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CARACTERIZAÇÃO FÍSICO-QUÍMICA, ANTIOXIDANTE E ANTIPROLIFERATIVA DE EXTRATOS DE VARIEDADES DE UVAS HÍBRIDAS EM LINHAGEM CELULAR HUMANA DE ADENOCARCINOMA DE PRÓSTATA

PHYSICOCHEMICAL, ANTIOXIDANT AND ANTIPROLIFERATIVE CHARACTERIZATION OF EXTRACTS OF HYBRID GRAPE VARIETIES IN HUMAN CELL LINE OF PROSTATE ADENOCARCINOMA

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Marta Angela de Almeida Sousa Cruz

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição, da Universidade Federal do Estado do Rio de Janeiro, como requisito para obtenção do título de Doutor em Ciência dos Alimentos e Nutrição.
Orientador: Dr. Anderson Junger Teodoro

RIO DE JANEIRO
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Dedico este trabalho a Deus, por me dar sustento e equilíbrio, direcionando os meus passos; e aos meus pais, marido e filhos, que nunca mediram esforços na realização dos meus sonhos, orgulhando-se a cada conquista alcançada na minha ânsia de evolução e melhoria.

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“Tudo tem a sua ocasião própria, e há tempo para todo propósito debaixo do céu. Há tempo de nascer, e tempo de morrer; tempo de plantar, e tempo de arrancar o que se plantou.”

Eclesiastes 3:1-2

RESUMO

Estudos têm buscado comprovar a associação da uva e de seus compostos à prevenção de câncer, no entanto, pouco se sabe sobre as variedades híbridas de uvas. Sendo assim, esse estudo teve como objetivo avaliar a capacidade antioxidante, composição volátil e fenólica do conteúdo de variedades híbridas *Vitis vinifera L.* de *Sweet Sapphire* e S*weet Surprise* e investigar a influência desses extratos de nas linhagens celulares de adenocarcinoma de próstata humano PC-3 e DU-145. Através da cromatografia líquida de alta eficiência/espectrometria de massas com ionização no ultravioleta e electronspray-tandem (HPLC-UV-/ESI-MS-MS), 86 compostos fenólicos foram detectados. Os potenciais antioxidantes foram avaliados por meio dos ensaios DPPH, ABTS, FRAP, ORAC e Folin-Ciocalteu. Os Compostos Orgânicos Voláteis (COV) foram isolados e analisados por GC/ME e GC/FID, que mostraram diferenças significativas entre as duas variedades. O extrato de acetona SA apresentou o maior conteúdo fenólico total (200,75 mg GAE/100 g) e potencial antioxidante por DPPH (1.393,19 μmol TEAC/g), FRAP (208,81 μmol Fe2SO4.g-1) e ORAC (341,01 μmolar de trolox eq./g). Na análise ABTS, o extrato aquoso de SA revelou o maior valor médio (549,37 μmol TEAC/g). As amostras de SA apresentaram valores elevados de antocianinas (23,04mg/100g) em relação às amostras de SU (9,43mg/100g). Malvidina-3-O-glicosídeo (14,46mg/100g) e peonidina-3-O-glicosídeo (3,77mg/100g) foram encontrados como compostos majoritários em SA. A fração volátil de SU foi mais rica que a de SA. O ensaio de MTT avaliou o efeito citotóxico de diferentes concentrações dos extratos, sobre as linhagens do adenocarcinoma de próstata em 24, 48 e 72 horas, demonstrando redução na viabilidade celular das células DU-145 (45 e 65%) e PC-3 (63 e 67%) após 48h de tratamento com SA e SU, respectivamente. Para analisar o impacto inibidor por citometria de fluxo, utilizou-se 24 e 48 horas, onde se obteve impacto inibitório no desenvolvimento devido à parada G2/M e aumentou o número de células apoptóticas em relação ao grupo controle. As antocianinas foram submetidas à aplicação *in silico*, com a concentração de cada antocianina estimada com base na dose máxima recomendada e na taxa de absorção intestinal. *In silico* nenhum dos compostos foi predito como hepatotóxico. Os resultados deste estudo destacam o potencial das variedades híbridas de *Vitis vinifera L*. estudadas, como uma importante fonte de antioxidantes naturais e seu efeito protetor contra as células do câncer de próstata, indicando-os como fontes potenciais para o desenvolvimento de nutracêuticos e alimentos funcionais.

Palavras-chave: *Vitis vinífera L.*; Antioxidantes; Compostos fenólicos; Antocianinas; Adenocarcinoma de próstata.

ABSTRACT

Studies have sought to prove the association of grapes and their compounds with cancer prevention; however, little is known about hybrid grape varieties. Thus, this study aimed to evaluate the antioxidant capacity, volatile and phenolic composition of the content of hybrid varieties Vitis vinifera L. de Sweet Sapphire and Sweet Surprise and to investigate the influence of these extracts on the cell lines of human prostate adenocarcinoma PC-3 and DU-145. Through high-performance liquid chromatography/ultraviolet ionization and electronspray-tandem mass spectrometry (HPLC-UV-/ESI-MS-MS), 86 phenolic compounds were detected. The antioxidant potentials were evaluated using the DPPH, ABTS, FRAP, ORAC and Folin-Ciocalteu assays. Volatile Organic Compounds (VOCs) were isolated and analyzed by GC/ME and GC/FID, which showed significant differences between the two varieties. The SA acetone extract showed the highest total phenolic content (200.75 mg GAE/100 g) and antioxidant potential by DPPH (1,393.19 μmol TEAC/g), FRAP (208.81 μmol Fe2SO4.g-1) and ORAC (341.01 μmolar trolox eq./g). The ABTS analysis, the aqueous extract of SA revealed the highest mean value (549.37 μmol TEAC/g). The SA samples showed high values of anthocyanins (23.04mg/100g) in relation to the SU samples (9.43mg/100g). Malvidine-3-O-glucoside (14.46mg/100g) and Peonidine-3-O-glucoside (3.77mg/100g) were found to be major compounds in SA. The volatile fraction of SU was richer than that of SA. The MTT assay evaluated the cytotoxic effect of different concentrations of extracts on prostate adenocarcinoma cell lines at 24, 48 and 72 hours, demonstrating a reduction in cell viability of DU-145 (45 and 65%) and PC-3 (63 and 67%) cells after 48 hours of treatment with SA and SU, respectively. To analyze the inhibitory impact by flow cytometry, 24 and 48 hours were used, where an inhibitory impact on development was obtained due to G2/M arrest and the number of apoptotic cells increased in relation to the control group. The anthocyanins were submitted to in silico application, with the concentration of each anthocyanin estimated based on the maximum recommended dose and the intestinal absorption rate. In silico none of the compounds were predicted to be hepatotoxic. The results of this study highlight the potential of the hybrid varieties of *Vitis vinifera L.* studied, as an important source of natural antioxidants and their protective effect against prostate cancer cells, indicating them as potential sources for the development of nutraceuticals and functional foods.

Keywords: *Vitis vinifera L.*; Antioxidants; Phenolic compounds; Anthocyanins; Prostatic adenocarcinoma.

SUMÁRIO

O presente trabalho segue as normas da tese no formato de artigo definido pelo Programa de Pós-Graduação em Alimentos e Nutrição em 14 de maio de 2019.

Assim esta tese, está dividida em 4 capítulos:

I. Artigo de revisão bibliográfica de frutas híbridas: **"Hybrid fruits as an alternative for improving health - a comprehensive review."**

II. Artigo de revisão bibliográfica de uvas híbridas: **"Recent Progress in grapes applications on human health: an overview with current knowledge."**

III. Artigo original que contempla resultados e discussão dos experimentos Bioquímicos do extrato das uvas híbridas estudadas: **"Evaluation of the antioxidant capacity, volatile composition and phenolic content of hybrid *Vitis vinifera L.* varieties *Sweet Sapphire* and *Sweet Surprise*."**

IV. Artigo original que contempla resultados e discussão dos experimentos dos extratos estudados em células de câncer de próstata: **"Antiproliferative activity and in vitro antioxidant effect of the hybrid varieties of *Vitis vinifera L.* Sweet *Sapphire* and *Sweet Surprise* on human prostate cancer cells."**

INTRODUÇÃO

Pesquisas realizadas pelo INCA (2022), apontam o impacto do câncer no mundo, em 2020, quando ocorreram 19,3 milhões de casos novos de câncer (18,1 milhões, excluindo os casos de câncer de pele não melanoma). Significando dizer que um em cada cinco indivíduos terão câncer durante sua vida (de Martel et al., 2020). Os dez principais tipos de câncer representam mais de 60% do total de casos novos. O câncer de próstata representa 7,3%, com 1,9 milhão de casos. É o segundo tipo de câncer mais comum entre os homens, com uma estimativa em valores absolutos de 704 mil novos casos de câncer no Brasil para cada ano do triênio 2023-2025, com destaque para as regiões Sul e Sudeste, que concentram cerca de 70% da incidência. O aumento dessa incidência no Brasil e no mundo também está relacionado ao envelhecimento populacional e como em outros países, à disseminação do Antígeno Prostático Específico (PSA) na década de 1990 no diagnóstico dessa neoplasia (Rotili, et al. 2022).

A maioria dos tratamentos existentes para o câncer de próstata podem ser citotóxicos ou causar efeitos secundários debilitantes, atuando nas células cancerosas e nas saudáveis ao mesmo tempo. Por esse motivo, busca-se cada vez mais novos tratamentos, mais seletivos ao câncer que comprometam menos as células saudáveis e apresentem eficácia no tratamento (Kucera et al., 2020).

Existem várias espécies e variedades de videiras no mundo, onde a colheita de seus frutos é valiosa, possuindo cultivos para diversas opções, tanto para consumo in natura, quanto para produtos processados, tais como vinho, geleia, gelatina, sucos, uva em passas, vinagre e óleo de semente de uva (Izadfar et al., 2023).

Por causa dos benefícios sobre a saúde humana, assim como a sua importância econômica, a uva é uma fruta amplamente cultivada e consumida em todo o mundo (Qin et al., 2022).

Historicamente, a produção e exportação de uvas eram controladas quase exclusivamente por países europeus tradicionais. No entanto, nos últimos anos, a América do Sul mostrou taxa significativa de crescimento na produção e exportação de uvas com duas safras por ano (Sousa et al., 2013; Qin et al., 2022; Izadfar et al., 2023). Embora no Brasil, a prática da viticultura seja recente quando comparada aos países europeus tradicionais, há uma melhora na qualidade da composição das cultivares de uva brasileiras devido ao uso de técnicas de hibridização (Kowalczyk et al., 2022).

As uvas híbridas são obtidas a partir do cruzamento de duas ou mais espécies de *Vitis*

que permitem a seleção de características de interesse, como alta resistência a doenças e patógenos. Além disso, os híbridos interespecíficos são caracterizados por diferentes composições químicas e são especialmente conhecidos por apresentarem alto conteúdo de compostos fenólicos e perfil específico de antocianinas, destacando-se também o seu potencial para produzir vinhos tintos de qualidade (Samoticha et al., 2016; Nicolini et al., 2020). No entanto, as variedades híbridas atualmente são pouco estudadas.

A hibridização das espécies *V. vinífera,* que, quando comparadas a outras espécies, já são fontes mais ricas de compostos fenólicos pertencentes a grupos como antocianinas, flavonóis, taninos, ácidos fenólicos e estilbenóides, resulta em novos metabólitos secundários e aumenta a atividade das vias de biossíntese existentes. Estudos realizados demonstram que os frutos de híbridos interespecíficos apresentam, em relação ao conteúdo total de compostos fenólicos e sua distribuição em órgãos vegetais individuais, traços aprimorados herdados de ambas as espécies parentais (Oledzki, 2022).

No Brasil, o cultivo de uvas híbridas, geneticamente adaptado para cultivado em condições climáticas diferentes das cultivares tradicionais, tem sido intensificado e novas variedades são sendo desenvolvido para a elaboração de sucos e vinhos (da Silva *et al*., 2019).

Observam-se variedades de videiras híbridas cultivadas por agricultores e produtores em todo o mundo. Assim como o grande interesse por parte dos consumidores aos efeitos dos alimentos consumidos. Estudos recentes revelam o potencial das frutas híbridas (de muitas espécies e variedades) em sintetizar compostos bioativos secundários, sendo consideradas como matéria-prima com efeitos terapêuticos e preventivos contra doenças civilizatórias, como doenças cardiovasculares (DCV) e câncer (Rätsep at al., 2020).

Os vinhos e sucos elaborados a partir de uvas híbridas apresentam sabores característicos e são muito apreciados por alguns consumidores, superando as características sensoriais de uvas europeias (Gomez et al., 2020).

O consumo de bebidas a partir de uvas híbridas têm sido correlacionado com o aumento da longevidade em algumas regiões brasileiras (Gomez et al., 2020). No entanto, há poucos relatórios dos sucos e vinhos elaborados a partir de uvas híbridas que demonstram a qualidade funcional (da Silva et al., 2019).

Os compostos fenólicos têm sido extensivamente estudados devido às suas propriedades antioxidantes, anti-inflamatórias e anticancerígenas potencialmente benéficas, que estimulam o interesse da indústria e dos consumidores por alimentos ricos em flavonoides. Uma ampla gama de atividades biológicas tem sido atribuída a esses compostos, indicando que algumas fontes de frutas podem fornecer mais do que nutrição. Alimentos naturais de alta qualidade nutricional

desempenham um papel importante na manutenção da saúde humana. Como resultado disso, muita atenção tem sido focada no uso de antioxidantes exógenos, especialmente antioxidantes naturais por inibir a oxidação de componentes celulares, protegendo assim dos danos causados pelos radicais livres (Aires et al., 2021; Bassanesi et al., 2020; Gonçalves et al., 2022).

Tem crescido o interesse na determinação de fontes alimentares adequadas de compostos fenólicos antioxidantes, porém existe pouco conhecimento sobre os compostos fenólicos de cultivares de uvas híbridas relatados na literatura: poucos relatos descrevem apenas as antocianinas para espécies híbridas (Zhu et al., 2021).

A química das cultivares de uva, especialmente o aroma varietal, tem um impacto significativo sobre o caráter das uvas, sua percepção sensorial e sua qualidade, impactando na aceitação do consumidor. O aroma varietal pode relacionar-se com um composto específico ou com um pequeno grupo de moléculas odoríferas, mas é normalmente atribuível à contribuição de vários compostos voláteis presentes nas uvas, em proporções que diferem de uma variedade para outra. Esses aromas incluem centenas de compostos orgânicos voláteis (COVs), compostos de diferentes grupos químicos, incluindo álcoois, ésteres, aldeídos, cetonas, monoterpenóides e outros. Os métodos para a extração de COVs incluem frequentemente extração líquido-líquido, destilação e extração simultâneas, técnicas de extração esportiva em barra de agitação (Del Rio et al., 2013; Nicolini et al., 2020; Wei et al., 2019).

Até o momento, pouco se conhece sobre a composição das várias uvas híbridas existentes, pois ainda há uma escassez de estudos fornecendo conhecimento das características bioquímicas de todas as espécies híbridas. Dessa forma, o presente trabalho tem como objetivo estudar os extratos das uvas híbridas *Sweet Sapphira* e *Sweet Surprise*, já comercializadas no nordeste brasileiro, em seu potencial bioquímico em sua atividade biológica como tratamento preventivo e coadjuvante do câncer de próstata.

CAPÍTULO I

Frutas híbridas como alternativa para melhorar a saúde. Uma revisão abrangente.

Hybrid fruits as an alternative for improving health - a comprehensive review.

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Hybrid fruits as an alternative for improving health – a comprehensive review

Several species of hybrid fruits, such as citrus, grapes, blueberries, apples, tomatoes, and lingonberries among others have attracted scientific attention in recent years, especially due to their reported antioxidant and anti-inflammatory properties. Bagasse, leaves, bark, and seeds, of these hybrid fruits, have large amounts of polyphenols, such as flavonoids, which act as potent antioxidants.

Several studies are carried out in cellular models of neurotoxicity of the extract of these fruits, to document the beneficial effects for human health, as well as to prove its action in the antiproliferative effect in cancer cells. In the present review, we demonstrate hybrid fruits as a source of antioxidant compounds, and bioactive compounds and the role of these substances in the inhibition of diseases such as cancer, diabetes, inflammatory and neurodegenerative diseases.

Keywords: Hybrid fruits, compounds, bioactive, antioxidants, health

1. Introduction

Brazil is the world's third-largest producer of fruits, behind China and India. with about 45 million tons per year. Most of this production is aimed at the domestic consumer market - only 2.5% is exported [1, 2].

World commercial fruit production in general, according to FAO data, in 2017 was about 865 million tons in an area of approximately 65 million hectares. China stands out with about 30% of all world fruit production and 24% of the area cultivated commercially in the world [1].

From north to south Brazil has more than 2.5 million hectares cultivated. The production estimate reaches 33 million reais in gross value and the sector holds about 16% of the Brazilian agribusiness workforce, with more than millions of jobs generated. However, regarding exports, the country occupies the 23rd position, as it exported only 3% of what was produced in 2022 [1, 3].

Fruit growing participates in various ways for the growth of the Brazilian economy, either as a source of food, bringing benefits to the population, or generating many jobs direct and indirect. Even with little performance the international market, Brazil had an increase in the generation of foreign exchange, in recent years, already with exports, both with fresh fruits and in concentrated juices, indicating an increase in this international performance [1, 2].

Brazil has the greatest biodiversity on the planet, with more than 15% of the total number of species, being about the variety of biota, with more than 30,000 different species of angiosperms (plants with flowers and fruits) scattered throughout the country[4].

Fruits are indispensable foods for the proper body function due to their nutritional composition whereby vitamins, carbohydrates, proteins, fibers, minerals and water[5].

The scientific evidence is based on the performance of its biochemical components in the prevention of cardiovascular diseases and various types of cancer, demonstrated in scientific literature.

The increased consumption of diets with low nutritional value and little variety, contributes to the appearance of growing problems of nutritional deficiencies and chronic diseases as a cause of malnutrition and unbalanced diet, demonstrating the need for advances in knowledge about the composition and beneficial properties for the health of neglected and underutilized Brazilian native species that are neglected and underutilized [4, 6].

Due to the recognition of the nutritional value of tropical fruits, which has aroused interest in the scientific community, their consumption has increased so much in local and international markets. To better understand the nutritional value of fruits, the quantification of their bioactive compounds is sought, as information that adds value to the products. Bioactive compounds have received significant attention, for their protective effect on the human body against oxidative stress and prevention of some noncommunicable-degenerative diseases [6].

Different clinical and epidemiological studies have shown results associated with the consumption of fruits and vegetables, the multiple health benefits and the decreased risk of cardiovascular diseases, diabetes, macular degeneration, age-related cataracts, and some cancers [6, 7]. The benefits happen due to nutrients such as dietary fiber and bioactive compounds, vitamins A, B, C, and E, polyphenols such as flavonoids, tocotrienols, alkaloids, saponins, terpenoids, phytosterols, organosulfur compounds, lactones sesquiterpenes, carotenoids, thiocyanate, and selenium [6, 8, 9].

The human consumption of wild species of fruits is an ancient habit. Industrialization, interbreeding, and natural changes have played a key role in the evolution and vegetative propagation, since they allowed morpho-functional variations in leaves, flowers, and berries, and have been improved in quality and increasing the number of existing cultivars by phenotypic plasticity [10].

From the point of view of fruit growers and producers, these new fruits must have some specific functional properties, such as higher yields, greater resistance to climatic factors, or lack of seeds. And, to attract consumers, better aroma and palatability [11].

Due to their high bioavailability that facilitates the absorption of other conjugated compounds (medicines, xenobiotics, fatty acids), natural antioxidants are of great interest in the cosmetic, pharmaceutical and food industries, which encourage research so that the production levels and consumption of these foods rich in natural antioxidants increase and are better accessible to the population [6].

The improvement of perennial fruit crops depends largely on conventional methods of introduction, selection, or hybridization using the cultivated genotypes of a species. However, in most crops, most cultivars are developed with relatively narrow genetic diversity. It is estimated that 75% of the genetic diversity of crops has been lost in the twentieth century [12]. When species do not present the desired characteristics, their creators impose these through methods such as mutation, polyploidization, or recombinant DNA technologies. The fundamental resources for the adaptation of the new species: agriculture to challenges posed by climate change will be given by the wild plant species related to them, which constitute the source of useful characteristics, such as agronomic, quality, biotic, and abiotic stresses, which are identified as critical components for food safety and environmental sustainability in the 21st century [13]. In this context, there is an increasing need to stimulate the implementation of measures and strategies, of sustainable systems of green bioprospecting and bioeconomy, to achieve the 17 Sustainable Development Goals (SDGs), foreseen in the 2030 Agenda, of the next major UN event, the United Nations Conference on Water.

The transfer of genes from one species to another allows the exchange of genomes, which results in changes in genotypes and phenotypes of progenies. In large-scale barriers, hybridization occurs as prezygotic and postzygotic barriers. Techniques of chromosome doubling, bridging species, protoplast/somatic fusion and embryo rescue are highly beneficial in recovering fertile progenies by overcoming various barriers in wide crossings [13].

Considering this, hybridization involving wild relatives and related taxa has been increasing every day in recent fruit improvement programs. Alternative technologies, such as CIS genesis and genome editing, can facilitate the development of genetically modified crop varieties with multiple favorable traits. Through crop improvement, introducing beneficial foreign gene(s) or silencing the expression of the endogenous gene(s) in cropping plants, genetic engineering, and plant transformation have played a key role in improving crops, introducing beneficial foreign gene(s), or silencing the expression of the endogenous gene(s) into crop plants [14].

2. Hybridization

2.1. Concept

Hybridization has an important role in the evolution of many lineages, with great phenotypic diversity, because it has great availability of genomic tools as well as innovations in genomic analysis, as it causes the divergent flow between taxa, leading to an adaptation of

these lineages to new environments, contributing to hybrid speciation [14, 15].

Since the beginning of human organizing as society, the improvement of livestock, farming and food supplying performance is a major concern. In search of a faster and more accurate way to modify food, scientists found in the 20th century a way to modify foods by altering their DNA, through genetic engineering, turning these foods into genetically modified organisms (GMOs). **Figure 1** shows the dates of development of genetically modified organisms which serve as food supply. In the 1940s, plant breeders used radiation or chemicals to randomly alter the DNA of an organism, basing in mendelian genetics knowledge. In 1953, based on Rosalind Franklin's findings, scientists James Watson and Francis Crick purposed the double-helix model of DNA structure.

