7.69 ± 0.02), and crude protein (50.33 ± 2.1) [26]. Only the grape seeds are also food residues and have in their composition in oil a mean 90% of unsaturated fatty acids (Table 2), mainly linoleic (65–75%) and oleic (20–40%) fatty acids, and 10% saturated fatty acids [8,9,14].

Table 2. Proportions of fatty acids in *Vitis vinifera* L. seed oil (%) [8,9,14].

Fatty Acids	Percentage (%)
C8:0 (Caprilic acid)	0.01
C12:0 (Lauric acid)	0.01
C14:0 (Myristic acid)	0.05
C15:0 (Pentadecilic acid)	0.01
C16:0 (Palmitic acid)	6.7
C17:0 (Heptadecanoic acid)	0.06
C18:0 (Estearic acid)	3.8
C20:0 (Arachidic acid)	0.16
C16:1 (n-7) (Palmitoleic acid)	0.2
C18:1 *cis* (n-9) (Oleic acid)	14.8
C20:1(n-9) (Gadoleic acid)	0.40
C18:2 *cis* (n-6) (Linolenic acid)	74.2
C18:3 (n-3) (α-Linolenic acid)	0.11
Saturated fatty acids (SFAs)	10.6
Monounsaturated fatty acids (MUFAs)	14.9
Polyunsaturated fatty acids (PUFAs)	74.3
n-3 PUFAs (ω-3)	0.2
n-6 PUFAs (ω-6)	74.7

Vitis vinifera seed oil presents a rich variety of macro- and microelements, including potassium (4347.8–9492.6 mg/1000 g), phosphorus (2277.6–3232.4 mg/1000 g) and calcium (1249.1–2073.9 mg/1000 g) (Table 3) [24].

Table 3. Macro- and microelements of *Vitis vinifera* L. seed oil (mg per 1000 g seeds; minimum and maximum values) [16,27–30].

Component	mg/1000 g
Potassium	4347.8–9492.6
Iron	29.9–73.8
Phosphorus	2277.6–3232.4
Calcium	1249.1–2073.9
Magnesium	249.1–2073.9
Zinc	8.2–15.9
Manganese	2.0–11.5
Sulphur	8.6–15.2

Regarding vitamins, Goufo et al. determined that 50 mg of vitamin E exists per 100 g of grape seed and noted that homologs α and β were the most abundant, representing 86–244 and 38–48 mg/1000 g, respectively, along with γ-tocopherols (17–29 mg/1000 g); regarding to-cotrienols, isomer γ (499–1575 mg/1000 g) was the most abundant (Table 4) [16]. Among the tocopherols in grape seeds, ÿ-tocopherol (47% tocochromanols) and ÿ-tocotrienol (155 µg/g oleoresin) were the most abundant [31]. A study by Harbeoui et al. [32] analyzed the unsaponifiable fraction of seed oil in three varieties (Merlot, Carign and Syrah), showing the presence of two triterpenic compounds (β-mirin, lanosterol), six phytosterols (campesterol, Δ 7-avenasterol, stigmasterol, β-sitosterol, β-sitostanol, cholesterol) and three tocopherols (α, β and γ). Dordevski et al. [14], exploring the functional properties of seed oil in Tamjanika, a subspecies of V. vinifera, found high quantities of tocotrienols (85.04 mg/100 g), predominating over tocopherols (8.37 mg/100 g), and also reported a carotenoid content of 0.27 mg/100 g, with lutein being the primary pigment.

Table 4. Main phytochemical compounds in *Vitis vinifera* L. seed oil [10,11,13,14].

Components	Quantity per 100 g
Flavonoids (mg) [10]	59.7
Epicatechin (mg) [10]	130.4
Catechins (mg) [11]	414.0
Procyanidins (mg) [11]	2.5
Phenolics (µg) [13]	73.0
Gallic acid (µg) [10]	77.0
Condensed tannins (mg CE) [14]	14.0
Vitamin E	
α-Tocopherols (mg)	86–244
β-Tocopherols (mg)	38–48
γ-Tocopherols (mg)	17–29
α-Tocotrienols (mg)	216–319
β-Tocotrienols (mg)	4–18
γ-Tocotrienols (mg)	499–1575
Vitamin C	46.0–179.2
Vitamin A	
Carotenoids (mg)	27.0–48.0
β-carotene (ppm)	33.9–59.8

Keskin et al. [33] reported a vitamin C level of 128.30 mg per 1000 g in pomace; however, in other studies, the content varied from 46.0 to 179.2 mg per 1000 g (Table 4) [34]. β-carotene content was reported to be between 33.9 and 59.8 ppm in the oil of grape seeds [35].

Evaluations of the phytochemical composition of grape seeds detected proanthocyanidins, i.e., oligomers and polymers of flavan-3-ol monomers, which presented only in the form of the procyanidins catechin, epicatechin and epicatechin gallate, and were linked mainly through C4–C6 or C4–C8 (type B) by differences in their constitutive units, the position of their connection and shape, and the polymerization degree [32]. Simpler and more common components of grape seeds include dimeric and trimeric procyanidins [36].

Bocsan et al. [13] identified a total polyphenol content in *V. vinifera* seed oil of 0.75 mg gallic acid equivalent (GAE)/100 g. By quantifying and qualifying the phenolic compounds extracted from the oil, they observed more catechins, epicatechins, gallic acid, quercetin, rutin, caffeic acid, procyanidins, and phenolic acids (Table 4) [37–39].

Mota et al. [16] analyzed the chemical profile of the seed oil of four cultivars of table grapes (*V. vinifera)*, and among the phenolic compounds identified 7.7 µg/g of gallic acid at a wavelength of 250 nm, and in the cultivars Cardinal (29.7 µg/g) and Muscat Hamburg (18.3 µg/g).

3. Medicinal Properties of *Vitis vinifera* L. Seed Oil

The medicinal properties attributed to grapes include the protective effects of antioxidant, antifungal, anti-inflammatory, and anti-obesity compounds and anticholinergic and cicatrizing actions [27]. Grape seed oil can function in the preservation of essential compounds to prevent oxidative stress. Goufo et al. [16] reported the preservation of micronutrients such as ascorbic acid, tocopherol, and β- carotene due to the presence of procyanidins in the plant's stems and leaves; thus, the presence of procyanidin in seeds can also indicate the antioxidant activity of the oil (Figure 1).

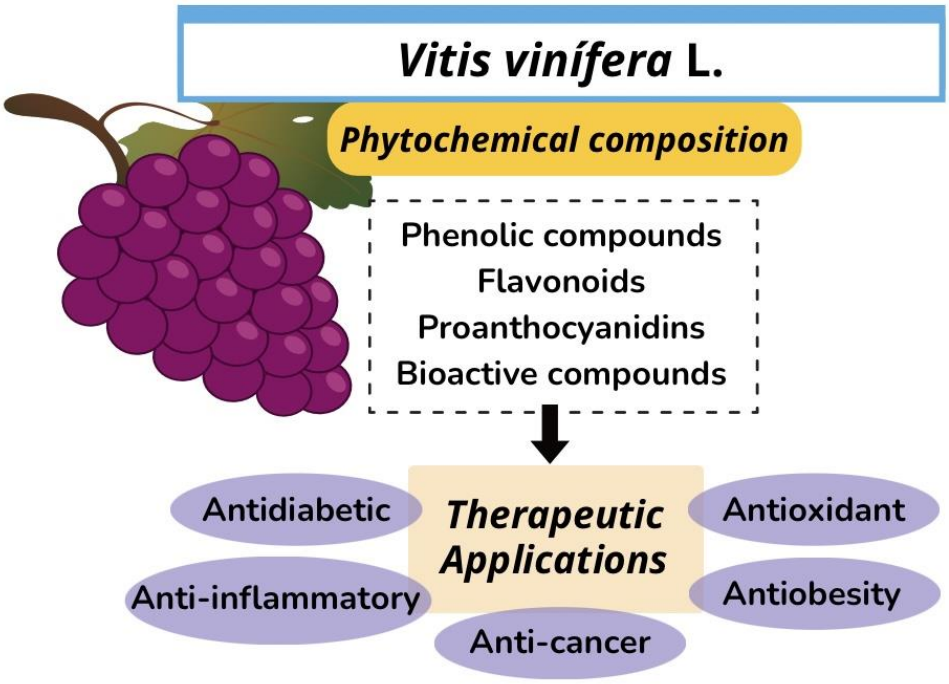

Figure 1. Phytochemical properties and therapeutic applications of grape (*Vitis vinífera* L.) seed oil.

Harbeoui et al. [32] used the unsaponifiable fraction to investigate cytotoxicity and cell viability. They observed a reduction from 10 to 15% of metabolically active cells in the cell culture in a time and dose-dependent manner and showed significant results in the DPPH trial after 24 h of incubation, demonstrating that the compounds present (ascorbic acid, tocopherol and β-carotene) in this fraction contribute to the potent and antiradical action of grape seed oil in addition to helping to protect against oxidative harm by modulating the production of nitric oxide (NO) and showing antioxidant activity.

Concerning the therapeutic properties, reports have indicated the use of seeds as well as leaves, stems, and fruits; however, the hypolipidemic action of grape seed oil stands out, with the dose for human consumption of one spoonful a day replacing fats and other oils [14].

Anti-inflammatory [40] and antidiabetic [41] activities have been reported since phenolic compounds (Figure 1), mainly catechins, play a relevant role in the inhibition and oxidation of cholesterol and low-density lipoprotein (LDL-c) and plate aggregation, increasing the concentration of antioxidant enzymes such as dismutases; in addition, the polyphenols of grape seeds can also inhibit inflammation and allergic reactions, through the action in some enzymes that catalyze histamine liberation [42].

Procyanidins present in grape seeds at concentrations of 2, 4, and 8 mL/kg contribute to the treatment of circulatory disturbances and promote anti-ulcerative activity by eliminating free radicals in the gut mucosa, inhibiting lesions [43].

Changes in fatty acid metabolism can promote the excessive lipidic peroxidation of LDL-c and the consequent development of cardiovascular disease. These oxidation products are also involved in the formation of thromboxane, which leads to increased plate

aggregation, affecting thrombosis. By contrast, polyphenols reduce cardiovascular risk by promoting LDL-c lipidic oxidation [44].

Garavaglia et al. [45] reported that the anti-inflammatory, antioxidant, cardioprotective, and anti-cancer properties of grape seed oil may be due to linoleic acid, tocopherol, carotenoids, and phytosterols along with polyphenolic compounds such as proanthocyanidins, resveratrol, and quercetin. Furthermore, these polyphenolic compounds protect against the oxidation of ascorbic acid, selenium, and carotenoids and act to inhibit the enzymatic systems producing free radicals associated with inflammatory reactions, primarily cumaric, caffeic, ferulic, chlorogenic, neo-chlorogenic, p-hydroxybenzoic, vanillic and gallic acids [46].

Nevertheless, despite the solid background in the literature regarding the benefits of consuming table grapes, it is challenging to attribute these advantages to a particular compound or group of compounds since each subspecies contains various levels of phenolic compounds [47].

Seeds of the variety Globo Vermelho, for example, are considered to be a source of anthocyanins, flavones, flavonols and stilbenes (resveratrol), in addition to having microbicide activity against six bacterial species (*Staphylococcus aureus*, *Micrococcus flavus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter cloacae*) and dermatomycetes, as well as a potential antifungal role [48].

4. Antioxidant and Anti-Inflammatory Effects of Grape Seed Oil

Free radicals are atoms or molecules that contain one or more mismatched electrons in their last layer and are constantly produced in organisms. They provide typical reactivity and can occur, such as in various situations of mitochondrial metabolism, in the uric acid pathway (the enzyme xanthine oxidase), peroxisome activity, inflammation, phagocyte activity, the ischemia process, and physical exercise [49,50]. Some external factors, such as tobacco smoke, pollution, radiation, medicaments, pesticides, and industrial solvents, influence free radical levels and, when in excess, lead to oxidative stress, harming the organism [51].

Oxidative stress is defined in the literature as an imbalance that occurs when there is a higher concentration of oxidants than antioxidants, which causes an interruption in redox signaling, hindering control and causing molecular damage, i.e., it is an adaptative response (Figure 2) [52]. Thus, the increased production of free radicals and reactive oxygen species in the organism is an amplification factor, i.e., an intensifier, for pathological alterations to appear, in addition to causing oxidative stress, intensifying the inflammatory process [53]. The inflammatory process is a natural defense mechanism to remove harmful stimuli from the body, such as pathogens, irritants, and damaged cells, and start the cicatrization process [54]. It can be classified as acute when referring to a beneficial process that helps to immobilize a lesioned region and allows the rest of the immune system to be activated to heal lesions, or it can be classified as chronic, which can become a problem and not a solution for lesions since chronically inflamed tissues normally induce immune cells in the bloodstream to amplify the inflammatory response, which can destroy healthy tissues in an erroneous attempt to start the repair process (Figure 2) [55].

Much effort is made in today's research to verify the consequences in biology and medicine, besides seeking ways to minimize or avoid the imbalance between oxidants and antioxidants in an attempt to fight oxidative stress and inflammation [54]. Among the endogenous antioxidant compounds that best combat oxidative stress there are dismutase peroxide, glutathione, peroxidase, glutathione reductase, and catalase; among the exogenous antioxidants are vitamin E, carotenoids, polyphenols, and vitamin C [56]. *Vitis vinifera* contains various bioactive compounds that can enhance antioxidant and anti-inflammatory actions, such as high levels of phenolic compounds gallic acid, catechin, epicatechin, procyanidins, and proanthocyanidins (Table 4) [57].

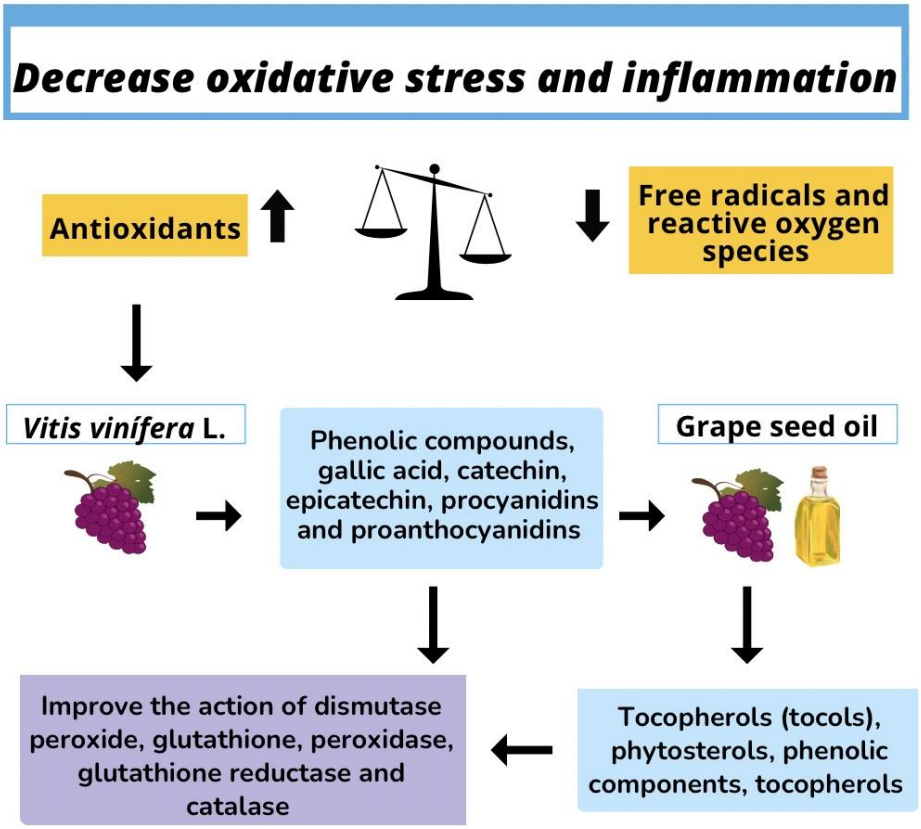

Figure 2. Decrease oxidative stress and inflammation applications of grape (*Vitis vinífera* L.) seed oil.

Kapcsándi et al. [58] reported the total polyphenol content in grape seed oil to be between 0.24 and 1.13 mg GAE/g and the total antioxidant content to be between 0.12 and 0.78 mg of the Trolox equivalent antioxidant capacity (TEAC)/g, demonstrating that it is a potent protector against oxidative stress. Mollica et al. [39] assessed the biological activity of the oil and found that it could inhibit the enzymes α-glycosidase, α-amylase, α-tyrosinase and cholinesterase (ChE), demonstrate anti-inflammatory activity by lipoxygenase (5-LOX) and stimulate the liberation of macrophages in trials of lipopolysaccharides (LPS). In addition, they reported a significant polyphenol (199.31 mg GAE/g), antioxidant (1036.98 mg TE/g), and enzymatic inhibitor (α-tyrosinase, 151.30 mg KAE/g) content, providing strong evidence for the potential use of grape seed oil as a functional food supplement in the human diet to mitigate alterations caused by cell stress and inflammation.

Wijekoon et al. [57] noted that grape seed can be an important source of anthocyanins, flavones, flavonols, and stilbenes (resveratrol), highlighting the relevance of consuming grape varieties with seeds as a functional food. Argon et al. [59] confirmed that grape seed oil contains bioactive components such as tocopherols (tocols), phytosterols, phenolic components, and other fat-soluble compounds; tocopherols are powerful natural liposoluble antioxidants, and carotenoids also have antioxidant effects.

Shaban et al. [60] administered 150 mg of grape seed oil per kg of body weight in mice with carcinoma cells. After 8 days of treatment, they observed the induction of apoptosis and potential redox, which reduced inflammation and oxidative stress. Concerning inflammation, Niknami et al. [43] tested grape seed oil at doses of 2, 4, and 8 mL/kg (via gavage) in male Wistar rats with ulcerative colitis. Administering the oil 2 h before the induction of colitis and continuing for 4 days, they observed significantly reduced colon weight, ulcer index, and total colitis index compared with the control group. The results demonstrate that grape seed oil has a potent anti-inflammatory effect and, thus, may be indicated to help in the treatment or prevention of ulcerative colitis.

Bocsan et al. [14] aimed to verify whether grape seed oil could have an anti-inflammatory effect on ischemia induced by isoproterenol in rats, and they observed that a 14-day dose of 4 mL/kg/day significantly reduced pro-inflammatory (IL-6 and TNF-α) and anti-inflammatory (IL-10) cytokines.

Millan-Linares et al. [61] investigated the effects of the unsaponifiable fraction of *V. vinifera* seed oil on oxidative and inflammatory responses using the fluorescence-activated cell sorting (FACS) analysis of reverse transcriptase followed by a real-time quantitative reverse-transcription polymerase chain reaction (RT-qPCR) and an enzyme-linked immunoassay (ELISA). They observed that at doses of 10–100 μg mL^{-1} of grape seed oil, there was a deviation in the plasticity of monocytes to the monocytes CD14+ and CD16 + +, non-classic anti-inflammatory monocytes, and the reduced inflammatory competence of human primary monocytes treated with LPS, lowering the gene expression and secretion of TNF-α, IL-1β, and IL-6. Furthermore, the unsaponifiable fraction of grape seed oil showed intense activity in eliminating reactive oxygen species (ROS), significantly lowering the nitrite levels, with considerably diminished gene No. 2 expression. The study of Zhoa et al. [62] also verified trunk cells derived from human adipose stem cells (hASCs) during the administration of 200 μM grape seed oil. On the tenth day, they observed a significant reduction in the pro-inflammatory gene expression induced by LPS in human adipocytes and the secretion of cytokines (IL-6 and IL-8).

5. Effects of *Vitis vinifera* L. Seeds on Obesity and Metabolic Alterations

It is known that adipose tissue has several physiological functions and participates in intense metabolic activity, contributing to energy balance and hormone regulation (insulin and catecholamines) in addition to the nutritional state under prolonged fasting or increased energy expenditure (Table 5) [63], mediated by biosynthesis, uptake and the storage of tryglycerides from food, and non-lipidic substrates such as carbohydrates, favoring anabolic action, also called lipogenic activity [64]. In addition, it contributes to the liberation of stored triglycerids, benefiting TG hydrolysis in long-chain fatty acids or glycerol, and can be mobilized to tissues and promote catabolic action, i.e., lipolitic activity [65].

Alterations in the energy homeostasis dynamics, i.e., more lipogenic activity and less lipolytic activity, can lead to abnormal fat buildup, causing hypertrophy and/or hyperplasia of adipocytes, and thus obesity [66]. This triggers alterations to the hormone regulators of metabolism and satiety (insulin and leptin) and increases the expression of pro-inflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1/CCL-2), tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) [65,67]. Inflammation is involved in many chronic multisystemic conditions, including obesity, atherosclerosis, and diabetes mellitus type 2; many studies have demonstrated the effects of the consumption of fatty acids, especially polyunsaturated linoleic and ÿ-linolenic acids, and its effects on the treatment of metabolic and pathological alterations caused by obesity (Table 6) [67].

Grape seed oil contains tocotrienols, with significative quantities of α- and γ-tocotrienol (T3), which significantly reduce the expression of mRNA protein, which is crucial for adipogenesis (e.g., PPARγ and aP2); it sharply reduces the pro-inflammatory gene expression (IL-6 and IL-8) [62]. In addition, the organic fatty acids in grape seed oil, such as malic, tartaric, and oxalic acids, help to lower LDL-c levels [13]. This function is further enhanced by the presence of polyphenols such as flavanols, catechin, and epicatechin, which may be correlated with the inhibition of arterial thrombosis, and compounds such as flavones, isoflavones, and anthocyanins, which have anti-inflammatory activity and are involved in the prevention of pathologies associated with weight gain (Figure 3) [68].

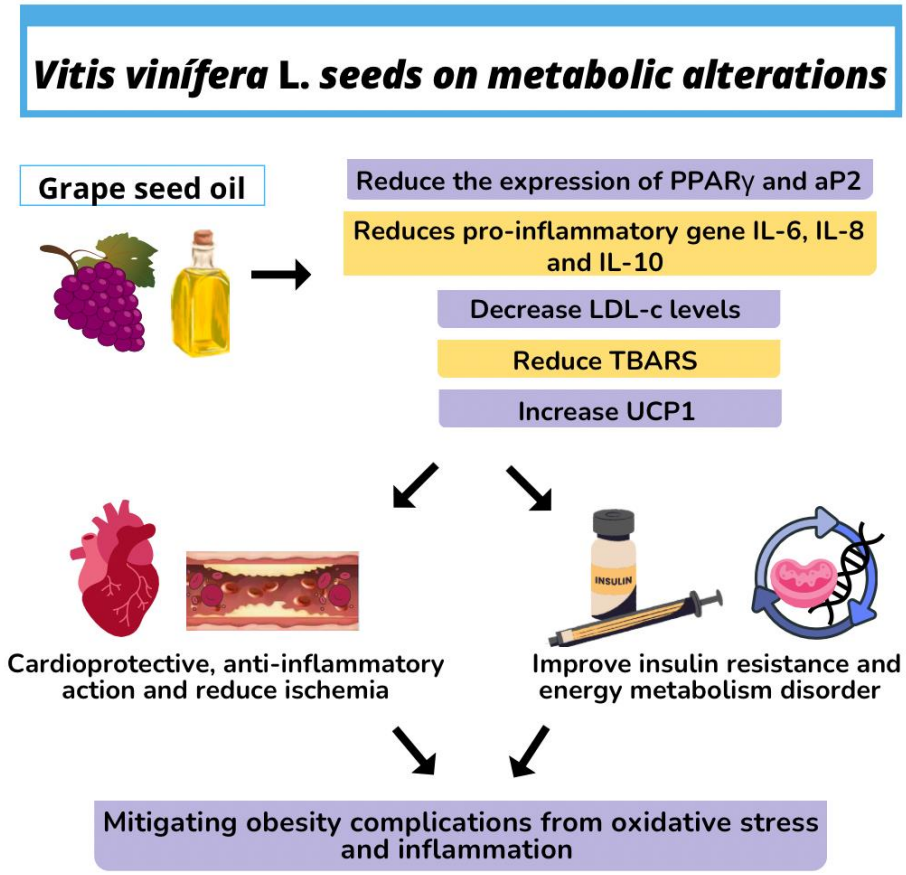

Figure 3. *Vitis vinífera* L. seeds on metabolic alterations applications of grape (*Vitis vinífera* L.) seed oil.