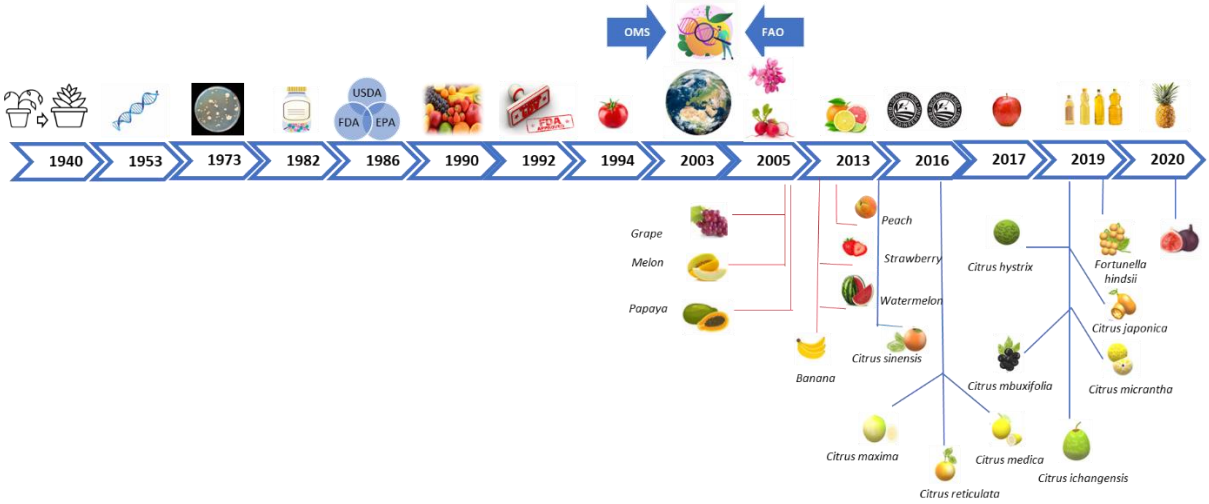

Figure 1 - Timeline on Genetic Modification in Agriculture, including some of the genomes of plants sequenced in the period, being indicated, below, citrus and related blue lines. And other crops, indicated with red lines [16–18]. Source: Authors, 2023.

In 1973, there was development of genetic engineering by inserting DNA from one bacterium into another, by biochemists Herbert Boyer and Stanley Cohen. In 1982, FDA approves the first GMO Human Insulin Product, developed through Genetic Engineering to treat diabetes. After the Asilomar Conference on recombinant DNA and mutagenesis, (CA, USA, 1975), the US federal rules are established for the Regulation of Biotechnology in 1986. This policy describes the safety regulations of GMOs jointly by the FDA, the USDA, and the EPA. In 1990, the products created through genetic engineering became available to consumers: summer squash, soybeans, cotton, corn, papaya, tomatoes, potatoes, and canola. However, not all of them are available for sale. In 1992, FDA policy stated that the safety standards for food from GMO plants must comply with the same requirements, including the same safety standards, such as derived foods from traditionally bred plants. The creation of the first GMO

product in 1994 through genetic engineering — a GMO tomato — become available for sale after studies evaluated by federal agencies, which proved to be as Safe as tomatoes raised traditionally. In 2001, they established in the laws of Brazil, the mandatory labeling for packaged foods, intended for human consumption, that contained or were produced with GMOs, with criteria on the existing limit, which underwent modifications in 2008, approved in 2015. In 2003, the Internationalization of Guidelines and Standards for determining the safety of genetically modified foods by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO). In 2005, alfalfa and genetically modified beets were available for sale in the USA. Due to the fragility of citrus species, most of them were domesticated from their wild ancestors or cross-bred. Congress passed law labeling, in 2016, for some food produced through genetic engineering and uses the term "bioengineer". In 2017, GMO apples were available for sale in the U.S. In 2019, FDA completes a consultation on the first food from a genome-edited plant. In 2020, GMO pink pineapple was made available to U.S. consumers [16–18].

Plant breeding is common across species, with the aim of, for example, increasing nutrient concentrations and some unique secondary metabolites; prolonging the shelf life of cut fruits, vegetables, and flowers; improving yield potential; and increasing tolerance to abiotic stresses and resistance to scourges and plant diseases [19].

Anthocyanins may be beneficial to human health as they are reported to inhibit certain cancers and degenerative diseases. In addition, anthocyanins are responsible for the coloration of flowers, fruits, and vegetables [20].

2.2. Hybridization Techniques

Over the past decade, green biotechnology has introduced a new generation of techniques, referred to as "new plant breeding techniques" (NPBTs), which it has been using as a powerful tool around the world. From many points of view, its potential is much greater compared to that of traditional reproduction and transgenesis (i.e., "classical" genetic engineering). The term NPBT comprises several techniques, the best-known being "cisgenesis" and "genome editing through site-directed nucleases" [21]. These alternative technologies can address many of these issues and facilitate the development of genetically modified crop varieties with multiple favorable traits [22]. A set of alternative technologies have been used for the development of crop improvements in recent years, in response to public concern and lower consumer acceptance of transgenic crops. Figure 2 provides a summary of these techniques.

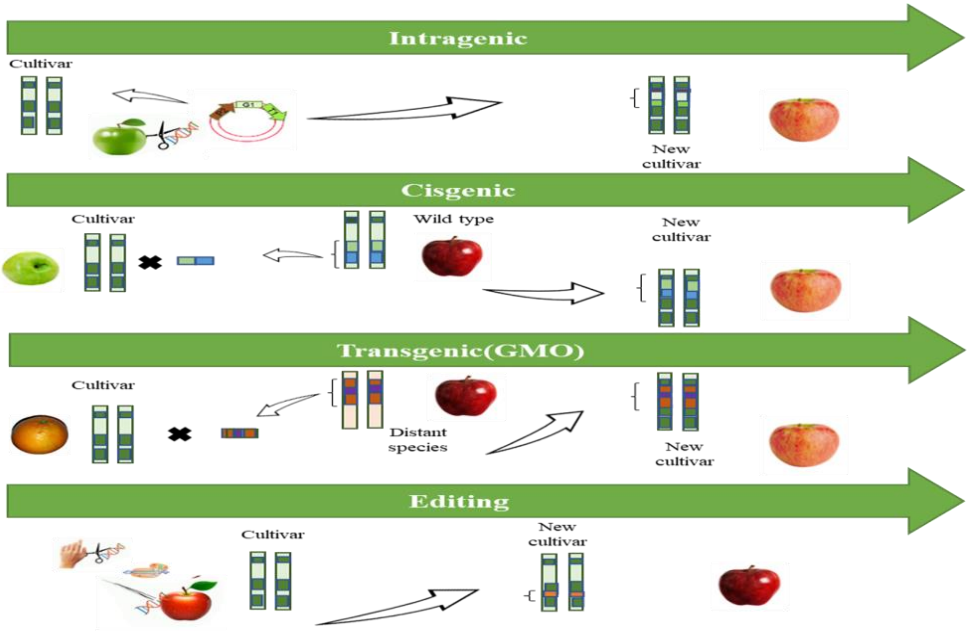

Figure 2 - Schematic illustration of the different types of hybridization techniques. Source: Authors, 2023.

Genetically modified (GM) crops are such crop plants whose genome is modified using genetic engineering techniques to improve the existing traits or for the introduction of a new trait that does not occur naturally in the given crop species [23].

The plants produced by the insertion of specific segments of foreign nucleic acid/gene sequence into their genome using transformation methods (such as *Agrobacterium*-mediated transformation or direct gene transfer) are known as transgenic plants. The inserted gene, also known as a transgene, may come from an unrelated plant, bacteria, virus, fungus, or animal species. Thus, the advent of genetic transformation overcomes the major limitation of conventional plant breeding in which sexual compatibility between species is a precondition to cross them [24, 25].

Such techniques produce cultivation plants genetically like developed plants through reproduction. Moreover, they can be used to develop improved crop plants. This occurs because they project the final products (genome of the modified culture) so that they do not contain any stranger's gene (transgene) [25].

Transgenesis and cisgenesis use the same molecular processes and techniques to transfer gene(s) to a plant, but that cisgenic plants will retain only species-specific genes that could also have been transferred by traditional breeding. Genome editing is responsible for the insertions or deletions of nucleotides at target sites that can cause genetic mutations, resulting in the silencing of the unwanted gene [14, 26]. Among the genome editing technologies, we have as the

most employed Zinc Finger Nuclease (ZFN), Activating Transcriptional Effector Nuclease (TALEN), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), in order of appearance. The CRISPR/Cas9 system is currently the most current tool for genome editing, due to its simple structure and applicability to a wide range of species [24, 27].

The 21st century has been known as the post-genomic era, with the availability of genomic sequence data for various crop plants that revolutionized plant-breeding programs. The implementation of comprehensive synthetic biology tools, known as "genome editing tools," integrates the desired traits into the genomes of crops. Synthetic biology uses the rational design of biological molecules to achieve a desired goal. Synthetic biological tools, through the rational design of synthetic biology, act with precision, accuracy, and predictability [18].

2.3. Nutrition and sensorial aspects of hybrid fruits

Hybrid plants can be created by crossbreeding individual varieties or species. Hybridization within one species can lead to a phenomenon known as heterosis. heterotic individuals are characterized by higher fertility, better lifespan, and higher fruitfulness. The newly created fruits, despite functional properties and health properties, also have new organoleptic attributes [18, 22, 28].

New secondary metabolites and increased activity of existing biosynthesis pathways result from the phenomenon of natural or planned generation of interspecific hybrid fruits, which studies have shown that concerning the total content of phenolic compounds and their distribution in individual plant organs, hybrids may present enhanced characteristics inherited from both parental species [29–31].

Studies conducted to date have proven that the phytochemical and organoleptic characteristics of interspecific hybrids may be better compared to parental species, whose fruits generally have an increased content of simple. In analyses with the hybrids of pummelo and oroblanco, a hybrid of pummelo, was demonstrated to have sweeter flavors, with smaller amounts of simple sugars, being healthier for diabetics, so that the hybridization process, in addition to improving the taste properties, resulted in a reduction of the sugar content in the oroblanco fruit compared to the parent fruit, the pummelo sugars [11, 14, 32, 33].

Results of physicochemical and biochemical studies revealed that substances such as stilbenes, normally abundant in *Vitaceae* spp., have health-promoting biological effects related to the prevention of coronary heart disease [30] and the reduction of the occurrence of cancer, for those who consume *V. vinifera*, aligned with the ability to inhibit the enzymatic activity of the

aromatase of the nuclear transcription factor NFκB crucial transcriptional mediator in cell activation pathways, involved in signaling of neoplastic, inflammatory and degenerative diseases[34–38].

Studies with hybrid citrus fruits (*Citrus aurantium)* have demonstrated one hundred and two chemical constituents that have been identified from their pulp and peel, including volatile oils, terpenoids, phenols, limonin, sugars, etc. [39, 40].

The production of hybrid fruits increases day-after-day, as well as their storage technologies. Its medicinal applications have attracted the attention of the scientific community, due to the various pharmacological effects already known, such as anti-inflammatories, antioxidants, antitumoral, hypolipidemic, and chemoprotective effects/properties on organs [41].

Tropical fruits and subtropical fruits are recognized as a source of high content of bioactive compounds and health-promoting properties due to their nutritional composition. These beneficial health effects are related to the content of several of these bioactive compounds, primarily flavonoids, and non-flavonoid phenolics. Many of these compounds are common in different tropical fruits, such as epicatechin in mango, pineapple, and banana, or catechin in pineapple, cocoa, or avocado [42, 43].

The aronia fruit also called Chokeberry had its hybrid *Aronia prunifolia* which presents more antioxidants than non-hybrid fruit [35, 44].

Most fruits of grape species (*Vitis*) are one of the richest sources of phenolic compounds belonging to groups such as anthocyanins, flavanols, tannins, phenolic acids, and stilbenes [45].

Studies demonstrate a relationship between the consumption of *V. vinifera* fruits and a reduction in the occurrence of cancer since it has been confirmed that the edible parts of the grape fruit (*V. vinifera*) show the ability to inhibit the activity of aromatase and the nuclear transcription factor NFκB, which are currently seen as crucial factors in the early stages of cancer development, e.g., skin, mucous membranes, and uveal membrane of the eye, i.e., malign melanoma [5, 19, 30].

However, it should be noted that the phenolic content varies between qualitative and quantitative intrinsic factors (genus, species, and cultivar) and extrinsic (environment, cultivation, handling, and storage), in addition to depending on factors such as extraction and quantification methods.

Depending on each region, with its particularities, the fruit will be differentiated in chemical composition, consequently having distinct biological activities, proving to be of fundamental importance the study of cultivars planted regionally. Research already carried out

shows the presence of important antioxidant and antitumor activity of the different hybrid cultivars cultivated in the Brazilian territory [46, 47].

Studies on the molecular mechanisms of antioxidant substances present in hybrid fruits that act as anti-inflammatories, point to modulation of the activity of various proteins and transcription factors, which are involved in metabolic pathways related to the synthesis of cytokines, chemokines and adhesion molecules. One of the possible pathways is related to the antioxidant capacity of these compounds, when they fight oxidative stress (caused by excess ROS) decreasing inflammation. It occurs when reactive oxygen species, capable of activating protein kinases (JNK, ERK and p38) and thus increasing the activity of inflammation-inducing transcription factors, are neutralized and may also inhibit the expression of cytokines by the transcription factors involved in the inflammatory response and cancer [48–50].

3. Antioxidants

New secondary metabolites and increased activity of existing biosynthesis pathways result from the phenomenon of natural or planned generation of hybrid fruits interspecific, which in studies conducted have shown that hybrid fruits, both in the total content of phenolic compounds and in their distribution in their plant organs, may present enhanced characteristics inherited from both parental species [8, 17, 51, 52].

Scientific studies have proven that the phytochemical and organoleptic characteristics of interspecific hybrids may be better compared to parental species, whose fruits usually have an increased content of simple sugars [11, 32].

Results of physicochemical and biochemical studies revealed that substances such as stilbenes, normally abundant in *Vitaceae* spp., have health-promoting biological effects related to the prevention of coronary heart disease [30], and the reduction of the occurrence of cancer [15, 34, 52, 53].

Flavonoids make up a large class of secondary metabolites of low molecular weight that are present in almost every compartment of plants, from the roots to the flowers and fruits [54]. Flavonoids are polyphenolic compounds subdivided into 6 groups: isoflavonoids, flavanones, flavanols, flavanols, flavones, and anthocyanidins found in a variety of plants [28, 37, 55].

Flavonoids have the properties of inhibiting auto-oxidation and scavenging free radicals. They can act as antioxidants because they can transfer electrons to free radicals and are catalysts for chelated metals [22, 56].

During biotic and abiotic stress conditions, such as drought, wounds, and metal toxicity, many flavonoid biosynthetic genes are induced, and flavonoid levels increase [18, 57, 58].

As can be seen in Figure 1, extracts of hybrid fruits rich in polyphenols, in contact with cancer cells, which, in addition to natural internal factors, have gone through external factors and have their ROS production increased, begin to present a reduction in cancer cells [37, 59].

Anthocyanins are of the flavonoid class, highly hydrophilic and are responsible for the red and blue colors in fruits, vegetables, and flowers [31]. In addition to being natural pigments, they are potent antioxidants [60] and can prevent lipid oxidation and eliminate free radicals [52]. It has been reported that dietary intake of fruits and vegetables has beneficial effects on human health, such as anti-cancer and anti-aging properties [37, 61]. Anthocyanins are also important for improving the nutritional values of processed foods [22].

Anthocyanins are being used as one of the most promising ingredients in the food, beverage, cosmetic and nutraceutical industries. The nutraceutical activities as well as the anticancer activities of anthocyanins and anthocyanidins have been extensively reviewed [8, 21, 62].

The abilities of anthocyanins to induce apoptosis and suppress angiogenesis have been explained as the reasons for the anticancer activities of anthocyanins, which cause the effect of cyanidin-3-glycoside (C3G) to block ethanol-induced activation of the ErbB2/cSrc/FAK pathway, preventing cell migration/invasion. This effect is beneficial for preventing ethanol-induced breast cancer metastases [38, 47, 60].

There is a great demand for natural antioxidants, arising from the desire to switch from synthetic to natural products, driving research in this area [60]. The antioxidant capacities of polyphenols in general and anthocyanins are evaluated by various methods. TEAC (Trolox Equivalent Antioxidant Capacity), FRAP (Ferric Reducing Ability of Plasma) e ORAC (Oxygen Radical Absorbance Capacity) are just a few among the many popular trials deployed by the scientific community. Table 1 describes the Experimental antioxidant capabilities of hybrid fruits, along with their total polyphenolic content.

Table 1 - Antioxidant capacity, total polyphenolic, flavonoids, and anthocyanins from hybrid fruits.

Hybrid fruits	TEAC (mmol Trolox/100 FW)	FRAP (mmol Fe^{2+}/100 g FW)	ORAC (mmol Trolox/100 g FW)	DPPH (mmol TE/100 g DM)	Total phenolics (mg GAE/100 g FW)	Total flavonoids (mg/100g)	Total Anthocyanins (mg/100g)	References
Strawberry	265.9	339	253.2	nd	6238	675	nd	[60, 91]
Red Plum	185.3	208.2	274.9	nd	352	nd	nd	[60]
Apple	447	402	578	nd	490	nd	nd	[60]
Tomato	269	501	459	nd	310	nd	620	[60, 92]
Mandarin	61	674,5	407.4	54.03	467	577	nd	[17, 33]
Sweet Orange	31.9	nd	nd	12.3	nd	nd	nd	[33]
Pummelo	24.9	7.31	449.1	10.1	1754	nd	nd	[17, 33]
Lemon	21.2	nd	nd	8.8	nd	nd	nd	[33]
Kumquat	15.7	nd	nd	2.9	nd	nd	nd	[33]
Orange	nd	755	244	16.1	1341	nd	nd	[17]
Chokeberry	nd	39.0	555.5	53.78	2340	556	256.4	[32]
Melon	nd	nd	nd	nd	715.8	nd	nd	[93]
Grape (*Vitis*)	nd	42.3	58.5	33.7	1015.2	122	3457.5	[30]
Grape (*Sweet jubilee*)	nd	63.7	nd	nd	2038	nd	nd	[14]

Values are expressed as the mean with standard deviation from literature collection. nd = not determined.

Table 1 illustrates the experimental antioxidant capacities of hybrid fruits, together with their total polyphenolic content, calculated as gallic acid equivalents.

Current scientific research has considered very carefully the content of fruits, especially in sugar, vitamins and minerals, so that they have better quality and meet the requirements of consumer needs. The hybridization techniques have presented, hybrid fruits, with chemical values in higher content of mineral elements and good quality for the native fruits, according to **Table 2**, with data collected in studies carried out in hybrid and native fruits.

Table 2 - Chemical and biochemical components of hybrid fruits with their natives.

Fruit	Hibrid fruit (H)/ Native Fruit (N)	ORAC (mmol TE/100g)	FRAP (µM Trolox/g FW)	DPPH (µM Trolox TE/g FW)	Total Polyphenols (mg GAE/100g)	Sugar (%)	Vitamin C (mg/100g) fresh content	References
	Sweet Orange (H)	468.96	nd	568.39	248.00	nd	49.90	[17, 66, 83, 94]
	Orange (N)	213.25	nd	333.76	206.00	nd	68.50	
	Pummelo (H)	319.01	nd	nd	275.50	nd	80.00	[17]
	Pummelo (N)	201.56	nd	nd	172.00	nd	57.00	
	Tomato (H)	855.83	nd	nd	52.21	nd	30.40	[29, 57]
	Tomato (N)	406.27	nd	nd	28.18	nd	21.20	
	Melon (H)	nd	378.80	nd	96.00	3.97	18.34	[95]
	Melon (N)	nd	493.80	nd	115.20	6.33	22.47	
	Grape (Vitis) (H)	341.00	nd	386.20	203.50	15.50	3.20	[5, 94, 96]
	Grape (RSG) (N)	146,57	nd	169.45	367.00	16.67	10.90	
	Apple (H)	nd	nd	72.20	588.90	12.34	9.80	[88]
	Apple (N)	nd	nd	131.60	83.00	nd	8.81	
	Mandarin (H)	357.54	nd	nd	211.10	nd	50.00	[17, 94]
	Mandarin (N)	163.88	nd	nd	195.80	nd	40.50	

(H)= hybrid fruits; (N)= natives' fruits; RSG= red seedless grape; nd = not determined.

The firmness of the fresh fruit and the sugar content are important quality factors, as they directly influence the purchasing power of consumers. The taste is the most complex to analyze since the organoleptic quality of the fresh fruit is composed of various organic acids, along with soluble sugars and aromas. Eleven organic acids were identified in the apple pulp and five more in the whole fruit, with malic acid, the predominant organic acid in apple fruits, which directly influences the flavor. The concentration of ascorbic acid in the fruit progressively decreases from the peel to the core of the fruit. Vitamin C is present in two forms in ascorbic acid and in its oxidized form, dehydroascorbic acid [63–65].

Vitamin C participates in the processes of regulating cell growth, cell signaling, apoptosis, antioxidants, and as cofactors for enzymes. Vitamin C comes mainly from the consumption of fruits and vegetables, being reduced by heat during processing; therefore, its nutritional value in raw foods is higher than in processed form. Vitamin C eliminates reactive oxygen species (ROS) and reactive nitrogen species (RNS) and regenerates α-tocopherol and coenzyme Q from α-tocopherol and coenzyme Q radical α. Studies postulated that they suggested that ascorbate induces the decomposition of lipid hydroperoxide into genotoxins in the absence of redox-active metal ions, leading to a reduction in the growth of aggressive tumor xenografts [8, 28, 29]. When performed in animal species, consumption of fruits rich in vitamin C

helped protect the body against cardiovascular disorders, gastrointestinal disorders, cancer, skin infections, and diabetes through reduced insulin glycation and an increase in glucose homeostasis [8].

Sugars, acids, and aromatic compounds are recognized as major components in fruit quality. These components vary greatly among the different varieties of fruits [44].

A comparative table with data from several studies, was presented, with the values of antioxidant capacity, vitamin C determined, total polyphenol amounts, percentage of sugar, and water of native cultivars, because are closely related to the quality of hybrid fruits produced.

The results of this table show that the nutritional values of the fruits can be altered by the application of hybridization, making them a qualitatively different product.

4. Health benefits of hybrid fruits

Tropical fruits and subtropical fruits are recognized as a source of high content of bioactive compounds and health-promoting properties due to their nutritional composition. These beneficial health effects are related to the content of several of these bioactive compounds, primarily flavonoids, and non-flavonoid phenolics. Many of these compounds are common in different tropical fruits, such as epicatechin in mango, pineapple, and banana, or catechin in pineapple, cocoa, or avocado [43, 48, 66].

The increased antioxidant capacity in the fruit increases its action against some diseases that compromise human health, as demonstrated during this study. Therefore, since hybridization increases the number of antioxidant compounds, as well as the antioxidant capacity of the fruit in the human body, we will describe some beneficial effects that the consumption of hybrid fruits can bring to those who use it in their diet [67].

In all countries, regardless of developed and developing countries, there is an increase in interest in "natural" products, their content of phytochemicals, and antioxidants [68] in the treatment of human diseases. Some of these natural products rich in antioxidants include tea, shrubs, wine, and fruit juice, when produced with the hybrid fruits will be much richer and may encourage increased production of these highly oxidizing fruits [5, 8, 68].

The preferential use of natural products occurs mostly for their minimal side effects and the growing preference for natural products used in preventive and therapeutic medicine. There is a great tendency to defend and use natural vitamins C and E as protection against ultraviolet radiation on the skin and other cosmetic products [8, 69].

Fruit juice and wine, processed food drinks, are currently supplemented with fruit-derived ascorbic acid. Natural dyes derived from carotenoids obtained from antioxidant-rich

fruits and vegetables are used in the replacement of synthetic artificial colors in the manufacture of food products [8].

In vitro health research on antioxidants contained in hybrid fruits, strengthened by existing studies on native fruits, is still being conducted in experimental models, with many of the claims about their therapeutic effects yet to be verified and confirmed, especially in humans. Research into phenolic compounds is of increasing interest due to the vital biological and pharmacological characteristics that these antioxidants have demonstrated in human health [8]. Bringing "bioactive" as the topic of discussion in most congresses related to food and health, highlighting the claim of functional properties of these food sources. This reinforces the need for more research to verify and substantiate these health claims and, especially, to determine the antioxidant contents of fresh peeled hybrid fruits, versus "*in natura*" (consumed with peel), versus processed (dried), as normally incorporated in other food products.

4.1. Anti-Cancer Effect

Cancer is a worldwide health concern this disease is caused by irregular cell growth with invasive potentials. The discovery of new bioactive components can be considered a new therapeutic strategy for cancer protocols [49, 70].

During the last two decades, plant studies have found bioactive compounds that have been reported as new health agents for prevention and/or mitigation of different human diseases such as cancer, inflammation, cardiovascular, and neurodegenerative diseases [71].

The production of free radicals (ROS) during various reactions of the human body, including respiration, is inevitable since the mitochondrial electron of the transport chain involves the direct reduction of oxygen by the free energy of electrons. They are also formed as part of the normal processes of metabolism and because of various external factors (Figure 3) such as smoke, pollution, exposure to chemicals, ozone, ultraviolet radiation, X-rays, pesticides, and certain drugs [8, 48].

Nitric oxide (NO) is a signaling molecule that plays an important role in both physiological processes and cancer promotion. Suggested that low levels of NO promote cancer, while high levels of NO protect against cancer. Both ROS and many species of reactive nitrogen (RNS) can be carcinogenic modifying the inflammatory state, as well as influencing cellular lipid structures, angiogenesis, and anti-apoptotic pathways, among others. For example, low concentrations of NO can cause redox imbalance, increased inflammation, and damage to subcellular components, accelerating the neoplastic process [48, 66, 67].

In the promotion of the tumor, paraneoplastic cells in active proliferation accumulate in a relatively long and reversible process. Progression, the final stage of neoplastic transformation, involves the growth of a tumor with invasive and metastatic potential [46, 72].

Research already carried out shows the presence of important antioxidant and antitumor activity of the different hybrid cultivars cultivated in the Brazilian territory.

The ability of flavonoids to scavenge free radicals, regulate cellular metabolism, and prevent diseases related to oxidative stress has been demonstrated in numerous studies [61, 73–76].

Cancer is a heterogeneous disease characterized by uncontrolled proliferation and impaired cell cycle leading to the growth of abnormal cells that invade and metastasize to other parts of the body [46].

Oxidative stress, hypoxia, genetic mutations, and lack of apoptotic function are the main internal causes of cancer, while external causes are related to increased exposure to stress, pollution, smoking, radiation, and ultraviolet rays [6, 57]. Altered metabolism, impaired cell cycles, mutations frequent, resistance to immune response, chronic inflammation, metastases, and induction of angiogenesis are the main features of cancer cells [63, 67]. Several studies link cancer as a metabolic disease determined by varying degrees of mitochondrial dysfunctions and metabolic changes [37] .

Mitochondria are essential for cellular energy supply, metabolism regulation, cell death signaling, and generation of reactive oxygen species (ROS). The main metabolic changes of tumor cells involve increased aerobic glycolysis, dysregulated pH, impaired lipid metabolism, increased ROS generation, and impaired enzyme activities [59] (Figure 3). In contrast, the extracellular environment becomes acidic and more favorable to inflammation, glutamine-driven lipid biosynthesis increases and upregulates the pathways involved in the onset of tumorigenesis and metastasis, cardiolipin levels decrease in membranes impairing enzyme activities and mitochondria are hyperpolarized, and this effect correlates with malignancy and invasiveness of cancer cells [46].

A study with luteolin, a flavonoid found in different plants, demonstrates that the flavonoid acts as an anticancer agent against various types of human malignancies, such as lung, breast, glioblastoma, prostate, colon, and pancreatic cancer. It also blocks the development of cancer *in vitro* and *in vivo* by inhibiting the proliferation of tumor cells, protecting against carcinogenic stimuli and activation of cell cycle arrest, and inducing apoptosis through different signaling pathways, which can also reverse the transition of epithelial-mesenchymal cancer cells, through a mechanism that involves shrinking the

cytoskeleton, inducing the expression of the epithelial biomarker E-cadherin and the negative regulation of mesenchymal biomarkers -cadherin, and vimentin [37, 46] (**Figure 3**).

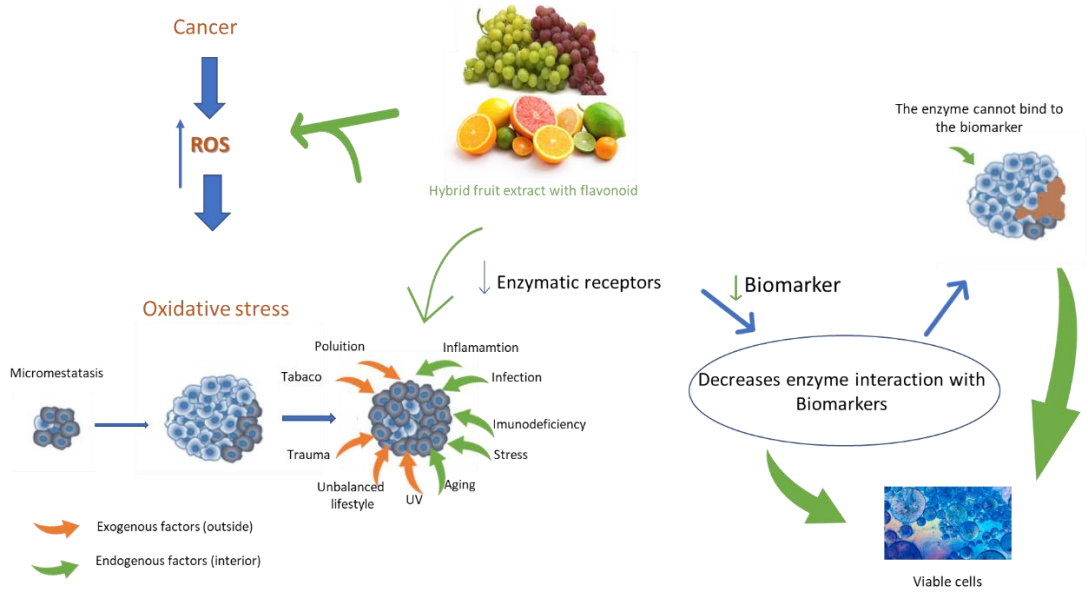

Figure 3 - The flavonoids of hybrids fruits protect against ROS induced by metabolic alteration occurring in tumor cells.

Flavonoids have several anticancer effects: they modulate enzymatic ROS-killing activities, participate in cell cycle disruption, induce apoptosis, and autophagy, and suppress the proliferation and invasion of cancer cells [38].

There is scientific evidence that there is a need for a balance between oxidants and antioxidants to maintain health, as changes in this balance can lead to pathological responses that result in functional disorders and diseases. Studies also suggest that a diet excluding vegetables and fruits may alter hormone production, metabolism, or action at the cellular level of the individual, thereby increasing the incidence of breast, colorectal, and prostate cancer [8].

4.1.1. Cytotoxic activity

For studies of carcinogenesis and mutagenesis, often cell lines such as MCF-7 (breast), Hep-2 (larynx), PC-3 (prostate), DU-145 (prostate), HeLa (cervix), HT-29 (colon), OVCAR03 (ovarian) are used, which are specifically derived from the transformation of cells of a type of carcinoma [77]. Most of these cell models have similar characteristics with rapid proliferation and a cell cycle of 18 to 24 hours, which may even influence the verification of similar cytotoxic activities between the various cell lines [47]. The drugs tested for possible cytotoxic activities in the face of this cellular model are kept in contact with the cells for a period of 24 to 72 hours,

determining the dose-effect curve and subsequently the proposed inhibition parameter [73].

Cell death can be classified according to characteristics morphological, apoptosis, necrotic, autophagic, or mitosis-associated [78, 79].

In necrosis, an "accidental" cell death occurs, as it is usually the result of an unintentional traumatic injury, which may be thermal, chemical, or due to lack of oxygen, where the cell enlarges its volume, causing cellular disruptions, randomly releasing fragmented in its surroundings [17].

Autophagy is an adaptive process evolutionarily conserved and genetically controlled, which occurs in response to metabolic stress resulting from the degradation of cellular components [31, 80].

Currently, the cytotoxic *in vitro* activity of tumor cell culture becomes important for the identification of anticancer agents. Cell cytotoxicity it has been evaluated by several methods. Among the methods to evaluate viability the MTT (3-(4,5-dimethylazol-2-yl)-2,5-diphenyltetrazolium bromide) assay highlights because it is an indirect, accurate, and rapid test, based on a colorimetric reaction. The MTT salt of yellow color, when incubated with metabolically active cells, enter the mitochondria and is reduced by the enzyme succinate dehydrogenase, producing crystals of formazan, with dark blue coloration, thus the resulting optical density is determined in a spectrophotometer [18, 33]. There are alternatives to MTT such as MTS (salt of 3-(4.5-dimethylthiazole-2-yl)-5-(3-carboxymethoxypenyl)-2-(4-sulfophenyl)-2H-tetrazoli) which presents basically the same principle, but with lower toxicity and higher solubility in water [33].

Aronia melanocarpa, *Aronia arbutifolia* and *Aronia prunifolia* demonstrated gradual inhibition in tumor cell proliferation after 48-h treatment, proving to be cytotoxic to HeLa, HepG2, HT-29 cells [35, 44].

Four hybrids of *Malus sieversii* (red pulp apple) were compared with Fuji apple and showed a higher amount of phenolic and flavonoid compounds, being the hybrids "A38" and "Meihong" of red pulp with higher antioxidant and antiproliferative activity in human breast cancer in the strains MCF-7 e MDA-MB-231[81].

About flavonoids, the extract of the leaf of atemoya (*Annona atemoya*) a hybrid of pinecone (*Annona squamosa*) and chirimoya (*Annona cherimola*) had the metabolite rutin identified in the regulation of the inhibitory effect in Alzheimer's disease by avoiding β-amyloid aggregation and neuronal cell death [82].

Grapefruit, a citrus fruit, resulting from the crossing of pomelo with orange, has as a secondary metabolite nootkatone, which tested in lung adenocarcinoma obtained results in

inhibiting cancer progression via cyclooxygenase (COX-2) and consequently inhibited the growth and decreased cell proliferation, by activating AMPK (AMP-activated protein kinase) [77].

Studies addressed in this review indicated the potential anticancer of antioxidant substances present in hybrid fruits, and in particular their capacity to inhibit the proliferation of cancer cells due to their effects on the cell cycle [5].

The food to be considered antiproliferative must have cell growth inhibition greater than 50% [38].

4.2. Anti-Diabetic Effect

Diabetes mellitus is a leading cause of mortality and morbidity. Recent data showed that approximately 150 million people have diabetes worldwide, and this number may double by the year 2025 [34]. Improvement in diabetes mellitus, digestive problems, immune disorders, cataracts, bronchitis, asthma, and other respiratory syndromes have been reported after regular intake of antioxidants from fruits and vegetables [8].

Like other chronic diseases, diabetes is an expensive disease for both an individual in terms of personal income and productivity for the community, due to the increasing and excessive burden on health care and rehabilitation facilities, causing the direct and indirect economic cost of diabetes to burden the health of the country with spending on care [61].

Studies with bitter orange extracts on liver antioxidant defense in diabetic mice have demonstrated a considerable drop in the blood glucose level of experimental diabetic mice compared to untreated diabetic mice. The activities of superoxide dismutase were increased in the liver of diabetic mice, while the activities of glutathione peroxidase, malondialdehyde, and nitric oxide were significantly reduced [40]. This hybrid orange extract was able to increase the antioxidant activity of the liver and acted in the reduction of liver damage when the histological analysis was performed in experimental diabetic mice in comparison with untreated diabetic mice [83].

Additional molecular and genomics efforts devoted to improving the nutritional value and other desirable traits are in intensive progress. In this way, we will be able to expand the uses of species and hybrids for the food and biotechnology industries [84].

4.3. Anti-inflammatory Effects

Inflammation causes edema, resulting from increased permeability of the endothelium tissue and the invasion of blood cells into the resulting space in response to an external stimulus. Macrophages, neutrophils, and epithelial cells usually mediate inflammation. The main pro-inflammatory mediators are prostaglandinsE2 and nitric oxide, which promote inflammation. Studies demonstrate hybrid citrus species, with various inhibitory metabolites of pro-inflammatory mediators, and report that flavonoids, coumarins, and essential oils found in some citrus species are effective against inflammation and have the potential to protect against inflammatory diseases [67, 72].

Research with methanolic extract of the leaves of the cajazeira (*Spondias mombin*), a hybrid fruit, rich in carotenoids and vitamin A, containing as the main carotenoid the β-cryptoxanthin, followed by lutein. Its leaves presented in phytochemical studies the presence of tannins, saponins, resins, sterols and triterpenes, flavonoids and alkaloids resulted activity antibacterial Against *Pseudomonas aeruginosa* and *Shigella dysenteries*, while extracts from the bark of the stem inhibited the growth of the bacteria *Escherichia coli* and *Klebsiella pneumoniae* [85, 86].

Acute toxicity studies were performed in rats, with induction of acute pancreatitis (AP) and with a diet composed of Granny Smith apple, which is a hybrid variety, reported as the second in the highest number of flavonoids and procyanidins among all varieties of apples. The results of the study indicated a protective effect on experimentally induced AP in rats by L-arginine. GSAE (Granny Smith Apple Extract) administration exhibited beneficial effects by reducing oxidative and nitrosative stress and modulating the inflammatory process [11, 87, 88].

4.4. Anti-degenerative disease effect

New epidemiological studies have proposed an inverse correlation between high antioxidant intake from fruits and vegetables and many degenerative diseases and aging [8].

Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), scrape induction disease, and amyotrophic lateral sclerosis (ALS), are neurodegenerative diseases, which share the abnormal accumulation of intraneuronal or extra neuronal proteins. For example, Alzheimer's disease (AD) is caused by the decrease or deficiency of acetylcholine, found in the synapses of the cortex, over a period [48, 67].

These diseases, as well as the concern with them, have been increasing with the aging of the population, making the topic relevant. Multiple pathways, such as apoptosis, autophagy, oxidative DNA damage, and repair, have been identified as different neurodegenerative diseases. Each one has such different mechanisms that are still far from being clarified, constituting a great challenge for the discovery of a potential therapy that can help delay the effects of aging and prevent these diseases [48, 62].

Fruit hybridization, which has been proven to increase the amount of antioxidant compounds, has become a topic of interest as a natural preventive/therapeutic strategy because these substances can protect neurons against oxidative stress, modulate cell signaling pathways and suppress neuroinflammation. Mitochondria influence the pathophysiology of inflammatory diseases, the production of free radicals for specific purposes in the cell (cell signaling and inflammatory process) and the detoxification of these same radicals in other situations [48, 51, 62].

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4.5. Drug-food synergy

During the prescription of medications, or formulation of herbs, supplements, or drugs, do not give importance to the drug-food synergy. This neglected fact, during consumption, can lead to health problems for those who seek a cure for a certain disease. Studies with patients treated with fruit juice and the drug donepezil. Sridharan and Sivaramakrishnan showed that the extract of *Citrus aurantium* L. who possessed nobiletin slowed the progression of Alzheimer's disease and that orange juice consumption did not affect the participant's plasma exposure when treated with cyclosporine [67, 89]. In addition, poly methoxy flavones from *Citrus sinensis* waste residue were found to possess a synergetic effect when it was combined with anethole. Citrus, psoralen-rich foods increase the risk factor for malignant melanoma [46, 67]. Various citrus fruits influence drug-metabolizing enzymes. Grapefruit contains a compound that inhibits (CYP3A4) cytochrome P4503A4, the main enzyme required for drug metabolism. Studies reports by De Castro et al. (2007) show that furanocoumarins in grape juice at the same time inhibited the intestine CYP3A4, increased the oral bioavailability of drugs such as felodipine and midazolam, which sequentially raise the level of toxic concentration, fatal rhabdomyolysis may occur [67, 90].

Pharmaceutical drugs such as donepezil and naturally formed galantamine are found to be effective therapeutic agents for AD. However, their adverse side effects on the gastrointestinal tract have been reported [8, 67].

5. Conclusions

The review demonstrates that research with different fruits in their hybrid forms has proven increased antioxidant activity, demonstrating functional and nutritional potential. When compared to their native forms, they demonstrate a more effective chemical composition for health, reinforcing that we need to continued studies need to devote to evaluate how hybrid plants can act on safety for consumers if their consumption increases.

Based on studies like this and with the many health claims attributed to the antioxidant content of hybrid fruits, their sustained and increased production will lead to increased consumption of this product by humans and a decreasing effect of degenerative diseases.

Thus, we suggest more studies, mainly in humans, with hybrid fruit extracts, as we need more research and clinical studies for a more in-depth analysis in search of understanding the underlying mechanisms, thus reducing the effect of diseases and side effects of medications.

Conflicts of Interest

The authors declare that they have no competing interests.

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6. References

[1] Christ, G. D.; Bernal, A. de O.; Galafassi, L. B.; Coronel, D. A. O Agronegócio Brasileiro No Comércio Internacional: Vulnerabilidade, Retrocesso, Oportunidade Perdida Ou Situação Ótima? Inf. GEPEC, 2022, 26 (2), 190–209. https://doi.org/10.48075/igepec.v26i2.28426.

[2] Landau, E. C.; Silva, G. A. da; Moura, L.; Hirsch, A.; Guimarães, D. P. Dinâmica Da

Produção Agroapecuária e Da Paisagem Natural Do Brasil Nas Últimas Décadas, 1st ed.; Embrapa: Brasília, Distrito Federal, 2020.

[3] Camargo, U. A.; Maia, J. D. G.; Ritschel, P. Embrapa Uva e Vinho; 2010.

[4] Schulz, M.; Katia, S.; Seraglio, T.; Brugnerotto, P.; Gonzaga, L. V.; Carolina, A.; Costa, O.; Fett, R. Composition and Potential Health Effects of Dark-Colored Underutilized Brazilian Fruits – A Review. Food Res. Int., 2020, 137 (September), 109744. https://doi.org/10.1016/j.foodres.2020.109744.

[5] Pascoal, G. de F. L.; Cruz, M. A. de A. S.; Abreu, J. P. de; Santos, M. C. B.; Fanaro, G. B.; Júnior, M. R. M.; Silva, O. F.; Moreira, R. F. A.; Cameron, L. C.; Ferreira, M. S. L.; et al. Evaluation of the Antioxidant Capacity, Volatile Composition and Phenolic Content of Hybrid Vitis Vinifera L. Varieties Sweet Sapphire and Sweet Surprise. Food Chem., 2022, 366 (July 2021). https://doi.org/10.1016/j.foodchem.2021.130644.

[6] Montero, M. L.; Rojas-Garbanzo, C.; Usaga, J.; Pérez, A. M. Nutritional Composition, Content of Bioactive Compounds, and Hydrophilic Antioxidant Capacity of Selected Costa Rican Fruits. Agron. Mesoam., 2022, 33 (2). https://doi.org/10.15517/am.v33i2.46175.

[7] Swallah, M. S.; Sun, H.; Affoh, R.; Fu, H.; Yu, H. Antioxidant Potential Overviews of Secondary Metabolites (Polyphenols) in Fruits. Int. J. Food Sci., 2020, 2020. https://doi.org/10.1155/2020/9081686.

[8] Jideani, A. I. O.; Silungwe, H.; Takalani, T.; Omolola, A. O.; Udeh, H. O.; Anyasi, T. A. Antioxidant-Rich Natural Fruit and Vegetable Products and Human Health. Int. J. Food Prop., 2021, 24 (1), 41–67. https://doi.org/10.1080/10942912.2020.1866597.

[9] Mallawaarachchi, M. A. L. N.; Madhujith, T.; Suriyagoda, L. D. B.; Pushpakumara, D. K. N. G. Antioxidant Efficacy of Selected Underutilized Fruit Species Grown in Sri Lanka. Trop. Agric. Res., 2021, 32 (1), 68. https://doi.org/10.4038/tar.v32i1.8443.

[10] Yu, H.; Li, J. Breeding Future Crops to Feed the World through de Novo Domestication. Nat. Commun., 2022, 13 (1), 8–11. https://doi.org/10.1038/s41467-022-28732-8.

[11] Ergün, Z. Determination of Biochemical Contents of Fresh, Oven-Dried, and Sun-Dried Peels and Pulps of Five Apple Cultivars (Amasya, Braeburn, Golden Delicious, Granny Smith, and Starking). J. Food Qual., 2021, 2021.

https://doi.org/10.1155/2021/9916694.

[12] Ganji, S. M.; Singh, H.; Friedman, M. Phenolic Content and Antioxidant Activity of Extracts of 12 Melon (Cucumis Melo) Peel Powders Prepared from Commercial Melons. No. c. https://doi.org/10.1111/1750-3841.14666.

[13] Dhanyasree, K.; Rafeekher, M. Wide Hybridization for Fruit Crop Improvement : A Review Anushma PL , Wide Hybridization for Fruit Crop Improvement : A Review. 2021, No. January. https://doi.org/10.22271/chemi.2021.v9.i1k.11317.

[14] Silva, H. New Plant Breeding Techniques in Citrus for the Improvement of Important Agronomic Traits . A Review. 2020, 11 (August), 1–15. https://doi.org/10.3389/fpls.2020.01234.

[15] Goulet, B. E.; Roda, F.; Hopkins, R. Hybridization in Plants : Old Ideas , New Techniques [OPEN]. 2017, 173 (January), 65–78. https://doi.org/10.1104/pp.16.01340.

[16] Zhu, L.; Li, X.; Hu, X.; Wu, X.; Liu, Y.; Yang, Y.; Zang, Y.; Tang, H. Características de Qualidade e Perfis de Antocianina de Diferentes Cultivares de Uva Vitis Amurensis e Híbridos Do Germoplasma Chinês. 2021, 26 (21). https://doi.org/10.3390/mol.