Bocsan et al. [13] evaluated the cardioprotective and anti-inflammatory effects of grape seed oil on ischemia induced by isoproterenol (ISO) in rats (4 mg/kg/day grape seed oil by gavage for 14 days). They observed reduced ventricular conduction, preventing alterations in ECG, changes in biological and inflammatory parameters after myocardial heart stroke induced by ISO, and cardioprotective effects in ischemia induced by ISO, indicating that it may be a potential option for the treatment of cardiovascular diseases (Figure 3).

Mohanna et al. [69] demonstrated for the first time in an experimental model that 12 weeks of supplementation with *V. vinifera* seed oil diminished the expression of the M1 marker and total F4/80 macrophages in white adipose tissue, significantly reduced pro-inflammatory adipokines in the serum and mRNA levels of inflammatory adipokines in the white adipose tissue, and increased the gene expression of uncoupling protein 1 (UCP1), which causes increased cell metabolism, thus elevating energy expenditure. These results indicate new beneficial effects and establish the potential use of grape seed oil to prevent obesity and its comorbidities.

Other in vivo studies have shown that Wistar rats with streptozotocin-induced diabetes treated with *V. vinifera* seed oil (25 mg/kg body weight) for 40 days presented a significant decline in serum glucose concentration, and in levels of plasma triglyceride (TG), low-density lipoprotein (LDL-c) and very-low-density lipoprotein (VLDL-c), demonstrating that grape seed oil can improve dyslipidemia and hyperglycemia in diabetic rats [70]. Li et al. [71] assessed the potential of grape seed oil to improve insulin resistance and the energy metabolism disorder in C57BL/6J mice when fed a fat-rich diet and observed that after supplementing with oil at 25.9% weight/total weight for 15 weeks, the animals showed increased energy. An effect on insulin resistance was also observed, which could be associated with the protective effect of hexokinase and α-glycosidase activity and improved leptin resistance, suggesting that polyphenols may be the most critical factor in regulating

insulin resistance and demonstrating that grape seed oil has a beneficial influence on the correlation between insulin resistance, energy metabolism, and hyperlipidemia.

Mice fed a hyperlipidic diet supplemented with 15.5 g grape seed oil per 100 g of food for 8 weeks showed lower body weight gain and white adipose tissue weight, as well as reduced plasma levels of glucose and total cholesterol and improved glucose tolerance. In addition, remarkable antioxidant properties were observed, with low plasma levels of TBARS (substances reactive to thiobarbituric acid, biomarkers of lipidic peroxidation), in addition to the reduced production of IL-6 and IL-10. This allows us to conclude that *V. vinifera* seed oil can be considered as a functional oil capable of mitigating obesity complications from oxidative stress and inflammation [72]. Studies on the use of grape seed oil in the treatment of obesity are scarce; however, despite there being little evidence regarding weight reduction, grape seed oil has an effect on blood lipid parameters and reduced blood glycemia in obese mice (Table 5) [73].

Table 5. Compounds related to antioxidant and anti-inflammatory effects attributed to *Vitis vinifera* seed oil.

Reference	Effects	Related Compounds	Main Results
[59]	Antioxidant Anti-inflammatory	- Total antioxidant content - Flavonoids - Vitamin E - Vitamin C	- TEAC: 0.14 and 1.16 mg/g - DPPH: 31.0 and 45.3%. - Total phenolic content: 48–360 mg GAE/kg - Phytosterols: β-sitosterol: 83.5–91.9 mg/100 g Stigmasterol: 30.5–32.6 mg/100 g Campesterol: 12.7–13.7 mg/100 g - Vitamin E: 223 mg α (5,7,8-trimetyltocol) β (5,8-dimetyltocol) γ (7,8-dimetyltocol) δ (8-metyltocol) - Carotenoids: 56.7 ppm β-carotene β-cryptoxanthine α-carotene
[58]	Antioxidant	Polyphenols Total antioxidant content	Polyphenols: 0.24–1.13, e.g., GAE/g Total antioxidants: 0.12 and 0.78 mg TEAC/g
[39]	Antioxidant Anti-inflammatory	Polyphenols Total antioxidant content	- Inhibit enzymes α-glycosidase, α-amylase, α-tirosinase and cholenisterase - Stimulate liberation of macrophages - Polyphenols: 199.31 mg GAE/g - Antioxidant content: 1036.98 mg TE/g
[57]	Antioxidant Anti-inflammatory	- Anthocyanins - Flavones - Flavanols - Stilbenes (resveratrol)	- 70% linoleic acid - Total cinnamic acid derivates: 89.2 μg/g - Total hydroxybenzoic acid: 31.9 μg/g - Total flavan-3-ols: 33.6 μg/g - Total flavanols: 85.6 μg/g - Total flavones: 19.7 μg/g - Total stilbenes (resveratrol): 13.9 μg/g - Total anthocyanins: 190.9 μg/g

Vitis vinifera seed oil was proven to significantly inhibit the proliferative growth of human colon cancer cells (HT-29) under different treatment times in vitro, and this protection is attributed to polyphenolic compounds, which have the capacity to inhibit kinase proteins, blocking cell proliferative transduction signals (Table 6).

Table 6. Summary of the effects of *Vitis vinifera* L seed oil on obesity and metabolic alterations.

Reference	Effects	Object/ Population	Period	Study Design	Main Results
[14]	- Cardioprotective - Antioxidant - Anti-inflammatory	Wistar rats	14 days	Group 1: Saline solution 0.4 mL/100 g Group 2: Saline solution 0.4 mL/100 g Group 3: *Nigella sativa* seed oil 0.4 mL/100 g Group 4: *Vitis vinifera* seed oil 0.4 mL/100 g	↓ ventricular conduction ↓ IL-6, IL-1 β and TNF-α ↓ CK-Mb Prevented cardiotoxic effect of ISO
[43]	- Anti-inflammatory - Antioxidant - Anticarcinogenic	In vitro	8 days	Evaluated inhibitory effect of 150 mg *Vitis vinifera* seed oil on growth of MCF-7 breast cancer cells	↑ apoptosis ↓ inflammation ↓ redox potential (E_h) ↓ CD44 cells
[61]	- Anti-inflammatory - Antioxidant	Newborn human monocytes	24 h	Newborn human monocytes used to analyze effects of unsaponifiable fraction of *Vitis vinifera* seed oil (10–100 µg/mL) on oxidative and inflammatory responses using FACS, RT-qPCR and ELISA	↓ CD14 ↑ surface expression of CD16 in human primary monocytes treated with LPS ↓ gene expression and secretion of TNF-α, IL-1β and IL-6
[62]	- Anti-obesogenic - Anti-inflammatory	In vitro	12 days	Trunk cells derived from primary human adipose tissue treated with 200 µM *Vitis vinifera* seed oil	↓ expression of mRNA ↓ adipogenic proteins (PPARγ and aP2)
[69]	- Anti-obesogenic - Anti-inflammatory	Swiss mice	12 weeks	Group 1: Diet A04-10 with 3% of energy as soybean oil Group 2: High-fat diet with 21% additional energy in milk cream fat Group 3: Diet + *Vitis vinifera* seed oil Group 4: Diet + *Vitis vinifera* seed oil enriched with 200 mg/kg/day resveratrol	↓ expression of marker M1 ↓ expression of macrophagss F4/80 in white adipose tissue ↓ pro-inflammatory adipokines of soro ↓ levels of mRNA from inflammatory adipokines in white adipose tissue ↑ gene expression of uncoupling protein 1 (UCP1)
[70]	- Glycemic control - Lipid control	Wistar rats	40 days	Group 1: Diabetic rats treated with 25 mg/kg *Vitis vinifera* seed oil Group 2: Diabetic rats treated with saline solution	↓ serum glucose ↓ triglycerides ↓ low-density liproprotein (LDL-c) ↓ very-low-density liproprotein (VLDL-c)
[71]	- Anti-obesogenic - Antioxidant - Anti-inflammatory - Glycemic control	C57BL/6J mice	15 weeks	Group 1: Control group Group 2: Diet rich in lard (25.93%) Group 2: Diet rich in corn oil (25.93%) Group 3: Diet rich in *Vitis vinifera* seed oil (25.93%)	↑ energy rate ↓ insulin resistance ↓ fasting glucose ↓ serum insulin ↓ glucagon concentration ↓ leptin resistance

Table 6. *Cont.*

Reference	Effects	Object/ Population	Period	Study Design	Main Results
[72]	- Anti-obesogenic - Antioxidant - Anti-inflammatory	Swiss mice	8 weeks	Group 1: control diet Group 2: High-fat diet (HFD) with 100% of lipidic content as lard Group 3: HFD with 50% of lipidic content as grape seed oil (HG) Group 4: HFD with 50% of lipidic content as SLs containing capric acid produced from grape seed oil (HG-MCT)	↓ body weight gain ↓ adiposity ↓ serum glucose ↓ total cholesterol ↓ plasma TBARS ↓ production of IL-6 and IL-10
[73]	- Anti-inflammatory - Antioxidant	Wistar rats	2 h before colitis induction, after 4 consecutive days	Group 1: Sham (normal) oral physiological serum/without colitis induction Group 2: Induced colitis (negative control), saline solution/colitis induction Groups 3, 4 and 5: Three doses (50, 100 and 200 mg/kg) of *Vitis vinifera* seed extract with colitis induction Groups 6, 7 and 8: Three doses (2, 4 and 8 mL/kg) of *Vitis vinifera* seed oil with colitis induction Groups 9 and 10: Reference (positive control), prednisone (4 mg/kg) or masalamine (100 mg/kg) with colitis induction	↓ colon weight ↓ ulcer index ↓ total colitis index ↓ oxidative stress ↓ inflammation
[74]	- Antioxidant - Antiproliferative	In vitro		Human HT-29 colorectal adenocarcinoma cells, 24 h incubation, using 2 g *Vitis vinifera* seed oil	↓ proliferation of human colon cancer cells (HT-29)
[75]	- Cardioprotective - Anti-inflammatory - Antidiabetic	Wistar rats	24 weeks	Group 1: Control diet Group 2: Experimental model of MetS Group 3: Diet-induced MetS treated with Grape-derived stilbene concentrate (GDSC) (from the 14th to the 24th week) Group 4: induced MS treated with GDSC (from the 19th to the 24th week)	↓ abdominal fat ↓ average glucose level ↓ triglycerides ↑ GLUT4 ↑ PPAR-γ (GDSC treatment from the 14th week of the experiment) ↓ PPAR-γ (GDSC treatment from the 24th week of the experiment) ↓ TLR4 concentration ↓ CRP level

Table 6. *Cont.*

Reference	Effects	Object/ Population	Period	Study Design	Main Results
[76]	- Cardioprotective - Antioxidant	Wistar rats	7 days	Group 1: Control diet Group 2: aqueous solution of cobalt chloride ($CoCL_2$) Group 3: Aqueous solution of cobalt chloride ($CoCL_2$) + 0.25 mL/kg of the extract of grape polyphenols together with 0.5 mL/kg of water Group 4: Aqueous solution of cobalt chloride ($CoCL_2$) + 2.5 mL/kg of red wine "health"	↓ free radical oxidation of lipids (TBA-AP) ↑ Catalase (CLA) ↑ Peroxidase (PLA) ↑ Enzyme superoxide dismutase (SOD)
[76]	- Cardioprotective - Antioxidant	rats		Group 1: Control diet Group 2: Metabolic syndrome + 0.5 g/L wine diluted with water Group 3: Metabolic syndrome + 1.0 g/L wine diluted with water Group 4: Metabolic syndrome + 2.5 g/L wine diluted with water	↓ free radical oxidation of lipids (TBA-AP) ↑ Enzyme superoxide dismutase (SOD) ↑ Peroxidase (PLA) ↑ Catalase (CLA)
[76]	- Cardioprotective - Antioxidant	Wistar rats	2 weeks	Group 1: Control diet Group 2: Standard food and drinking water and were undergo bloodletting within the first week of the experiment Group 3: Undergo bloodletting within the first week of the experiment and received sparkling red wine (0.5 mL/100 g of body weight) diluted with water Group 4: Did not undergo bloodletting and received sparkling red wine (0.5 mL/100 g of body weight) diluted with water	↑ free radical oxidation of lipids (TBA-AP) ↑ Enzyme superoxide dismutase (SOD) ↑ Peroxidase (PLA)

↑: increased; ↓: decreased.

6. Conclusions

The wine industry generates large quantities of residues, with grape seeds standing out among them since their extracted oil presents antioxidant and anti-inflammatory activities. Seed oil primarily comprises polyunsaturated fatty acids such as linoleic acid, vitamin E, and phytosterols, along with hydrophilic phenols, and shows promise as a nutritional compound and a valuable therapeutical substance, described in the literature as exhibiting antiobesogenic, antidiabetogenic, and anti-cancer activity.

Nevertheless, studies confirming such therapeutic qualities based on clinical experiments remain scarce. Most results are based on the nutritional and phytochemical composition of *Vitis vinifera* seed oil and not exclusively on the findings from original studies. Thus, more studies are needed to confirm such results and determine the mechanisms of action involved in the indicated therapeutical properties.

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References

1. Migicovsky, Z.; Sawler, J.; Gardner, K.M.; Aradhya, M.K.; Prins, B.H.; Schwaninger, H.R.; Bustamante, C.D.; Buckler, E.S.; Zhong, G.Y.; Brown, P.J.; et al. Patterns of genomic and phenomic diversity in wine and table grapes. *Hortic. Res.* **2017**, *4*, 17035. [CrossRef]
2. Parihar, S.; Sharma, D. A brief overview on *Vitis vinifera*. *Sch. Acad. J. Pharm.* **2021**, *12*, 231–239. [CrossRef]
3. Ramos-Madrigal, J.; Runge, A.K.W.; Bouby, L.; Lacombe, T.; Samaniego Castruita, J.A.; Adam-Blondon, A.F.; Figueiral, I.; Hallavant, C.; Martínez-Zapater, J.M.; Schaal, C.; et al. Palaeogenomic insights into the origins of French grapevine diversity. *Nat. Plants* **2019**, *5*, 595–603. [CrossRef] [PubMed]
4. Arroyo-García, R.; Ruiz-García, L.; Bolling, L.; Ocete, R.; López, M.A.; Arnold, C.; Ergul, A.; Söylemezoğlu, G.; Uzun, H.I.; Cabello, F.; et al. Multiple origins of cultivated grapevine (*Vitis vinifera* L. ssp. sativa) based on chloroplast DNA polymorphisms. *Mol. Ecol.* **2006**, *15*, 3707–3714. [CrossRef] [PubMed]
5. Radulescu, C.; Buruleanu, L.C.; Nicolescu, C.M.; Olteanu, R.L.; Bumbac, M.; Holban, G.C.; Simal-Gandara, J. Phytochemical Profiles, Antioxidant and Antibacterial Activities of Grape (*Vitis vinifera* L.) Seeds and Skin from Organic and Conventional Vineyards. *Plants* **2020**, *9*, 1470. [CrossRef] [PubMed]
6. Marrano, A.; Micheletti, D.; Lorenzi, S.; Neale, D.; Grando, M.S. Genomic signatures of different adaptations to environmental stimuli between wild and cultivated *Vitis vinifera* L. *Hortic. Res.* **2018**, *5*, 34. [CrossRef] [PubMed]
7. Martin, M.E.; Grao-Cruces, E.; Millan-Linares, M.C.; Montserrat-de la Paz, S. Grape (*Vitis vinifera* L.) Seed Oil: A Functional Food from the Winemaking Industry. *Foods* **2020**, *9*, 1360. [CrossRef] [PubMed]
8. Fernandes, L.; Casal, S.; Cruz, R.; Pereira, J.A.; Ramalhosa, E. Seed oils of ten traditional Portuguese grape varieties with interesting chemical and antioxidant properties. *Food Res. Int.* **2013**, *50*, 161–166. [CrossRef]
9. Lachman, J.; Hejtmánková, A.; Táborský, J.; Kotíková, Z.; Pivec, V.; Střalková, R.; Vollmannová, A.; Bojňanská, T.; Dědina, M. Evaluation of oil content and fatty acid composition in the seed of grapevine varieties. *LWT Food Sci. Technol.* **2015**, *63*, 620–625. [CrossRef]
10. Mota, A.; Pinto, J.; Fartouche, I.; Correia, M.J.; Costa, R.; Carvalho, R.; Aires, A.; Oliveira, A.A. Chemical profile and antioxidante potential of four table grape (*Vitis vinifera*) cultivars grown in Doura region, Portugal. *Chemistry* **2018**, *33*, 125–135. [CrossRef]
11. Guaita, M.; Bosso, A. Caracterização polifenólica de peles de uva e sementes de quatro cultivares vermelhas italianas na colheita e após maceração fermentativa. *Foods* **2019**, *8*, 395. [CrossRef]

12. Mahanna, M.; Millan-Linares, M.C.; Grao-Cruces, E.; Claro, C.; Toscano, R.; Rodriguez-Martin, N.M.; Montserrat-de la Paz, S. Resveratrol-enriched grape seed oil (*Vitis vinifera* L.) protects from white fat dysfunction in obese mice. *J. Funct. Foods* **2019**, *62*, 103546. [CrossRef]
13. Bocsan, I.C.; Pop, R.M.; Sabin, O.; Sarkandy, E.; Boarescu, P.M.; Rosian, S.H.; Leru, P.M.; Chedea, V.S.; Socaci, S.A.; Buzoianu, A.D. Comparative protective effect of *Nigella sativa* oil and *Vitis vinífera* seed oil in na experimenral modelo isoproterenol-induced acute myocardial ischemia in rats. *Molecules* **2021**, *26*, 3321. [CrossRef] [PubMed]
14. Dordevski, N.; Stojkovic, D.; Zivkovic, J.; Pljevljakusic, D.; Ristanovic, E.; Nikolic, B.; Ciric, A. Tamjanika, a Balkan native variety of *Vitis vinifera* L.: Chemical characterization, antibacterial, and anti-dermatomycosis potential of seed oil. *Food Sci. Nutr.* **2022**, *10*, 1312–1319. [CrossRef] [PubMed]
15. Sargin, S.A.; Selvi, S.; López, V. Ethnomedicinal plants of Sarigöl district (Manisa), Turkey. *J. Ethnopharmacol.* **2015**, *2*, 64–84. [CrossRef]
16. Goufo, P.; Singh, R.K.; Cortez, I. A Reference List of Phenolic Compounds (Including Stilbenes) in Grapevine (*Vitis vinifera* L.) Roots, Woods, Canes, Stems, and Leaves. *Antioxidants* **2020**, *9*, 398. [CrossRef]
17. Keller, M. *The Science of Grapevines—Anatomy and Physiology*; Elsevier: Prosser, WA, USA, 2020.
18. Lima, R.M.M.; Felgueiras, M.L.; Cunh, A.; Chicau, G.; Ferreres, F.; Dias, C.P.A. Differential phenolic production in leaves of *Vitis vinifera* cv. Alvarinho affected with esca disease. *Plant Physiol. Biochem.* **2017**, *112*, 45–52. [CrossRef]
19. Gorena, T.; Saez, V.; Mardones, C.; Vergara, C.; Winterhalter, P.; Von Baer, D. Influence of post-pruning storage on stil benoid levels in *Vitis vinifera*. L. canes. *Food Chem.* **2014**, *155*, 256–263. [CrossRef]
20. Shinagawa, F.B.; De Santana, F.C.; Torres, L.R.O.; Mancini-Filho, J. Óleo de semente de uva: Um potencial alimento funcional? *Rev. Nutr.* **2015**, *35*, 399–406. [CrossRef]
21. Handoussa, H.; Hanafi, R.; El-Khatib, A.; Linscheid, M.; Mahran, L.; Ayoub, N. Computer-assisted HPLC method development using DryLab for determination of major phenolic components in *Corchorus olitorius* and *Vitis vinifera* by using HPLC-PDA-ESI-TOF-MS^n. *Res. Rev. J. Bot. Sci.* **2017**, *6*, 9–16.
22. Unterkofler, J.; Muhlack, R.A.; Muhlack, J.W. Processes and purposes of extraction of grape components during winemaking: Current state and perspectives. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 4737–4755. [CrossRef] [PubMed]
23. Karaman, S.; Karasu, S.; Tornuk, F.; Toker, O.S.; Gecgel, U.; Sagdic, O.; Ozcan, N.; Gul, O. Recovery potential of cold press by products obtained from the edible oil industry: Physicochemical, bioactive, and antimicrobial properties. *J. Agric. Food Chem.* **2015**, *63*, 2305–2313. [CrossRef] [PubMed]
24. Santi, A.C.; Simon, K.M.; Silva, A.J.M.; Balbi, M.E.; Monteiro, C.S. Characteristics of chemical composition and nutritional seed grape (*Vitis vinifera*, Vitaceae) cv. Cabernet sauvignon. *Visão Académica* **2015**, *16*, 98–107. [CrossRef]
25. Averilla, J.N.; Oh, J.; Kim, H.J.; Kim, J.S.; Kim, J.S. Potential health benefits of phenolic compounds in grape processing by-products. *Food Sci. Biotechnol.* **2019**, *28*, 1607–1615. [CrossRef]
26. Ali, S.M.; Imran, A.; Arshad, M.U.; Ahmed, R.S.; Imran, M. Physicochemical, antioxidant and enzymes activities of grape fruit peel and pomace enriched functional drinks. *Cell. Mol. Biol.* **2021**, *67*, 125–131. [CrossRef]
27. Al Juhaimi, F.; Geçgel, Ü.; Gülcü, M.; Hamurcu, M.; Özcan, M.M. Bioactive properties, fatty acid composition and mineral contents of grape seed and oils. *S. Afr. J. Enol. Vitic.* **2017**, *38*, 103–108. [CrossRef]
28. Katsarou, A.I.; Kaliora, A.C.; Chiou, A.; Kalogeropoulos, N.; Papalois, A.; Agrogiannis, G.; Andrikopoulos, N.K. Amelioration of oxidative and inflammatory status in hearts of cholesterol-fed rats supplemented with oils or oil-products with extra virgin olive oil components. *Eur. J. Nutr.* **2016**, *55*, 1283–1296. [CrossRef]
29. Böger, B.R.; Salviato, A.; Valezi, D.F.; Di Mauro, E.; Georgetti, S.R.; Kurozawa, L.E. Optimization of ultrasound-assisted extraction of grape-seed oil to enhance process yield and minimize free radical formation. *J. Sci. Food Agric.* **2018**, *98*, 5019–5026. [CrossRef]
30. Castejón, N.; Luna, P.; Señoráns, F.J. Alternative oil extraction methods from *Echium plantagineum* L. seeds using advanced techniques and green solvents. *Food Chem.* **2018**, *244*, 75–88. [CrossRef]
31. Luo, L.; Bai, R.; Zhao, Y.; Li, J.; Wei, Z.; Wang, F.; Sun, B. Protective Effect of Grape Seed Procyanidins against H_2O_2-Induced Oxidative Stress in PC-12 Neuroblastoma Cells: Structure-Activity Relationships. *J. Food Sci.* **2018**, *83*, 2622–2628. [CrossRef]
32. Harbeoui, H.; Dakhlaoui, S.; Wannes, W.A.; Bourgou, S.; Hammami, M.; Khan, N.A.; Tounsi, M.S. Does unsaponifiable fraction of grape seed oil attenuate nitric oxide production, oxidant and cytotoxicity activities. *J. Food Biochem.* **2019**, *43*, e12940. [CrossRef] [PubMed]
33. Keskin, N.; Bilir Ekbic, H.; Kaya, O.; Keskin, S. Antioxidant activity and biochemical compounds of *Vitis vinifera* L. (cv.'Katıkara') and *Vitis labrusca* L. (cv.'Isabella') grown in Black Sea Coast of Turkey. *Erwerbs-Obstbau* **2021**, *63*, 115–122. [CrossRef]
34. Pinna, C.; Morazzoni, P.; Sala, A. Proanthocyanidins from Vitis vinifera inhibit oxidative stress-induced vascular impairment in pulmonary arteries from diabetic rats. *Phytomedicine* **2017**, *25*, 39–44. [CrossRef]
35. Shinagawa, F.B.; de Santana, F.C.; Araujo, E.S.; Purgatto, E.; Mancini-Filho, J. Chemical composition of cold pressed Brazilian grape seed oil. *Food Sci. Technol.* **2018**, *38*, 164–171. [CrossRef]
36. Nunes, M.A.; Pimentel, F.; Costa, A.S.; Alves, R.C.; Oliveira, M.B.P. Cardioprotective properties of grape seed proanthocyanidins: An update. *Trends Food Sci. Technol.* **2016**, *57*, 31–39. [CrossRef]
37. Chedea, V.S.; Pop, R.M. Total polyphenols content and antioxidant DPPH assays on biological samples. In *Polyphenols in Plants*; Academic Press: Cambridge, MA, USA, 2019; Volume 12, pp. 169–183. [CrossRef]

38. Román, G.C.; Jackson, R.E.; Gadhia, R.; Román, A.N.; Reis, J. Mediterranean diet: The role of long-chain ω-3 fatty acids in fish; polyphenols in fruits, vegetables, cereals, coffee, tea, cacao and wine; probiotics and vitamins in prevention of stroke, age-related cognitive decline, and Alzheimer disease. *Rev. Neurol.* **2019**, *175*, 724–741. [CrossRef]
39. Mollica, A.; Scioli, G.; Della Valle, A.; Cichelli, A.; Novellino, E.; Bauer, M.; Kamysz, W.; Llorent-Martínez, E.J.; Fernández-de Córdova, M.L.; Castillo-López, R.; et al. Phenolic Analysis and In Vitro Biological Activity of Red Wine, Pomace and Grape Seeds Oil Derived from *Vitis vinifera* L. cv. Montepulciano d'Abruzzo. *Antioxidants* **2021**, *10*, 1704. [CrossRef]
40. Simonetti, G.; D'auria, F.D.; Mulinacci, N.; Innocenti, M.; Antonacci, D.; Angiolella, L.; Santamaria, A.R.; Valletta, A.; Donati, L.; Pasqua, G. Anti-dermatophyte and anti-malassezia activity of extracts rich in polymeric flavan-3-ols obtained from vitis vinifera seeds. *Phytother. Res.* **2017**, *31*, 124–131. [CrossRef]
41. Tkacz, K.; Wojdyło, A.; Nowicka, P.; Turkiewicz, I.; Golis, T. Characterization in vitro potency of biological active fractions of seeds, skins and flesh from selected *Vitis vinifera* L. cultivars and interspecific hybrids. *J. Funct. Foods* **2019**, *56*, 353–363. [CrossRef]
42. Rasines-Perea, Z.; Teissedre, P.L. Grape polyphenols' effects in human cardiovascular diseases and diabetes. *Molecules* **2017**, *22*, 68. [CrossRef]
43. Niknami, E.; Sajjadi, S.E.; Talebi, A.; Minaiyan, M. Protective Effect of *Vitis vinifera* (Black Grape) Seed Extract and Oil on Acetic Acid-Induced Colitis in Rats. *Int. J. Prev. Med.* **2020**, *11*, 102. [CrossRef] [PubMed]
44. Raj, P.; Zieroth, S.; Netticadan, T. An overview of the efficacy of resveratrol in the management of ischemic heart disease. *Ann. N. Y. Acad. Sci.* **2015**, *1348*, 55–67. [CrossRef] [PubMed]
45. Garavaglia, J.; Markoski, M.M.; Oliveira, A.; Marcadenti, A. Grape Seed Oil Compounds: Biological and Chemical Actions for Health. *Nutr. Metab. Insight* **2016**, *9*, 59–64. [CrossRef] [PubMed]
46. Sabra, A.; Netticadan, T.; Wijekoon, C. Grape bioactive molecules, and the potential health benefits in reducing the risk of heart diseases. *Food Chem.* **2021**, *12*, 100149. [CrossRef]
47. Bordiga, M.; Meudec, E.; Williams, P.; Montella, R.; Travaglia, F.; Arlorio, M.; Coïsson, J.D.; Doco, T. The impact of distillation process on the chemical composition and potential prebiotic activity of different oligosaccharidic fractions extracted from grape seeds. *Food Chem.* **2019**, *285*, 423–430. [CrossRef]
48. Carvalho, É.L.S.; Bomfim, E.M.S.; da Costa Silva, M.; Lima, L.C.; de Jesus Marques, E.; Vale, V.L.C. Antibacterial Activity, Antioxidant and Phenolic Compounds of Honeys Produced by *Nannotrigona testaceicornis* Lepeletier (Apidae, Meliponini). *Res. Soc. Dev.* **2021**, *10*, e48101018424. [CrossRef]
49. Bashir Dar, K.; Hussain Bhat, A.; Amin, S.; Masood, A.; Afzal Zargar, M.; Ahmad Ganie, S. Inflammation: A multidimensional insight on natural anti-inflammatory therapeutic compounds. *Curr. Med. Chem.* **2016**, *23*, 3775–3800. [CrossRef]
50. Alkadi, H. A review on free radicals andantioxidants. *Infect. Disord.-Drug Targets* **2020**, *20*, 16–26. [CrossRef]
51. Khomich, O.A.; Kochetkov, S.N.; Bartosch, B.; Ivanov, A.V. Redox biology of respiratory viral infections. *Viruses* **2018**, *10*, 392. [CrossRef]
52. Sies, H. Oxidative stress: Eustress and distress in redox homeostasis. In: Stress: Physiology, biochemistry, and pathology. *Annu. Rev. Biochem.* **2017**, *86*, 153–163. [CrossRef]
53. Nascimento-Gavioli, M.C.A.; Rockenbach, M.F.; Welter, L.J.; Guerra, M.P. Histopathological study of resistant (*Vitis labrusca* L.) and susceptible (*Vitis vinifera* L.) cultivars of grapevine to the infection by downy mildew. *J. Hortic. Sci. Biotechnol.* **2020**, *95*, 521–531. [CrossRef]
54. Brassea-Pérez, E.; Hernández-Camacho, C.J.; Labrada-Martagón, V.; Vázquez-Medina, J.P.; Gaxiola-Robles, R.; Zenteno-Savín, T. Oxidative stress inducedbyphthalates in mammals: State of the art and potential biomarkers. *Environ. Res.* **2022**, *206*, 112636. [CrossRef] [PubMed]
55. Aghasafari, P.; George, U.; Pidaparti, R. A review of inflammatory mechanism in airway diseases. *Inflamm. Res.* **2019**, *68*, 59–74. [CrossRef]
56. Khan, M.; Liu, H.; Wang, J.; Sun, B. Inhibitory effect of phenolic compounds and plant extracts on the formation of advance glycation end products: A comprehensive review. *Food Res. Int.* **2020**, *130*, 108933. [CrossRef]
57. Wijekoon, C.; Netticadan, T.; Siow, Y.L.; Sabra, A.; Yu, L.; Raj, P.; Prashar, S. Potential associations among bioactive molecules, antioxidant activity and resveratrol production in Vitis vinifera fruits of North America. *Molecules* **2022**, *27*, 336. [CrossRef] [PubMed]
58. Kapcsándi, V.; Lakatos, E.H.; Sik, B.; Linka, L.Á.; Székelyhidi, R. Characterization of fatty acid, antioxidant, and polyphenol content of grape seed oil from different *Vitis vinifera* L. varieties. *OCL* **2021**, *28*, 30. [CrossRef]
59. Argon, Z.U.; Celenk, V.U.; Gumus, Z.P. Cold pressed grape (*Vitis vinifera*) seed oil. In *Cold Pressed Oils*; Academic Press: Cambridge, MA, USA, 2020; Volume 12, pp. 39–52. [CrossRef]
60. Shaban, N.Z.; El-Faham, A.A.; Abu-Serie, M.M.; Habashy, N.H. Targeting apoptosis in MCF-7 and Ehrlich ascites carcinoma cells by saponifiable fractions from green and black *Vitis vinifera* seed oil. *Biomed. Pharmacother.* **2023**, *157*, 114017. [CrossRef] [PubMed]
61. Millan-Linares, M.C.; Bermudez, B.; Martin, M.E.; Muñoz, E.; Abia, R.; Millan, F.; Montserrat-de la Paz, S. Unsaponifiable fraction isolated from grape (*Vitis vinifera* L.) seed oil attenuates oxidative and inflammatory responses in human primary monocytes. *Food Funct.* **2018**, *9*, 2517–2523. [CrossRef]
62. Zhao, L.; Yagiz, Y.; Xu, C.; Lu, J.; Chung, S.; Marshall, M.R. Muscadine grape seed oil as a novel source of tocotrienols to reduce adipogenesis and adipocyte inflammation. *Food Funct.* **2015**, *6*, 2293–2302. [CrossRef]

63. Noh, D.; Choi, J.G.; Huh, E.; Oh, M.S. Tectorigenin, a flavonoid-based compound of leopard lily rhizome, attenuates UV-B-induced apoptosis and collagen degradation by inhibiting oxidative stress in human keratinocytes. *Nutrients* **2018**, *10*, 1998. [CrossRef]
64. Chin, S.O.; Keum, C.; Woo, J.; Park, J.; Choi, H.J.; Woo, J.T.; Rhee, S.Y. Successful weight reduction and maintenance by using a smartphone application in those with overweight and obesity. *Sci. Rep.* **2016**, *6*, 34563. [CrossRef] [PubMed]
65. Sarapio, E.; De Souza, S.K.; Model, J.F.; Trapp, M.; Da Silva, R.S. Stanniocalcin-1 and-2 effects on glucose and lipid metabolism in white adipose tissue from fed and fasted rats. *Can. J. Physiol. Pharmacol.* **2019**, *97*, 916–923. [CrossRef] [PubMed]
66. Flaherty, S.E.; Grijalva, A.; Xu, X.; Ables, E.; Nomani, A.; Ferrante Jr, A.W. A lipase-independent pathway of lipid release and immune modulation by adipocytes. *Science* **2019**, *363*, 989–993. [CrossRef] [PubMed]
67. Carpentier, A.C.; Blondin, D.P.; Virtanen, K.A.; Richard, D.; Haman, F.; Turcotte, É.E. Brown adipose tissue energy metabolism in humans. *Front. Endocrinol.* **2018**, *9*, 447. [CrossRef] [PubMed]
68. Gomes, J.M.G.; De Assis Costa, J.; Alfenas, R.D.C.G. Metabolic endotoxemia and diabetes mellitus: A systematic review. *Metab. Clin. Exp.* **2017**, *68*, 133–144. [CrossRef]
69. Filip, A.; Clichici, S.; Daicoviciu, D.; Catoi, C.; Bolfa, P.; Postescu, I.D.; Gal, A.; Baldea, I.; Gherman, C.; Muresan, A. Chemopreventive effects of Calluna vulgaris and Vitis vinifera extracts on UVB-induced skin damage in SKH-1 hairless mice. *J Physiol. Pharmacol.* **2011**, *62*, 385–392.
70. Shiri-Shahsavar, M.R.; Alijani, S.; Parsamanesh, N.; Nazari, S.S.; Moazzen, S.; Majnouni, A.; Rasouli, A. The effect of grape-seed oil on diabetes-related hyperglycemia, dyslipidemia, and inflammation in streptozotocin-induced diabetic rats. *Obes. Med.* **2023**, *37*, 100476. [CrossRef]
71. Li, X.; Shen, Y.; Zhu, J.; Xiao, J.; Cong, R.; Zhang, H.; Qi, X. Virgin Grape Seed Oil Alleviates Insulin Resistance and Energy Metabolism Disorder in Mice Fed a High-Fat Diet. *Eur. J. Lipid Sci. Technol.* **2020**, *122*, 1900158. [CrossRef]
72. Martínez-Galán, J.P.; Ontibón-Echeverri, C.M.; Costa, M.C.; Batista-Duharte, A.; Batista, V.G.; Mesa, V.; Baviera, A.M. Enzymatic synthesis of capric acid-rich structured lipids and their effects on mice with high-fat diet-induced obesity. *Food Res. Int.* **2021**, *148*, 110602. [CrossRef]
73. Montserrat-de la Paz, S.; Fernández-Arche, M.A.; Ángel-Martín, M.; García-Giménez, M.D. Phytochemical characterization of potential nutraceutical ingredients from Evening Primrose oil (*Oenothera biennis* L.). *Phytochem. Lett.* **2014**, *8*, 158–162. [CrossRef]
74. Alves, E.; Simoes, A.; Domingues, M.R. Fruit seeds and their oils as promising sources of value-added lipids from agro-industrial byproducts: Oil content, lipid composition, lipid analysis, biological activity and potential biotechnological applications. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 1305–1339. [CrossRef] [PubMed]
75. Kubyshkin, A.; Shevandova, A.; Petrenko, V.; Fomochkina, I.; Sorokina, L.; Kucherenko, A.; Gordienko, A.; Khimich, N.; Zyablitskaya, E.; Makalish, T.; et al. Anti-inflammatory and antidiabetic effects of grape-derived stilbene concentrate in the experimental metabolic syndrome. *J. Diabetes Metab. Disord.* **2020**, *19*, 1205–1214. [CrossRef] [PubMed]
76. Kubyshkin, A.; Ogai, Y.; Fomochkina, I.; Chernousova, I.; Zaitsev, G.; Shramko, Y. Polyphenols of red grape wines and alcohol-free food concentrates in rehabilitation technologies. In *Polyphenols*; IntechOpen: London, UK, 2018. [CrossRef]

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Comparative Analysis of Grape Seed Oil, Linseed Oil, and a Blend: In Vivo Effects of Supplementation

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Abstract: Grape seeds are rich in bioactive substances, including polyphenols, terpenoids, and phytosterols. Linseed (*Linum usitatissimum* L.) boasts a high concentration of polyunsaturated fatty acids (PUFAs), lignans, phytoestrogens, and soluble fibers, all contributing to its therapeutic potential. In this study, we pioneered the formulation of an oil blend (GL) combining grape seed oil (G) and golden linseed oil (GL) in equal volumes (1:1 (*v*/*v*)) and we evaluated in terms of the nutritional, physical, and chemical properties and their influence in an in vivo experimental model. We analyzed the oils by performing physical–chemical analyses, examining the oxidative stability using Rancimat; conducting thermal analyses via thermogravimetry/derivative thermogravimetry (TG/DTG) and differential scanning calorimetry (DSC), performing optical UV–vis absorption analyses; examining the fluorescence emission–excitation matrix, total carotenoids, and color, and conducting metabolic assessments in an in vivo experimental trial. The fatty acid profile presented a higher fraction of linoleic acid (C18:2) in G and GL and alpha-linolenic acid (C18:3) in L. The acidity and peroxide indices were within the recommended ranges. The TG/DTG, DSC, and Rancimat analyses revealed similar behaviors, and the optical analyses revealed color variations caused by carotenoid contents in L and GL. In the in vivo trial, G (G2: 2000 mg/kg/day) promoted lower total consumption, and the blend (GL: 2000 mg/kg/day) group exhibited less weight gain per gram of consumed food. The group with G supplementation (G2: 2000 mg/kg/day) and GL had the highest levels of HDL-c. The group with L supplementation (L2: 2000 mg/kg/day) had the lowest total cholesterol level. The L2, G1 (1000 mg/kg/day), and G2 groups exhibited the lowest MCP-1 and TNF-α values. Additionally, the lowest adipocyte areas occurred in G and GL. Our results suggest that this combination is of high quality for consumption and can influence lipid profiles, markers of inflammation, and antioxidant status.

Keywords: fatty acids; linolenic acid; seed oil; alpha-linolenic acid; cytokines

1. Introduction

Vineyards of the grape species *Vitis vinifera* L. have a high economic impact, and they are traditionally grown in temperate regions, primarily those in Europe, northern Africa, and West Asia [1].

Grape seeds are rich in bioactive substances, including polyphenols, terpenoids, and phytosterols, and other compounds [2]. Due to their chemical profile, they have potential applications in the pharmaceutical and food industries, ranging from oil extraction to the developing food supplements and cosmetic products [3].

The seeds are byproducts of the vine and juice industry, and they are generally discarded during the production processes [3]. There is a growing interest in grape residues and techniques, aiming to achieve the full use of foods because studies have emphasized the therapeutic properties of the stems, peels, seeds, and leaves [4]. Extracted grape seed oil (*V. vinifera*) represents 6–20% of the seed composition [5], and extraction via cold pressing ensures the highest quality as well as preservation of its antioxidant properties [4,6].

The grape seed oil composition presents an average of 90% unsaturated fatty acids, with linoleic acid (65–75%) and oleic acid (20–40%) being among them, and only 10% saturated fatty acids [5]. Additionally, it demonstrates inflammatory action on reactive species of oxygen (RSO) and on the reduction in inflammatory markers, such as the secretion of TNF-α, IL-1β, and IL-6, as well as other pro-inflammatory molecules, acting in the health–disease process [5].

Linseed (*Linum usitatissimum* L.), another well-researched seed, is renowned for the quality of its oil, which boasts a high concentration of polyunsaturated fatty acids (PUFAs), accounting for 73% of its composition. Notably, it contains 60% alpha-linolenic acid (C18:3), besides a significant level of monounsaturated fatty acids (MUFAs) at 18%. Additionally, linseed is a rich source of lignans, phytostroegens, and soluble fibers, all of which contributing to its therapeutic potential [7,8]. Linseed oil is also obtained via cold pressing and contains only 9% saturated fatty acids, like grape seed oil [5,6].

Linseed oil's composition is instrumental in regulating blood lipid levels, helping to lower total cholesterol, low-density lipoprotein (LDL-c), and serum triglycerides [8]. It also plays a role in reducing tissue inflammation caused by oxidative stress and decreasing the occurrence of primary cardiovascular events due to its high concentration of α-linolenic acid [9].

There is a consensus that a healthy diet with a moderate consumption of vegetable oils preferred over animal fats because oils extracted from seeds improve metabolic profiles, such as glycemia and serum lipoproteins, and reduce inflammatory cytokines. The diet also has various components such as a fatty acid profile with a high concentration of MUFAs and PUFAs, relevant to human health [10].

Nutritional interventions are the best option compared with the high cost and adverse effects of drugs [11,12], with the incorporation of protective foods acting on the metabolism being crucial. Therefore, an evaluation of the bioavailability of the nutraceutical substances present in seed oils is necessary, as different degrees of fruit ripening, in addition to soil fertility, climate, and temperature, among other influences of natural origin, impact the nutritional quality of the seeds and their extracted oils [13].

Considering these facts, linseed oil and grape seed oil, each possessing distinct fatty acid profiles and unique nutritional, physical, and chemical properties, present an intriguing opportunity for blending [14,15]. Combining these oils in equal proportions (1:1, *v*/*v*) creates a composition that does not occur naturally. Therefore, conducting a comprehensive assessment of grape seed oil, linseed oil, and their blended mixture in terms of nutritional and physicochemical properties is crucial [14,15]. Additionally,

extended investigations into their compounds and impacts on in vivo experimental models are essential. Furthermore, predicting conditions for their application can contribute to economic growth and advancements in nutrition and technology [16].

2. Materials and Methods

2.1. Raw Material

In this study, cold-pressed vegetable oils from seeds of *Vitis vinifera* L. *and Linum usitatissimum* L. seeds were sourced from reputable suppliers at Indústria Pazze Alimentos™, Panambi (RS), Brazil. These oils were obtained by using the cold pressing technique, ensuring low acidity and peroxide indices, as verified by the manufacturer. This process preserved the oils' purity and uniformity, which are critical for the integrity of this research.

To develop a consistent oil blend, the meticulous blending of equal volumes of grape seed oil and golden linseed oil was conducted using volumetric flasks to establish a precise 1:1 (*v*/*v*) ratio. Delicate agitation was employed to avoid introducing of air bubbles that could compromise the blend's stability. Subsequently, the oil blend was stored in an airtight and sterile container, shielded from direct solar exposure, and kept at a stable temperature to preserve its quality and inhibit oxidative deterioration.

Extra virgin olive oil of the Andorinha Portugal™ Ferreira do Alentejo, Portugal brand was used in this study for the oil-supplemented control group, with its identity and quality details specified on the label.

2.2. Fatty Acid Profile

Fatty acids were esterified using a method adapted from Maya and Rodriguez-Amaya (1993). The resulting fatty acid methyl esters (FAMEs) were analyzed via gas chromatography (GC 2010, Shimadzu, Japan) to identify their peaks. The FAME standard consisted of a mixture of 37 components that mimic the fatty acid composition found in many food samples. FAMEs characterize the lipid fraction in foods and determine the total fat and trans fat contents. This technique involves the separation of fatty acids on a gas chromatography column [17].

The apparatus featured a flame ionization detector (FID) and a BPX-70 capillary column with precise dimensions and film thickness. Both the injector and detector were heated to 250 °C. Initially, the column temperature was held at 80 °C for three minutes, increased to 140 °C at 10 °C/min, and finally raised to 240 °C at 5 °C/min, where it remained for five minutes. By comparing the relative retention times to those of a Supelco C22 FAME pattern (99% pure), we pinpointed the FAME peaks. The fatty acid content was quantified by integrating the areas of these peaks, and the results are presented as grams of fatty acids per gram of oil extracted.

Additionally, we utilized the atherogenicity index (Equation (1)) and the thrombogenicity index (Equation (2))[18].

$$\text{Atherogenic index} = \frac{[(C12{:}0 + (4 \times C14{:}0) + C16{:}0)]}{(\sum \text{MUFA} + \sum \omega 6 + \sum \omega 3)} \quad (1)$$

$$\text{Thrombogenicity index} = \frac{(C14{:}0 + C16{:}0 + C18{:}0)}{[(0.5 \times \sum \text{MUFA}) + (0.5 \times \sum \omega 6) + (3 \times \sum \omega 3) + (\omega 3/\omega 6)]} \quad (2)$$

Here, MUFA is the sum of the monounsaturated fatty acids of the studied oils.

2.3. Basic Quality Parameters and Chemical–Physical Analyses

2.3.1. Acidity Index

The oil acidity index was determined by adding a neutralized ether–alcohol (1:1) solution and phenolphthalein to indicate color change. We utilized potassium hydroxide (KOH) 0.1 N as a titrant until the color changed to pink. The results are expressed as mgKOH/g and g of oleic acid/100 g oil [19].

2.3.2. Peroxide Index

The peroxide value was assessed by adding 5.0 mL of an acetic acid–chloroform solution (3:2), 0.1 mL of a saturated potassium iodide solution, and 0.1 mL of a 1% soluble starch solution as an indicator to the oils. The mixture was then allowed to stand, shielded from light. Titration was conducted using a 0.01 N sodium thiosulfate solution, and the results are expressed as milliequivalents of oxygen per kilogram (mEqO2/kg) [18,20].

2.3.3. Refraction Index

The refraction index of the oils was obtained with an Abbé refractometer (RL3, Tecnal, Brazil) calibrated with distilled water, with a refraction index of 13.330, at 27 °C, temperature-corrected to 40 °C [18,20].

2.3.4. Iodine Index

The iodine index was obtained by adding carbon tetrachloride and Wijs solution to the oils, and standard sodium thiosulphate was used as a titrant until the color changed from black to pink. The results are expressed as g I_2/100 g [18,20].

2.3.5. Saponification Index

We determined the saponification index by adding a solution of alcohol and potassium hydroxide (KOH) at 4% (m/v) to the samples and refluxing for 1 h. Next, we used phenolphthalein to indicate color change and titration with chloridric acid (HCl) 0.5 N. The results are expressed as mgKOH/g [18,20].