[17] Wang, Y.; Qian, J.; Cao, J.; Wang, D.; Liu, C.; Yang, R.; Li, X. Capacidade Antioxidante , Capacidade Anticética e Composição de Flavonoides de 35 Variedades Cítricas (Citrus Reticulata Blanco) Abstrair. 2017, 22 (7).

[18] Daldoul, S.; Boubakri, H.; Gargouri, M.; Mliki, A. Recent Advances in Biotechnological Studies on Wild Grapevines as Valuable Resistance Sources for Smart Viticulture. Mol. Biol. Rep., 2020, 47 (4), 3141–3153. https://doi.org/10.1007/s11033-020-05363-0.

[19] Hallauer, A. R. Evolution of Plant Breeding. Crop Breed. Appl. Biotechnol., 2011, 11 (3), 197–206. https://doi.org/10.1590/s1984-70332011000300001.

[20] Blesso, C. N. Dietary Anthocyanins and Human Health. Nutrients, 2019, 11 (9), 10–13. https://doi.org/10.3390/nu11092107.

[21] Costa, L. D.; Malnoy, M.; Gribaudo, I. Criação de Frutos de Árvores de Próxima Geração : Desafios Técnicos e Legais Introduçã O. 2017. https://doi.org/10.1038/hortres.2017.67.

[22] Sirohi, R.; Tarafdar, A.; Singh, S.; Negi, T.; Gaur, V. K.; Gnansounou, E.; Bharathiraja, B. Green Processing and Biotechnological Potential of Grape Pomace: Current Trends and Opportunities for Sustainable Biorefinery. Bioresour. Technol., 2020, 314, 123771. https://doi.org/10.1016/j.biortech.2020.123771.

[23] Santos, A. M. M. dos; Santos, P. R. dos; Costa, K. D. da S.; Nascimento, M. R.; Martins, C. dos S. R.; Michelon, G. K.; Lima, R. S. R. de. Melhoramento e Caracterização de Recursos Genéticos Da Videira : Uma Revisão de Literatura. In Annais da XXI Encontro Latino Americano de Iniciação Científica, XVII Encontro Latino Americano de Pós-Graduação e VII Encontro de Iniciação à Docência; Universidade do Vale do Paraíba: São José dos Campos, São Paulo, 2017; pp 1–6.

[24] Abdallah, N. A.; Prakash, C. S.; Mchughen, A. G. EDITOR ' S CORNER Genome Editing for Crop Improvement : Challenges and Opportunities. 2015, 183–205. https://doi.org/10.1080/21645698.2015.1129937.

[25] Zecca, G.; Labra, M.; Grassi, F. Untangling the Evolution of American Wild Grapes: Admixed Species and How to Find Them. Front. Plant Sci., 2020, 10. https://doi.org/10.3389/fpls.2019.01814.

[26] Lobato-gómez, M.; Hewitt, S.; Capell, T.; Christou, P.; Dhingra, A.; Girón-calva, P. S. Transgenic and Genome-Edited Fruits : Background , Constraints , Bene Fi Ts , and Commercial Opportunities. Hortic. Res., 2021. https://doi.org/10.1038/s41438-021-00601-3.

[27] Mashilo, J.; Shimelis, H.; Ngwepe, R. M.; Thungo, Z. Genetic Analysis of Fruit Quality Traits in Sweet Watermelon (Citrullus Lanatus Var . Lanatus): A Review. 2022, 13 (March), 1–14. https://doi.org/10.3389/fpls.2022.834696.

[28] Chen, J.; Luo, M.; Li, S.; Tao, M.; Ye, X.; Duan, W.; Zhang, C.; Qin, Q.; Xiao, J.; Liu, S. A Comparative Study of Distant Hybridization in Plants and Animals. 2017. https://doi.org/10.1007/s11427-017-9094-2.

[29] Palmitessa, O. D.; Durante, M.; Caretto, S.; Milano, F.; Imperio, M. D.; Serio, F.; Santamaria, P. Supplementary Light Differently Influences Physico-Chemical Parameters and Antioxidant Compounds of Tomato Fruits Hybrids. 2021, 1–15.

[30] Olędzki, R.; Lutosławski, K.; Nowicka, P.; Wojdyło, A.; Harasym, J. Non-Commercial Grapevines Hybrids Fruits as a Novel Food of High Antioxidant Activity. Foods, 2022,

11 (15), 2216. https://doi.org/10.3390/foods11152216.

[31] Gratl, V.; Sturm, S.; Zini, E.; Letschka, T.; Stefanini, M.; Vezzulli, S.; Stuppner, H. Comprehensive Polyphenolic Profiling in Promising Resistant Grapevine Hybrids Including 17 Novel Breeds in Northern Italy. J. Sci. Food Agric., 2021, 101 (6), 2380–2388. https://doi.org/10.1002/jsfa.10861.

[32] Samoticha, J.; Wojdyło, A.; Golis, T. Phenolic Composition, Physicochemical Properties and Antioxidant Activity of Interspecific Hybrids of Grapes Growing in Poland. Food Chem., 2016. https://doi.org/10.1016/j.foodchem.2016.07.147.

[33] Chen, Y.; Pan, H.; Hao, S.; Pan, D.; Wang, G.; Yu, W. Evaluation of Phenolic Composition and Antioxidant Properties of Different Varieties of Chinese Citrus. Food Chem., 2021, 364 (January), 130413. https://doi.org/10.1016/j.foodchem.2021.130413.

[34] Rasines-Perea, Z.; Teissedre, P. L. Grape Polyphenols' Effects in Human Cardiovascular Diseases and Diabetes. Molecules, 2017, 22 (1), 1–19. https://doi.org/10.3390/molecules22010068.

[35] Gill, N. K.; Rios, D.; Osorio-camacena, E.; Mojica, B. E.; Kaur, B.; Soderstrom, M. A.; Gonzalez, M.; Poblete, C.; Kaur, N.; Singh, H.; et al. Anticancer Effects of Extracts from Three Different Chokeberry Species Anticancer Effects of Extracts from Three Different Chokeberry Species. Nutr. Cancer, 2020, 0 (0), 1–7. https://doi.org/10.1080/01635581.2020.1789679.

[36] Gustavo, L.; Ponte, S.; Carolina, I.; Pavan, B.; Camargo, M.; Mancini, S.; Guilherme, L.; Morelli, A. P.; Severino, M. B.; Maria, R.; et al. The Hallmarks of Flavonoids in Cancer. 2021, 1–55.

[37] Kopustinskiene, D. M.; Jakstas, V.; Savickas, A.; Bernatoniene, J. Flavonoids as Anticancer Agents. 2020, No. C, 1–25.

[38] Xia, L.; Xu, C.; Huang, K.; Lu, J.; Zhang, Y. Evaluation of Phenolic Compounds, Antioxidant and Antiproliferative Activities of 31 Grape Cultivars with Different Genotypes. J. Food Biochem., 2019, 43 (6), 1–13. https://doi.org/10.1111/jfbc.12626.

[39] Yang, L.; Gao, S.; Su, Z.; Qin, X.; Li, Z. Journal of Pharmaceutical and Biomedical Analysis Identification of the Constituents and the Cancer-Related Targets of the Fruit of Solanum Nigrum Based on Molecular Docking and Network Pharmacology. J. Pharm. Biomed. Anal., 2021, 200, 114067. https://doi.org/10.1016/j.jpba.2021.114067.

[40] Maksoud, S.; Abdel-massih, R. M.; Rajha, H. N.; Louka, N.; Chemat, F.; Barba, F. J. Citrus Aurantium L. Active Constituents, Biological Effects and Extraction Methods. An Updated Review. 2021, 1–18.

[41] Fakhri, S.; Khodamorady, M.; Naseri, M.; Farzaei, M. H.; Khan, H. The Ameliorating Effects of Anthocyanins on the Cross-Linked Signaling Pathways of Cancer Dysregulated Metabolism. Pharmacol. Res., 2020, 159, 104895. https://doi.org/10.1016/j.phrs.2020.104895.

[42] Carlos, A.; Pinto, D. Q.; Hugo, V.; Ramos, V. Boletim de Pesquisa. 2004.

[43] da Silva, M. J. R.; da Silva Padilha, C. V.; dos Santos Lima, M.; Pereira, G. E.; Filho, W. G. V.; Moura, M. F.; Tecchio, M. A. Grape Juices Produced from New Hybrid Varieties Grown on Brazilian Rootstocks – Bioactive Compounds, Organic Acids and Antioxidant Capacity. Food Chem., 2019, 289 (September 2018), 714–722. https://doi.org/10.1016/j.foodchem.2019.03.060.

[44] Perera, S.; Silva, A. B. G.; Amarathunga, Y.; Silva, S. De; Jayatissa, R.; Gamage, A.; Merah, O.; Madhujith, T. Nutritional Composition and Antioxidant Activity of Selected Underutilized Fruits Grown in Sri Lanka. 2022.

[45] Balasubramani, S. P. Ação Sinérgica de Stilbenes No Extrato de Amora de Uva Muscadina Mostra Melhor Potencial Citotóxico Contra Células Cancerígenas Do Que Resveratrol Sozinho. 2019, 7 (4). https://doi.org/10.3390/biomedicinas7040096.

[46] Oskarsson, T. Stress-Induced Metastatic Niches in Breast Cancer. Mol. Cell. Oncol., 2020, 00 (00). https://doi.org/10.1080/23723556.2020.1780105.

[47] Montenegro, J.; dos Santos, L. S.; de Souza, R. G. G.; Lima, L. G. B.; Mattos, D. S.; Viana, B. P. P. B.; da Fonseca Bastos, A. C. S.; Muzzi, L.; Conte-Júnior, C. A.; Gimba, E. R. P.; et al. Bioactive Compounds, Antioxidant Activity and Antiproliferative Effects in Prostate Cancer Cells of Green and Roasted Coffee Extracts Obtained by Microwave-Assisted Extraction (MAE). Food Res. Int., 2021, 140 (December 2020). https://doi.org/10.1016/j.foodres.2020.110014.

[48] Kelly, E.; Vyas, P.; Weber, J. T. Biochemical Properties and Neuroprotective Effects of Compounds in Various Species of Berries. Molecules, 2018, 23 (1). https://doi.org/10.3390/molecules23010026.

[49] Olivati, C.; de Oliveira Nishiyama, Y. P.; de Souza, R. T.; Janzantti, N. S.; Mauro, M.

A.; Gomes, E.; Hermosín-Gutiérrez, I.; da Silva, R.; Lago-Vanzela, E. S. Effect of the Pre-Treatment and the Drying Process on the Phenolic Composition of Raisins Produced with a Seedless Brazilian Grape Cultivar. Food Res. Int., 2019, 116 (April 2018), 190–199. https://doi.org/10.1016/j.foodres.2018.08.012.

[50] Tabeshpour, J.; Mehri, S.; Shaebani Behbahani, F.; Hosseinzadeh, H. Protective Effects of Vitis Vinifera (Grapes) and One of Its Biologically Active Constituents, Resveratrol, against Natural and Chemical Toxicities: A Comprehensive Review. Phyther. Res., 2018, 32 (11), 2164–2190. https://doi.org/10.1002/ptr.6168.

[51] Nassiri-Asl, M.; Hosseinzadeh, H. Review of the Pharmacological Effects of Vitis Vinifera (Grape) and Its Bioactive Constituents: An Update. Phyther. Res., 2016, No. April, 1392–1403. https://doi.org/10.1002/ptr.5644.

[52] Ladeia, D. N.; Costa, A. luiza melo lima; Vieira, A. L. C.; Ramos, D. S.; Coelho, J. L.; Lima, L. R. de O.; Santos, L. P. de F.; Violante, P. M.; Bravim, P. M. V.; Lopes, A. G. Fatores de Proteção e Ações de Prevenção Para o Câncer de Colo de Útero: Uma Revisão Integrativa Dos Últimos 10 Anos. Revista Eletrônica Acervo Saúde. 2020, p e3923. https://doi.org/10.25248/reas.e3923.2020.

[53] Mitchell, N.; Campbell, L. G.; Ahern, J. R.; Paine, K. C.; Giroldo, A. B.; Whitney, K. D. Correlates of Hybridization in Plants. 2019, 570–585. https://doi.org/10.1002/evl3.146.

[54] Orsavova, J.; Misurcova, L.; Vavra 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 (6), 12871–12890. https://doi.org/10.3390/ijms160612871.

[55] Klinder, A.; Shen, Q.; Heppel, S.; Lovegrove, J. A. Function Impact of Increasing Fruit and Vegetables And. 2016. https://doi.org/10.1039/c5fo01096a.

[56] Zhang, Y.; Sun, Y.; Xi, W.; Shen, Y.; Qiao, L.; Zhong, L.; Ye, X.; Zhou, Z. Phenolic Compositions and Antioxidant Capacities of Chinese Wild Mandarin (Citrus Reticulata Blanco) Fruits Key Laboratory of Horticulture Science for Southern Mountainous Regions ,. FOOD Chem., 2013. https://doi.org/10.1016/j.foodchem.2013.08.012.

[57] Blando, F.; Berland, H.; Maiorano, G.; Durante, M.; Mazzucato, A.; Picarella, M. E.; Nicoletti, I.; Gerardi, C.; Mita, G.; Andersen, Ø. M. Nutraceutical Characterization of

Anthocyanin-Rich Fruits Produced by “ Sun Black ” Tomato Line Plant Material (Genetic Characteristics Of. 2019, 6 (August). https://doi.org/10.3389/fnut.2019.00133.

[58] Mahmoud, A. M.; Bautista, R. J. H.; Sandhu, M. A.; Hussein, O. E. Review Article Beneficial Effects of Citrus Flavonoids on Cardiovascular and Metabolic Health. 2019, 2019. https://doi.org/10.1155/2019/5484138.

[59] Stevens, J. F.; Revel, J. S.; Maier, C. S. Mitochondria-Centric Review of Polyphenol Bioactivity in Cancer Models. Antioxidants Redox Signal., 2018, 29 (16), 1589–1611. https://doi.org/10.1089/ars.2017.7404.

[60] Alappat, B.; Alappat, J. Anthocyanin Pigments: Beyond Aesthetics. Molecules, 2020, 25 (23). https://doi.org/10.3390/molecules25235500.

[61] Fraga, C. G.; Croft, K. D.; Kennedy, D. O.; Tomás-Barberán, F. A. The Effects of Polyphenols and Other Bioactives on Human Health. Food Funct., 2019, 10 (2), 514–528. https://doi.org/10.1039/c8fo01997e.

[62] Mattioli, R.; Francioso, A.; Mosca, L.; Silva, P. Anthocyanins: A Comprehensive Review of Their Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative Diseases. Molecules. 2020. https://doi.org/10.3390/molecules25173809.

[63] Campeanu, G.; Neata, G.; Darjanschi, G. Chemical Composition of the Fruits of Several Apple Cultivars Growth as Biological Crop. Not. Bot. Horti Agrobot. Cluj-Napoca, 2009, 37 (2), 161–164.

[64] Bassanesi, G.; Andrade Touguinha, L. B.; Salvador, M. Capacidade Antioxidante de Sucos e Néctares de Uvas Do Rio Grande Do Sul. Rev. Int. Ciências, 2020, 10 (2), 55–67. https://doi.org/10.12957/ric.2020.48081.

[65] Wei, Z.; Liu, X.; Huang, Y.; Lu, J.; Zhang, Y. Volatile Aroma Compounds in Wines from Chinese Wild/Hybrid Species. J. Food Biochem., 2019, 43 (10). https://doi.org/10.1111/jfbc.12684.

[66] Sommella, E.; Pagano, F.; Pepe, G.; Ostacolo, C.; Manfra, M.; Chieppa, M.; Sanzo, D.; Carabetta, S.; Russo, M. Flavonoid Composition of Tarocco (Citrus Sinensis L . Osbeck) Clone “ Lempso ” and Fast Antioxidant Activity Screening By. 2017, No. April. https://doi.org/10.1002/pca.2701.

[67] Raghavan, S.; Gurunathan, J. Citrus Species – a Golden Treasure Box of Metabolites

That Is Beneficial against Disorders. J. Herb. Med., 2021, 28 (September 2019), 100438. https://doi.org/10.1016/j.hermed.2021.100438.

[68] Sanjust, E.; Mocci, G.; Zucca, P.; Rescigno, A. Mediterranean Shrubs as Potential Antioxidant Sources. Nat. Prod. Res., 2008, 22 (8), 689–708. https://doi.org/10.1080/14786410801997125.

[69] Dembitsky, V. M.; Poovarodom, S.; Leontowicz, H.; Leontowicz, M.; Vearasilp, S.; Trakhtenberg, S.; Gorinstein, S. The Multiple Nutrition Properties of Some Exotic Fruits: Biological Activity and Active Metabolites. Food Res. Int., 2011, 44 (7), 1671–1701. https://doi.org/10.1016/j.foodres.2011.03.003.

[70] Yu, Y.; Guo, D.; Li, G.; Yang, Y.; Zhang, G.; Li, S.; Liang, Z. The Grapevine R2R3-Type MYB Transcription Factor VdMYB1 Positively Regulates Defense Responses by Activating the Stilbene Synthase Gene 2 (VdSTS2). BMC Plant Biol., 2019, 19 (1). https://doi.org/10.1186/s12870-019-1993-6.

[71] Aguiñiga-s, I.; Soto-hern, M.; Cadena-iñiguez, J.; Mendoza-n, M.; Santiago-osorio, E. Anti-Inflammatory Capacity of the Extracts of Fruits of the Sechium Hybrid. 2020, 10.

[72] Aguiñiga-Sánchez, I.; Soto-Hernández, M.; Cadena-Iñiguez, J.; Suwalsky, M.; Colina, J. R.; Castillo, I.; Rosado-Pérez, J.; Mendoza-Núñez, V. M.; Santiago-Osorio, E. Phytochemical Analysis and Antioxidant and Anti-Inflammatory Capacity of the Extracts of Fruits of the Sechium Hybrid. Molecules, 2020, 25 (20). https://doi.org/10.3390/molecules25204637.

[73] Gorinstein, S.; Poovarodom, S.; Leontowicz, H.; Leontowicz, M.; Namiesnik, J.; Vearasilp, S.; Haruenkit, R.; Ruamsuke, P.; Katrich, E.; Tashma, Z. Antioxidant Properties and Bioactive Constituents of Some Rare Exotic Thai Fruits and Comparison with Conventional Fruits In Vitro and in Vivo Studies. FRIN, 2011, 44 (7), 2222–2232. https://doi.org/10.1016/j.foodres.2010.10.009.

[74] Liang, N. N.; Pan, Q. H.; He, F.; Wang, J.; Reeves, M. J.; Duan, C. Q. Phenolic Profiles of Vitis Davidii and Vitis Quinquangularis Species Native to China. J. Agric. Food Chem., 2013, 61 (25), 6016–6027. https://doi.org/10.1021/jf3052658.

[75] Tassoni, A.; Zappi, A.; Melucci, D.; Reisch, B. I.; Davies, P. J. Seasonal Changes in Amino Acids and Phenolic Compounds in Fruits from Hybrid Cross Populations of American Grapes Differing in Disease Resistance. Plant Physiol. Biochem., 2018.

https://doi.org/10.1016/j.plaphy.2018.11.034.

[76] Gazzola, R.; Dalla Porta Gründling, R.; Araújo Aragão, A. A Produção E O Comércio Internacional De Uva. Revista Brasileira de Agrotecnologia. 2020, pp 68–74. https://doi.org/10.18378/rebagro.v10i3.8011.

[77] Correa, M. G.; Couto, J. S.; Trindade, B. B.; Abreu, J. P.; Nakajima, V. M.; Oliveira, F. L.; Farah, A.; Teodoro, A. J. Antiproliferative Effect of Guava Fruit Extracts in MDA-MB-435 and MCF- 7 Human Breast Cancer Cell Lines. 2020, 92, 1–20. https://doi.org/10.1590/0001-3765202020191500.

[78] Correa, M. G.; Couto, J. S.; Teodoro, A. J. Anticancer Properties of Psidium Guajava - a Mini-Review. Asian Pacific J. Cancer Prev., 2016, 17 (9), 4199–4204.

[79] Soares, N. da C. P.; Teodoro, A. J.; Lotsch, P. F.; Granjeiro, J. M.; Borojevic, R. Anticancer Properties of Carotenoids in Prostate Cancer. A Review. Histol. Histopathol., 2015, 30 (10), 1143–1154. https://doi.org/10.14670/HH-11-635.

[80] Yaman, M.; Uzun, A. Evaluation of Superior Hybrid Individuals with Intra and Interspecific Hybridization Breeding in Apricot Evaluation of Superior Hybrid Individuals with Intra and Interspecific Hybridization Breeding in Apricot. Int. J. Fruit Sci., 2020, 20 (3), 2045–2055. https://doi.org/10.1080/15538362.2020.1852151.

[81] Spada, V.; Di Stasio, L.; Ferranti, P.; Addeo, F.; Mamone, G.; Picariello, G. Differential Protein Expression in Berry Skin from Red Grapes with Varying Hybrid Character. Int. J. Mol. Sci., 2022, 23 (3). https://doi.org/10.3390/ijms23031051.

[82] López-Caamal, A.; Tovar-Sánchez, E. Genetic, Morphological, and Chemical Patterns of Plant Hybridization. Revista Chilena de Historia Natural. 2014, pp 1–14. https://doi.org/10.1186/s40693-014-0016-0.

[83] Alamu, E. O.; Dixon, B. M.; Menkir, A.; Ogunlade, A. O. Harvesting Time and Roasting Effects on Colour Properties , Xanthophylls , Phytates , Tannins and Vitamin C Contents of Orange Maize Hybrid. Sci. Rep., 2020, 1–7. https://doi.org/10.1038/s41598-020-78433-9.

[84] Tel-Zur, N. Breeding an Underutilized Fruit Crop: A Long-Term Program for Hylocereus. Horticulture Research. 2022, pp 1–13. https://doi.org/10.1093/hr/uhac078.

[85] Li, A. N.; Li, S.; Zhang, Y. J.; Xu, X. R.; Chen, Y. M.; Li, H. Bin. Resources and Biological Activities of Natural Polyphenols. Nutrients, 2014, 6 (12), 6020–6047.

https://doi.org/10.3390/nu6126020.

[86] Colombo, M.; Masiero, S.; Rosa, S.; Caporali, E.; Toffolatti, S. L.; Mizzotti, C.; Tadini, L.; Rossi, F.; Pellegrino, S.; Musetti, R.; et al. NoPv1: A Synthetic Antimicrobial Peptide Aptamer Targeting the Causal Agents of Grapevine Downy Mildew and Potato Late Blight. Sci. Rep., 2020, 10 (1). https://doi.org/10.1038/s41598-020-73027-x.