2.3.6. Relative Density at 25 °C/25 °C

We determined the relative density by using the pycnometer method, with prior taring in an oven at 105 °C. Then, we added a mixture of alcohol and water at 20–23 °C and placed it in a bath at a constant temperature (25 ± 0.1 °C). After 30 min, we adjusted it to the water level and weighed it on an analytic scale. We repeated the same procedures with the oils. The results are expressed as mg/mL [18,20].

2.4. Oxidative Stability: Rancimat

Oxidative stability was obtained through the induction period (PI) resulting from the Rancimat test, following the EN 14112 method, utilizing Rancimat equipment (893 Professional Biodiesel Rancimat, Metrohm, Brazil). Analyses were performed with 3.0 g of oil without dilution at 110 °C, with a constant airflow of 10 L/h through the samples, followed by a recipient containing 50 mL of deionized water, and the conductivity produced by the volatile products during the degradation of the vegetable oils was measured as a function of time [18,21].

2.5. Thermal Analysis

2.5.1. Thermogravimetry and Derivative Thermogravimetry (TG/DTG)

For TGA/DTG curves, we utilized 4.0 mg of each oil in a TGA Q50 system (TA Instruments, EUA) under a nitrogen atmosphere, with a flow of 60 mL/min in the oven and a heating rate of 10 °C/min at temperatures varying between ambient and 700 °C, with platinum crucibles used as supports [21].

2.5.2. Differential Scanning Calorimetry (DSC)

We evaluated the crystallization and fusion processes of the oils by generating DCS curves with a sample mass of around 3 mg in a DSC (Q20 TA Instruments, EUA) equipped with a double-stage cooling system (RCS 90). Cooling and heating curves were programmed in cycles. Initially, the temperature was equilibrated at 60 °C, followed by an isotherm of 10 min and a cooling ramp until reaching −60 °C, at a pace of 5 °C/min,

completing Cycle 1. In sequence, the temperature was equilibrated at −60 °C, followed by an isotherm for 10 min and a heating ramp at 5 °C/min until the final temperature of 60 °C was reached, completing Cycle 2. The total analysis time was close to 70 min.

2.6. Optic Analyses

UV–Vis Absorption and Excitation–Emission Matrix (EEM) Fluorescence Spectroscopy

The samples were diluted in hexane (spectroscopic grade, Sigma-Aldrich > 99%) at concentrations of 1.0, 5.0, and 90 g/dm^3. We obtained the ultraviolet and visible (UV–vis) absorption spectra in the 200 to 600 nm range with the aid of a spectrometer (UV–vis Lambda 265, PerkinElmer®), using a quartz cuvette with an optic length of 10 mm and a capacity of 3.5 mL. We determined the excitation–emission matrix (EEM) fluorescence spectra of the samples at a concentration of 90 g/dm^3 using a bench spectrofluorimeter (FluoroMate FS-2, Scinco®). The spectrofluorimeter consisted of excitation and emission monochromators, a 150 W Xenon excitation lamp, and an R-928 PMT detector. The analyses were performed using a 90° angle geometry between excitation and emission, using a quartz cuvette with four polished faces and a 1 cm optical path length, with the excitation and emission slits set to 5 nm. All analyses were performed at room temperature.

2.7. Color

Colors were determined by using a portable spectrometer (Konica Minolta® model CM-2300d). The results are expressed as *L**, *a**, and *b**, according to the color space L* a* b* and applying the scale CIE *L*a* b**, where L* represents the light, varying between 0 (without light or black) and 100 (white); a* represents the colors from red (+a*) to green (−a*); and b* represents the color range from yellow (+b*) to blue (−b*). From the results, we obtained the *Hue* angle indices (Equation (3)), and it was then possible to define the tone in degrees and chroma (C*) (Equation (4)), which indicate the color saturation of samples.

$$Hue = b * a * \quad (3)$$

Here, the Hue value is the color tone defined in degrees, b* indicates the chromaticity on the axis varying from yellow to blue, and a* shows the chromaticity on the axis varying from red to green (Minolta Corporation™, 1994).

$$C * = a * 2 + b * 2 \quad (4)$$

Here, the C* value (chroma) indicates the color saturation, b* indicates the chromaticity on the axis varying from yellow to blue, and a* shows the chromaticity on the axis varying from red to green (Minolta Corporation™, 1994).

2.8. Determination of Carotenoids

The samples of G, L, and GL were initially vacuum-filtered in an environment protected from light to remove possible impurities. The method was performed according to recommendations by Rodriguez-Amaya (1999) [22] and Maldonade et al. (2021), with some modifications depending on the oil purity. Several tests were conducted to define the proportions of oil and petroleum ether P.A. (dynamic) in order to obtain absorbances between 0.2 and 0.8. Thus, aliquots of 3 to 5 g of oil were mixed with 3 to 5 mL of petroleum ether P.A., and the volume was adjusted with the same solvent in a volumetric flask (10 mL) for subsequent reading on a spectrophotometer. The subsequent steps followed the methodology described by Rodriguez-Amaya (1999) [22]. We carried out all steps with aluminium foil protecting from light, avoiding carotenoid photodegradation. We performed three dilutions of each sample and conducted absorbance readings on a UV–visible spectrophotometer (Biochrom Libra S60PC) at a wavelength of 450 nm, using quartz cuvettes. We used P.A. petroleum ether as the blank. The carotenoid content was

calculated using Equation (5), with a β-carotene absorptivity value of 2592. Absorbance readings were taken for each sample in triplicate, with 3 repetitions in each dilution. The result is expressed as micrograms of carotenoids per hundred grams of oil (μg/100 g).

$$Carotenoids\left(^{\mu g}/_{g}\right) = \frac{A \times V \times 10^4}{E^{1\%}_{1cm} \times m} \quad (5)$$

Here, A is the absorbance at the maximum absorption peak, V is the final sample volume (mL), m is the sample mass (g), and $E^{1\%}{}_{1cm}$ is the extinction coefficient (β-carotene = 2592 in petroleum ether).

2.9. In Vivo Experiment

2.9.1. Animals

We developed this study according to the ethics precepts of Law n. 11.794 of 8 October 2008, of Decree n. 6.899 of 15 July 2009, and the rules edited by the National Council of Control of Animal Experimentation (Concea) and approved by the Ethics Commission in the Use of Animals (CEUA) of UFMS (n. 1339/2022).

One hundred and twenty-six Swiss Webster strain mice (*Mus musculus*), adult males aged 12 weeks, with an average weight of 36 g, were used. They were supplied by the Central Vivarium of the Federal University of Mato Grosso do Sul. The acclimatization period occurred 7 days before the start of the experimental period. During this time, the animals were kept in an environment with a controlled temperature (71.6 ± 33.8 °F), under a 12 h light/dark cycle, with ad libitum access to food and water, with four to five animals per cage.

2.9.2. Experimental Design

This study included two standard control groups: one supplemented with distilled water (C) and the other supplemented with olive oil (O). Additionally, there were five experimental groups (L1, L2, G1, G2, GL). The experimental design, as depicted in Figure 1, drew inspiration from previous studies by Torres et al. (2020) [23], Marcelino et al. (2022) [24], and Silva et al. (2023) [25]. These studies utilized the gavage administration of *Caryocar brasiliense* Cambess oil, *Mauritia flexuosa* oil, and *Acrocomia aculeata* oil in *Swiss* mice at corresponding dosages of 1000 mg/kg/day and 2000 mg/kg/day, demonstrating efficacy in their results. Following the acclimation period, daily dosages were administered for 11 weeks (see Table 1 and Figure 1).

Table 1. Determination of experimental groups, supplementation, and dosages.

Groups	Supplementation	Dosages (mg/kg/animal)
Control (C)	Distilled water	1000
Olive oil (O)	Olive oil	1000
Linseed oil (L1)	Golden linseed oil	1000
Linseed oil (L2)	Golden linseed oil	2000
Grape seed oil (G1)	Grape seed oil	1000
Grape seed oil (G2)	Grape seed oil	2000
Blend oil (GL) *	Blend oil 1:1 (*v*/*v*) *	2000

* Blend oil: Grape seed oil + Linseed oil 1:1 (*v*/*v*).

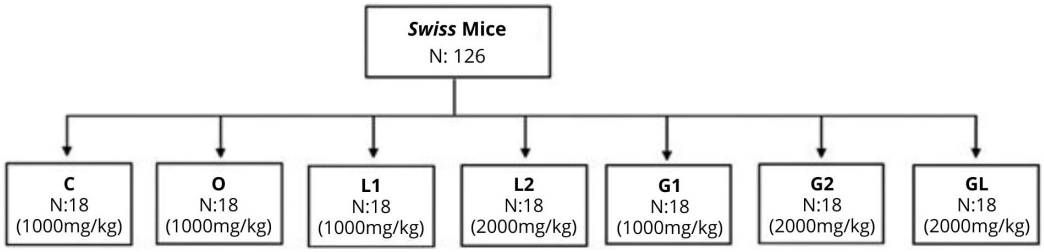

Figure 1. Flowchart of the experimental groups after 7 days of adaptation. C: control group (distilled water at dose of 1000 mg/kg/animal); O: extra virgin olive oil group (1000 mg/kg/animal); L1: Golden linseed oil group (1000 mg/kg/animal); L2: Golden linseed oil group (2000 mg/kg/animal); G1: Grape seed oil group (1000 mg/kg/animal); G2: Grape seed oil group (2000 mg/kg/animal); GL: blend oil (2000 mg/kg/animal).

2.9.3. Food Ingestion and Weight Gain

All animals received a standard commercial normocaloric diet (Nuvital®) ad libitum, and food consumption was measured according to the weekly consumption of the individual animals, making it possible to assess the average weekly consumption of the mice.

Supplementation was administered daily via gavage throughout the 11-week study period. Dosage adjustments for each group were performed by changes in body weight, which were assessed thrice weekly (Table 1 and Figure 1).

We evaluated food consumption and weight gain weekly, using the food efficiency coefficient (FEC) (Equation (6)) to determine the extent to which 1 g of food ingested promotes body weight gain [26].

$$\mathrm{FEC} = (\mathrm{FW} - \mathrm{IW}) \div \mathrm{TFA} \quad (6)$$

Here, FW is the final body weight in grams, IW is the initial body weight in grams, and TFA represents the total amount of ingested food in grams [26].

We calculated the coefficient of weight gain by caloric intake (CWGCI) to measure animal weight gain in order to obtain the animal capacity to convert consumed food energy into body weight (Equation (7)).

$$\mathrm{CWGCI} = (\mathrm{FW} - \mathrm{IW}) \div \mathrm{kcal\ ingested} \quad (7)$$

Here, FW represents the final body weight in grams, IW is the initial body weight in grams, and kcal ingested is the caloric value of the ingested diet [26].

The normocaloric commercial feed (Nuvital®) composition is shown in Table 2, describing the ingredients, percentage of macronutrients (carbohydrates, proteins, and lipids), and number of calories per gram.

Table 2. Composition of the commercial normocaloric feed (g/kg feed).

Ingredients (g/kg)	Nuvital®
Starch	725.67
Casein (≥82% protein)	40.00
DL-methionine	100.00
Soy oil	40.00
Cellulose	100.00
Mineral mix **	35.00
Vitamin mix **	10.00
L-cystine	1.80
Choline bitartrate	2.50
Tertbutyl hydroquinone	0.008
Energy (kcal/kg)	4360.00

Carbohydrates (%)	75.75%
Proteins (%)	16.00%
Lipids (%)	8.25%
Calories/g diet	4.36

** Mix of vitamins and minerals according to the manufacturer.

2.9.4. Euthanasia

Euthanasia was performed on the animals after a fasting period of six hours, with water available ad libitum. Anesthesia was conducted with a lethal dose of isoflurane, followed by exsanguination through the posterior vena cava for tests of serum parameters. We removed five visceral sites of fat (epididymal, mesenteric, omental, retroperitoneal, and perirenal) and the liver, all weighed on an electronic scale (Bel Diagnóstica®) in milligrams (mg). The epididymal tissue and liver were stored in 10% formaldehyde for histological analyses.

The adiposity index (%) was calculated using the formula adapted from Taylor and Phillips (1996) [27] (Equation (8)):

$$\text{Adiposity Index (\%)} = \frac{(\text{Sum of visceral fat sites}) \times 100}{\text{Final body weight}} \quad (8)$$

2.9.5. Serum Parameters

In this study, blood samples were collected from the posterior vena cava and transferred to tubes containing a separator gel. After centrifugation at 5000 rpm for 10 min using a refrigerated centrifuge (model universal 320, Hettich), serum was obtained. The enzymatic colorimetric methodology, with visible light reading through a green filter, was employed to determine serum parameters. Glucose (code 277), triglyceride (code 643), total cholesterol (code 167), high-density lipoprotein (HDL-c) (code 166), low-density lipoprotein (LDL-c), and very-low-density lipoprotein (VLDL-c) levels were analyzed following the manufacturer's instructions (Labtest®, Lagoa Santa, Minas Gerais, Brazil).

We determined total cholesterol (TC) using the cholesterol esterase methodology. For high-density lipoprotein (HDL-c), we used the selective precipitation method with magnesium chloride to separate the other lipoproteins (VLDL-c, LDL-c, and IDL-c) and then used the cholesterol esterase method for determination. For triglycerides (TGs), we used the methodology according to Trinder—the action of lipase to isolate glycerol and the action of glycerol kinase, with hydrogen peroxide as the main product, which reacts by coupling with 4-chlorophenol and 4-amino antipyrine, with the product being anti-pyrylquinonemine, which has a red color (cherry). Fasting glycemia was measured utilizing the enzymatic colorimetric method, and for spectrophotometry measurements, the glucose oxidase method was used [28]. All analyses were performed simultaneously with quality control.

We calculated low-density lipoprotein (VLDL-c) (Equation (9)), low-density lipoprotein (LDL-c) (Equation (10)), and cholesterol non-HDL (non-HDL-c) (Equation (11)) [28] as follows:

$$\text{VLDL Cholesterol Values} = \text{Triglycerides}/5 \quad (9)$$

$$\text{LDL Cholesterol} = \text{Total cholesterol} - (\text{HDL} - \text{c} + \text{VLDL} - \text{c}) \quad (10)$$

$$\text{Non} - \text{HDL cholesterol} = \text{Total cholesterol} - \text{HDL} - \text{c} \quad (11)$$

2.9.6. Cytokines

The adipokine bead panel, which includes IL-6, MCP-1, TNF-α, PAI-1, insulin, leptin, and resistin, was quantified using the commercial kit MAD-KMAG-71K (Merck-Sigma Aldrich, São Paulo, Brazil). The plates were analyzed on a Luminex MAGPIX System (Luminex Corporation, Austin, TX, USA) and the data were generated with xPONENT software 4.3. Luminex®. We obtained the concentration values in pg/mL from blood serum centrifuged in a tube with a separator gel. We vortexed the serum for 30 s and centrifuged it at 6000 rpm for 10 min. Next, 10 µL of the serum of each animal was placed on a plate with 96 wells, together with 10 µL of an assay buffer solution and 25 µL of a solution containing seven adipokines. We also prepared the blank, standard, and control parameters by following the instructions (Milliplex® MAP kit, Billerica, MA, USA). Afterwards, the plate was read on a Luminex® using MAGPIX® software, and concentration values were obtained in pg/mL.

2.9.7. Histology of the Epididymal Adipose Tissue and Liver

After euthanasia, the liver was removed for histological study and fixed in a 10% formaldehyde solution until embedded in paraffin. Then, 7 µm thick sections were introduced using a microtome, with subsequent mounting on glass slides.

To examine the treatment effects on the hepatocytes, pathologists analyzed the liver, utilizing the Kleiner et al. system (2005) [29] which evaluates the degree of steatosis (<5%, 5 to 33%, 34 to 66%, >66%), microvesicular steatosis (absent or present), lobular inflammation (absent, <1 focus/field, 2–4 foci/field, or >4 foci/field), ballooning (absent, few cells, or many cells), Mallory's hyaline (absent or present), glycogenated nucleus (none/rare or some), and apoptosis (absent or present). The epididymal adipose tissue area was analyzed according to the method described by Jernås et al. (2006) [30].

2.10. Statistical Analyses

We performed analyses utilizing the Jandel Sigma Stat software, version 3.5 (Systat Software, Inc., San Jose, CA, USA), and Sigma Plot, version 12.5 (Systat Software Inc., San Jose, CA, USA), obtaining mean ± standard deviation (DP) values. We compared the groups by utilizing an ANOVA on ranks followed by Tukey's post-test and the Kruskal–Wallis test followed by Dunn's test ($p < 0.05$). Histological data are described in absolute (n) and relative frequencies (%). The chi-square test was utilized to evaluate the association in the histological analysis, followed by Bonferroni correction, using the statistical program Biostat 5.0. Significance was considered at $p < 0.05$.

3. Results and Discussion

Individually, both grape seed oil and linseed oil show potential for enhancing human health due to their compositions, making possible the improvements in the domains of cardiovascular health and anti-inflammatory effects. Despite the anticipated scientific support for the individual use and commercialization of grape seed oil and linseed oil, the fusion of these oils (*blend*) results in an oil rich in polyunsaturated fatty acids, with thepossible presence of antioxidants and bioactive compounds derived from both oils. This blend combines the complementary profiles of both seeds. Our quality analyses, including optical techniques, lipid stability, and oxidative stability, evaluated the nutritional quality and behavior of this blend compared with those of the individual oils.

The fatty acid profile demonstrated the stability and nutritional quality of the studied oils [31]. In the analysis detailed in Table 3, we identified 14 fatty acids in the grape seed oil (G), 15 fatty acids in the linseed oil (L), and 14 fatty acids in the blend oil (GL). Polyunsaturated fatty acids (PUFAs) were the predominant type in these oils, with linoleic acid (C18:2) being the most abundant in G and GL at 52.40% and 42.53%, respectively, and alpha-linolenic acid (C18:3) constituting 53.30% of L. As for monounsaturated fatty acids (MUFAs), oleic acid (C18:1) was found to be the highest in all oils, at 27.94% in G, 25.57%

in GL, and 18.31% in L. Among the saturated fatty acids, palmitic acid (C16:0) was the most prevalent, observed at 13% in G, 12.45% in GL, and 9.9% in L.

The content of total saturated fatty acids was low in all oils, with values of 13%, 9.9%, and 12.45% for G, L, and GL. The total content of unsaturated fatty acids was the highest in G, L, and GL at 82.61%, 89.63%, and 86.86%, respectively. The atherogenicity and thrombogenicity indices were similar between the oils. The content of total saturated fatty acids was low in all oils, with values of 13%, 9.9%, and 12.45% for G, L, and GL. The total content of unsaturated fatty acids was the highest in G, L, and GL at 82.61%, 89.63%, and 86.86%, respectively. The atherogenicity and thrombogenicity indices were similar between the oils.

Table 3. Fatty acid profile (%) of grape seed oil, linseed oil, and blend oil.

Fatty Acids	Grape Seed Oil (G) (%)	Linseed Oil (L) (%)	Blend Oil (GL) (%)
Myristic acid (C14:0)	0.07 ± 0.01	0.1 ± 0.01	0.06 ± 0.02
Palmitic acid (C16:0)	9.2 ± 0.1	5.8 ± 0.05	8.47 ± 0.07
Heptadecanoic acid (C17:0)	0.07 ± 0.06	0.1 ± 0.02	0.07 ± 0.01
Stearic acid (C18:0)	3.66 ± 0.02	3.9 ± 0.06	3.91 ± 0.07
Σ SATURATED	13 ± 0.18	9.9 ± 0.15	12.45 ± 0.17
Palmitoleic acid (C16:1ω7)	0.07 ± 0.01	0.1 ± 0.01	0.07 ± 0.01
Oleic acid (C18:1ω-9)	27.94 ± 0.8	18.31 ± 1.0	25.57 ± 0.07
Elaidic acid (C18:1*trans*-9)	1.12 ± 0.05	0.1 ± 0.04	1.0 9± 0.03
Gadoleic acid (C20:1ω-9)	0.03 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
Σ MONOUNSATURATED	29.16 ± 0.9	18.71 ± 1.06	26.93 ± 0.12
Linoleic acid (C18:2ω-6)	52.40 ± 1.34	14.32 ± 1.00	42.53 ± 0.2
Gamma-Linolenic acid (C18:3ω-6)	0.14 ± 0.01	0.2 ± 0.01	0.16 ± 0.01
Alpha-linolenic acid (C18:3ω-3)	0.37 ± 0.04	53.30 ± 0.03	16.76 ± 0.06
Cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5ω-3)	0.04 ± 0.01	3 ± 0.01	0.1 ± 0.03
Arachidonic acid (C20:4ω-6)	0.5 ± 0.04	0.1 ± 0.02	0.38 ± 0.01
Σ POLY-UNSATURATED	53.45 ± 1.4	70,92 ± 1.07	59.93 ± 0.31
Σ TOTAL UNSATURATED FATTY ACIDS	82.61%	89.63%	86.86%
Atherogenicity index	0.11	0.7	0.1
Thrombogenicity index	0.3	0.05	0.14

Values expressed as mean ± standard deviation.

Grape seed oil (G) is rich in polyunsaturated fatty acids, mainly linoleic acid (C18:2) (Table 3). In our study, G was composed of a mix of *Vitis vinifera* seeds, resulting in a percentage of linoleic fatty acid (C18:2) between those of the varieties Sangiovese (47.34%) and Cinsaut (72.91%) [14,31]. The tested grape seed oil (Table 3) had a higher presence of oleic fatty acid (C18:1) than 10 grape varieties (Chardonnay, Muscadine, Rubi, Sangiovese, Concord, Ada-karası, Cinsaut, Cabernet Sauvignon, Gamay, and Narince), with the oleic acid (C18:1) content ranging between 13.35% (Concord) and 26.30% (Sangiovese) [14,31]. The total unsaturated fatty acid content in the grape seed oil (82.61%) (Table 3) is comparable to the result of 82.35% reported by Hussein and Abdrabba (2015) [32].

Other vegetable oils are sources of mono- and PUFAs, such as L, which had a total unsaturated fatty acid content of 89.63% (Table 3), with the polyunsaturated alpha-linolenic acid constituting 53.3% of this fraction; this level is within the reported variation of 45% to 55% [33]. Thus, the oleic acid (C18:1) content in L (Table 3) agrees with the range of 12% to 30% (C18:1) found in other varieties [7,33]. Additionally, L had a total saturated fatty acid content of 9.9% (Table 3), within the expected value of <11% [7], having the lowest percentage of total saturated fatty acids among the studied oils.

The blend oil (GL) exhibited a fatty acid profile that was intermediate compared to those of the grape seed (*V. vinifera*) and linseed (*Linum usitatissimum*) oils. GL had an

unsaturated fatty acid content of 86.86%, with notable amounts of the polyunsaturated fatty acids linoleic acid (C18:2) and alpha-linolenic acid (C18:3), alongside the presence of oleic acid (C18:1) and a total saturated fatty acid content of 12.45% (Table 3). G had a higher concentration of oleic acid (C18:1) (52.40%) than GL and L, but its concentration was lower than that of peanut oil and almond oil [14]. Around 10% of the total contents of saturated fatty acids in G, L, and GL are in congruence with those in other reports, with high levels of palmitic acid (C16:0), followed by stearic acid (C18:0), in all tested oils [34].

Linseed oil and grape seed oil, each with distinct fatty acid profiles, offer a compelling opportunity for blending. Linseed oil boasts a high content of alpha-linolenic acid (ALA), an essential omega-3 fatty acid. In contrast, grape seed oil is rich in linoleic acid, an omega-6 fatty acid. Blending these oils in equal proportions creates a novel composition that nature does not provide and that has a unique fatty acid profile, potential health advantages, culinary versatility, and use in supplementation [10,12]

MUFAs and PUFAs acquired from food affect cell and tissue responsivity, regulating antioxidant signaling pathways and modulating inflammatory processes [35]. These fatty acids are energy sources; they compose the cell membrane phospholipids and present signaling specific to hormone interactions [36]. Therefore, the moderate consumption of vegetable oils rich in MUFAs and PUFAs is recommended to prevent cardiovascular and metabolic diseases, such as type 2 diabetes, inflammatory conditions, and cancer [35,36].

Two leading families of PUFAs are relevant to health: omega-3 and omega-6. In most diets, these fatty acids are obtained from plants, such as seeds, nuts, and vegetable oils [36]. α-linolenic (C18:3) and linoleic fatty acids (C18:2) participate in intracellular signaling, such as in transcription factors and gene expression [35]. Therefore, a deficiency of essential fatty acids causes dermatitis, disturbances in mitochondrial activity, cardiovascular diseases, cognitive deficits, arthritis, and other health conditions [37].

The metabolic conversion of linoleic fatty acid (C18:2) into arachidonic acid (C20:4) and α-linolenic acid (C18:3) in eicosapentaenoic acids (C20:5) occurs through the same enzymes; therefore, a high presence of these fatty acids in an organism relates to substrate availability [38]. Linoleic acid (C18:2) acts as a substrate for the synthesis of γ-linolenic acid (C18:3) and dihomo-γ-linolenic acid (C20:3), the same way that it incorporates phospholipids into the cell membrane [38].

Table 3 describes the atherogenicity index (AI) and the thrombogenicity index (TI) for the oils studied. There are no recommended values for these indices; however, their low values across all oils indicate the quality of the fatty acids, with proportions favorable to vascular health. This demonstrates the absence of correlations with atherosclerotic and thrombogenic events [33].

The quality and identity indices of the oils are shown in Table 4. The acidity index was the highest in L (2.8 mg KOH/g), followed by GL (1.3 mg KOH/g) and G (1.2 mg KOH/g). G presented the highest level of peroxides (6.5 mEqO_2/kg), followed by GL (4.6 mEqO_2/kg), while L had the lowest peroxide content (2.0 mEqO_2/kg). The refraction index values were 1.470 and 1.477 in G and L, respectively. GL exhibited results between the G and L oils because of its composition being 1:1 (*v*/*v*) (Table 4). The iodide index and relative density of G (122.71 and 0.910, respectively) were closer to those of GL (128.59 and 0.918) than those of L (175 and 0.927, respectively). GL was similar to G in terms of the saponification index and was close to L.

Table 4. Quality and identity scores of grape seed oil, linseed oil, and blend oil 1:1 (*v*/*v*).

Indices	Grape Seed Oil (G)	Linseed Oil (L)	Blend Oil (GL)
Acidity (mg KOH/g)	1.2	2.8	1.3
Peroxide (mEqO_2/kg)	6.5	2.0	4.6
Refraction (40 °C)	1.470	1.477	1.473
Iodide (g I_2/100 g)	122.71	175	128.59
Saponification (mg KOH/g)	192	196	192

Relative density (mg/mL)	0.910	0.927	0.918

Values expressed as the mean ± standard deviation of the mean.

The quality and identity of G, L, and GL (Table 4) fall within the recommended acidity index for cold-pressed oils (<4.0 mg KOH/g), indicating low levels of free fatty acids and the absence of deterioration [39]. The peroxide index is related to the presence or absence of hydroperoxides, which form in the initial steps of lipid auto-oxidation [39]. For this index (Table 4), all samples are within the limit recommended for cold-pressed virgin oils (<15 $mEqO_2$/kg), indicating the absence of oxidative processes in the analyzed oils.

These results can be attributed to the antioxidant components in G, L, and GL, such as the phenolic compounds, carotenoids, and tocopherols, which help prevent oxidative events, thus improving oil stability [40].

The refraction index is related to the viscosity of the vegetable oil, and high indices indicate a deteriorated product or a deterioration process [39]. Concerning the refraction index, the grape seed oil and linseed oil are within the recommended ranges of 1.467–1.477 and 1.472–1.487, respectively [40]. The blend oil has an index between the grape seed oil and the linseed oil (Table 4) because of its composition being 1:1 (*v*/*v*), showing coherence with the integrity of its component oils and values within the recommended range for grape seed oil and linseed oil.

The iodine index reveals the unsaturations of fatty acids in vegetable oils and their tendency to oxidize; thus, the higher the number of unsaturations, the higher the predisposition to oxidation [24,39]. G presented an iodine index below the expected range of 128–150 g I_2/100 g [40]. Variations in this index are related to the fatty acid profile of different varieties of grapes (*V. vinifera*), which result in the extracted oils having individual characteristics [9,14]. Therefore, the literature offers figures above the recommendations, e.g., 165.5 I_2/100 g and 194 I_2/100 g [32,37], which, observed together with other analyses, result in an oil with good stability [41]. L and GL have iodine indices within the indicated range of 170–211 I_2/100 g [7], similar to the cold-pressed G [3,24].

The saponification index indicates the presence of low-molecular-weight fatty acids in oils, inversely proportional to the presence of high-molecular-weight (long-chain) fatty acids. The obtained saponification indices (Table 4) were within the limits of 188–194 mg KOH/g for G and 185–197 mg KOH/g for L [40], and GL stayed within the recommended range. Thus, the levels of low-molecular-weight fatty acids were within the indicated patterns for all samples.

In contrast, the relative density of G (Table 4) was below the recommended range of 0.920 to 0.926 mg/mL, while that of L fell within the expected range of 0.925 to 0.935 mg/mL. However, GL had a value of 0.918 mg/mL, which is below the recommended limits for both G and L [40].

Some analyses showed results divergent from those in the literature because the specific cultivars of the tested oils were unknown, as they originated from various varieties of *V. vinifera* and *L. usitatissimum*. It is known that differences in maturation degrees, soil fertility, climate, and temperature, among other natural influences, impact the nutritional quality of fruits and seeds [42,43].

The Rancimat test, shown in Figure 2, indicates oxidative stability through the induction period (IP). It is a direct indication of the oxidation of vegetable oils during heating, with inversely proportional results; thus, the lower the content of PUFAs, the higher the IP value [27,44].

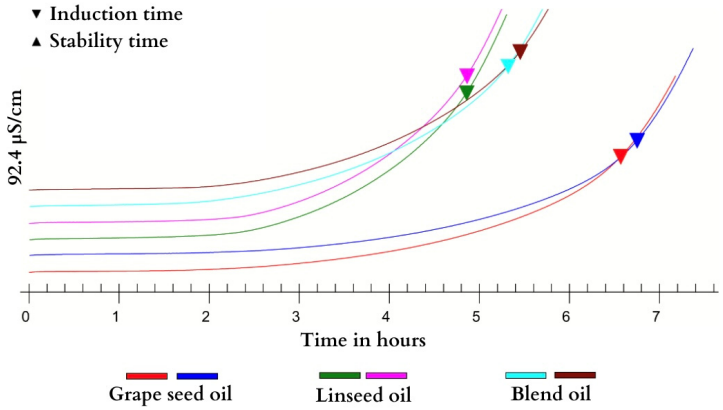

Figure 2. Oxidative stability of grape seed oil (G), linseed oil (L), and blend oil (GL) determined using the Rancimat method. The arrows indicate the formation of the graph originated by the analysis

The grape seed oil (G1 and G2) obtained an IP of 6.66 h, the linseed oil (L1 and L2) obtained an IP of 4.86 h, and the blend oil (GL1 and GL2) obtained an IP of 5.39 h. The estimate of oxidative stability demonstrated by IP allowed us to detect of alterations caused by the increased temperature (range of 100 °C) and heat conductivity of the sample under controlled conditions and a constant airflow.

Vegetable oils are susceptible to oxidative events because of their high content of unsaturated fatty acids, and polyunsaturated fatty acids are the most susceptible to loss during the first heating step due to their high number of double bonds [45]. G contained less PUFAs and more saturated fatty acids than GL and L (Table 3) [41]. Therefore, the oxidative stability (Figure 2) of G (G1 and G2) was higher than that of GL (GL1 and GL2) and L (L1 and L2). G demonstrated stability similar to O [46]. Such coincident behavior is due to the 12.20% content of total saturated fatty acids in O [20], with percentages similar to those of G (Table 3 and Figure 2). The oil with the second best thermal stability was GL (Figure 2) because it had a higher content of total saturated fatty acids and a lower presence of PUFAs than L (Table 3). GL presented an intermediate IP compared with that of G and L (Figure 2), with oxidative stability adequate for the behavior of pure oils.

L demonstrated a low oxidative stability (Figure 2) because of its high content of PUFAs. In particular, α-linolenic acid is more susceptible to degradation and has lower oxidative stability [44]. Nevertheless, some linseed cultivars present induction period (PI) values of 2.11–3.3 h [47], lower than what we found. Therefore, we suggest that linseed oil should not be utilized at high temperatures because of the loss of nutritional content and that it is best used as supplementation in capsules or added to cold preparations [45,46].

Thermogravimetry/Derivative Thermogravimetry (TG/DTG) and Differential Scanning Calorimetry (DSC) analyses show the thermal decomposition and transitions caused by a controlled variation in temperature [48]. The TG/DTG curves show the thermal decomposition process in G, L, and GL (Figure 3). In G, two steps of consecutive mass loss were observed at temperatures between 205 °C and 598 °C. For the correlation of the thermal decomposition profile, we calculated the mass losses in intervals. The first loss was 75.06%, starting at 210.92 °C until temperatures around 430.39 °C. The second loss was 24.72%, and occurred until the temperature of 594.64 °C. In L, we observed that the first decomposition step started at 207.70 °C until temperatures around 444.38 °C, with a mass loss of 78.01% and a second loss of 21.53% until the temperature of 590.69 °C. The first mass loss was observed in GL starting at 205.01 °C until 433.62 °C, with a loss of 78.85% and a final step of loss of 20.89% until 598.2 °C. The curves show similar thermal decomposition profiles; however, GL presented losses closer to those of L, overlapping the behavior of G.

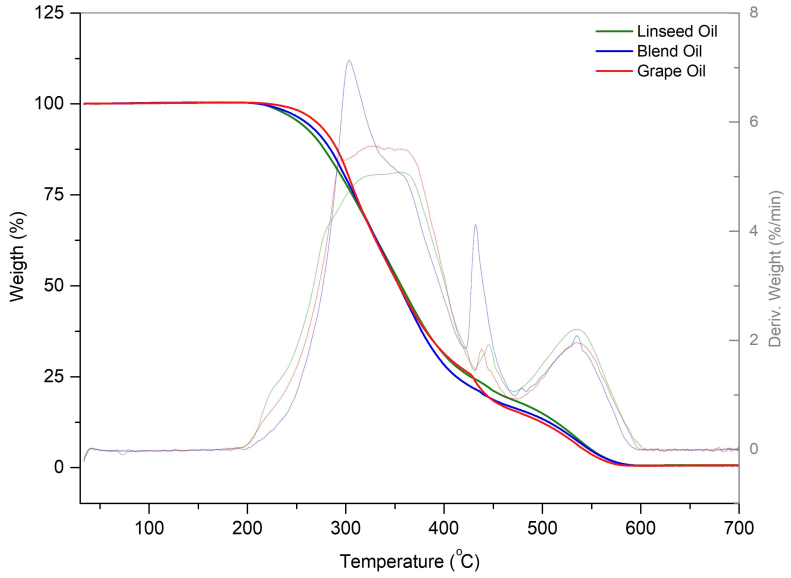

Figure 3. TG/DTG curves of grape seed oil (G), linseed oil (L), and blend oil (GL).

Thus, no significant differences occurred in the mass losses. However, by comparing the decomposition profiles, we found that the first mass loss of G occurred at higher temperatures than that of the GL and L oils because of the higher content of saturated fatty acids (Table 3), which increased thermal stability and reduced mass loss [48,49]. GL presented a mass loss similar to that of L in both steps (Figure 3) because it had a higher presence of polyunsaturated fatty acids and a lower content of saturated fatty acids than G (Table 3). L from the province of Hebei, China showed low decomposition, with weight losses of 29.14% at 389 °C, 59.78% at 389 °C to 470 °C, and 11.08% at 470 °C to 600 °C [50], necessary to correlate the mass losses with the temperature intervals [51].

The DSC curves show endothermic and exothermic peaks and the corresponding temperatures and absorbed or liberated energies during crystallization and fusion events in two steps (Figure 4). Our results show that GL has an intermediate crystallization temperature when compared with G and L.

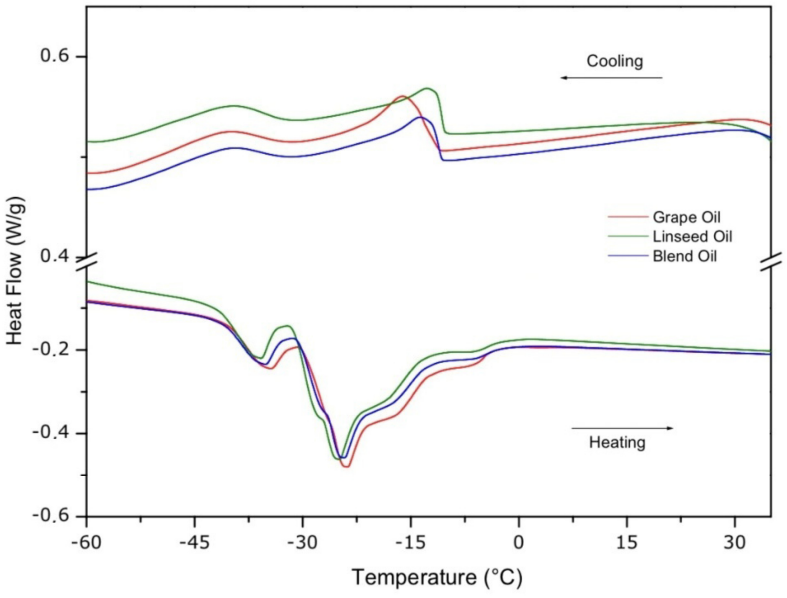

Figure 4. DSC curves of grape seed oil (G), linseed oil (L), and blend oil (GL).

In the cooling curve of G, we observed the first peak in the temperatures at −16.01 °C, corresponding to the crystallization temperature of saturated fatty acids, with an energy consumption of 4.864 J/g. Next, the second peak occurred at −42.36 °C, corresponding to the crystallization of unsaturated fatty acids, with an energy consumption of 3.152 J/g. In the linseed oil curve, the first crystallization peak occurred at −12.53 °C, with an energy consumption of 4.004 J/g for saturated fatty acids, and the second peak occurred at −41.53 °C, with an energy consumption of 3.549 J/g for unsaturated fatty acids. In the GL curve, the same events occurred at −13.66 °C, with an energy of 4305 J/g for saturated fatty acids, and the second peak occurred at a temperature of −41.68 °C, relative to the crystallization of unsaturated fatty acids, with an energy of 2.972 J/g.

Regarding the heating processes, we observed the corresponding temperatures and energies of the fusion peaks. In G, the first peak occurred at −40.72 °C, with a liberation of 5.370 J/g for unsaturated fatty acids, and the second peak occurred at a temperature of −28.37 °C, with a liberation of 38.74 J/g for saturated fatty acids. L showed the first peak at temperatures of −41.99 °C, with 6.330 J/g for unsaturated fatty acids, and the second peak occurred at −31.10 °C, with 40.22 J/g for saturated fatty acids. GL presented the first peak at a temperature of −41.17 °C, with 5.293 J/g for unsaturated fatty acids, and the second peak occurred at −28.89 °C, with 36.05 J/g for saturated fatty acids. The DSC curves allow us to conclude that GL had a reduced crystallization temperature and an increased fusion time compared with G, demonstrating similarities to the intervals of L.

The crystallization events alter the physical properties related to the oxidation process of vegetable oils, which are relevant quality parameters [52]. The heating curves serve to determine the functionality of these oils when incorporated into several food products. A safety interval for the manipulation of vegetable oils around 10 °C, close to the steps of crystallization and fusion, is recommended to conserve their physical–chemical properties [53]. The results for G, L, and GL did not indicate oxidative processes, demonstrating the integrity of their fatty acids (Table 3), as well as the quality and integrity of their composition (Table 4) [54]. Thus, under faster heating rates, L showed a crystallization profile with three exothermic peaks, with the highest peak at −63.54 °C and the two lower peaks at temperatures similar to those in our study of −40.11 °C and −15.37 °C [55]. Concerning the fusion, we found that L had characteristics similar to the peaks of −38.39 °C and −24.81 °C reported by Zhang et al. (2022) [55]. Other linseed cultivars show fusion peaks at −31,73 °C and −10.26 °C [56]. By comparing the fusion peaks of G and peanut oil, we found that the fusion temperature of G was lower, around −24.64 °C, with an enthalpy of 23.15 J/g [57]. These results are similar to the second fusion peak of G (Figure 4), correlated with the presence of antioxidants in the cultivated grape [14].

Antioxidant substances affect only the initial decomposition stage, preventing the formation of hydroperoxides [58]. The DSC curves of GL show accordance with the fatty acid profile (Table 3) and the analyses of identity and quality (Table 4), with values intermediate compared to those of G and L, with this pattern also observed in the thermal analyses.

Concerning the UV–vis absorption analyses, shown in Figures 5 and 6, the results demonstrate a trend for the presence of carotenoids in L because of the higher value for this band, followed by the intermediate value of GL, which also presents results characteristic of the antioxidant group of carotenoids [59]; thus, they have a higher carotenoid content than G, as proven in the coloration analyses of each sampled oil (Table 5).

The measured absorption spectra were between 5 g/dm^3 and 90 g/dm^3 (Figure 5A, B). Different concentrations provided evidence of the UV–vis absorption bands of G, L, and GL. Regarding absorbance in the 250–290 nm band detected at 5 g/dm^3 (Figure 5A), G demonstrated the highest value, followed by GL with intermediate absorbance behavior.

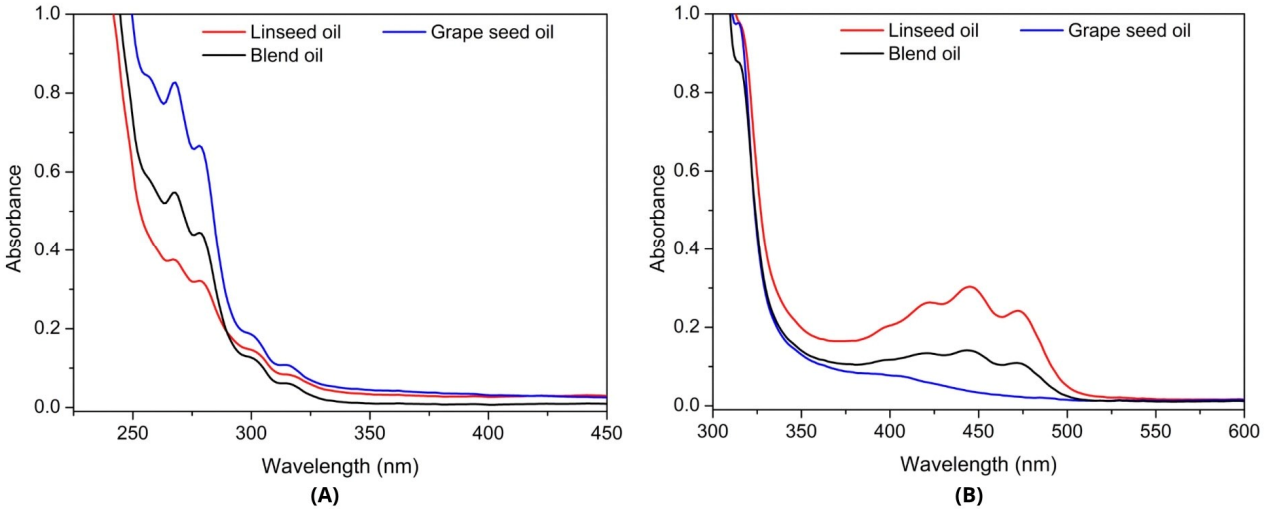

Figure 5. UV–vis absorption curves of grape seed oil (G), linseed oil (L), and blend oil (GL) (at (A) 5 g/dm^3 and (B) 90 g/dm^3 diluted in hexane).

The 400–500 nm band was detected for the 90 g/dm^3 concentration of G, L and GL (Figure 5B). L presented the highest value in this band, followed by GL with an intermediate value; thus, the presence of carotenoids in L and GL was superior to that in G.

Concerning the fluorescence analysis of the vegetable oils (Figure 6), it was possible to note that fluorescence centered at 330 nm with excitation at 300 nm (first band), demonstrating the highest intensity for L (a), followed by GL (c). In the emission region of 350 to 470 nm with excitation between 300 and 400 nm (second band), G (b), L, and GL presented similar emission profiles, with, however, different fluorescence intensities.

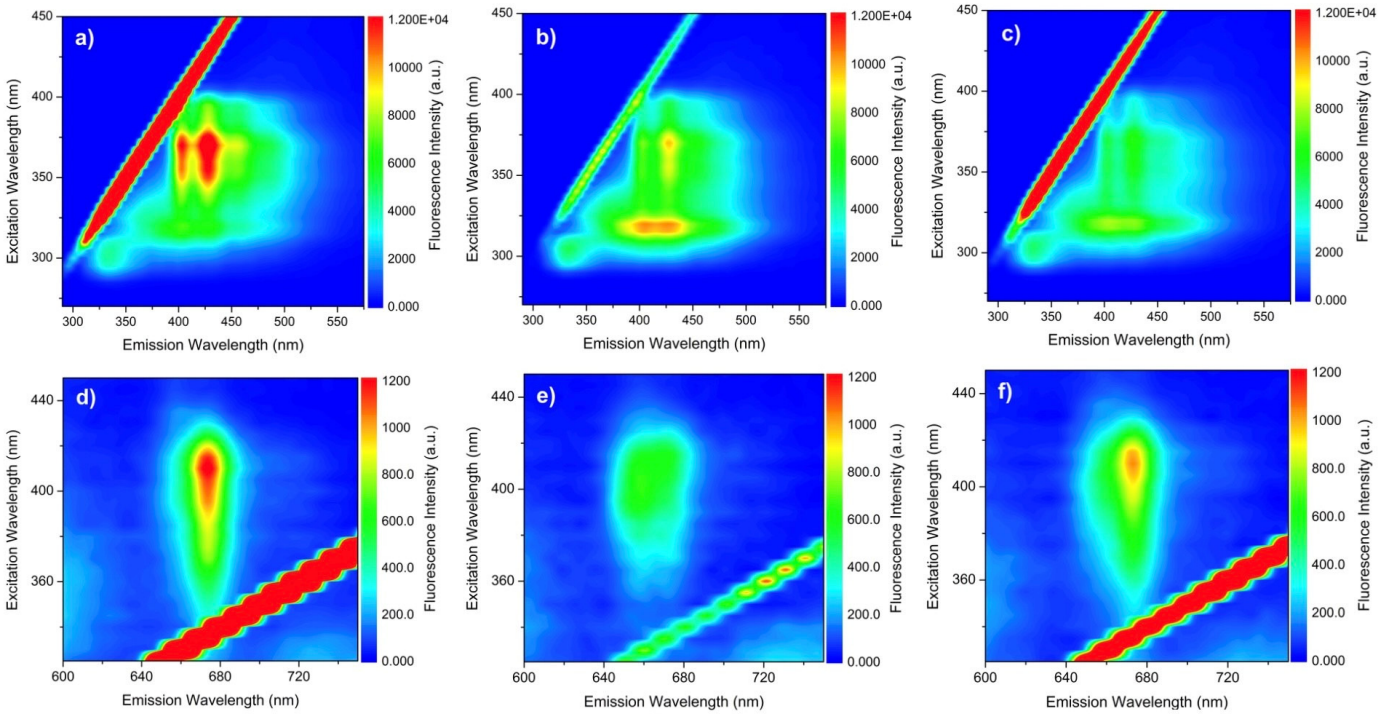

Figure 6. Excitation–emission maps of (**a**) linseed oil, (**b**) grape seed oil, and (**c**) blend oil in the 300-500 nm emission range and (**d**) linseed oil, (**e**) grape seed oil, and (**f**) blend oil in the 600-750 nm emission range.