[87] Veena, G.; Challa, S. R.; Palatheeya, S.; Prudhivi, R.; Kadari, A. Granny Smith Apple Extract Lowers Inflammation and Improves Antioxidant Status in L-Arginineinduced Exocrine Pancreatic Dysfunction in Rats. Turkish J. Pharm. Sci., 2021, 18 (3), 262–270. https://doi.org/10.4274/tjps.galenos.2020.92145.

[88] Wolfe, K.; Wu, X.; Liu, R. H. Antioxidant Activity of Apple Peels. J. Agric. Food Chem., 2003, 51 (3), 609–614. https://doi.org/10.1021/jf020782a.

[89] Sridharan, K.; Sivaramakrishnan, G. Interaction of Citrus Juices with Cyclosporine: Systematic Review and Meta-Analysis. Eur. J. Drug Metab. Pharmacokinet., 2016, 41 (6), 665–673. https://doi.org/10.1007/s13318-016-0351-4.

[90] de Castro, W. V.; Mertens-Talcott, S.; Derendorf, H.; Butterweck, V. Grapefruit Juice–Drug Interactions: Grapefruit Juice and Its Components Inhibit P-Glycoprotein (ABCB1) Mediated Transport of Talinolol in Caco-2 Cells. J. Pharm. Sci., 2007, 96 (10), 2808–2817. https://doi.org/10.1002/jps.20975.

[91] El-Hawary, S. S.; Mohammed, R.; El-Din, M. E.; Hassan, H. M.; Ali, Z. Y.; Rateb, M. E.; Bellah El Naggar, E. M.; Othman, E. M.; Abdelmohsen, U. R. Comparative Phytochemical Analysis of Five Egyptian Strawberry Cultivars (Fragaria × Ananassa Duch.) and Antidiabetic Potential of Festival and Red Merlin Cultivars. RSC Adv., 2021, 11 (27), 16755–16767. https://doi.org/10.1039/D0RA10748D.

[92] Tommonaro, G.; Speranza, G.; De Prisco, R.; Iodice, C.; Crudele, E.; Abbamondi, G. R.; Nicolaus, B. Antioxidant Activity and Bioactive Compound Contents before and after in Vitro Digestion of New Tomato Hybrids. J. Sci. Food Agric., 2017, 97 (15), 5241–5246. https://doi.org/10.1002/jsfa.8408.

[93] Madeira, P. M. R.; Macedo, G. R. de. Agregação de Valor Ao Resíduo de Melão: Caracterização, Avaliação de Atividade Antioxidante, Antiproliferativa, Potencial Prebiótico e Produção de Enzimas, Universidade Federal do Rio Grande do Norte, 2017.

[94] Bernardi, D. M.; Mello, H. R. L. S.; Almeida, L. T.; Marangon, L. M. Composiçoes e Propriedades Funcionais de Frutas, Bebidas e Temperos, 1st ed.; Cascavel, Paraná, 2019.

[95] Kolayli, S.; Kara, M.; Tezcan, F.; Erim, F. B.; Sahin, H.; Ulusoy, E.; Aliyazicioglu, R. Comparative Study of Chemical and Biochemical Properties of Different Melon Cultivars: Standard, Hybrid, and Grafted Melons. J. Agric. Food Chem., 2010, 58 (17), 9764–9769. https://doi.org/10.1021/jf102408y.

[96] Musa, C. I.; Weber, B.; Gonzatti, H. C.; Biguelini, C. B.; Souza, C. F. V. de; Oliveira, E. C. Avaliação Do Teor De Vitamina C Em Morangos De Diferentes Cultivares Em Sistemas De Cultivo Distintos No Município De Bom Princípio/Rs. Ciência e Nat., 2015, 37 (2), 368–373. https://doi.org/10.5902/2179460x16810.

CAPÍTULO II

Progressos recentes em aplicações de uvas na saúde humana: uma visão geral com o conhecimento atual

Recent Progress in grapes applications on human health: an overview with current knowledge.

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Abstract

Grapes, both as a fruit and derivative products, have long been associated with numerous health benefits. Rich in antioxidants, polyphenols, vitamins, and minerals, grapes have played a pivotal role in traditional medicines and have more recently attracted scientific interest due to their potential therapeutic and preventative properties. This review aims to consolidate recent advancements in the understanding of grapes and their effects on human health, highlighting

the latest scientific evidence that underscores their potential benefits and mechanisms of action. A comprehensive search of databases, including PubMed/Medline, Scopus, and Web of Science, was performed. Peer-reviewed articles focusing on *in vivo, in vitro*, and clinical studies related to grape consumption and its correlation with human health were included. Studies focusing solely on wine or other grape-derived alcoholic beverages were excluded. Findings indicate a positive correlation between grape consumption and a reduced risk of chronic diseases including cardiovascular disease, type II diabetes, and certain types of cancer. Grape compounds, particularly resveratrol and other polyphenols, demonstrated potential neuroprotective effects, improved endothelial function, and antioxidant properties. Additionally, new evidence suggests that grapes can influence gut microbiota composition, which in turn can have beneficial effects on metabolic health and immunity.

Grapes, with their rich phytochemical content, present promising health benefits. The recent findings offer substantial support for the continued exploration of grapes in dietary and therapeutic applications. While a significant correlation has been established between grape consumption and enhanced health outcomes, further long-term and large-scale clinical studies are warranted to solidify recommendations and understand the breadth of their health-promoting properties.

KEYWORDS: grapes, polyphenols, antioxidants, phenolic compounds, cardioprotective, antioxidant, chronic diseases, health benefits

1. INTRODUCTION

The Portuguese brought the first varieties of grapes to Brazil. They cultivated the fine grape (*Vitis vinifera*) in Europe and selected it through the knowledge of the European winemakers. Brazilian viticulture, however, was only consolidated in the mid-nineteenth century, when Italian immigrants brought the American grape Isabel (*Vitis labrusca*), replacing the vineyards of European grapes. The first cycle of expansion of Brazilian viticulture, therefore, was based on the cultivation of American grapes, rustic and adapted to local soil and climate conditions. This phase established new directions for the technification of the national viticulture, mainly aiming to prevent the attack of pests and diseases (Camargo et al., 2010). In the twentieth century, fine grapes returned to production to produce wines and for consumption in natura. Commercial-scale production initiatives of fine table grapes in the northeastern semiarid region mark the beginning of tropical viticulture in Brazil. New production centers of fine table grapes in tropical conditions appear in the regions of the North of Paraná, Northwest of São Paulo and

North of Minas Gerais, which stimulated the appearance of new planting areas, indicating an expansion trend of the culture in the country, supporting the development and the adoption of new technologies that contribute to the establishment of viticulture as an economically profitable activity in the country (Camargo et al., 2010; Wijekoon et al., 2022). Due to selection, accidental crosses, or breeding processes between domesticated grapes and native wild grapes, some American cultivars have been developed. For example, native grape cultivars such as *V. labrusca* and *V. aestivalis* were used to develop Concord and Norton grapes, respectively. Seedless grape varieties were also developed to meet the demand for table grapes based on consumer preference, but researchers are now discovering that many health benefits of grapes may come from the seeds, as well as due to their enriched phytochemical composition and bioactive molecules (Wijekoon et al., 2022). Varieties of European origin, the *Vitis vinifera*, can be producers of table grapes, such as the Italian, known as lady's finger and the Ruby, or producers of wines such as the famous, Cabernet sauvignon, Merlot, Chardonnay, Sauvignon Blanc, Moscato and Pinot noir among many others that worldwide are used to produce wines and sparkling wines called fine. These varieties are difficult to grow, have low productivity and require special climate conditions for their fruits to give rise to differentiated products (Sabo et al., 2022). A major limiting factor to the growth of exports from the São Francisco Vale in the mid-1990s was the production concentrated on cultivars of table grapes with seeds, especially the cultivar Italy. The need for introduction, adaptation and technical and economic viability of cultivars of seedless grapes became, at that time, the biggest challenge for producers, companies and research institutions. The research conducted by Brazilian Agricultural Research Corporation (EMBRAPA) Semiarid and partner Institutions aiming the introduction and evaluation of new cultivars was intensified from 1994 (M. C. Ribeiro, 2016). The first commercial seedless grape cultivars 'Thompson Seedless', 'Sugraone' and 'Crimson Seedless' presented limiting characteristics such as low bud fertility, low productivity, sensitivity to berry cracking and diseases, and production concentrated in one crop per year with a high degree of risk. The significant reduction in the economic profitability of grape production of these cultivars, a consequence of the high cost of production, crop losses caused by rain and diseases, combined with unstable economic scenarios, brought a second challenge to the productive sector and research institutions: the need to introduce and develop new cultivars adapted to produce two crops per year and with high and stable yields (Ribeiro, 2016; Table et al., 2020; Zecca et al., 2020). Thus, in the last decade, major changes have been observed in the production chain of table grapes, especially the diversification of cultivars and the increase in the supply of seedless grapes in the domestic market. More than 20 cultivars of table grapes

developed by EMBRAPA and international private breeding companies are currently cultivated (Ribeiro, 2016). The foreign table grape cultivars introduced in the region play an important role in the changes observed in the production system, with positive impact on economic profitability and strengthening of the production chain. However, the lack of adaptation of part of the selections and cultivars to the environmental conditions of the tropical semi-arid region, the susceptibility to disease, the cost of the license (royalties) for their production, and the restrictions on the size of cultivated areas and number of companies licensed, imposed by private breeding companies, emphasize the need for technological independence of the country through the development of Brazilian table grape cultivars adapted to the environmental conditions of the main producing regions(Camargo et al., 2010; de Freitas Laiber Pascoal et al., 2022; M. C. Ribeiro, 2016; Table et al., 2020). Studies analyzed for this review suggest as the main cultivars developed by different international breeding companies, found in the main producing countries of table grapes: • White grapes: Array 15®(Grape), Sugar Crisp®, *Sweet* Globe®, Cotton Candy® (International Fruit Genetics – IFG) e Autumn Crisp® (Sun Word). • Red grapes: *Sweet* Celebration®, Candy Snaps® (IFG), Timko® (She gene), Scar lotta Seedless® (Sun World) • Black grapes: *Sweet Sapphire*® (IFG), Sable®, Midnight Beauty® (Sun World).

They can be cited as examples, since more than 70 cultivars developed by these companies are available to licensed producers (Camargo et al., 2010; da Silva et al., 2019). The grape is the third most exported fruit in Brazil, after mango and melon, with the São Francisco Vale accounting for 99% of total Brazilian grape exports since the year 2002, with volumes reaching 45,000 tons in 2019. Besides being economically important, its cultivation contributes to society in the generation of jobs, generating up to five direct jobs per hectare (Landau, 2020). In such a large production, there is not to be alarmed the existence of waste, which represents 40% (Almeida et al., 2019), resulting from the non-following of the quality parameters determined by the exporters causes the discarding of the non-suitable ones. They take as determinants of the standard, characteristics such as: coloration, caliber (mm), acidity, size of the bunch, quantity of bunches inside the bowl, weight of the bowl, presence of physical contaminants and rotten berries, organization of the bowls in the box (Rotili et al., 2022). Being a great challenge for the industry, the transformation of this waste into value-added by-products, destining them to reprocessing, so that they do not generate negative impacts. According to Caldas et al. (2018), the waste generated from wine and juice production in 2013, almost 3.8 tons of grape pomace, caused environmental, social and economic problems. This destination of by-products being, a major challenge for wine industry Sugars (glucose and fructose),

organic acids (malic, tartaric, citric, lactic, acetic, and succinic acids), amino acids (arginine, proline, alanine, ammonium, g-amino butyric acid, cystathionine, and glutamic acid), peptides, proteins, vitamins (thiamine, riboflavin, pyridoxine, α- tocopherol, choline, folate, niacin, and ascorbic acid), carotenoids (lutein, β-carotene, neochrome, neoxanthin, violaxanthin, luteoxanthin, flavaxanthin, and zeaxanthin), flavor components (β-ionone, β- damascene, furaneol, and 2-phenylethanol), and phenolic compounds (iso flavonoids, anthocyanins, flavanols, flavanols [quercetin,] proanthocyanins, hydroxycinnamic and hydroxybenzoic acids, stilbenes, lineoids, coumarins, anthocyanins, catechin, and epicatechin) are the chemical composition of grapes (Tabeshpour et al., 2018). Grapes can have different profiles of phenolic compounds, which in wines, can be altered by their processing or type (Gomez et al., 2020; Nassiri-Asl & Hosseinzadeh, 2016; Tabeshpour et al., 2018; Zhu et al., 2021). Thus, some wines may be genetically richer in polyphenols than others. Therefore, quantitative and qualitative analysis on the distribution of polyphenols in seeds and skins among grape varieties should be conducted separately about their health benefits.

2. REVIEW METHODOLOGY

The primary aim of this review was to collate and present recent progress regarding the applications of grapes in human health, highlighting their composition and potential health benefits. To gather the requisite data, we systematically searched the following databases: PubMed/Medline, Web of Science, Wiley, Cielo using the following Medical Subject Headings (MeSH) terms to ensure a comprehensive search: “Antioxidants/analysis”, “Flavonoids/analysis”, “Health Promotion”, “Humans”, “Phenols/analysis”, “Phytochemicals/analysis”, “Plant Extracts/chemistry”, “Vitis/chemistry”, “Wine”. The search was limited to articles published from 2018 up to August 08, 2022. Articles were considered for inclusion if they were full-length articles, were available in English, Spanish, or Portuguese; could be accessed freely or through payment. Exclusion criteria: abstracts of any kind; papers presented at conferences, symposia, and those included in annals; graduation course conclusion papers; articles published in languages other than English, Spanish, or Portuguese. After the search was completed, the identified papers that met the inclusion criteria, specifically those evaluating studies on hybrid grape composition, were added to a spreadsheet for more manageable data analysis and synthesis. The outcome of this review aimed to provide an up-to-date overview of the current knowledge on grapes' applications in human health, emphasizing their chemical compositions and associated health benefits and the most representative data were summarized in figures and tables.

3. BIOACTIVE COMPOUNDS FROM GRAPES

The grape can be used for table consumption, such as juices, oils, jellies and, mainly, in the form of wine, which has been closely associated with food, particularly in Mediterranean countries, and for many years, moderate and regular wine consumption was associated with health benefits (Daldoul et al., 2020; Kowalczyk et al., 2022; Olivati et al., 2019). In the last two decades, several epidemiological and clinical studies around the world have pointed out that moderate drinking produces positive effects on antioxidant capacity (Barbalho et al., 2020; Muñoz-Bernal et al., 2021; Yu et al., 2019) lipid profile, and the coagulation system, which may be associated with decreased incidence of cardiovascular disease, overall mortality, and other chronic diseases (Rasines-perea & Teissedre, 2017; Zhu et al., 2021). Most studies focus their attention on red wine components (mainly polyphenols and especially resveratrol) with the aim of explaining the observed relationship between wine consumption and cardiovascular disease incidence (Sabra et al., 2021; Zhu et al., 2021). Although the chemical components of grapes and wine may vary, similar beneficial effects have been observed in red wine varieties related to their higher polyphenol content (Rasines-perea & Teissedre, 2017).

Polyphenols form a group of bioactive compounds. They have in their chemical structure multiple units of phenol and external elements that link these rings together. Thus, distinctions are made between phenolic receptors, stilbenes (resveratrol), chlorogenic acid, coumarins, lignin and flavonoids. Flavonoids are the most abundant polyphenols in our diets. They can be divided into several classes according to their common structure composed of 2 aromatic rings joined by 3 carbon atoms that form an oxygenated heterocycle. It can be divided into subclasses depending on the type of heterocycle involved: flavones, flavanols, isoflavones, flavanones, anthocyanidins, catechins and proanthocyanins (Hou et al., 2019; Muñoz-Bernal et al., 2021; Nassiri-Asl & Hosseinzadeh, 2016; Sabra et al., 2021). Dietary intake of polyphenols is highly variable, and it is difficult to achieve an accurate estimate of dietary intake of polyphenols due to poor characterization of polyphenols in foods and the large variability in polyphenol content within foods (Rasines-perea & Teissedre, 2017). Several factors can affect the content of polyphenols in daily foods, such as environmental conditions, storage, and food processing. For example, sun exposure, rainfall, different crop types, and degree of ripeness can affect polyphenol concentrations and proportions in different ways (Jideani et al., 2021). Generally, phenolic acid concentrations decrease during ripening, while anthocyanin concentrations increase (Swallah et al., 2020; Xu et al., 2011; Zhu et al., 2021).

In addition to polyphenol compounds, grape seed oil also contains healthy fatty acids, particularly unsaturated fatty acids such as linoleic and oleic acids that increase the nutritional

value of grape oil when used in food or dietary supplements (Jing et al., 2014; Orsavova et al., 2015; Tabeshpour et al., 2018). Studies have shown that grape seed oil displays anti-inflammatory, antioxidant, cardioprotective, and anticancer properties, which may be due to the occurrence of linoleic acid, tocopherol, carotenoids, phytosterol, in addition to some polyphenols compounds such as proanthocyanins, resveratrol, and quercetin (Dave et al., 2023; Vislocky & Fernandez, 2010).

The different chemical groups of polyphenols identified in different parts of fruits were summarized in **Figure 1**.

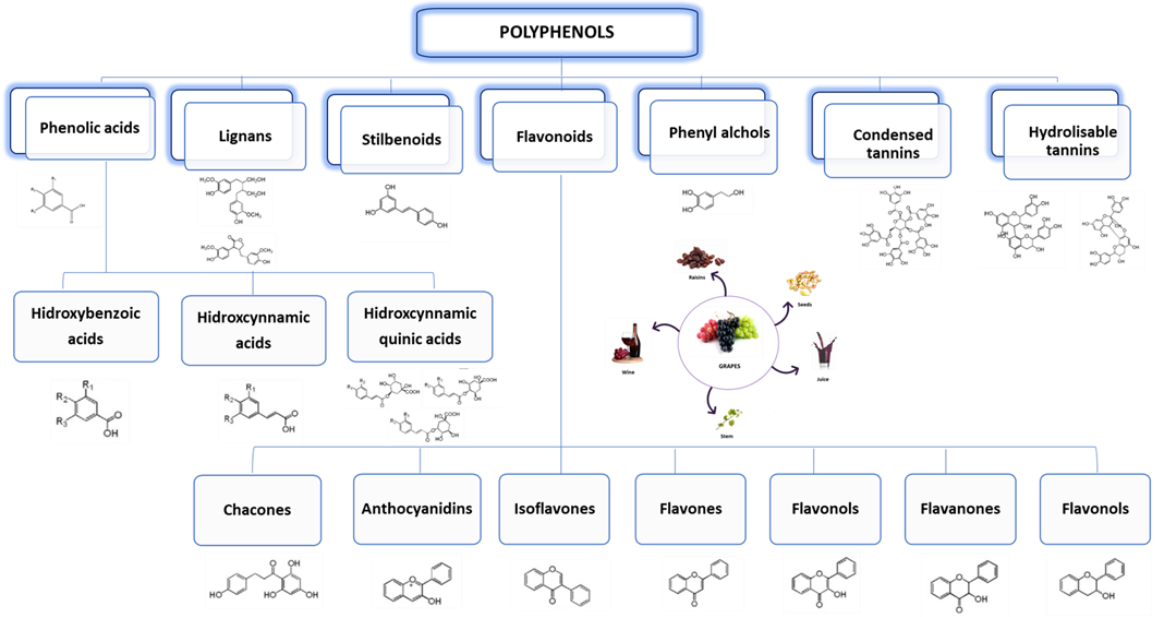

FIGURE 1 Chemical groups of polyphenols identified in different fruit parts (L. Zhang et al., 2021).

4. BIOAVAILABILITY, ABSORPTION, METABOLISM, AND EXCRETION OF BIOACTIVE COMPOUNDS FROM GRAPES

Polyphenols are the most numerous and widely distributed group of bioactive inclusions (Abbas et al., 2017) and present different sets of biological activities attributed to their particular characteristics and structural complexity, which interfere with the limit and speed of absorption in the intestines (Liu et al., 2019).

Flavones, isoflavones, flavanols and anthocyanins are frequently glycosylated (H. Zhang et al., 2014) in the gastrointestinal mucosa and colonic microflora. Anthocyanins are absorbed without de-glycosylation in the stomach, via specific transporters, such as sodium-dependent

glucose co-transporter 1 and facilitative glucose transporters 1, while in the small intestine, they are mainly absorbed as aglycones. High polymeric anthocyanins are easily degraded into low-polymeric forms or smaller phenolic acids by colonic microbiota, which improves their absorption (Panchal & Brown, 2023). In this way, we understand that polyphenols pass through the small intestine without being absorbed. Going through extensive metabolism and complex reactions, catalyzed by the intestinal microbiota, being biologically transformed into their relatively more bioavailable metabolites (Iannone et al., 2017). Derived metabolites circulate in plasma and can penetrate tissues (Del Rio et al., 2013; Manach et al., 2004). According to Williamson et al (2017) the maximum dosage of polyphenols in postprandial blood are generally less than 1uM, however for intestinal catabolic the maximum concentrations can reach 100 times more than the original compound. The elimination of polyphenols and their derivatives is carried out mainly via urinary and biliary excretion. Studies report that the half-life of these compounds in plasma varies from 2 to 3 hours for anthocyanins and flavanols, 4 to 8 hours for isoflavones and can reach 28 hours for quercetin. Demonstrating that the regular and frequent consumption of foods with these chemical compounds elevated these metabolites in the plasma (Percival & West, 2013). Anthocyanins for having modulatory effects on several signaling pathways involved in the cell cycle are seen in scientific circles as promising anticancer and antimutagenics therapies. On the other hand, due to the rapid oxidation of phenolic hydroxyl groups in quinones, anthocyanins are particularly unstable hydrophilic compounds. They also suffer the influence of external factors such as pH or temperature that can negatively affect their biological activities, which to be overcome, along with their pharmacokinetic limitations, designing new drugs release systems are of utmost importance to exert their potential health-promoting effects. Fruits and plants rich in anthocyanins indicated anticancer properties when targeting multiple cross-linked signaling pathways in cancer metabolism, including oxidative stress, inflammation, angiogenesis, and apoptosis (Fakhri et al., 2020).