It is possible to observe fluorescence centered at 330 nm with excitation at 300 nm (first band), which is associated with the emission of tocopherols and polyphenols [60]. The emission region of 350 to 470 nm with excitation between 300 and 400 nm can be attributed to the emissions of chlorophyll and methyl esters [60,61], with variations in the higher-intensity band due to differences in the compositions of antioxidants of the fatty acids of the oils. The second fluorescence region with a lower intensity was detected at 330 nm with excitation at 300 nm, characteristic of chlorophyll emission [62]. The highest presence of this compound was observed in G, followed by in GL (Table 5). The phenolic compounds protect vegetable oils against the free radicals involved in lipid peroxidation and act directly on oxidative reactions, impeding the action of pro-oxidant agents [63].

A second fluorescence region on had a lower intensity than the first region, presenting a maximum emission intensity at around 675 nm when excited at 410 nm. The fluorescence of L in this region was more intense than that of G, while GL demonstrated a fluorescence intensity intermediate compared to that of G and L.

Other analyses must be carried out to complement the identification and quantification of phenolic compounds, such as the applications of mass spectrometry systems (MS, MS/MS), use of mass detectors, liquid chromatography coupled to mass spectrometry (LC-MS/MS), high-performance liquid chromatography (HPLC), paper chromatography and thin layer chromatography (TLC), high-speed countercurrent chromatography (HSCCC), capillary electrophoresis (CE), and supercritical fluid chromatography (SFC). In addition, high-resolution systems such as time of flight (TOF-MS) and ion trap-MS focus on unknown compounds [64,65].

Regarding the colorimetric parameters of G, L, and GL (Table 5), G and GL showed a darker color (L^*) than L. Concerning color saturation (C^*), G demonstrated the lowest concentration of pigments, and L was similar to GL, presenting more intense colors. Regarding a^* (red axe (+)/green (−)), G presented a greenish color; L presented an a* positive result, indicating coloration with orange tones; and GL stayed a yellowish color. For b^* (yellow axe (+)/blue (−)), all results were positive, indicating yellow tones for all oils, with G showing less yellow than L and GL.

Table 5. Colorimetric parameters of grape seed oil, linseed oil, and blend oil.

Parameters	Grape Seed Oil (G) (%)	Linseed Oil (L) (%)	Blend Oil (GL) (%)
L^*	50.32	38.42	47.15
C^*	22.31	38.70	39.27
Hue (°)	−70.23	82.40	88.72
a^*	−7.55	5.12	0.88
b^*	20.99	38.36	39.26

* The means were determined from triplicates. Values are expressed as the mean ± standard deviation of the mean.

When the Hue angle (°) was observed, an indicator of tone, G exhibited a greyish yellow–green color, L showed a yellow color with orange tones, and GL demonstrated a bright yellow color.

The colorimetric parameters of G indicated greyish coloration because of the low content of yellow and orange pigments (Table 5), but L and GL had a significant presence of these pigments of bright colors. Yellow to reddish pigments indicate carotenoids, which were mainly observed in L and GL, with β-carotene being the most abundant, as shown in Tables 5 and 6. The presence of β-carotene in vegetable oils benefits utilization and conservation, as it counteracts the reactive species of oxygen and free radicals [66].

Table 6. Carotenoid content in grape seed oil, linseed oil, and blend oil.

Oil	Carotenoids (μg/g)	CV *
Grape seed oil (G)	1.16 ± 0.15 [c]	0.13
Linseed oil (L)	13.67 ± 1.74 [a]	0.13
Blend oil (GL)	6.23± 0.54 [b]	0.88

* CV: coefficient of variation. The means were determined from triplicates. Values are expressed as the mean ± standard deviation. The same letters in the same column mean no significant difference at the 5% level.

The quantification of carotenoids in the oils was directly related to the emission–excitation (Figure 6), coloration (Table 5), quality, and identity analyses (Table 4), confirming the results [21]. A comparison of the total carotenoid contents of G, L, and GL (Table 6) showed that linseed oil had the highest carotenoid levels, followed by GL and G.

The high CV values are caused by the easy degradation of carotenoids under exposure to light and air oxygen, as well as solvent evaporation during the absorbance determination.

The carotenoids associated with the chlorophyll in plants are punctuated by values of "a", a blue-greenish color, and "b", a greenish-yellow color (Table 6) [67]. Carotenoids are liposoluble compounds of vegetable oils [63]. Thus, the coloration (Table 5) confirms the presence of β-carotene (Table 6) mainly in L, followed by in GL. β-carotene is the primary precursor of vitamin A [67].

Nonetheless, G, even with low concentrations of carotenoids, contains polyphenols and vitamin E, which enhance wound healing and reduce systemic inflammation [68]. Compared with other vegetable oils, G possesses a higher content of vitamin E isomers, such as tocotrienol, known for their antioxidant, anti-inflammatory, and antitumor properties, and this distinguishes the oil in terms of antioxidant activity [69]. Furthermore, the easy degradation of carotenoids under exposure to light, oxygen, and solvent evaporation during oil analysis and extraction from seeds can alter their quantification [66,70]. Variations in carotenoid content are influenced by the genetic characteristics of the plant variety, environmental conditions, maturation degree, and oil extraction method [71].

Likewise, flavonoids, phenolic acids, and tocopherols are unsaponifiable products with anti-inflammatory properties and regenerative effects [49], isolated from G and L, which demonstrate anti-inflammatory properties and regenerative effects [49]. Additionally, we found that GL has intermediate results compared to both crude G and L, with possible beneficial actions to health for attributions specific to each oil, demonstrating a synergic effect.

Concerning the animal experimentation, while both linseed oil and grape seed oil have garnered scientific attention and commercial viability, their prominence in research emerged primarily in the last century. In contrast, olive oil has a rich historical tradition, having been consumed and studied for centuries within Mediterranean cultures and globally [15,31].

The health benefits, historical use, scientific support, culinary versatility, and global popularity of olive oil contributed to the decision to use this oil in the control group supplemented with oil (o). Olive oil is rich in polyphenols, particularly hydroxytyrosol and oleuropein, with antioxidant properties. These compounds act by scavenging free radicals and mitigating oxidative damage to cells, exerting anti-inflammatory effects through the modulation of inflammatory pathways associated with cardiovascular conditions and cancer [72].

Concerning the total weight gain of animals, as presented in Table 7, the experimental groups did not show significant differences in total weight gain between the start of the experiment ($p = 0.877$) and the experiment end ($p = 0.290$). After eleven weeks of treatment, the control group © exhibited a significantly higher weight gain than the groups that received the grape seed oil (G1 and G2) and linseed oil at a dosage of 2000 mg/kg/day (L2)

($p < 0.05$). The GL group showed a lower total weight gain than the C and O groups ($p < 0.001$ and $p < 0.05$, respectively).

Table 7. Behavioral parameters of weight, food consumption, FEC, and CWGCI of animals after 11 weeks of the experiment.

Parameters	C	O	L1	L2	G1	G2	GL
Starting weight (g)	35.94 ± 4.34	35.50 ± 3.54	35.33 ± 3.16	36.50 ± 4.46	35.12 ± 2.98	36.65 ± 3.35	36.83 ± 4.14
Final weight (g)	43.31 ± 6.16	40.83 ± 5.49	41.39 ± 4.65	40.06 ± 5.68	39.06 ± 5.46	40.47 ± 4.06	39.44 ± 4.49
Total weight gain (g)	7.37 ±3.48	5.33 ± 3.07	6.06 ± 3.64	3.56 ± 3.09 [a]	2.94 ± 3.15 [a]	3.82 ± 2.45 [a]	2.61 ± 2.93 [Ab]
Total consumption (g) [1]	459.67 ± 68.96	346.78 ± 29.27 [ac]	398.22 ± 36.84	355.61 ± 45.18 [a]	376.47 ± 34.49 [a]	327.59 ± 18.58 [acde]	383.11 ± 40.70
FEC	1.65 × 10^{-2} ± 0.8 × 10^{-2}	1.49 × 10^{-2} ± 0.7 × 10^{-2}	1.55 × 10^{-2} ± 0.8 × 10^{-2}	1.05 × 10^{-2} ± 0.8 × 10^{-2}	0.8 × 10^{-2} ± 0.7 × 10^{-2}	1.18 × 10^{-2} ± 0.7 × 10^{-2}	0.7 × 10^{-2} ± 0.7 × 10^{-2} [ac]
CWGCI	0.38 × 10^{-2} ± 0.1 × 10^{-2}	0.34 × 10^{-2} ± 0.1 × 10^{-2}	0.35 × 10^{-2} ± 0.2 × 10^{-2}	0.24 × 10^{-2} ± 0.1 × 10^{-2}	0.19 × 10^{-2} ± 0.1 × 10^{-2} [a]	0.27 × 10^{-2} ± 0.1 × 10^{-2}	0.16 × 10^{-2} ± 0.1 × 10^{-2} [ac]

FEC: feed effectiveness coefficient; CWGCI: weight gain per caloric consumption index; C: control group (distilled water at dose of 1000 mg/kg/animal); O: extra virgin olive oil group (1000 mg/kg/animal); L1: Golden linseed oil group (1000 mg/kg/animal); L2: Golden linseed oil group (2000 mg/kg/animal); G1: Grape seed oil group (1000 mg/kg/animal); G2: Grape seed oil group (2000 mg/kg/animal); GL: blend oil (2000 mg/kg/animal). Mean ± standard deviation from the mean. Different letters in the same line indicate significant differences in relation to C ($p < 0.05$: a; $p < 0.001$: A), O ($p < 0.05$: b), L1 ($p < 0.05$: c), G1 ($p < 0.05$: d), and GL ($p < 0.05$: e). Kruskal–Wallis/Dunn's tests [1]; ANOVA/Tukey's tests).

Concerning total feed consumption in the 11-week experiment (Table 7), which refers to the total feed intake of each mouse for the experimental period of 11 weeks, C showed more consumption than the O, L2, G1, and G2 groups ($p < 0.05$). The L1 group also showed more consumption than the O and G2 groups ($p < 0.05$), and G1 and GL showed more consumption than G2 ($p < 0.05$).

After the eleven-week experimental period, we evaluated the feed effectiveness coefficient (FEC) and weight gain per caloric consumption (GWGCI) according to the intake of each group (as detailed in Table 7). Notably, the GL group exhibited significantly lower weight gain ($p < 0.05$) than both C and L1 groups, whether measured per gram of consumed feed or in terms of total caloric consumption. Total feed consumption was the lowest in G2 and O groups (also indicated in Table 7), and total consumption was the lowest in G2 and O groups. Nevertheless, GL had higher total consumption than G2 but lower final weight gain when compared to C and L1.

It is known that supplementation with vegetable oils does not influence calorie intake. The oil dosages in ml ranged from 0.03 ± 0.02 mL/day to 0.05 ± 0.03 mL/day, with weekly adjustment according to weight evolution, so the additional calories from the supplement varied from 0.27 ± 0.1 kcal/day to 0.44 ± 0.2 kcal/day, according to the dosages of 1.000 mg/ kg/day and 2.000 mg/kg/day.

Our results confirm that vegetable oils supplemented with nutritional and effective functional properties can act on an organism as nutraceuticals [13]. Linoleic acid (C18:2) and α-linolenic fatty acid (C18:3) induce the lengthening of carbon chains (more than 22 C atoms) and are described as "essential fatty acids" because they must be in the diet [73,74]. Thus, the supplementation of GL, composed of a 1:1 (*v*/*v*) ratio of G and L, ratifies the significant presence of both fatty acids (Table 3) and shows the beneficial utilization of macronutrients in the ingested feed, resulting in the total weight gain, CEA, and CGPCC being lower than in the other groups.

Compared with L and GL, the higher satiety and lower weight gain caused by the supplementation of G at a dosage of 2000 mg/kg/day (G2) can be justified by the eminent exposure of the animals to quantities of monounsaturated fatty acids (Table 3). Additionally, the higher total consumption of group C can be attributed to the satiety and caloric

percentage of the distilled water supplementation being lower than those of the vegetable oil supplementation [75].

Concerning serum parameters, shown in Figure 7, fastening glycemia did not show significant differences ($p = 0.153$) between the experimental groups. Similarly, triglyceride levels did not show differences between the groups ($p = 0.052$); however, L1 had the highest values. Regarding total cholesterol, group C showed the lowest value compared with G2 and GL ($p < 0.001$), and O and L2 also had lower total cholesterol than G2 and GL ($p < 0.05$) (Figure 7). This demonstrates that, in C, O and L2, fewer changes occurred in the transport of cholesterol and triglycerides and the reverse transport of cholesterol to the liver, where they are metabolized [76].

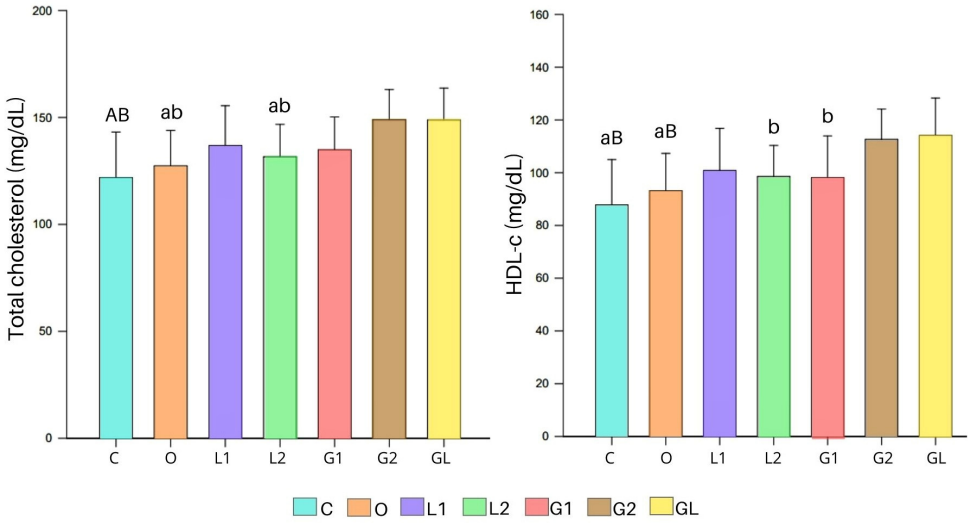

Figure 7. Serum parameters of total cholesterol and HDL-c at the end of the 11-week experiment. C: Control group (distilled water at dose of 1000 mg/kg/animal); O: Extra virgin olive oil group (1000 mg/kg/animal); L1: Golden linseed oil group (1000 mg/kg/animal); L2: Golden linseed oil group (2000 mg/kg/animal); G1: Grape seed oil group (1000 mg/kg/animal); G2: Grape seed oil group (2000 mg/kg/animal); GL: Blend oil (2000 mg/kg/animal). Mean ± standard deviation from the mean. Different letters indicate significant differences in relation to G2 ($p < 0.05$: a; $p < 0.001$: A) and GL ($p < 0.05$: b; $p < 0.001$: B), determined via one-way ANOVA with Tukey's post-test.

HDL-c levels were higher in GL and G2 than in C ($p < 0.001$) and O (GL: $p < 0.001$; G2: $p < 0.05$); additionally, group GL exhibited higher HDL-c levels than G1 ($p < 0.05$) (Figure 7). Demonstrating that, in C and O, fewer changes occurred in the transport of cholesterol and triglycerides and the reverse transport of cholesterol to the liver, where they are metabolized [76]. Concerning LDL-c, VLDL-c, and non-HDL cholesterol, we did not observe significant differences between the groups ($p = 0.531$, $p = 0.052$, and $p = 0.082$, respectively).

The experimental groups, mainly G2 and GL, despite not receiving a significant amount of calories from the oil, showed the lowest consumption and weight gain, which can be attributed to the greater satiety and sensation of food intake from the oil [75]. Furthermore, the supplementation of oils may have impacted cholesterol levels, leading to a different metabolic response related to their lipid profile compared with the control group [77]. The increase in HDL-c cholesterol levels, referred to as "good cholesterol", mainly observed in G and GL, can be attributed to the composition of fatty acids, the presence of antioxidant compounds, and the unique physicochemical characteristics of the supplemented oils contributing to this favorable outcome [78].

Group C, which did not receive oil supplementation, showed the highest food consumption and a significant total weight gain (Table 3). However, this group showed the lowest total cholesterol levels, without changes in the blood lipid profile [76,78].

The L2 and O groups showed significantly lower total cholesterol values than the G2 and GL groups. Although the olive oil supplementation at 1000 mg/kg/day did not influence on weight control, it did affect total cholesterol levels. The monounsaturated fatty acids (MUFAs) present in olive oil play a crucial role in increasing nitric oxide production, impacting endothelial function [79]. Although an increase in HDL-c was not demonstrated in this study, Millman et al. (2021) [72] found that olive oil consumption reduces low-density lipoprotein (LDL-c) cholesterol levels while increasing high-density lipoprotein (HDL-c) cholesterol levels.

Additionally, the significant reduction in cholesterol in the L2 group could have had cardio-protective, antitumoral, and anti-inflammatory effects via the metabolic action of α-linolenic fatty acid (C18:3), with this group showing a trend of reduced levels of triglycerides and LDL-c [80,81]. This predisposition was not noted for the L1 group.

In this study, the G2 and GL groups exhibited higher levels of high-density lipoprotein cholesterol (HDL-c), while the HDL-c of GL surpassed that of G1 (as shown in Figure 7). Thus, in addition to promoting better weight maintenance, GL significantly elevated HDL-c levels. These findings demonstrate that improved treatments at the doses of 2000 mg/kg/day administered to the G2 and GL groups exhibited anti-inflammatory characteristics, leading to weight control and increased plasma HDL-c levels [79].

The GL dosage suggests that the relative proportions of the fatty acids in the grape seed oil and linseed oil complement each other, resulting in an antioxidant effect. Combined, these oils may offer enhanced health benefits due to their unique fatty acid profiles [79,80]. While grape seed oil is rich in linoleic acid, linseed oil provides a significant amount of alpha-linolenic acid (ALA), both contributing to overall health and oxidative protection and increase HDL-c. The synergy between these oils underscores their potential as nutraceuticals [80]. In this study, it was observed that grape seed oil (G) at the highest dosage of 2000 mg/kg/day and GL at the same dosage were the most effective supplements for increasing high-density lipoprotein cholesterol (HDL-c) levels and for causing the lowest weight gain in the studied animals. Interestingly, the high total cholesterol levels in these groups did not suggest any disease-related changes.

The concentrations of cytokines measured in the blood serum of the experimental groups, shown in the Figure 8, showed lower MCP1 levels in L2, G2, and GL than in C ($p < 0.05$). Regarding IL-6, we did not detect significant differences between the groups ($p = 0.199$). The TNF-α values were lower in L2, GL ($p < 0.001$), L1, and G1 ($p < 0.05$) than in C (Figure 8). The L2 and GL groups also presented lower levels than O (Figure 8).

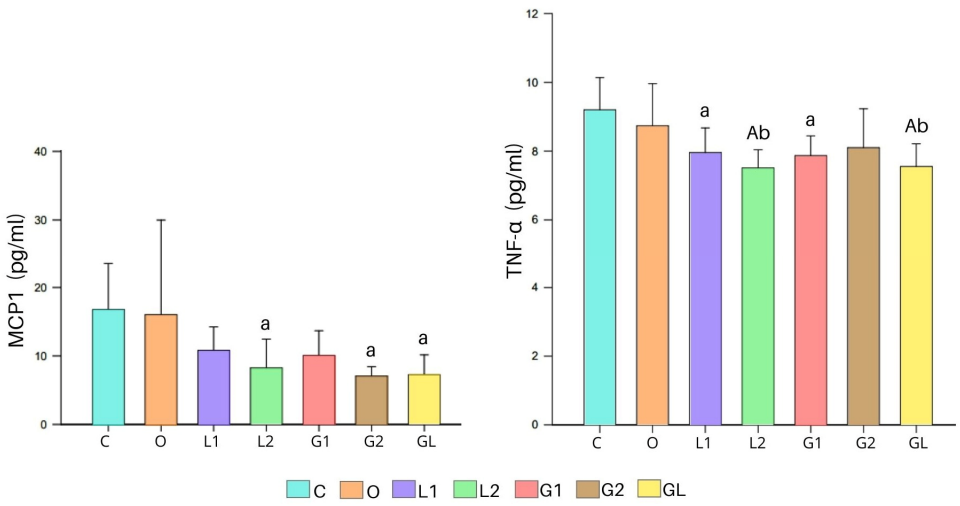

Figure 8. Concentrations of MCP1 and TNF-α cytokines at the end of 11-week experiment. C: Control group (distilled water at dose of 1000 mg/kg/animal); O: Extra virgin olive oil group (1000 mg/kg/animal); L1: Golden linseed oil group (1000 mg/kg/animal); L2: Golden linseed oil group (2000 mg/kg/animal); G1: Grape seed oil group (1000 mg/kg/animal); G2: Grape seed oil group (2000

mg/kg/animal); GL: Blend oil (2000 mg/kg/animal). Mean ± standard deviation from the mean. Different letters in the same bar indicate significant differences in relation to C ($p < 0.05$: a; $p < 0.001$: A) and O ($p < 0.05$: b), determined via one-way ANOVA with Tukey's post-test. MCP1 determined via Kruskal–Wallis test followed by Dunn's test (Kruskal–Wallis/Dunns).

The PAI-1 values did not show differences between the groups ($p = 0.697$). Additionally, regarding the hormones of insulin, leptin, and resistin, we did not detect a significant difference between the groups ($p = 0.165$, $p = 0.121$, and $p = 0.091$, respectively), with the lowest insulin levels in group L1, lowest leptin values in group O, and lowest resistin levels in group C.

The L2, G2, and GL groups exhibited reduced levels of pro-inflammatory cytokines (MCP-1 and TNF-α), highlighting the anti-inflammatory action of their supplementation. This effect can be partly attributed to the fatty acid (FA) profiles of the oils, particularly the presence of linoleic acid (LA) at percentages of 52.4%, 14.32%, and 42.53% for L2, G2, and GL, respectively.

LA, a common omega-6 fatty acid, undergoes metabolism to other omega-6 polyunsaturated fatty acids (PUFAs). Among these, arachidonic acid (ARA) significantly contributes to the composition of membrane phospholipids in cells involved in inflammation. ARA serves as a precursor to pro-inflammatory mediators, including prostaglandins and leukotrienes, which are targeted by anti-inflammatory pharmaceuticals for inflammation control [82].

However, an excessive omega-6 intake can intensify inflammatory processes. Conditions such as hypertension, increased pain in arthritis cases, and even inflammatory bowel diseases may result from an imbalance. Interestingly, studies in healthy adults have shown that increased LA consumption does not necessarily increase the concentrations of many inflammatory markers. Indeed, epidemiological evidence has suggested that LA might be associated with reduced inflammation [83].

Ferrucci and colleagues observed that total n-6 PUFA plasma concentrations were inversely associated with serum CRP, IL-6, IL-6r, IL-1ra, and TNF in a cross-sectional analysis of 1123 Italian adults [80]. Another study found an inverse and/or no association between plasma or dietary LA and a variety of markers of chronic inflammation, and other explanations for the proinflammatory LA hypothesis have been offered by several investigators [83].

In contrast, the dietary supplementation had beneficial effects on the L1, L2, GL, and G1 groups, as evidenced by the lower tumor necrosis factor-alpha (TNF-α) levels, shown in Figure 8. This reduction indicates a possible decreased activation of the NF-κB pathway and an improved endothelial cell integrity due to a reduced pro-inflammatory cytokine expression [83,84] that correspond to decreased TNF binding to its receptor TNFR1, causing lesser activation of inflammatory genes [85]. However, in the C and O groups, the results, potentially linked to the greater weight gain during the 11-week experiment and differences in adipocyte size, may indicate low-grade chronic inflammation [84,86]

Concerning the histology of the hepatic tissue (Table 8), all groups presented an absence of steatosis (<5%) and microvesicle steatosis (both $p = 1.000$). Regarding lobular inflammation, we did not find significant differences between the groups ($p = 0.528$). For the analysis of ballooning hepatocytes (Table 8 and Figure 9), G2 exhibited a prevalence of many cells compared with the C, L1, and GL groups ($p = 0.008$). However, all groups presented an absence of Mallory's hyaline ($p = 0.421$), apoptosis ($p = 0.143$), and glycogenated nuclei ($p = 0.249$).