5. HEALTH BENEFITS AND THERAPEUTIC POTENTIAL OF HYBRID GRAPES

5.1. Antioxidant and anti-inflammatory

Oxidative stress occurs by an imbalance between the systemic manifestation of reactive oxygen species and the ability of a biological system to promptly detoxify the reactive intermediates to repair the resulting damage(Rasines-perea & Teissedre, 2017; Zhu et al., 2021). This leads to the production of free radicals such as superoxide (O2.-), hydrogen peroxide (H2O2), hydroxyl radical (OH), peroxin trite (ONO-2-), and nitric oxide is necessary as they

have roles in growth, repair, and immune functions essential to human cells. In contrast, these molecules also can oxidize signaling molecules, DNA, macromolecules, and cellular structures, such as lipid membranes of healthy cells, all to the detriment of these cells (Rasines-perea & Teissedre, 2017; Zhu et al., 2021). Long-term oxidative stress has been associated with various diseases such as diabetes, chronic obstructive pulmonary disease, cardiovascular disease, cancer, and asthma. Several human studies have documented reductions in these diseases following grape supplementation. Studies have reported that aqueous extracts of *V. vinifera L*. have the potential to increase the antioxidant capacity of human keratinocytes NCTC 2544, which need sufficient antioxidant defenses, when exposed to oxidative stress (Li et al., 2014; Stevens & Revel, 2018). The oxidative activity of *V. vinifera L.* grapes has a direct relationship between total phenolic compounds and flavonoids (Yu et al., 2019). Polyphenols may affect vascular inflammation and injury not only as antioxidants but also as modulators of inflammatory redox signaling pathways(Li et al., 2014; Tabeshpour et al., 2018). One of the important anti-inflammatory mechanisms is the inhibition of eicosanoid-generating enzymes, including phospholipase A2 and cyclooxygenase. Polyphenols act by modulating cyclooxygenase-2 activity and gene expression in different cell types (Fraternale et al., 2011; Rocha et al., 2012; Tabeshpour et al., 2018).

Nitric oxide (NO) is an essential component in the maintenance of vascular health, and is a key intravascular antithrombotic factor, but causes inflammatory response if converted to peroxin trite in the presence of free radicals (Orsavova et al., 2015). Ribeiro (2018) measured the antioxidant capacity with different methodologies in in vitro tests, in several samples of grape pomace and demonstrated that the different samples had a high antioxidant capacity based on the DPPH, ABTS, FRAP methods and auto-oxidation by the β- system carotene/linoleic acid. About biological assays, the extracts of all grape marc samples reduced the formation of reactive oxygen species when evaluated in mitochondria isolated from the liver of Holtzman rats. The effect of the antioxidant potential can be verified through the tendency to restore the levels of oxidative stress in rats with induced arthritis. Proving to be an alternative source for functional foods, supplements or nutraceutical formulations (Bortolini et al., 2022). In this last study, no differences in superoxide dismutase activity were observed in the group with red wine or grape juice consumption, when compared to all groups(V. M. Ribeiro et al., 2018). A human study using a high-fat diet associated with wine consumption found the same results in variation in the activity of this enzyme (Di Renzo et al., 2014). Both the study by Hogan et al., (2010) on inflammation in Diet-Induced Obese Mice and the study by Nishiumi et al., (2012) on liver inflammation, observed that the use of grape products inhibited the activation of NF-κB, which

represents an inflammatory factor with a central role in several pro-inflammatory signaling pathways, the study demonstrated the suppression of the expression of the proteins COX-2 (cyclooxygenase-2) and iNOS (inducible nitric oxide synthase). iNOS leads to an excess of NO, which could act as a free radical, and COX-2 produces prostacyclin and prostaglandins, which are pro-inflammatory mediators. Thus, both suppressive actions demonstrate the anti-inflammatory activity of grape metabolites (Bocsan et al., 2022; Cruz-Machado, 2010; Gao & Zhang, 2021).

5.2. Antibacterial, antiviral and antifungal effects

Quercetin and resveratrol, both active polyphenols in grape muscadine skin extracts, have inhibitory effects against *H. pylori* with minimum bactericidal concentrations of 256 μg/mL and 128 μg/mL, respectively. Polyphenols in grape seed extract have antibacterial effects at a concentration of 3 mg/mL against methicillin-resistant Staphylococcus aureus, which may be by disrupting the cell wall or cell membrane (Nassiri-Asl & Hosseinzadeh, 2016), and also reduced ergosterol biosynthesis correlated with inhibition of folate pathways (Simonetti et al., 2020). There are relationships between antiviral activities of resveratrol and grapes (Blesso, 2019; Dave et al., 2023; Systems & Leporatti, 2022). Berardi et al. (2009) studied the effect of resveratrol against polyomavirus, which later in 2011 also showed anti-influenza activity (Dembitsky et al., 2011). The ethanolic extract of *V. vinifera L*. exhibits antifungal activities against *Fusarium,* demonstrating that high amounts of polyphenols in this plant play an important role on the fungal control (Gratl et al., 2021; Kelly et al., 2018). Study with cell culture, from monkeys, in vitro, suggests that grape juice may exhibit antiviral activity. The researchers reported reductions in infectivity to undetectable levels within minutes of exposure to 25–2,000 mg/mL of proanthocyanins from grape juice. There was also a great reduction in viral activity caused by the proanthocyanins in grape juice (Vislocky & Fernandez, 2010).

5.3. Anticancer effects

Grape seeds are rich in proanthocyanins, which have been shown to induce cancer cell apoptosis, reduce inflammation, and prevent the proliferation of cancer cells (Wang et al., 2020). These compounds have shown promise in preventing skin, breast, and colon cancer in pre-clinical models(Wang et al., 2019). They have also been studied for their potential to mitigate the side effects of chemotherapy. Found in dark-colored grapes, anthocyanins can interfere with the signaling pathways that cancer cells use to grow, communicate, and spread

(Posadino et al., 2023). Their potential anticancer effects have been observed against several cancer types, including leukemia, and liver and lung cancers.(Câmara et al., 2022). Flavonoids such as Quercetin, Myricetin can modulate several signaling pathways in cancer cells, affecting cell cycle arrest, apoptosis, and inflammation and their anticancer properties have been observed in different types of cancers of the breast, colon, and liver, among others (Sehitoglu et al., 2014). Resveratrol, a stilbene found primarily in grape skins, has been extensively studied for its anticancer effects (Ko et al., 2017). It's believed to act by modulating pathways related to cell division and apoptosis, inflammation, and angiogenesis. Studies have demonstrated the potential of resveratrol to inhibit the growth of multiple cancer types, including breast, prostate, lung, and colorectal cancers (Ko et al., 2017). Additionally, it has shown synergy when combined with traditional chemotherapeutic agents (Ko et al., 2017).

In a recent study, grape seed extract showed anticancer effects and induced apoptosis in colon cancer cell lines. Demonstrated that grape seed extract not only enhanced the anticancer effect of 5-fluorouracil in a dose-dependent manner *in vitro*, but also reduced 5-fluorouracil-induced mucositis in mice after chemotherapy, and its protective effect was more obvious in the proximal jejunum than in the distal small intestine (Cheah et al., 2014). The anticancer effects of resveratrol metabolites, including resveratrol-3-O-sulfate, resveratrol-3-O-glucuronide and resveratrol-4-O-glucuronide on colon cancer cells have been established. At a concentration of 30 μM, they inhibited the proliferation of metastatic colon cancer cells and caused strong cell accumulation in the S phase of the cell cycle. At concentrations of 10 or 20 μM, they showed synergistic chemotherapeutic effects with SN38 and oxaliplatin in metastatic colon cancer cells (SW620) (Nassiri-Asl & Hosseinzadeh, 2016; Soares et al., 2015). Udenigwe et al. (2008) reported the positive effects of bioactive grape compounds, mainly resveratrol exhibiting several physiological activities, especially anticancer and anti-inflammatory activities analyzed in vitro and in experimental animal and human models. The anticancer activity of this compound is mainly due to the induction of apoptosis by various pathways, as well as the alteration of gene expression, leading to a decrease in tumor initiation, promotion and progression. Other effects of anti-inflammatory activity are through modulation of enzymes and pathways that produce inflammation mediators and also induction of programmed cell death in activated immune cells (Aires et al., 2021; Ghate et al., 2014). A study comparing the effects of a polyphenol-rich grape pomace extract on redox status using *in vitro* and *in vivo* models demonstrated that the extract has potent antioxidant and chemo-preventive properties in vitro, as it scavenges free radicals (DPPH• or ABTS•+) and prevents DNA damage induced by ROO• and OH• radicals. It is established that ROO•. are the main initiating factors of the

cascade reactions of lipid peroxidation, causing the extract, in low concentrations, to be considered as a chemo preventive agent with ROO• and lipid peroxidation causes mutations in DNA. It also confirms that the extract has in vitro chemo preventive properties against the effects of UV radiation, since UV radiation is one of the main producers of OH•. These findings confirm the potent antioxidant and chemo preventive properties in vitro from other grape extracts of the Vitis vinifera species (Veskoukis et al., 2012).

5.4. Cardioprotective

Emerging studies have unveiled potential cardioprotective effects on hybrid grapes, possibly attributing to their polyphenolic content (Otręba et al., 2021). Resveratrol is known for its antioxidant properties; it aids in the prevention of LDL oxidation, thus preventing atherosclerosis (Otręba et al., 2021). Moreover, resveratrol improves nitric oxide production, promoting vasodilation and improved blood flow. Several clinical studies have demonstrated the ability of resveratrol to reduce inflammatory markers, improve lipid profiles, and enhance endothelial function, all of which are crucial for cardiovascular health(Fan et al., 2022, Otręba et al., 2021). Proanthocyanins are known to strengthen capillaries and reduce oxidative stress; they also play a role in reducing blood pressure and inhibiting platelet aggregation. Studies on patients with cardiovascular risks have shown that grape seed extracts can lead to reduced blood pressure and improved arterial function (Huang, 2023). Anthocyanins, present in dark-colored grapes, possess strong antioxidant capacities and they have been linked to reduced arterial stiffness, decreased LDL oxidation, and enhanced HDL levels (Câmara et al., 2022). Daily consumption of anthocyanin-rich foods has been associated with a reduced risk of myocardial infarction in some cohort studies (Xu et al., 2021). Flavonoids from grapes, such as quercetin, have been recognized for their potential to reduce inflammation, improve vascular function, modulate blood lipid metabolism and have anti-atherogenic properties(Kozłowska and Szostak-Węgierek, 2022). Clinical studies have showcased the potential of flavonoids to reduce systolic blood pressure and improve overall lipid profiles(Kozłowska and Szostak-Węgierek, 2022).

Platelet aggregation is involved in the development of atherosclerotic coronary artery disease, and inhibition of platelet aggregation is an accepted mechanism in cardio protection (Sabra et al., 2021). Polyphenols affect Apolipoprotein (apo) A and B, which are emerging as risk factors for cardiovascular disease, modify very low-density lipoprotein (VLDL) particles and reduce triglyceride (TG) levels due to the possible increase in lipoprotein (LPL) activity, which leads to decreased LDL activity in the circulation (Ladeia et al., 2020; Rasines-perea & Teissedre, 2017; Sabra et al., 2021; Wijekoon et al., 2022). Polyflavan-3-Ols present in *V.*

vinifera L. act efficiently in inhibiting human platelet aggregation by being of low-density lipoprotein oxidation in vitro (Gomez et al., 2020).

In a clinical study, it has been investigated the effect of grape juice consumption in 20 individuals diagnosed with coronary heart disease for 14 days and observed that there was a significant reduction in CD40L protein, which is associated with increased production of free radicals, expression of adhesion molecules and expression of pro-inflammatory cytokines (Albers et al., 2004). Observed in another in vivo study, in humans, showed antihypertensive effects and increased the levels of antioxidant agents (Vaisman & Niv, 2015). In the study by Maggi-Capeyron et al. (2001) phenolic acids (gallic, caffeic, p-coumaric, synaptic and ferulic) present in wine showed, in vitro, the ability to negatively modulate AP-1, which is an inflammatory marker. Noratto et al. (2011) treated vascular endothelial cells with inflammation induced by LPS (bacterial lipopolysaccharides) with grape extracts (20 mg gallic acid equivalent/L) and showed that there was an inhibition of the translation of interleukins 6 and 8, mediated by the inhibition of the activity of NF-kB (Maggi-Capeyron et al., 2001; Noratto et al., 2011). In the study conducted with grape extract powder (GPE), which is an organic solvent extract of whole freeze-dried table grapes, researchers demonstrated that the treatment caused growth inhibition and reduced the ability of colony formation and migration of DU-145 and PC-3M prostate cancer cells. Less aggressive DU-145 cells showed greater sensitivity to the antiproliferative and anti-colony effects of GPE than to PC-3M cells(Kumar et al., 2018). *Vitis* vinifera extract (50 μg mL−1, 4-h incubation) significantly reduced the increase of oxaliplatin-dependent superoxide anion and lipid peroxidation in rat astrocytes, not interfering with oxaliplatin mortality in HT-29 cancer cells. Vitis vinifera reduced oxidative damage by maintaining the anti-cancer activity of oxaliplatin (Micheli et al., 2018).

5.5. Antidiabetic

Polyphenols, especially those found in grape by-products, used for wine (mainly red) and juice production, demonstrate antidiabetic activity. Studies show that different proteins and enzymes are involved in these antidiabetic activities (Li et al., 2014; Rasines-perea & Teissedre, 2017). Schmatz et al. (2021) carried out in vitro tests with the polyphenol's resveratrol, quercetin, rutin, caffeic acid and gallic acid on the activity of ectonucleotides, adenosine deaminase and platelet aggregation in control and diabetic rats. The results obtained demonstrated an increase in the levels of lipid peroxidation in the liver and kidney of diabetic rats and the treatment with resveratrol (10 and 20 mg/kg) prevented this increase (Systems &

Leporatti, 2022). A study conducted to evaluate the role of grape seed extract (GSE) administration during MSC transplantation in streptozotocin-induced type I diabetes (STZ), and also to test some of the components of GSE [procyanidins (P)-B1 and P-C1] in conjunction with MSCs, in vivo, with the intention of determining whether one of them was more effective in alleviating the measured attributes of diabetes than the entire GSE, showed that GSE/MSC therapy in type I-induced diabetic rats dramatically controlled the homeostasis of glucose and insulin secretion; Along with, improvement in levels of inflammatory markers and oxidative stress, demonstrating that co-treatment with GSE and MSCs in vivo regenerates beta cells in type I-induced diabetic rats (Farid et al., 2022). *In vitro* studies, using intestinal cell models, demonstrate that quercetin reduces blood glucose levels, proving the effect of the polyphenol content of grape pomace (grape seeds and skins) on diabetes (Hegedüs et al., 2022).

5.6. Neuroprotective

Oligomers of resveratrol, including (+) -vitisinol, (+)-ε-viniferin, (+)-ampelopsin A, (+)-vitisin A and (-)-viticin B, that were isolated from a stembark extract of *V. vinifera*, have inhibitory effects on BACE-1 (beta-site APP-cleaving enzyme 1) in vitro. BACE-1 inhibition is an important target for the treatment of Alzheimer's diseases as β-secretase in neurons is essential to produce beta-amyloid (Nassiri-Asl & Hosseinzadeh, 2016). Studies with resveratrol demonstrate positive results for treatment of mice with Alzheimer's related to age, disease, better cognition impairment, and increasing the life span of the animals (Nassiri-Asl & Hosseinzadeh, 2016; Systems & Leporatti, 2022; Yu et al., 2019). Administration of grape seed proanthocyanins (500 mg/kg) could improve abnormal peripheral nerve functions and impaired nervous tissues in the spinal cord of rats with type 2 diabetes mellitus. In addition, this dose showed no inhibitory effect on Ca^{2+} overload in sciatic nerves (Li et al., 2014; Rasines-perea & Teissedre, 2017). Singh et al. (2013) demonstrated in their study that resveratrol has a direct antioxidant role in the central nervous system, through a protective mechanism through the increase of endogenous cellular antioxidant defenses, which trigger a cascade of parallel neuroprotective pathways, through studies in vitro with human cells and in vivo evidence with several rodent models, which indicate that resveratrol acts through multiple pathways and reduces ischemic damage in vital organs such as the heart and brain. Most of resveratrol's protective biological actions are associated with its antioxidant, anti-inflammatory and anti-apoptotic properties and other indirect pathways (Singh et al., 2013; Xia et al., 2019).

5.7. Hepatoprotective effects

Resveratrol has been observed to attenuate liver damage caused by toxins and oxidative stress. It functions by upregulating antioxidant enzymes, inhibiting pro-inflammatory cytokines, and preventing fibrosis by decreasing the activation of hepatic stellate cells (Alshehri and Alorfi, 2023). Animal models with induced liver injury have shown that resveratrol treatment can reduce markers of liver damage such as ALT and AST, pointing towards its potential hepatoprotective effect(Alshehri and Alorfi, 2023). Proanthocyanidins have demonstrated the ability to scavenge free radicals and reduce oxidative stress in the liver; they also modulate liver enzyme levels and improve overall liver function. In vivo experimental models with drug-induced liver injuries, grape seed extracts rich in proanthocyanidins have been shown to reduce liver damage and fibrosis(Amer et al., 2022). Anthocyanins exert their hepatoprotective effects primarily through their antioxidant capacities(Câmara et al., 2022). They mitigate oxidative stress in hepatocytes, thereby preventing liver damage. Studies on rodents have suggested that anthocyanin supplementation can alleviate liver injury by decreasing oxidative stress markers and improving liver enzyme profiles (Sangsefidi et al., 2021). Polyphenols have demonstrated abilities to reduce inflammation in the liver and improve its regenerative capacity (Rudrapal et al., 2022). Dietary supplementation with grape-derived polyphenols in animal models has resulted in decreased levels of pro-inflammatory markers and improvement in the histological appearance of the liver(Rana et al., 2022).

Through a diet, in rats, that included 15% grape seed powder, their tissues were protected, including the liver, against oxidative stress induced by 20% ethanol (Dembitsky et al., 2011). In this study, it was suggested that the intake of functional foods helps prevent chronic degenerative liver diseases. Study with groups of hamsters fed a hyper lipidic diet and flour with grape compost for 28 days showed that the diet did not interfere with the reverse transport of cholesterol and significantly improved the antioxidant status, exhibiting high activities of SOD and Cat enzymes even after administration of a hyperlipidemic diet, avoiding oxidative stress and the consequent inflammation and steatosis of the hepatic tissue, which was verified by normal levels of AST and ALT proteins in the groups (Ishimoto & Vicente, 2020). Even with the excessive consumption of dietary fat, the results show that the treatment with grape juice protected the liver of the animals, maintaining the percentage of viable cells, apoptotic cells and non-apoptotic cells without alteration (V. M. Ribeiro et al., 2018).

The most representative mechanisms and biological effects of grapes and their bioactive compounds are summarized in **Table 1**.

TABLE 1 The most representative mechanisms and biological effects of grapes.

Biological property	Tested Bioactive compounds from grapes	Model	Doses	Mechanisms	Effects	References
Antioxidante antiinflammatory	Flavonoids and polyphenols	In vivo (Human)	Daily diet 750 ml red wine in association whith McDonald´s and Mediterranean Meal on ox-LDL.	Daily oral dose, randomized crossover trial with six arms.	Confirmed the antioxidant potential of flavonoids and polyphenols in red wine in the fight against chronicity non-communicable diseases linked to inflammation.	Renzo ent al., 2014
Antioxidante antiinflammatory	Total phenolics and flavonoids.	In vitro (cell culture - HepG2)	150mg/mL of grape extract.	Cell treatment.	It presented high antioxidant and kidnapper capacity of peroxyl radicals that caused inhibition of cell proliferation, ranging from 25 to 82%.	Li et al., 2014
Antioxidante antiinflammatory	Resveratrol	In vivo (animal)	Daily doses =>(CG)= 4% fat, (DH)= 20% fat, (GJ)= 20% fat + 15ml red grape juice, (PR)= 20% fat + 10 ml red wine and (RS)= 15ml solution.	Daily oral dose. Plasma ORAC and DPPH.	There was no significant difference in CAT activity between HD and CG, but when associating the drink with high polyphenol content, it increased enzymatic activity by 125% in GJ, 82% in RS, 196% in HFD and 273% in RW when compared to CG.	Ribeiro, 2018
Antibacterial, antiviral and antifungal	Resveratrol	In vivo (animal)	Ethanolic extract of grape skin and flesh 2.5 g/kg. p.o, 12 w	Daily oral dose.	Reduced oxidative stress and alteration in immune function and angiogenesis.	Nassiri-Asl & Hosseinzadeh, 2016
Neuroprotective	Quercitin	In vivo (animal)	grape juice, 1208 ± 43.00 µg/mL as the gallic acid equivalent and 5.2 ± 0.19 µg/mL as the quercitin equivalent, 2 mL/kg, 28 d	Cell treatment.	Increased antioxidant capacity, protected against LDL oxidation and showed neuroprotective effects	Pirinc¸c¸ioglu,2012
Neuroprotective	Resveratrol	In vitro (animal)	1, 3 µM ou 30 µM	Cell treatment.	Neuroprotection	Singh et al. (2013)
Neuroprotective	Resveratrol	In vivo (Human) In vitro (human)	100 mg/kg body weight	Daily oral dose	It negatively regulated the expression of certain cytokines and chemokines.	Singh et al. (2013)
Antibacterial	Quercetin and resveratrol	In vivo (Human)	3mg/mL in grape seed extract	Treatment cells	It presented antibacterial effects against methicillin-resistant Staphylococcus aureus.	Nassiri-Asl & Hosseinzadeh (2016)
Antifungal	gallic acid and quercitrin	In vivo (Human)	Not reported	Daily oral dose	It reduced ergosterol biosynthesis correlated with inhibition of folate pathways.	Simonetti et al., 2020
Hepatoprotective. Cardioprotective.	Phenolic compounds	In vivo (animal). In vitro.	control (C), H (hyperlipidemic), V (hyperlipidemic supplemented with 20% wine pomace flour) and S (hyperlipidemic supplemented with 20% juice pomace flour)	Daily oral dose. Cell treatment.	The antioxidant status was improved with decreased oxidative stress, inflammation and steatosis of the liver tissue. presented high amounts of trans-resveratrol and high enzymatic activity, also presenting cardioprotective effect.	Ishimoto & Vicente, 2020
Cardioprotective	Phenolic acids, flavonoids, anthocyanins, stilbenes and lipids	In vivo (animal)	250-375mg/Kg/dia	Daily oral dose	Reduce severely elevated blood pressure.	Sabra et al., 2021
Cardioprotective	Phenolic acids, flavonoids, anthocyanins, stilbenes and lipids	In vivo (human)	200 or 400 mg or placebo	Daily oral dose	Significant effect of grape powder on endothelial function and a positive effect on oxidative stress.	Vaisman & Niv, 2015
Antidiabetic	Polyphenols	In vitro (Human)	0.26 mg. mL−1 or 10 mg. mL−1	Cell treatment.	Inhibition of α-amylase and α-glucosidase activity.	Campos et al., 2021
Anticancer	Resveratrol	In vivo (animal)	5 mg/kg, 15 mg/kg, 45 mg/kg resveratrol and 200 mg/kg N-acetyl-L-cysteine (NAC).	Daily injecting dose	Resveratrol reduced oxidative injury .	Bohara et al., 2022

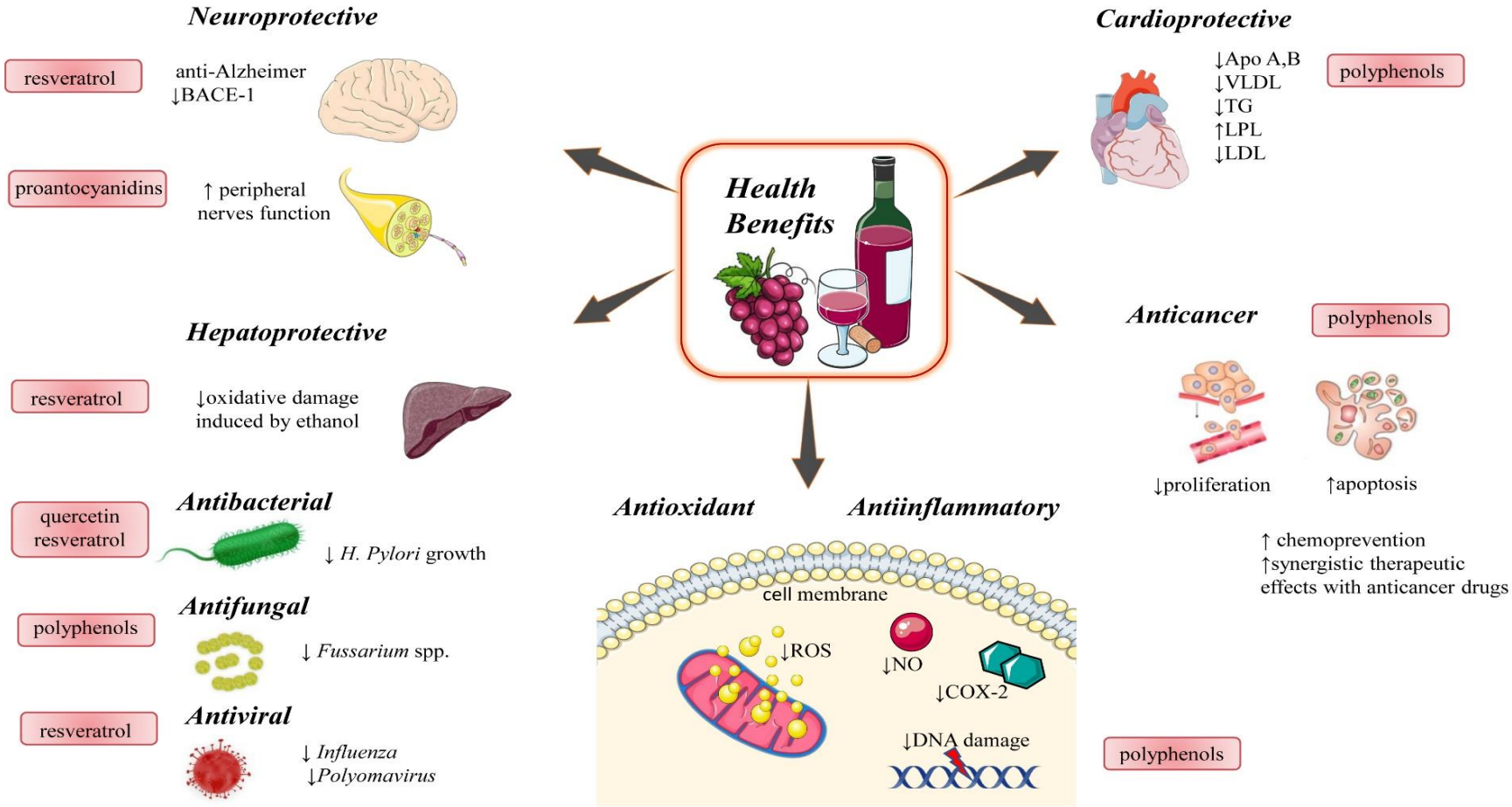

FIGURE 2 Summarized scheme with mechanisms of the main pharmacological properties of bioactive compounds from grapes. Abbreviations and symbols: ↑increase, decrease, BACE-1 (beta-site APP-cleaving enzyme 1), Nitric oxide (NO), cyclooxygenase- 2 (COX-2), Apolipoprotein (apo) A, B, very low-density lipoprotein (VLDL), triglycerides (TG) levels, lipoprotein (LPL).

6. HUMAN CLINICAL STUDIES

Many human intervention studies have been reported with grape-derived beverages, such as red wine and whole red grape juice, featuring a complex array of polyphenols, including anthocyanins and resveratrol. In a review of human intervention studies carried out between 2010 and 2023, the main results analyzed were the antioxidant effect, acting to prevent oxidative reactions and ROS formation, and also its anti-proliferative and anti-inflammatory properties, benefiting various cellular processes (Nguyen, 2023; Springer & Moco, 2019; Tomé-Carneiro et al., 2012). A study carried out by McGill et al. (2013) analyzed the relationship between the consumption of grapes and non-alcoholic grape products (raisins and whole juice) with two different age groups from 2 to 19 years old (n = 9622) and adults aged 20 or more years (n = 12251), the surprising results that the consumption of grape derivatives is associated with a healthier eating pattern, with lower fat intake and added sugar, in both groups (Ergün, 2021; Percival & West, 2013). Fandy (2023) analyzed 13 articles, 12 studies controlled in vivo and 1 controlled in vivo and in vitro and the results satisfied positive results with the use of Grape Seed Extract as a pre-treatment to prevent or delay Parkinson's disease and post-treatment to relieve or reduce the symptoms of Parkinson's disease. Demonstrating significantly reduced damage caused by oxidative stress and improved antioxidant status in all subjects (Nguyen, 2023).

7. SYNERGISTIC EFFECTS OF COMBINATORY TREATMENT OF GRAPES' BIOACTIVE COMPOUNDS AND CONVENTIONAL DRUGS

The combination of natural compounds and conventional drugs is becoming an intriguing approach in therapeutic strategies. Grapes, a rich source of bioactive compounds, have been researched for their potential synergistic interactions with various drugs, enhancing therapeutic outcomes or attenuating side effects.

Resveratrol and Statins

Both resveratrol and statins have cholesterol-lowering effects. Resveratrol enhances endothelial nitric oxide production, promoting vasodilation, while statins inhibit HMG-CoA reductase, a key enzyme in cholesterol synthesis(Soner and Sahin, 2014). Studies have shown that the combination of resveratrol and statins results in a more pronounced reduction in LDL cholesterol and improvement in endothelial function than either agent alone(Soner and Sahin, 2014).

Proanthocyanins and Antihypertensive drugs

Proanthocyanins are known to enhance endothelial function and reduce blood pressure, while conventional antihypertensives act via various mechanisms, like ACE inhibition or calcium channel blockade(Odai et al., 2019). Some studies indicate that grape seed extracts, rich in proanthocyanins, can potentiate the blood pressure-lowering effect of certain antihypertensive medications(Odai et al., 2019).

Anthocyanins and Diabetes Medications

Anthocyanins have been shown to improve insulin sensitivity and reduce blood glucose levels. When combined with conventional diabetes drugs like metformin, which reduces hepatic glucose production, a potential synergistic glucose-lowering effect is suggested(Solverson, 2020). Preliminary clinical studies have demonstrated improved glycemic control in patients consuming anthocyanin-rich foods alongside standard diabetes treatments(Burton-Freeman et al., 2019).

Polyphenols and Non-steroidal Anti-inflammatory Drugs (NSAIDs)

Grape-derived polyphenols exhibit anti-inflammatory properties by inhibiting pro-inflammatory cytokines. When combined with NSAIDs, which inhibit COX enzymes, there is potential for enhanced anti-inflammatory action with reduced side effects(González-Ponce et al., 2018). In animal models, the combination of grape polyphenols and NSAIDs resulted in more pronounced anti-inflammatory effects with reduced gastrointestinal toxicity typically seen with NSAIDs(González-Ponce et al., 2018).

Flavonoids and Chemotherapeutic Agents

Grape-derived flavonoids, like quercetin, have been observed to induce apoptosis in cancer cells. In combination with chemotherapeutic drugs, there might be an enhanced anti-cancer effect and possibly reduced resistance to chemotherapy(Zhai et al., 2021). *In vitro* pharmacological studies with cancer cell lines have shown that the combined treatment of grape flavonoids and certain chemotherapeutic drugs can result in an enhanced reduction in cell viability(Zhai et al., 2021, Liskova et al., 2021).

Tenore et al. (2012) demonstrated in freeze-dried red grape juice, in doses up to 0.01 µg, cardioprotective effects against doxorubicin-induced toxicity in heart-derived H9c2 myocytes. In contrast, at doses of 0.01 µg to 0.05 µg, it increased oxidative stress in heart cells, probably due to pro-oxidant effects of juice, as indicated primarily by the increase of reactive nitrogen and antioxidant species enzymatic levels (Nassiri-Asl & Hosseinzadeh, 2016). In contrast, Boccalandro et al. (2011) suggested that melatonin is an antioxidant present in grapes, after finding an inverse relationship between melatonin and malondialdehyde (MDA) levels in the

fruit of V. vinifera cv. Malbec(Boccalandro et al., 2011). Polyphenols in abundance, present in grape pomace extract, have shown dual effects both in vitro and in vivo. *In vitro*, the extract eliminated free radicals and inhibited peroxyl-induced DNA damage and Hydroxyl radicals, but *in vivo*, induced oxidative stress by increasing the carbonyl groups of the protein in erythrocytes and cardiac cells, increasing plasma Thiobarbituric acidic reactive substances, and decreased concentration of glutathione in the liver (Veskoukis et al., 2012). Liang et al. (2014) conducted a study with 24 grape cultivars V. vinifera, where they found a direct relationship between total phenolic compounds and flavonoids and antioxidant activity (Liang et al., 2014). The literature reports on the comparative effect of sitagliptin with and without resveratrol on clear cell kidney cancer, demonstrating that renal function was significantly improved by sitagliptin and/or resveratrol, while significantly increasing tissue antioxidant defenses when administered simultaneously, reinforcing the hypothesis that the combination of sitagliptin and resveratrol may be an appropriate treatment method to improve clear cell kidney cancer (Almatroodi et al., 2022).

8. TOXICOLOGY AND SAFETY DATA

Most of the evidence on disease prevention using bioactive compounds from grapes comes from in vitro or animal experiments, which generally use very high doses compared to what humans consume through diet. There is no consensus regarding the recommended effective dose of all bioactive compounds found in grapes to achieve the various beneficial health effects. According to Usnish (2010) in an experimental study, doses of 2.5 or 5.0 mg/kg of resveratrol recorded the expression of enzymes involved in cell survival signaling pathways, while higher doses of 25 and 50 mg/kg, potentiated signs of cell death. Other authors claim that doses of 5g/day of resveratrol help prevent diseases such as cancer, metabolic syndrome, Alzheimer's disease, among others (Genovese et al., 2008; Li et al., 2014). Khadem-Ansari et al (2011), Raffoul et al (2012) and Ben Youssef et al (2021) relate the effects of grape vitamins, mostly, to the phenolic compounds that are part of its composition. However, the occurrence of these compounds in a relevant concentration in the grape and its derivatives does not guarantee the effective action in the organism, being important to evaluate aspects such as isomerism, conjugation with other descendent in the product or in the organism, in addition to the transformation during processing, among others, that may influence the bio accessibility, bioavailability and bioactivity of phenolic compounds (Ben Youssef et al., 2021; Khadem-Ansari et al., 2011; Li et al., 2014; Rocha et al., 2012). Studies have shown that resveratrol at doses of 0.5 and 1 g was completely safe and what gastrointestinal adverse effects appeared

with doses of 2.5 and 5 g. Maximal plasma levels (CMax) and areas under the curve (AUC) of the metabolites were greater than those of resveratrol. Resveratrol has chemo preventive effects by decreasing circulating levels of insulin-like growth factor1 and insulin-like growth factor-3 binding protein (Nassiri-Asl & Hosseinzadeh, 2016).

9. THERAPEUTIC PERSPECTIVES, LIMITATIONS AND CLINICAL PITFALLS

As evidenced in several studies, the bioactive compounds in grapes have anti-inflammatory, antimicrobial, anticancer properties, help with glycemic and cholesterol control, among other effects. The articles assume that a sufficient dose for an effect is needed with each consumption and that, unlike minerals and vitamins, the active component is not temporarily stored or retained in the body. The active component is not temporarily stored or retained in the body. On the other hand, for components such as grape flavonoids, which are not stored in the body, the magnitude of the effect is dose dependent. A truly important question in this area would be what value is needed for the smallest biologically significant effect that would be effective and observable over an adequate period, also considering (Blesso, 2019; Gratl et al., 2021; Olędzki et al., 2022; Olivati et al., 2019; Sabra et al., 2021). the sub-compounds that accumulate and become apparent over weeks, months or even years. Therefore, one proposition is that balanced daily intake of food sources is recommended to achieve desirable levels of these compounds under normal physiological conditions (Springer & Moco, 2019).

Grapes, both as whole fruits and as sources of bioactive compounds, have been linked to an array of health benefits, from cardiovascular protection to anti-cancer properties. Yet, while the scientific literature provides promising insights, there are limitations and clinical gaps that warrant consideration. Much of the evidence on grapes' health benefits stems from in vitro studies. The behavior of bioactive compounds in cell cultures does not necessarily translate directly to complex physiological systems in living organisms. The therapeutic potential of grape-derived compounds can be limited by their bioavailability. While these compounds may exhibit strong therapeutic effects in studies, the fraction that becomes available for use by the body's cells after consumption can be minimal. The optimal dose of grape-derived compounds for therapeutic purposes remains unclear. Variability in study dosages makes it challenging to determine a universally recommended intake.

Grapes come in various species, each with a distinct profile of bioactive compounds. This heterogeneity can lead to inconsistencies in research findings, depending on the grape species or variety used. Many studies on grapes' health benefits are of short duration and long-term effects, particularly concerning chronic disease outcomes, are less documented. While

numerous animal and in vitro studies have showcased the benefits of grapes, large-scale randomized controlled trials in humans are fewer; these are critical for establishing causality and real-world effectiveness. The interactions between grape-derived compounds and conventional medications remain under-researched. This gap is crucial as many populations of interest (e.g., the elderly) are often on multiple medications. The biological effects of grapes and their compounds may vary based on genetics, age, gender, and health status, tailored research to understand these differential effects is sparse. Much of the current research focuses predominantly on certain compounds like resveratrol, potentially overshadowing other beneficial compounds in grapes.

The potential health benefits of grapes and their derived compounds are undoubtedly promising. However, the existing limitations and clinical gaps underscore the need for continued, rigorous research. Addressing these clinical pitfalls will provide a more comprehensive understanding, paving the way for more definitive recommendations and applications in clinical settings.

10. CONCLUSION AND FUTURE DIRECTIONS

This review demonstrated that hybrid grapes are rich in antioxidant compounds and exhibit various pharmacological effects of extracts due to their active constituents. Mainly because it has anti-inflammatory, antimicrobial, anti-cancer properties, aids in glycemic and cholesterol control, among other effects. Although the beneficial effects of grapes on health have been reported in experimental studies on cell lines in humans and animals, conducting clinical studies with grapes, few bioavailability studies, mainly on human cells, are needed to confirm the effects of its assets on humans. As grapes are an important component in our daily diet, continued study of the role of grapes and their active constituents in health prevention is recommended. Based on this study, we also suggest studies that inform the chemical composition of existing varieties. Because we know of the existence of several fruits not yet cataloged in their chemical composition, and of others still unknown, that could be useful for the health of the population, both in food and in the prevention and treatment of diseases.

ABBREVIATION LIST

ABTS: (2,2 AZINO BIS [3-ethylbenzo thiazoline 6 sulfonic acid])

BACE-1: (Beta-site APP-cleaving enzyme 1)

DPPH: (2,2-Diphenyl-1-picril-hidrazil)

EMBRAPA: The Brazilian Agricultural Research Corporation

FRAP: Ferric Reducing Antioxidant Power

LPL: Lipoprotein

MeSH: Medical Subject Headings

VLDL: Very low-density lipoprotein

AUTHOR CONTRIBUTIONS

Marta Angela de Almeida Sousa Cruz: Conceptualization; validation; writing-original draft. **Monique de Barros Elias**: Conceptualization; writing-review and editing. **Anderson Junger Teodoro**: Conceptualization; Validation; writing-review and editing; supervision. **Javad Sharifi-Rad:** Conceptualization; validation; writing-review and editing; supervision**. Daniela Calina**: Conceptualization; Writing-review and editing.

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REFERENCES

Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, T., Bashir, M. S., Ishtiaq, A., Hussain, S., & Suleria, H. A. R. (2017). Natural polyphenols: An overview. *International Journal of Food Properties*, *20*(8), 1689–1699. https://doi.org/10.1080/10942912.2016.1220393

Aires, M. V. L., Modesto, R. M. G., & Santos, J. S. (2021). Os benefícios da uva na saúde humana: uma revisão. *Research, Society and Development*, *10*(14), e281101421825. https://doi.org/10.33448/rsd-v10i14.21825

Albers, A. R., Varghese, S., Vita, J. A., & Freedman, J. E. (2004). Letters to the Editor The Antiinflammatory Effects of Purple. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *24*(11), e179–e180.

Alshehri, F. S. & Alorfi, N. M. (2023). Protective role of resveratrol against VCM-induced hepatotoxicity in male wistar rats. *Frontiers in Pharmacology,* 14.

Almatroodi, S. A., Alsahli, M. A., Aljohani, A. S. M., Alhumaydhi, F. A., Babiker, A. Y., Khan, A. A., & Rahmani, A. H. (2022). Potential Therapeutic Targets of Resveratrol, a Plant Polyphenol, and Its Role in the Therapy of Various Types of Cancer. *Molecules*, *27*(9).

https://doi.org/10.3390/molecules27092665

Amer, M. A., Othman, A. I., El-missiry, M. A., Farag, A. A. & Amer, M. E. (2022). Proanthocyanidins attenuated liver damage and suppressed fibrosis in CCl4-treated rats. *Environ Sci Pollut Res Int,* 29**,** 91127-91138. https://doi.org/10.1007/s11356-022-22051-7

Barbalho, S. M., Bueno Ottoboni, A. M. M., Fiorini, A. M. R., Guiguer, É. L., Nicolau, C. C. T., Goulart, R. de A., & Flato, U. A. P. (2020). Grape juice or wine: which is the best option? *Critical Reviews in Food Science and Nutrition*, *60*(22), 3876–3889. https://doi.org/10.1080/10408398.2019.1710692

Ben Youssef, S., Brisson, G., Doucet-Beaupré, H., Castonguay, A. M., Gora, C., Amri, M., & Lévesque, M. (2021). Neuroprotective benefits of grape seed and skin extract in a mouse model of Parkinson's disease. *Nutritional Neuroscience*, *24*(3), 197–211. https://doi.org/10.1080/1028415X.2019.1616435

Blesso, C. N. (2019). Dietary Anthocyanins and Human Health. *Nutrients*, *11*(9), 10–13. https://doi.org/10.3390/nu11092107

Boccalandro, H. E., González, C. V., Wunderlin, D. A., & Silva, M. F. (2011). Melatonin levels, determined by LC-ESI-MS/MS, fluctuate during the day/night cycle in Vitis vinifera cv Malbec: Evidence of its antioxidant role in fruits. *Journal of Pineal Research*, *51*(2), 226–232. https://doi.org/10.1111/j.1600-079X.2011.00884.x

Bocsan, I. C., Măgureanu, D. C., Pop, R. M., Levai, A. M., Macovei, Ștefan O., Pătrașca, I. M., Chedea, V. S., & Buzoianu, A. D. (2022). Antioxidant and Anti-Inflammatory Actions of Polyphenols from Red and White Grape Pomace in Ischemic Heart Diseases. *Biomedicines*, *10*(10), 1–29. https://doi.org/10.3390/biomedicines10102337

Bortolini, D. G., Maciel, G. M., Fernandes, I. de A. A., Rossetto, R., Brugnari, T., Ribeiro, V. R., & Haminiuk, C. W. I. (2022). Biological potential and technological applications of red fruits: An overview. *Food Chemistry Advances*, *1*(February). https://doi.org/10.1016/j.focha.2022.100014

Burton-freeman, B., Brzeziński, M., Park, E., Sandhu, A., Xiao, D. & Edirisinghe, I. (2019). A Selective Role of Dietary Anthocyanins and Flavan-3-ols in Reducing the Risk of Type 2 Diabetes Mellitus: A Review of Recent Evidence. *Nutrients,* 11. https://doi.org/10.3390/nu11040841

Câmara, J. S., Locatelli, M., Pereira, J. A. M., Oliveira, H., Arlorio, M., Fernandes, I., Perestrelo, R., Freitas, V. & Bordiga, M. (2022). Behind the Scenes of Anthocyanins-From the Health Benefits to Potential Applications in Food, Pharmaceutical and Cosmetic Fields. *Nutrients,* 14, 5133. https://doi.org/10.3390/ nu14235133

Camargo, U. A., Maia, J. D. G., & Ritschel, P. (2010). *Embrapa Uva e Vinho*.