Table 8. Distribution of changes observed in the liver of experimental animals.

Parameters	C		O		L1		L2		G1		G2		GL	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Steatosis														
<5%	12	100	12	100	12	100	12	100	12	100	12	100	12	100
Microvesicle steatosis														
Absent	12	100	12	100	12	100	12	100	12	100	12	100	12	100
Lobular inflammation (p = 0.528)														
Absent/<2 foci per field/200X	12	100	12	100	11	91.7	12	100	12	100	11	91.7	12	100
2–4 foci per field/200X	0	0	0	0	1	8.3	0	0	0	0	1	8.3	0	0
Ballooning (p = 0.008)														
Absent/few cells	12	100	11	91.7	12	100	10	83.3	8	66.7	7	58.3 [a,b,c]	12	100
Many cells	0	0	1	8.3	0	0	2	16.7	4	33.3	5	41.7	0	0
Mallory's hyaline (p = 0.421)														
Absent	9	75	10	83.3	9	75	10	83.3	6	50	7	58.3	7	58.3
Present	3	25	2	16.7	3	25	2	16.7	6	50	5	41.7	5	41.7
Apoptosis (p= 0.143)														
Absent/few cells	12	100	10	83.3	12	100	8	66.7	8	66.7	9	75	10	83.3
Present	0	0	2	16.7	0	0	4	33.3	4	33.3	3	25	2	16.7
Glycogenated nuclei (p = 0.249)														
None/rare	8	66.7	9	75	9	75	9	75	7	58.3	11	91.7	8	66.7
Some	4	33.3	3	25	3	25	3	25	5	41.7	1	8.3	4	33.3

C: Control group (distilled water at dose of 1000 mg/kg/animal); O: extra virgin olive oil group (1000 mg/kg/animal); L1: Golden linseed oil group (1000 mg/kg/animal); L2: Golden linseed oil group (2000 mg/kg/animal); G1: Grape seed oil group (1000 mg/kg/animal); G2: Grape seed oil group (2000 mg/kg/animal); GL: Blend oil (2000 mg/kg/animal). The data are presented as absolute and relative frequencies. Different letters in the same line indicate significant differences calculated using Bonferroni correction with respect to C ($p < 0.05$: a), L1 ($p < 0.05$: b), and GL ($p < 0.05$: c).

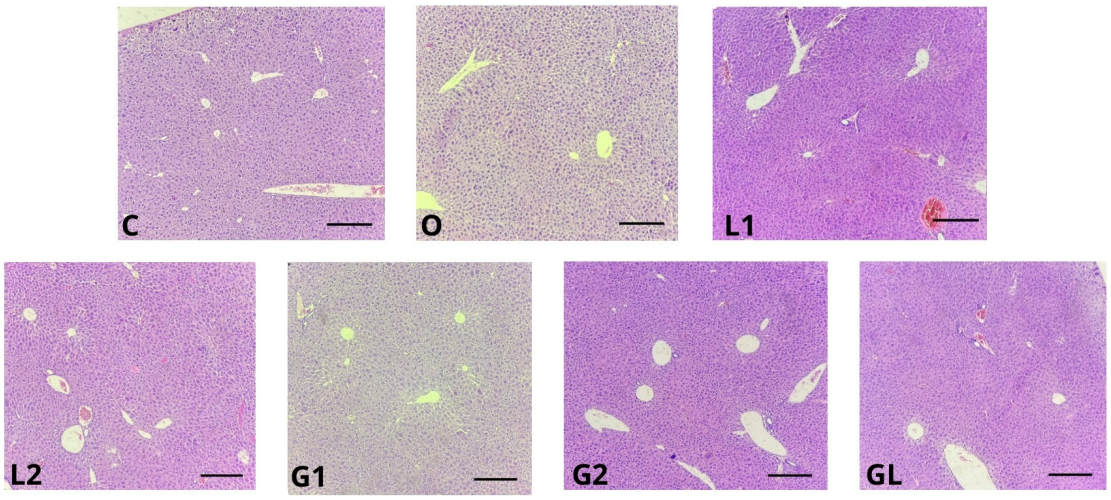

Figure 9. Histology of animal hepatocytes 100x. C: Control group (distilled water at dose of 1000 mg/kg/animal); O: Extra virgin olive oil group (1000 mg/kg/animal); L1: Golden linseed oil group (1000 mg/kg/animal); L2: Golden linseed oil group (2000 mg/kg/animal); G1: Grape seed oil group (1000 mg/kg/animal); G2: Grape seed oil group (2000 mg/kg/animal); GL: Blend oil (2000 mg/kg/animal). Mean ± standard deviation from the mean. Different letters in the same bars indicate significant differences in relation to L1 ($p < 0.05$: a). Kruskal–Wallis/Dunn's tests. Scale bar = 100 μm; original magnification × 100.

Furthermore, steatosis (fatty liver) was not detected in any group (Table 8 and Figure 9). This finding suggests that, in both the control group receiving olive oil (O) and the experimental groups supplemented with grape seed oil (G1 and G2), linseed oil (L1 and L2), and blend oil (GL), liver damage was not induced. Neither group C (control) nor the experimental animals exhibited microvesicular steatosis or lobular inflammation.

In our study, we did not observe notable swollen hepatocytes with cytoplasm fat droplets, a characteristic feature of hepatocellular ballooning [86]. While significant differences were detected in this analysis (as shown in Table 8 and Figure 9), other hepatic parameters such as the presence of Mallory's hyaline, apoptosis, and glycogenated nuclei remained unaltered. Notably, the identification of ballooned hepatocytes in hematoxylin and eosin-stained slides has limitations, including sampling variability and inconsistent lesion distributions among observers [85,87].

The dietary supplementations did not significantly impact the hepatic function. However, certain diet components can positively influence metabolic and hepatic health. For instance, vitamin E, known for its antioxidant properties, and polyphenols, which reduce oxidative stress and liver inflammation, may offer hepatoprotective effects [76,88].

By assessing the adipocyte area of the epididymal adipose tissue, Figure 10, we found that the G1 and GL groups presented lower areas than the L1 group ($p < 0.05$) (Figure 11).

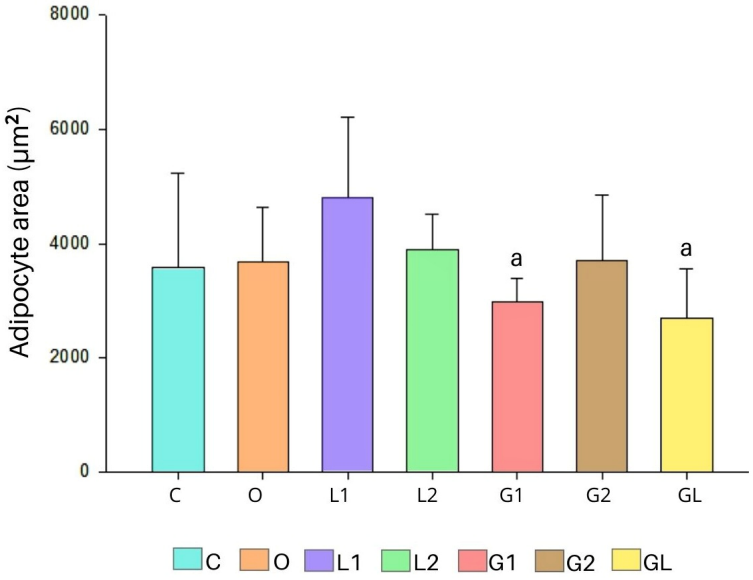

Figure 10. Adipocyte area (µm²) of animals at the end of the 11-week experiment. C: Control group (distilled water at dose of 1000 mg/kg/animal); O: extra virgin olive oil group (1000 mg/kg/animal); L1: Golden linseed oil group (1000 mg/kg/animal); L2: Golden linseed oil group (2000 mg/kg/animal); G1: Grape seed oil group (1000 mg/kg/animal); G2: Grape seed oil group (2000 mg/kg/animal); GL: Blend oil (2000 mg/kg/animal). Mean ± standard deviation from the mean. Different letters indicate significant differences in relation to L1 ($p < 0.05$: a). Kruskal–Wallis/Dunn's tests.

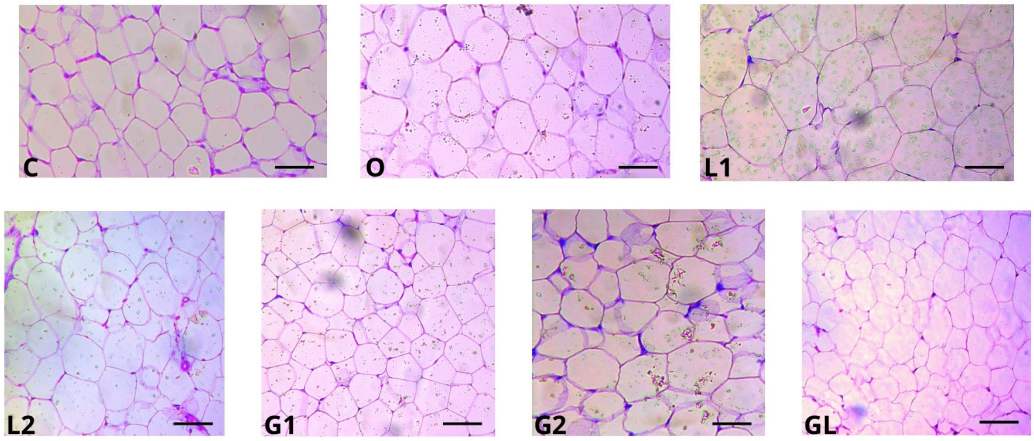

Figure 11. Histopathological analyses of the epididymal adipose tissue of the animals at the end of the 11-week experiment. G: Control group (distilled water at dose of 1000 mg/kg/animal); O: Extra virgin olive oil group (1000 mg/kg/animal); L1: Golden linseed oil group (1000 mg/kg/animal); L2: Golden linseed oil group (2000 mg/kg/animal); G1: Grape seed oil group (1000 mg/kg/animal); G2: Grape seed oil group (2000 mg/kg/animal); GL: Blend oil (2000 mg/kg/animal). Histopathological analyses of the adipose tissue via hematoxylin and eosin (H&E); 20x magnification; bar scale: 100 μm.

In our investigation of epididymal adipose tissue (as illustrated in Figures 10 and 11), we observed that both the G1 and GL groups exhibited smaller adipocyte areas than the L1 group. Adipose tissue, functioning as an energy reservoir, plays a crucial role in maintaining metabolic homeostasis by storing excess energy derived from the diet. However, when there is a positive energy balance, this tissue undergoes reorganization, increasing both adipocyte number and size. Notably, the L1 group, which gained more weight, displayed a larger adipose tissue area. Furthermore, we found that L1 also produced lower levels of TNF levels (tumor necrosis factor) and MCP-1 (monocyte chemoattractant protein-1) compared with the other groups. These findings underscore the intricate relationship between adipose tissue expansion, cytokine production, and weight gain, with larger adipocytes potentially accumulating more fat due to cell hypertrophy.

In our assessment of epididymal adipose tissue (as depicted in Figures 10 and 11), we observed that the G1 and GL groups exhibited smaller adipocyte areas than L1. Adipose tissue serves as an energy reservoir, maintaining homeostasis by storing excess energy from the diet. When there is a positive energy balance, this tissue undergoes reorganization, leading to an increase in both adipocyte number and size. Larger adipocytes can grow fatter due to cell hypertrophy and higher weight gain in the L1 group [89,90].

Di Pietro et al. (2023) [13] emphasized the relevance of grape seed oil as a therapeutic compound with active properties for health, pointing out several in vitro and in vivo studies that show the modulation of the expression of antioxidant enzymes, anti-inflammatory and anti-atherosclerotic effects, and protection against cell oxidative damage. Thus, our results of the excellent therapeutic fraction of 2.000 mg/kg/day of G2 and its combination with L in GL agreed with the results of that report [13].

The response to oxidative stress and the improved antioxidant system provided by the blend oil are observed in the reduced size of adipocytes. Hence, the beneficial effects of the moderate consumption of vegetable oils, as well as their combination, can help reduce obesity given their physical–chemical quality, the presence of bioactive compounds, and their antioxidant and anti-inflammatory properties [21,30].

Grape seeds offer significant health benefits due to their high antioxidant potential. These benefits include protection against oxidative damage, anti-diabetic effects, cholesterol regulation, and anti-platelet properties [13]. Grape seed oil, rich in natural antioxidants, finds application in the food industry, where it extends the shelf life of various food products. This natural alternative is particularly valuable given the potential carcinogenic

and toxic effects associated with synthetic antioxidants, such as tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT). Notably, grape seeds have been used as food additives in Japan [91].

Additionally, grape seed extracts find application in cosmetic formulations for their anti-aging properties. These extracts, abundant in proanthocyanidins, exhibit robust free radical scavenging capabilities. Recent studies have explored topical applications of grape seed extracts [92,93]

Linseeds also offer specific nutritional advantages. Notably, they are rich in omega-3 fatty acids and contain extraordinarily high levels of alpha-linolenic acid (ALA). Additionally, linseeds provide a significant lignan content and mucilage gums, making them a desirable commodity in the food industry [94].

This study aimed to comprehensively evaluate the combination of grape seed oil and linseed oil. Analytical techniques provided insights into the oils' physical behavior, which impacts storage stability and use in supplementation. Gas chromatography–mass spectrometry verified the oil blend composition, confirming the specific fatty acids and bioactive compounds, with carotenoids and color observations complementing these analyses.

By intentionally utilizing an in vivo experimental model with eutrophic feeding, we specifically investigated the physiological effects of the oil mixture. Despite the widespread use of grape seed oil and linseed oil as dietary supplements, robust evidence supporting the physiological benefits of their joint use remains elusive. Our findings indicate that this combination may indeed impact lipid profiles, indicators of inflammation, and antioxidant status.

4. Conclusions

The predominant polyunsaturated fatty acid in the grape seed oil and blend oil is linoleic acid (C18:2), while that in the linseed oil is alfa-linolenic acid (C18:3). In all oils, oleic acid (C18:1) is the dominant monounsaturated fatty acid, and palmitic (C16:0) was the leading saturated fatty acid. The physical–chemical indices of the studied oils are within the recommended ranges, indicating the absence of oxidation. Oxidative stability and thermal analyses (TGA/DTA and DSC) revealed similar behaviors, and optical analyses showed color variations caused by pigments (carotenoids).

The grape seed oil at a dosage of 2000 mg/kg/day (G2) promotes lower food consumption, and the oil blend (GL) causes a lower weight gain per gram of consumed feed and total caloric consumption. Both oils induce high lipoprotein HDL-c levels, while linseed oil (2000 mg/kg/day) (L2) reduce total cholesterol levels. Furthermore, L2, G2, and GL show reduced levels of pro-inflammatory cytokines (MCP-1 and TNF-α), highlighting the anti-inflammatory effects of their supplementation; G2 and GL exhibit the smallest adipocyte areas.

Future studies should be conducted on the application of grape seed, linseed, and blend oils, as their nutraceutical properties have potential uses in the food, pharmaceutical, and cosmetic industries.

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Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee for Animal Use (protocol no. 1339/2022), which is essential in the International Guiding Principles for Biomedical Research Involving Animals (CIOMS), Genebra, 1985; the Universal Declaration of Animal Rights (UNESCO), Bruxelles, Belgium, 1978; and the guidelines of the National Health Institutes on the use and care of laboratory animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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

References

1. Parihar, S.; Sharma, D. A Breif Overview on Vitis Vinifera. *Sch. Acad. J. Pharm.* **2021**, *10*, 231–239. https://doi.org/10.36347/sajp.2021.v10i12.005.
2. Sławińska, N.; Prochoń, K.; Olas, B. A Review on Berry Seeds—A Special Emphasis on Their Chemical Content and Health-Promoting Properties. *Nutrients* **2023**, *15*, 1422. https://doi.org/10.3390/nu15061422.
3. Cecchi, L.; Innocenti, M.; Urciuoli, S.; Arlorio, M.; Paoli, P.; Mulinacci, N. In Depth Study of Phenolic Profile and PTP-1B Inhibitory Power of Cold-Pressed Grape Seed Oils of Different Varieties. *Food Chem.* **2019**, *271*, 380–387. https://doi.org/10.1016/j.foodchem.2018.07.140.
4. Lau, K.Q.; Sabran, M.R.; Shafie, S.R. Utilization of Vegetable and Fruit By-Products as Functional Ingredient and Food. *Front. Nutr.* **2021**, *8*, 661693. https://doi.org/10.3389/fnut.2021.661693.
5. Millan-Linares, M.C.; Bermudez, B.; Martin, M.E.; Muñoz, E.; Abia, R.; Millan, F.; Muriana, F.J.G.; Montserrat-de La Paz, S. Unsaponifiable Fraction Isolated from Grape (*Vitis Vinifera* L.) Seed Oil Attenuates Oxidative and Inflammatory Responses in Human Primary Monocytes. *Food Funct.* **2018**, *9*, 2517–2523. https://doi.org/10.1039/C8FO00063H.
6. Karaman, S.; Karasu, S.; Tornuk, F.; Toker, O.S.; Geçgel, Ü.; Sagdic, O.; Ozcan, N.; Gül, O. Recovery Potential of Cold Press Byproducts Obtained from the Edible Oil Industry: Physicochemical, Bioactive, and Antimicrobial Properties. *J. Agric. Food Chem.* **2015**, *63*, 2305–2313. https://doi.org/10.1021/jf504390t.
7. Deme, T.; Haki, G.D.; Retta, N.; Woldegiorgis, A.; Geleta, M. Fatty Acid Profile, Total Phenolic Content, and Antioxidant Activity of Niger Seed (*Guizotia Abyssinica*) and Linseed (*Linum Usitatissimum*). *Front. Nutr.* **2021**, *8*, 674882. https://doi.org/10.3389/fnut.2021.674882.
8. Campos, J.R.; Severino, P.; Ferreira, C.S.; Zielinska, A.; Santini, A.; Souto, S.B.; Souto, E.B. Linseed Essential Oil—Source of Lipids as Active Ingredients for Pharmaceuticals and Nutraceuticals. *Curr. Med. Chem.* **2019**, *26*, 4537–4558. https://doi.org/10.2174/0929867325666181031105603.
9. Kanikowska, D.; Malińska, A.; Mickiewicz, A.; Zawada, A.; Rutkowski, R.; Pawlaczyk, K.; Sato, M.; Bręborowicz, A.; Witowski, J.; Korybalska, K. Effect of Flaxseed (*Linum Usitatissimum* L.) Supplementation on Vascular Endothelial Cell Morphology and Function in Patients with Dyslipidaemia—A Preliminary Observation. *Nutrients* **2022**, *14*, 2879. https://doi.org/10.3390/nu14142879.
10. Izar, M.C.D.O.; Lottenberg, A.M.; Giraldez, V.Z.R.; Santos, R.D.D.; Machado, R.M.; Bertolami, A.; Assad, M.H.V.; Saraiva, J.F.K.; Faludi, A.A.; Moreira, A.S.B.; et al. Posicionamento Sobre o Consumo de Gorduras e Saúde Cardiovascular—2021. *Arq. Bras. Cardiol.* **2021**, *116*, 160–212. https://doi.org/10.36660/abc.20201340.
11. Lyons, C.; Kennedy, E.; Roche, H. Metabolic Inflammation-Differential Modulation by Dietary Constituents. *Nutrients* **2016**, *8*, 247. https://doi.org/10.3390/nu8050247.
12. Mirabelli, M.; Chiefari, E.; Arcidiacono, B.; Corigliano, D.M.; Brunetti, F.S.; Maggisano, V.; Russo, D.; Foti, D.P.; Brunetti, A. Mediterranean Diet Nutrients to Turn the Tide against Insulin Resistance and Related Diseases. *Nutrients* **2020**, *12*, 1066. https://doi.org/10.3390/nu12041066.
13. Di Pietro Fernandes, C.; Santana, L.F.; Dos Santos, J.R.; Fernandes, D.S.; Hiane, P.A.; Pott, A.; Freitas, K.D.C.; Bogo, D.; Do Nascimento, V.A.; Filiú, W.F.D.O.; et al. Nutraceutical Potential of Grape (*Vitis Vinifera* L.) Seed Oil in Oxidative Stress, Inflammation, Obesity and Metabolic Alterations. *Molecules* **2023**, *28*, 7811. https://doi.org/10.3390/molecules28237811.
14. Martin, M.E.; Grao-Cruces, E.; Millan-Linares, M.C.; Montserrat-de La Paz, S. Grape (*Vitis Vinifera* L.) Seed Oil: A Functional Food from the Winemaking Industry. *Foods* **2020**, *9*, 1360. https://doi.org/10.3390/foods9101360.
15. Nowak, W.; Jeziorek, M. The Role of Flaxseed in Improving Human Health. *Healthcare* **2023**, *11*, 395. https://doi.org/10.3390/healthcare11030395.
16. Falade, A.; Oboh, G.; Okoh, A. Potential Health Implications of the Consumption of Thermally-Oxidized Cooking Oils—A Review. *Pol. J. Food Nutr. Sci.* **2017**, *67*, 95–105. https://doi.org/10.1515/pjfns-2016-0028.

17. David, F.; Sandra, P.; Vickers, A.K. *Column Selection for the Analysis of Fatty Acid Methyl Esters*; Nota de Aplicação Da Agilent Technologies: Palo Alto, CA, USA, 2005.
18. Ulbricht, T.L.V.; Southgate, D.A.T. Coronary Heart Disease: Seven Dietary Factors. *Lancet* **1991**, *338*, 985–992. https://doi.org/10.1016/0140-6736(91)91846-M.
19. Läubli, M.W.; Bruttel, P.A. Determination of the Oxidative Stability of Fats and Oils: Comparison between the Active Oxygen Method (AOCS Cd 12-57) and the Rancimat Method. *J. Am. Oil Chem. Soc.* **1986**, *63*, 792–795. https://doi.org/10.1007/BF02541966.
20. Filoda, P.F.; Chaves, F.C.; Hoffmann, J.F.; Rombaldi, C.V. Olive Oil: A Review on the Identity and Quality of Olive Oils Produced in Brazil. *Rev. Bras. Frutic.* **2021**, *43*, e847. https://doi.org/10.1590/0100-29452021847.
21. Meireles, B.R.L.D.A.; Alcântara, M.A.; Polari, I.D.L.B.; Souza, A.G.D.; Santos, N.A.D.; Grisi, C.V.B.; Cordeiro, A.M.T.D.M. Catole Coconut (*Syagrus Cearensis*) Oil: Physicochemical Characterization and Thermo-Oxidative Stability by TG/DTG/DTA and Rancimat. *J. Therm. Anal. Calorim.* **2022**, *147*, 3591–3598. https://doi.org/10.1007/s10973-021-10789-0.
22. Rodriguez-Amaya, D.B. Changes in Carotenoids during Processing and Storage of Foods. *Arch. Latinoam. Nutr.* **1999**, *49*, 38S–47S.
23. Torres Silva, G.; Di Pietro Fernandes, C.; Hiane, P.A.; Freitas, K.D.C.; Figueiredo, P.S.; Inada, A.C.; Filiú, W.F.; Maldonade, I.R.; Nunes, Â.A.; Oliveira, L.C.S.D.; et al. Caryocar Brasiliense Cambess. Pulp Oil Supplementation Reduces Total Cholesterol, LDL-c, and Non-HDL-c in Animals. *Molecules* **2020**, *25*, 4530. https://doi.org/10.3390/molecules25194530.
24. Marcelino, G.; Hiane, P.; Pott, A.; De Oliveira Filiú, W.; Caires, A.; Michels, F.; Júnior, M.; Santos, N.; Nunes, Â.; Oliveira, L.; et al. Characterization of Buriti (*Mauritia Flexuosa*) Pulp Oil and the Effect of Its Supplementation in an In Vivo Experimental Model. *Nutrients* **2022**, *14*, 2547. https://doi.org/10.3390/nu14122547.
25. Silva, L.P.R.D.; Rodrigues, E.L.; Hiane, P.A.; Nunes, Â.A.; Filiú, W.F.; Cavalheiro, L.F.; Nazário, C.E.D.; Asato, M.A.; Freitas, K.D.C.; Bogo, D.; et al. Bocaiuva (*Acrocomia Aculeata*) Nut Oil: Composition and Metabolic Impact in an Experimental Study. *Food Sci. Technol.* **2023**, *43*, e43522. https://doi.org/10.1590/fst.43522.
26. Nery, C.D.S.; Pinheiro, I.L.; Muniz, G.D.S.; Vasconcelos, D.A.A.D.; França, S.P.D.; Nascimento, E.D. Medidas Murinométricas e Eficiência Alimentar Em Ratos Provenientes de Ninhadas Reduzidas Na Lactação e Submetidos Ou Não Ao Exercício de Natação. *Rev. Bras. Med. Esporte* **2011**, *17*, 49–55. https://doi.org/10.1590/S1517-86922011000100010.
27. Taylor, B.A.; Phillips, S.J. Detection of Obesity QTLs on Mouse Chromosomes 1 and 7 by Selective DNA Pooling. *Genomics* **1996**, *34*, 389–398. https://doi.org/10.1006/geno.1996.0302.
28. Burtis, C.A.; Bruns, D.E. *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*, 7th ed.; Elsevier: Rio de Janeiro, Brazil, 2016.
29. Kleiner, D.E.; Brunt, E.M.; Van Natta, M.; Behling, C.; Contos, M.J.; Cummings, O.W.; Ferrell, L.D.; Liu, Y.-C.; Torbenson, M.S.; Unalp-Arida, A.; et al. Design and Validation of a Histological Scoring System for Nonalcoholic Fatty Liver Disease. *Hepatology* **2005**, *41*, 1313–1321. https://doi.org/10.1002/hep.20701.
30. Jernås, M.; Palming, J.; Sjöholm, K.; Jennische, E.; Svensson, P.-A.; Gabrielsson, B.G.; Levin, M.; Sjögren, A.; Rudemo, M.; Lystig, T.C.; et al. Separation of Human Adipocytes by Size: Hypertrophic Fat Cells Display Distinct Gene Expression. *FASEB J.* **2006**, *20*, 1540–1542. https://doi.org/10.1096/fj.05-5678fje.
31. Al Juhaimi, F.; Geçgel, Ü.; Gülcü, M.; Hamurcu, M.; Özcan, M.M. Bioactive Properties, Fatty Acid Composition and Mineral Contents of Grape Seed and Oils. *S. Afr. J. Enol. Vitic.* **2017**, *38*, 103–108. https://doi.org/10.21548/38-1-1042.
32. Hussein, S.; Abdrabba, S. Physico-Chemical Characteristics, Fatty Acid, Composition of Grape Seed Oil and Phenolic Compounds of Whole Seeds, Seeds and Leaves of Red Grape in Libya. *Int. J. Appl. Sci. Math.* **2015**, *2*, 5, 2394-2894.
33. Yadav, R.K.; Singh, M.; Roy, S.; Ansari, M.N.; Saeedan, A.S.; Kaithwas, G. Modulation of Oxidative Stress Response by Flaxseed Oil: Role of Lipid Peroxidation and Underlying Mechanisms. *Prostaglandins Other Lipid Mediat.* **2018**, *135*, 21–26. https://doi.org/10.1016/j.prostaglandins.2018.02.003.
34. Orsavova, J.; Misurcova, L.; Ambrozova, J.; Vicha, R.; Mlcek, J. Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. *Int. J. Mol. Sci.* **2015**, *16*, 12871–12890. https://doi.org/10.3390/ijms160612871.
35. Oppedisano, F.; Macrì, R.; Gliozzi, M.; Musolino, V.; Carresi, C.; Maiuolo, J.; Bosco, F.; Nucera, S.; Caterina Zito, M.; Guarnieri, L.; et al. The Anti-Inflammatory and Antioxidant Properties of n-3 PUFAs: Their Role in Cardiovascular Protection. *Biomedicines* **2020**, *8*, 306. https://doi.org/10.3390/biomedicines8090306.
36. Sakai, C.; Ishida, M.; Ohba, H.; Yamashita, H.; Uchida, H.; Yoshizumi, M.; Ishida, T. Fish Oil Omega-3 Polyunsaturated Fatty Acids Attenuate Oxidative Stress-Induced DNA Damage in Vascular Endothelial Cells. *PLoS ONE* **2017**, *12*, e0187934. https://doi.org/10.1371/journal.pone.0187934.
37. Mercola, J.; D'Adamo, C.R. Linoleic Acid: A Narrative Review of the Effects of Increased Intake in the Standard American Diet and Associations with Chronic Disease. *Nutrients* **2023**, *15*, 3129. https://doi.org/10.3390/nu15143129.
38. Beyer, M.P.; Videla, L.A.; Farías, C.; Valenzuela, R. Potential Clinical Applications of Pro-Resolving Lipids Mediators from Docosahexaenoic Acid. *Nutrients* **2023**, *15*, 3317. https://doi.org/10.3390/nu15153317.
39. Codex Alimentarius Commission. Edible Fats and Oils Not Covered by Individual Standards. Available online: https://www.fao.org/3/y2774e/y2774e03.htm. (accessed on 19 July 2024).
40. Endo, Y. Analytical Methods to Evaluate the Quality of Edible Fats and Oils: The JOCS Standard Methods for Analysis of Fats, Oils and Related Materials (2013) and Advanced Methods. *J. Oleo Sci.* **2018**, *67*, 1–10. https://doi.org/10.5650/jos.ess17130.

41. Carmona-Jiménez, Y.; Igartuburu, J.M.; Guillén-Sánchez, D.A.; García-Moreno, M.V. Fatty Acid and Tocopherol Composition of Pomace and Seed Oil from Five Grape Varieties Southern Spain. *Molecules* **2022**, *27*, 6980. https://doi.org/10.3390/molecules27206980.
42. Kasote, D.M.; Badhe, Y.S.; Hegde, M.V. Effect of Mechanical Press Oil Extraction Processing on Quality of Linseed Oil. *Ind. Crops Prod.* **2013**, *42*, 10–13. https://doi.org/10.1016/j.indcrop.2012.05.015.
43. Dabetic, N.M.; Todorovic, V.M.; Djuricic, I.D.; Antic Stankovic, J.A.; Basic, Z.N.; Vujovic, D.S.; Sobajic, S.S. Grape Seed Oil Characterization: A Novel Approach for Oil Quality Assessment. *Eur. J. Lipid Sci. Technol.* **2020**, *122*, 1900447. https://doi.org/10.1002/ejlt.201900447.
44. Yang, K.-M.; Hsu, F.-L.; Chen, C.-W.; Hsu, C.-L.; Cheng, M.-C. Quality Characterization and Oxidative Stability of Camellia Seed Oils Produced with Different Roasting Temperatures. *J. Oleo Sci.* **2018**, *67*, 389–396. https://doi.org/10.5650/jos.ess17190.
45. Mueed, A.; Shibli, S.; Korma, S.A.; Madjirebaye, P.; Esatbeyoglu, T.; Deng, Z. Flaxseed Bioactive Compounds: Chemical Composition, Functional Properties, Food Applications and Health Benefits-Related Gut Microbes. *Foods* **2022**, *11*, 3307. https://doi.org/10.3390/foods11203307.
46. Redondo-Cuevas, L.; Castellano, G.; Torrens, F.; Raikos, V. Revealing the Relationship between Vegetable Oil Composition and Oxidative Stability: A Multifactorial Approach. *J. Food Compos. Anal.* **2018**, *66*, 221–229. https://doi.org/10.1016/j.jfca.2017.12.027.
47. Symoniuk, E.; Ratusz, K.; Krygier, K. Comparison of the Oxidative Stability of Linseed (*Linum Usitatissimum* L.) Oil by Pressure Differential Scanning Calorimetry and Rancimat Measurements. *J. Food Sci. Technol.* **2016**, *53*, 3986–3995. https://doi.org/10.1007/s13197-016-2398-2.
48. Saad, B.; Wai, W.T.; Lim, B.P. Comparative Study on Oxidative Decomposition Behavior of Vegetable Oils and Its Correlation with Iodine Value Using Thermogravimetric Analysis. *J. Oleo Sci.* **2008**, *57*, 257–261. https://doi.org/10.5650/jos.57.257.
49. Poljšak, N.; Kočevar Glavač, N. Vegetable Butters and Oils as Therapeutically and Cosmetically Active Ingredients for Dermal Use: A Review of Clinical Studies. *Front. Pharmacol.* **2022**, *13*, 868461. https://doi.org/10.3389/fphar.2022.868461.
50. Zhang, S.; Dong, Q.; Zhang, L.; Xiong, Y. Effects of Water Washing and Torrefaction on the Pyrolysis Behavior and Kinetics of Rice Husk through TGA and Py-GC/MS. *Bioresour. Technol.* **2016**, *199*, 352–361. https://doi.org/10.1016/j.biortech.2015.08.110.
51. D'Eusanio, V.; Malferrari, D.; Marchetti, A.; Roncaglia, F.; Tassi, L. Waste By-Product of Grape Seed Oil Production: Chemical Characterization for Use as a Food and Feed Supplement. *Life* **2023**, *13*, 326. https://doi.org/10.3390/life13020326.
52. Miyagawa, Y.; Adachi, S. Analysis of Nonisothermal Crystallization of Rapeseed Oil by Deconvolution of Differential Scanning Calorimetry Curve. *J. Oleo Sci.* **2019**, *68*, 1215–1222. https://doi.org/10.5650/jos.ess19252.
53. Jiménez Márquez, A.; Beltrán Maza, G. Application of Differential Scanning Calorimetry (DSC) at the Characterization of the Virgin Olive Oil. *Grasas Aceites* **2003**, *54*, 403–409. https://doi.org/10.3989/gya.2003.v54.i4.228.
54. Tangolar, S.G.; Özoğul, Y.; Tangolar, S.; Torun, A. Evaluation of Fatty Acid Profiles and Mineral Content of Grape Seed Oil of Some Grape Genotypes. *Int. J. Food Sci. Nutr.* **2009**, *60*, 32–39. https://doi.org/10.1080/09637480701581551.
55. Zhang, Z.; Wei, Y.; Guan, N.; Li, B.; Wang, Y. Changes in Chemical Composition of Flaxseed Oil during Thermal-Induced Oxidation and Resultant Effect on DSC Thermal Properties. *Molecules* **2022**, *27*, 7135. https://doi.org/10.3390/molecules27207135.
56. Morya, C.P.; Punia, M. Impact of Urbanization Processes on Availability of Ecosystem Services in National Capital Region of Delhi (1992–2010). *Environ. Dev. Sustain.* **2022**, *24*, 7324–7348. https://doi.org/10.1007/s10668-021-01748-8.
57. Campos-Lozada, G.; Pérez-Marroquín, X.A.; Callejas-Quijada, G.; Campos-Montiel, R.G.; Morales-Peñaloza, A.; León-López, A.; Aguirre-Álvarez, G. The Effect of High-Intensity Ultrasound and Natural Oils on the Extraction and Antioxidant Activity of Lycopene from Tomato (*Solanum Lycopersicum*) Waste. *Antioxidants* **2022**, *11*, 1404. https://doi.org/10.3390/antiox11071404.
58. Chambre, D.R.; Tociu, M.; Stanescu, M.D.; Popescu, C. Influence of Composition on the Thermal Behavior of Oils Extracted from the Seeds of Some Romanian Grapes. *J. Sci. Food Agric.* **2019**, *99*, 6324–6332. https://doi.org/10.1002/jsfa.9909.
59. Kuznetsova, V.; Fuciman, M.; Polívka, T. Relaxation Dynamics of High-Energy Excited States of Carotenoids Studied by UV Excitation and Pump–Repump–Probe Transient Absorption Spectroscopy. *Phys. Chem. Chem. Phys.* **2023**, *25*, 22336–22344. https://doi.org/10.1039/D3CP02485G.
60. Baltazar, P.; Hernández-Sánchez, N.; Diezma, B.; Lleó, L. Development of Rapid Extra Virgin Olive Oil Quality Assessment Procedures Based on Spectroscopic Techniques. *Agronomy* **2019**, *10*, 41. https://doi.org/10.3390/agronomy10010041.
61. Domenici, V.; Ancora, D.; Cifelli, M.; Serani, A.; Veracini, C.A.; Zandomeneghi, M. Extraction of Pigment Information from Near-UV Vis Absorption Spectra of Extra Virgin Olive Oils. *J. Agric. Food Chem.* **2014**, *62*, 9317–9325. https://doi.org/10.1021/jf503818k.
62. Bielski, B.H.J.; Arudi, R.L. Preparation and Stabilization of Aqueous/Ethanolic Superoxide Solutions. *Anal. Biochem.* **1983**, *133*, 170–178. https://doi.org/10.1016/0003-2697(83)90239-7.
63. Ibiapina, A.; Gualberto, L.D.S.; Dias, B.B.; Freitas, B.C.B.; Martins, G.A.D.S.; Melo Filho, A.A. Essential and Fixed Oils from Amazonian Fruits: Proprieties and Applications. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 8842–8854. https://doi.org/10.1080/10408398.2021.1935702.
64. Silva, A.S.L.D.; Silva, A.D.J.; Latif, A.L.O.; Santos Júnior, A.D.F.; Benevides, C.M.D.J. Uso de Metodologias Analíticas Para Determinação de Compostos Fenólicos Em Alimentos No Brasil: Avanços e Fragilidades. *Res. Soc. Dev.* **2022**, *11*, e1311225193. https://doi.org/10.33448/rsd-v11i2.25193.
65. Bongiorno, D.; Di Stefano, V.; Indelicato, S.; Avellone, G.; Ceraulo, L. Bio-phenols Determination in Olive Oils: Recent Mass Spectrometry Approaches. *Mass Spectrom. Rev.* **2023**, *42*, 1462–1502. https://doi.org/10.1002/mas.21744.

66. Speranza, P.; de Oliveira Falcao, A.; Macedo, J.A.; Da Silva, L.H.; Rodrigues, A.M.; Macedo, G.A. Amazonian Buriti Oil: Chemical Characterization and Antioxidant Potential. *Grasas Aceites* **2016**, *67*, e135. https://doi.org/10.3989/gya.0622152.
67. Mezzomo, N.; Ferreira, S.R.S. Carotenoids Functionality, Sources, and Processing by Supercritical Technology: A Review. *J. Chem.* **2016**, *1*, 3164312. https://doi.org/10.1155/2016/3164312.
68. Vitale, S.; Colanero, S.; Placidi, M.; Di Emidio, G.; Tatone, C.; Amicarelli, F.; D'Alessandro, A.M. Phytochemistry and Biological Activity of Medicinal Plants in Wound Healing: An Overview of Current Research. *Molecules* **2022**, *27*, 3566. https://doi.org/10.3390/molecules27113566.
69. Lu, Y.; Zhang, Y.; Xu, D.; Wang, Y.; Pan, D.; Wang, P.; Xia, J.; Yin, S.; Liao, W.; Wang, S.; et al. Tocotrienol-Rich Fractions Offer Potential to Suppress Pulmonary Fibrosis Progression. *Int. J. Mol. Sci.* **2022**, *23*, 14331. https://doi.org/10.3390/ijms232214331.
70. Shinagawa, F.B.; Santana, F.C.D.; Araujo, E.; Purgatto, E.; Mancini-Filho, J. Chemical Composition of Cold Pressed Brazilian Grape Seed Oil. *Food Sci. Technol.* **2017**, *38*, 164–171. https://doi.org/10.1590/1678-457x.08317.
71. Marcelino, G.; Donadon, J.R.; Caires, A.R.; Michels, F.S.; Oliveira, L.C.; Cortes, M.R.; Maldonade, I.R.; Cavalheiro, L.F.; Nazário, C.E.; Maróstica Júnior, M.R.; et al. Characterization and Oxidative Stability of Oils and Bioactive Compounds of the Fruits of *Byrsonima Cydoniifolia* A. Juss. at Different Ripening Stages. *J. Sci. Food Agric.* **2019**, *99*, 2855–2864. https://doi.org/10.1002/jsfa.9498.
72. Millman, J.F.; Okamoto, S.; Teruya, T.; Uema, T.; Ikematsu, S.; Shimabukuro, M.; Masuzaki, H. Extra-Virgin Olive Oil and the Gut-Brain Axis: Influence on Gut Microbiota, Mucosal Immunity, and Cardiometabolic and Cognitive Health. *Nutr. Rev.* **2021**, *79*, 1362–1374. https://doi.org/10.1093/nutrit/nuaa148.
73. Gitea, M.A.; Bungau, S.G.; Gitea, D.; Pasca, B.M.; Purza, A.L.; Radu, A.-F. Evaluation of the Phytochemistry–Therapeutic Activity Relationship for Grape Seeds Oil. *Life* **2023**, *13*, 178. https://doi.org/10.3390/life13010178.
74. Joshi, A.; Hegde, M.; Zanwar, A. Flaxseed Oil and Palm Olein Blend to Improve Omega-6: Omega-3 Ratio. *J. Food Sci. Technol.* **2022**, *59*, 498–509. https://doi.org/10.1007/s13197-021-05033-4.
75. De Carvalho, C.; Caramujo, M. The Various Roles of Fatty Acids. *Molecules* **2018**, *23*, 2583. https://doi.org/10.3390/molecules23102583.
76. Santana, L.F.; Do Espirito Santo, B.L.S.; Tatara, M.B.; Negrão, F.J.; Croda, J.; Alves, F.M.; De Oliveira Filiú, W.F.; Cavalheiro, L.F.; Nazário, C.E.D.; Asato, M.A.; et al. Effects of the Seed Oil of Carica Papaya Linn on Food Consumption, Adiposity, Metabolic and Inflammatory Profile of Mice Using Hyperlipidic Diet. *Molecules* **2022**, *27*, 6705. https://doi.org/10.3390/molecules27196705.
77. Wu, Z.; Pu, H.; Sun, D.-W. Fingerprinting and Tagging Detection of Mycotoxins in Agri-Food Products by Surface-Enhanced Raman Spectroscopy: Principles and Recent Applications. *Trends Food Sci. Technol.* **2021**, *110*, 393–404. https://doi.org/10.1016/j.tifs.2021.02.013.
78. Isaakidis, A.; Maghariki, J.E.; Carvalho-Barros, S.; Gomes, A.M.; Correia, M. Is There More to Olive Oil than Healthy Lipids? *Nutrients* **2023**, *15*, 3625. https://doi.org/10.3390/nu15163625.
79. Popeijus, H.E.; Zwaan, W.; Tayyeb, J.Z.; Plat, J. Potential Contribution of Short Chain Fatty Acids to Hepatic Apolipoprotein A-I Production. *Int. J. Mol. Sci.* **2021**, *22*, 5986. https://doi.org/10.3390/ijms22115986.
80. Kontush, A. HDL and Reverse Remnant-Cholesterol Transport (RRT): Relevance to Cardiovascular Disease. *Trends Mol. Med.* **2020**, *26*, 1086–1100. https://doi.org/10.1016/j.molmed.2020.07.005.
81. Ferrucci, L.; Cherubini, A.; Bandinelli, S.; Bartali, B.; Corsi, A.; Lauretani, F.; Martin, A.; Andres-Lacueva, C.; Senin, U.; Guralnik, J.M. Relationship of Plasma Polyunsaturated Fatty Acids to Circulating Inflammatory Markers. *J. Clin. Endocrinol. Metab.* **2006**, *91*, 439–446. https://doi.org/10.1210/jc.2005-1303.
82. Katsiki, N.; Pérez-Martínez, P.; Lopez-Miranda, J. Olive Oil Intake and Cardiovascular Disease Prevention: "Seek and You Shall Find". *Curr. Cardiol. Rep.* **2021**, *23*, 64. https://doi.org/10.1007/s11886-021-01496-1.
83. Zhu, L.; Sha, L.; Li, K.; Wang, Z.; Wang, T.; Li, Y.; Liu, P.; Dong, X.; Dong, Y.; Zhang, X.; et al. Dietary Flaxseed Oil Rich in Omega-3 Suppresses Severity of Type 2 Diabetes Mellitus via Anti-Inflammation and Modulating Gut Microbiota in Rats. *Lipids Health Dis.* **2020**, *19*, 20. https://doi.org/10.1186/s12944-019-1167-4.
84. Yoneyama, S.; Miura, K.; Sasaki, S.; Yoshita, K.; Morikawa, Y.; Ishizaki, M.; Kido, T.; Naruse, Y.; Nakagawa, H. Dietary Intake of Fatty Acids and Serum C-Reactive Protein in Japanese. *J. Epidemiol.* **2007**, *17*, 86–92. https://doi.org/10.2188/jea.17.86.
85. Tiniakos, D.G. Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis: Histological Diagnostic Criteria and Scoring Systems. *Eur. J. Gastroenterol. Hepatol.* **2009**, *22*, 6, 643-50. https://doi.org/10.1097/MEG.0b013e32832ca0cb.
86. Papatheodoridi, M.; Cholongitas, E. Diagnosis of Non-Alcoholic Fatty Liver Disease (NAFLD): Current Concepts. *Curr. Pharm. Des.* **2019**, *24*, 4574–4586. https://doi.org/10.2174/1381612825666190117102111.
87. Younossi, Z.M.; Gramlich, T.; Liu, Y.C.; Matteoni, C.; Petrelli, M.; Goldblum, J.; Rybicki, L.; McCullough, A.J. Nonalcoholic Fatty Liver Disease: Assessment of Variability in Pathologic Interpretations. *Mod. Pathol. Off. J. U. S. Can. Acad. Pathol. Inc* **1998**, *11*, 560–565.
88. Miller, E.F. Nutrition Management Strategies for Nonalcoholic Fatty Liver Disease: Treatment and Prevention. *Clin. Liver Dis.* **2020**, *15*, 144–148. https://doi.org/10.1002/cld.918.
89. Santillana, N.; Astudillo-Guerrero, C.; D'Espessailles, A.; Cruz, G. White Adipose Tissue Dysfunction: Pathophysiology and Emergent Measurements. *Nutrients* **2023**, *15*, 1722. https://doi.org/10.3390/nu15071722.
90. Pellegrinelli, V.; Carobbio, S.; Vidal-Puig, A. Adipose Tissue Plasticity: How Fat Depots Respond Differently to Pathophysiological Cues. *Diabetologia* **2016**, *59*, 1075–1088. https://doi.org/10.1007/s00125-016-3933-4.

91. Sochorova, L.; Prusova, B.; Cebova, M.; Jurikova, T.; Mlcek, J.; Adamkova, A.; Nedomova, S.; Baron, M.; Sochor, J. Health Effects of Grape Seed and Skin Extracts and Their Influence on Biochemical Markers. *Molecules* **2020**, *25*, 5311. https://doi.org/10.3390/molecules25225311.
92. Huber, K.L.; Fernández, J.R.; Webb, C.; Rouzard, K.; Healy, J.; Tamura, M.; Stock, J.B.; Stock, M.; Pérez, E. AGSE: A Novel Grape Seed Extract Enriched for PP2A Activating Flavonoids That Combats Oxidative Stress and Promotes Skin Health. *Molecules* **2021**, *26*, 6351. https://doi.org/10.3390/molecules26216351.
93. Michailidis, D.; Angelis, A.; Nikolaou, P.E.; Mitakou, S.; Skaltsounis, A.L. Exploitation of Vitis Vinifera, Foeniculum Vulgare, Cannabis Sativa and Punica Granatum By-Product Seeds as Dermo-Cosmetic Agents. *Molecules* **2021**, *26*, 731. https://doi.org/10.3390/molecules26030731.
94. Rafique, M.; Hussain Shah, S.N.; Hussain, I.; Javed, I.; Nisar, N.; Riaz, R. Development of Grape Seed Extract Based Formulations by Using Non-Invasive Biophysical Technique and Its Impact on Skin Aging. *Pak. J. Pharm. Sci.* **2021**, *34*, 1621–1628.