Cheah, K. Y., Howarth, G. S., Bindon, K. A., Kennedy, J. A., & Bastian, S. E. P. (2014). Low molecular weight procyanidins from grape seeds enhance the impact of 5-Fluorouracil chemotherapy on Caco-2 human colon cancer cells. *PLoS ONE*, *9*(6). https://doi.org/10.1371/journal.pone.0098921

Cruz-Machado, S. da S. (2010). Lipopolissacarídeo (LPS): ativador e regulador da transcrição gênica via fator de transcrição NFKB. In *Revista da Biologia* (Vol. 4, pp. 40–43). https://doi.org/10.7594/revbio.04.08

da Silva, M. J. R., da Silva Padilha, C. V., dos Santos Lima, M., Pereira, G. E., Filho, W. G. V., Moura, M. F., & Tecchio, M. A. (2019). Grape juices produced from new hybrid varieties grown on Brazilian rootstocks – Bioactive compounds, organic acids and antioxidant capacity. *Food Chemistry*, *289*(September 2018), 714–722. https://doi.org/10.1016/j.foodchem.2019.03.060

Daldoul, S., Boubakri, H., Gargouri, M., & Mliki, A. (2020). Recent advances in biotechnological studies on wild grapevines as valuable resistance sources for smart viticulture. *Molecular Biology Reports*, *47*(4), 3141–3153. https://doi.org/10.1007/s11033-020-05363-0

Dave, A., Beyoğlu, D., Park, E. J., Idle, J. R., & Pezzuto, J. M. (2023). Influence of grape consumption on the human microbiome. *Scientific Reports*, 1–19. https://doi.org/10.1038/s41598-023-34813-5

de Freitas Laiber Pascoal, G., de Almeida Sousa Cruz, M. A., Pimentel de Abreu, J., Santos, M. C. B., Bernardes Fanaro, G., Júnior, M. R. M., Freitas Silva, O., Moreira, R. F. A., Cameron, L. C., Simões Larraz Ferreira, M., & Teodoro, A. J. (2022). Evaluation of the antioxidant capacity, volatile composition and phenolic content of hybrid Vitis vinifera L. varieties *Sweet Sapphire* and *Sweet Surprise*. *Food Chemistry*, *366*(July 2021). https://doi.org/10.1016/j.foodchem.2021.130644

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants and Redox Signaling*, *18*(14), 1818–1892. https://doi.org/10.1089/ars.2012.4581

Dembitsky, V. M., Poovarodom, S., Leontowicz, H., Leontowicz, M., Vearasilp, S., Trakhtenberg, S., & Gorinstein, S. (2011). The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites. *Food Research International*, *44*(7), 1671–1701. https://doi.org/10.1016/j.foodres.2011.03.003

Di Renzo, L., Carraro, A., Valente, R., Iacopino, L., Colica, C., & De Lorenzo, A. (2014). Intake of red wine in different meals modulates oxidized LDL level, oxidative and inflammatory gene expression in healthy people: A randomized crossover trial. *Oxidative Medicine and Cellular Longevity*, *2014*. https://doi.org/10.1155/2014/681318

Ergün, Z. (2021). Determination of Biochemical Contents of Fresh, Oven-Dried, and Sun-Dried Peels and Pulps of Five Apple Cultivars (Amasya, Braeburn, Golden Delicious, Granny Smith, and Starking). *Journal of Food Quality*, *2021*. https://doi.org/10.1155/2021/9916694

Fakhri, S., Khodamorady, M., Naseri, M., Farzaei, M. H., & Khan, H. (2020). The ameliorating effects of anthocyanins on the cross-linked signaling pathways of cancer dysregulated metabolism. *Pharmacological Research*, *159*, 104895. https://doi.org/10.1016/j.phrs.2020.104895

Fan, D., Liu, C., Zhang, Z., Huang, K., Wang, T., Chen, S. & Li, Z. (2022). Progress in the Preclinical and Clinical Study of Resveratrol for Vascular Metabolic Disease. *Molecules,* 27. https://doi.org/ 10.3390/molecules27217524

Farid, A., Haridyy, H., Ashraf, S., Ahmed, S., & Safwat, G. (2022). Co-treatment with grape seed extract and mesenchymal stem cells in vivo regenerated beta cells of islets of Langerhans in pancreas of type I-induced diabetic rats. *Stem Cell Research and Therapy*, *13*(1), 1–18. https://doi.org/10.1186/s13287-022-03218-y

Fraternale, D., De Bellis, R., Calcabrini, C., Potenza, L., Cucchiarini, L., Mancini, U., Dachà, M., & Ricci, D. (2011). Aqueous extract from Vitis vinifera tendrils is able to enrich keratinocyte antioxidant defences. *Natural Product Communications*, *6*(9), 1315–1319. https://doi.org/10.1177/1934578x1100600925

Gao, W., & Zhang, Y. (2021). Depression of lncRNA MINCR antagonizes LPS-evoked acute injury and inflammatory response via miR-146b-5p and the TRAF6-NFkB signaling. *Molecular Medicine (Cambridge, Mass.)*, *27*(1), 124. https://doi.org/10.1186/s10020-021-00367-3

Genovese, M. I., Da Silva Pinto, M., De Souza Schmidt Gonçalves, A. E., & Lajolo, F. M. (2008). Bioactive compounds and antioxidant capacity of exotic fruits and commercial frozen pulps from Brazil. *Food Science and Technology International*, *14*(3), 207–214. https://doi.org/10.1177/1082013208092151

Ghate, N. B., Hazra, B., Sarkar, R., & Mandal, N. (2014). In vitro anticancer activity of Spondias pinnata bark on human lung and breast carcinoma. *Cytotechnology*, *66*(2), 209–218. https://doi.org/10.1007/s10616-013-9553-7

Gomez, H. A. G., Marques, M. O. M., Borges, C. V., Minatel, I. O., Monteiro, G. C., Ritschel,

P. S., Zanus, M. C., Diamante, M. S., Kluge, R. A., & Lima, G. P. P. (2020). Biogenic Amines and the Antioxidant Capacity of Juice and Wine from Brazilian Hybrid Grapevines. *Plant Foods for Human Nutrition*, *75*(2), 258–264. https://doi.org/10.1007/s11130-020-00811-5

González-ponce, H. A., Rincón-sánchez, A. R., Jaramillo-juárez, F. & Moshage, H. (2018). Natural Dietary Pigments: Potential Mediators against Hepatic Damage Induced by Over-The-Counter Non-Steroidal Anti-Inflammatory and Analgesic Drugs. *Nutrients,* 10. http://doi:10.3390/nu10020117

Gratl, V., Sturm, S., Zini, E., Letschka, T., Stefanini, M., Vezzulli, S., & Stuppner, H. (2021). Comprehensive polyphenolic profiling in promising resistant grapevine hybrids including 17 novel breeds in northern Italy. *Journal of the Science of Food and Agriculture*, *101*(6), 2380–2388. https://doi.org/10.1002/jsfa.10861

Hegedüs, I., Andreidesz, K., Szentpéteri, J. L., Kaleta, Z., Szabó, L., Szigeti, K., Gulyás, B., Padmanabhan, P., Budan, F., & Máthé, D. (2022). The Utilization of Physiologically Active Molecular Components of Grape Seeds and Grape Marc. *International Journal of Molecular Sciences*, *23*(19). https://doi.org/10.3390/ijms231911165

Hou, C. Y., Tain, Y. L., Yu, H. R., & Huang, L. T. (2019). The effects of resveratrol in the treatment of metabolic syndrome. *International Journal of Molecular Sciences*, *20*(3), 1–15. https://doi.org/10.3390/ijms20030535

Huang, P. (2023). Proanthocyanidins may be potential therapeutic agents for the treatment of carotid atherosclerosis: A review. *J Int Med Res,* 51**,** 3000605231167314.http://doi: 10.1177/03000605231167314

Iannone, M., Mare, R., Paolino, D., Gagliardi, A., Froiio, F., Cosco, D., & Fresta, M. (2017). Characterization and in vitro anticancer properties of chitosan-microencapsulated flavan-3-ols-rich grape seed extracts. *International Journal of Biological Macromolecules*, *104*, 1039–1045. https://doi.org/10.1016/j.ijbiomac.2017.07.022

Ishimoto, E., & Vicente, S. J. V. (2020). Efeitos Hipolipidêmico E Antioxidante Em Hamsters Pela Administração De Subprodutos Do Processamento De Uvas. *Avanços Em Ciência e Tecnologia de Alimentos - Volume 2*, 179–201. https://doi.org/10.37885/201102217

Jideani, A. I. O., Silungwe, H., Takalani, T., Omolola, A. O., Udeh, H. O., & Anyasi, T. A. (2021). Antioxidant-rich natural fruit and vegetable products and human health. *International Journal of Food Properties*, *24*(1), 41–67. https://doi.org/10.1080/10942912.2020.1866597

Jing, P., Zhao, S., Ruan, S., Sui, Z., Chen, L., Jiang, L., & Qian, B. (2014). Quantitative studies on structure – ORAC relationships of anthocyanins from eggplant and radish using 3D-

QSAR. *Food Chemistry*, *145*, 365–371. https://doi.org/10.1016/j.foodchem.2013.08.082

Kelly, E., Vyas, P., & Weber, J. T. (2018). Biochemical properties and neuroprotective effects of compounds in various species of berries. *Molecules*, *23*(1). https://doi.org/10.3390/molecules23010026

Khadem-Ansari, M. H., Rasmi, Y., & Ramezani, F. (2011). Effects of Red Grape Juice Consumption on High Density Lipoprotein-Cholesterol, Apolipoprotein AI, Apolipoprotein B and Homocysteine in Healthy Human Volunteers. *The Open Biochemistry Journal*, *4*, 96–99. https://doi.org/10.2174/1874091x01004010096

Kozłowska, A. & Szostak-węgierek, D. (2022). Targeting Cardiovascular Diseases by Flavonols: An Update. *Nutrients, 14***,** 1439. https://doi.org/10.3390/ nu14071439

Kowalczyk, B., Bieniasz, M., & Kostecka-Gugała, A. (2022). The Content of Selected Bioactive Compounds in Wines Produced from Dehydrated Grapes of the Hybrid Variety 'Hibernal' as a Factor Determining the Method of Producing Straw Wines. *Foods*, *11*(7). https://doi.org/10.3390/foods11071027

Kumar, A., D'silva, M., Dholakia, K., & Levenson, A. S. (2018). In vitro anticancer properties of table grape powder extract (GPE) in prostate cancer. *Nutrients*, *10*(11), 1–12. https://doi.org/10.3390/nu10111804

Ladeia, D. N., Costa, A. luiza melo lima, Vieira, A. L. C., Ramos, D. S., Coelho, J. L., Lima, L. R. de O., Santos, L. P. de F., Violante, P. M., Bravim, P. M. V., & Lopes, A. G. (2020). Fatores de proteção e ações de prevenção para o câncer de colo de útero: uma revisão integrativa dos últimos 10 anos. In *Revista Eletrônica Acervo Saúde* (Issue 56, p. e3923). https://doi.org/10.25248/reas.e3923.2020

Landau, E. C. . et al. (2020). Dinâmica da produção agroapecuária e da paisagem natural do Brasil nas últimas décadas. *Embrapa*, *11*(3), 55. https://www.m-culture.go.th/mculture_th/download/king9/Glossary_about_HM_King_Bhumibol_Adulyadej's_Funeral.pdf

Li, A. N., Li, S., Zhang, Y. J., Xu, X. R., Chen, Y. M., & Li, H. Bin. (2014). Resources and biological activities of natural polyphenols. *Nutrients*, *6*(12), 6020–6047. https://doi.org/10.3390/nu6126020

Liang, Z., Cheng, L., Zhong, G. Y., & Liu, R. H. (2014). Antioxidant and antiproliferative activities of twenty-four Vitis vinifera grapes. *PLoS ONE*, *9*(8). https://doi.org/10.1371/journal.pone.0105146

Liu, C., Tong, P., Yang, R., You, Y., Liu, H., & Zhang, T. (2019). Solidified phospholipid-TPGS as an effective oral delivery system for improving the bioavailability of resveratrol. *Journal*

of Drug Delivery Science and Technology, *52*(July 2018), 769–777. https://doi.org/10.1016/j.jddst.2019.02.025

Liskova, A., Samec, M., Koklesova, L., Brockmueller, A., Zhai, K., Abdellatif, B., Siddiqui, M., Biringer, K., Kudela, E., Pec, M., Gadanec, L. K., Šudomová, M., Hassan, S. T. S., Zulli, A., Shakibaei, M., Giordano, F. A., Büsselberg, D., Golubnitschaja, O. & Kubatka, P. 2021. Flavonoids as an effective sensitizer for anti-cancer therapy: insights into multi-faceted mechanisms and applicability towards individualized patient profiles. *EPMA Journal, 12*, 155-176. https://doi.org/ 10.1007/s13167-021-00242-5

Maggi-Capeyron, M. F., Ceballos, P., Cristol, J. P., Delbosc, S., Le Doucen, C., Pons, M., Léger, C. L., & Descomps, B. (2001). Wine phenolic antioxidants inhibit AP-1 transcriptional activity. *Journal of Agricultural and Food Chemistry*, *49*(11), 5646–5652. https://doi.org/10.1021/jf010595x

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition*, *79*(5), 727–747. https://doi.org/10.1093/ajcn/79.5.727

Micheli, L., Mattoli, L., Maidecchi, A., Pacini, A., Ghelardini, C., & Di Cesare Mannelli, L. (2018). Effect of Vitis vinifera hydroalcoholic extract against oxaliplatin neurotoxicity: in vitro and in vivo evidence. *Scientific Reports*, *8*(1), 1–14. https://doi.org/10.1038/s41598-018-32691-w

Muñoz-Bernal, Ó. A., Coria-Oliveros, A. J., de la Rosa, L. A., Rodrigo-García, J., del Rocío Martínez-Ruiz, N., Sayago-Ayerdi, S. G., & Alvarez-Parrilla, E. (2021). Cardioprotective effect of red wine and grape pomace. *Food Research International*, *140*(July 2020). https://doi.org/10.1016/j.foodres.2020.110069

Nassiri-Asl, M., & Hosseinzadeh, H. (2016). Review of the Pharmacological Effects of Vitis vinifera (Grape) and its Bioactive Constituents: An Update. *Phytotherapy Research*, *April*, 1392–1403. https://doi.org/10.1002/ptr.5644

Nguyen, H. D. (2023). Resveratrol, Endocrine Disrupting Chemicals, Neurodegenerative Diseases and Depression: Genes, Transcription Factors, microRNAs, and Sponges Involved. *Neurochemical Research*, *48*(2), 604–624. https://doi.org/10.1007/s11064-022-03787-7

Noratto, G. D., Angel-Morales, G., Talcott, S. T., & Mertens-Talcott, S. U. (2011). Polyphenolics from Açaí (Euterpe oleracea Mart.) and red muscadine grape (Vitis rotundifolia) protect human umbilical vascular endothelial cells (HUVEC) from glucose- and lipopolysaccharide (LPS)-induced inflammation and target microRNA-126. *Journal of Agricultural and Food Chemistry*, *59*(14), 7999–8012. https://doi.org/10.1021/jf201056x

Odai, T., Terauchi, M., Kato, K., Hirose, A. & Miyasaka, N. (2019). Effects of Grape Seed Proanthocyanidin Extract on Vascular Endothelial Function in Participants with Prehypertension: A Randomized, Double-Blind, Placebo-Controlled Study. *Nutrients,* 11. http://doi:10.3390/nu11122844

Olędzki, R., Lutosławski, K., Nowicka, P., Wojdyło, A., & Harasym, J. (2022). Non-Commercial Grapevines Hybrids Fruits as a Novel Food of High Antioxidant Activity. *Foods*, *11*(15), 2216. https://doi.org/10.3390/foods11152216

Olivati, C., de Oliveira Nishiyama, Y. P., de Souza, R. T., Janzantti, N. S., Mauro, M. A., Gomes, E., Hermosín-Gutiérrez, I., da Silva, R., & Lago-Vanzela, E. S. (2019). Effect of the pre-treatment and the drying process on the phenolic composition of raisins produced with a seedless Brazilian grape cultivar. *Food Research International*, *116* (April 2018), 190–199. https://doi.org/10.1016/ j.foodres.2018.08.012

Orsavova, J., Misurcova, L., Vavra Ambrozova, J., Vicha, R., & Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International Journal of Molecular Sciences*, *16*(6), 12871–12890. https://doi.org/10.3390/ijms160612871

Otręba, M., Kośmider, L. & Rzepecka-Stojko, A. (2021). Polyphenols' Cardioprotective Potential: Review of Rat Fibroblasts as Well as Rat and Human Cardiomyocyte Cell Lines Research. *Molecules,* 26. https://doi.org/10.3390/molecules26040774

Panchal, S. K., & Brown, L. (2023). Potential Benefits of Anthocyanins in Chronic Disorders of the Central Nervous System. *Molecules*, *28*(1), 1–14. https://doi.org/10.3390/molecules28010080

Percival, S. S., & West, R. L. (2013). Effect of Health-promoting Properties of Grapes, Including Resveratrol. *Bioactives in Fruit*, 197–214. https://doi.org/10.1002/ 9781118635551.ch9

Posadino, A. M., Giordo, R., Ramli, I., Zayed, H., Nasrallah, G. K., Wehbe, Z., Eid, A. H., Gürer, E. S., Kennedy, J. F., Aldahish, A. A., Calina, D., Razis, A. F. A., Modu, B., Habtemariam, S., Sharifi-rad, J., Pintus, G. & Cho, W. C. (2023). An updated overview of cyanidins for chemoprevention and cancer therapy. *Biomedicine & Pharmacotherapy,* 163**,** 114783. https://doi.org/10.1016/j.biopha.2023.114783

Rana, A., Samtiya, M., Dhewa, T., Mishra, V. & Aluko, R. E. 2022. Health benefits of polyphenols: A concise review. *J Food Biochem***,** e14264.

Rasines-perea, Z., & Teissedre, P. (2017). *Efeitos dos polifenó is da uva em doenças cardiovasculares humanas e diabetes*. *22*(1).

Rasouli, H., Farzaei, M.H. & Khodarahmi, R. (2017). Polyphenols and their benefits: A review. *International journal of food properties* , 20, 1700-41. https:// www.tandfonline.com/loi/ljfp20

Ribeiro, M. C. (2016). Brazilian Science and Technology Policy and the Case of Embrapa Semiarid. *Contexto Internacional*, *38*(2), 685–710. https://doi.org/10.1590/s0102-8529.2016380200008

Ribeiro, V. M., Bedê, T. P., Rocha, G. S., Barroso, S., Valença, S., & de Azeredo, V. B. (2018). Efectos de dieta con alto contenido de grasa y bebidas ricas en polifenoles en la actividad antioxidante enzimática y no enzimática. *Nutricion Hospitalaria*, *35*(1), 169–175. https://doi.org/10.20960/nh.1219

Rocha, L. D., Monteiro, M. C., & Teodoro, A. J. (2012). Anticancer Properties of Hydroxycinnamic Acids -A Review. *Cancer and Clinical Oncology*, *1*(2), 109–121. https://doi.org/10.5539/cco.v1n2p109

Rotili, M. C. C., Villa, F., da Silva, D. F., Rosanelli, S., Braga, G. C., & Ritter, G. (2022). Bioactive compounds, bromatological and mineral characterization of blackberries in a subtropical region. *Revista Ceres*, *69*(1), 13–21. https://doi.org/10.1590/0034-737X202269010003

Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., Alshabrmi, F. M., Palai, S., Deb, P. K. & Devi, R. (2022). Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action. *Front Pharmacol,* 13**,** 806470. http://doi: 10.3389/fphar.2022.806470

Sabo, J., Farkasov, S., Droppa, M., & Žiarovsk, J. (2022). *of Selected Vitis vinifera L . Varieties*.

Sabra, A., Netticadan, T., & Wijekoon, C. (2021). Grape bioactive molecules, and the potential health benefits in reducing the risk of heart diseases. *Food Chemistry: X*, *12*. https://doi.org/10.1016/j.fochx.2021.100149

Sangsefidi, Z. S., Mozaffari-khosravi, H., Sarkhosh-khorasani, S. & Hosseinzadeh, M. (2021). The effect of anthocyanins supplementation on liver enzymes: A systematic review and meta-analysis of randomized clinical trials. *Food Sci Nutr,* 9**,** 3954-3970. http:// doi: 10.1002/fsn3.2278

Sehitoglu, M. H., Farooqi, A. A., Qureshi, M. Z., Butt, G. & Aras, A. (2014). Anthocyanins: targeting of signaling networks in cancer cells. *Asian Pac J Cancer Prev,* 15**,** 2379-81. http://dx.doi.org/10.7314/APJCP.2014.15.5.2379

Simonetti, G., Brasili, E., & Pasqua, G. (2020). Antifungal Activity of Phenolic and

Polyphenolic Compounds from Different Matrices of Vitis vinifera L. Against Human Pathogens. *Molecules*, *25*(16). https://doi.org/10.3390/molecules25163748

Singh, N., Agrawal, M., & Doré, S. (2013). Neuroprotective properties and mechanisms of resveratrol in in vitro and in vivo experimental cerebral stroke models. *ACS Chemical Neuroscience*, *4*(8), 1151–1162. https://doi.org/10.1021/cn400094w

Soares, N. da C. P., Teodoro, A. J., Lotsch, P. F., Granjeiro, J. M., & Borojevic, R. (2015). Anticancer properties of carotenoids in prostate cancer. A review. *Histology and Histopathology*, *30*(10), 1143–1154. https://doi.org/10.14670/HH-11-635

Solverson, P. (2020). Anthocyanin Bioactivity in Obesity and Diabetes: The Essential Role of Glucose Transporters in the Gut and Periphery. *Cells,* 9.http:// doi:10.3390/cells9112515

Soner, B. C. & Sahin, A. S. (2014). Cardiovascular effects of resveratrol and atorvastatin treatments in an H(2)O(2)-induced stress model. *Exp Ther Med,* 8**,** 1660-1664. http://doi: 10.3892/etm.2014.1956

Springer, M., & Moco, S. (2019). Resveratrol and its human metabolites—effects on metabolic health and obesity. *Nutrients*, *11*(1), 1–17. https://doi.org/10.3390/nu11010143

Stevens, J. F., & Revel, J. S. (2018). *Mitochondria-Centric Review of Polyphenol*. *29*(16), 1589–1611. https://doi.org/10.1089/ars.2017.7404

Swallah, M. S., Sun, H., Affoh, R., Fu, H., & Yu, H. (2020). Antioxidant Potential Overviews of Secondary Metabolites (Polyphenols) in Fruits. *International Journal of Food Science*, *2020*. https://doi.org/10.1155/2020/9081686

Systems, N., & Leporatti, S. (2022). *Molecules-27-05154.Pdf*. 1–17.

Tabeshpour, J., Mehri, S., Shaebani Behbahani, F., & Hosseinzadeh, H. (2018). Protective effects of Vitis vinifera (grapes) and one of its biologically active constituents, resveratrol, against natural and chemical toxicities: A comprehensive review. *Phytotherapy Research*, *32*(11), 2164–2190. https://doi.org/10.1002/ ptr.6168

Table, S., Production, G., The, I. N., & Semi, B. (2020). *Semiárido brasileiro*. 70–81.

Tomé-Carneiro, J., Gonzálvez, M., Larrosa, M., García-Almagro, F. J., Avilés-Plaza, F., Parra, S., Yáñez-Gascón, M. J., Ruiz-Ros, J. A., García-Conesa, M. T., Tomás-Barberán, F. A., & Espín, J. C. (2012). Consumption of a grape extract supplement containing resveratrol decreases oxidized LDL and ApoB in patients undergoing primary prevention of cardiovascular disease: A triple-blind, 6-month follow-up, placebo-controlled, randomized trial. *Molecular Nutrition and Food Research*, *56*(5), 810–821. https://doi.org/10.1002/mnfr.201100673

Vaisman, N., & Niv, E. (2015). Daily consumption of red grape cell powder in a dietary dose

improves cardiovascular parameters: A double blind, placebo-controlled, randomized study. *International Journal of Food Sciences and Nutrition*, *66*(3), 342–349. https://doi.org/10.3109/09637486.2014.1000840

Veskoukis, A. S., Kyparos, A., Nikolaidis, M. G., Stagos, D., Aligiannis, N., Halabalaki, M., Chronis, K., Goutzourelas, N., Skaltsounis, L., & Kouretas, D. (2012). The antioxidant effects of a polyphenol-rich grape pomace extract in vitro do not correspond in vivo using exercise as an oxidant stimulus. *Oxidative Medicine and Cellular Longevity*, *2012*. https://doi.org/10.1155/2012/185867

Vislocky, L. M., & Fernandez, M. L. (2010). Biomedical effects of grape products. *Nutrition Reviews*, *68*(11), 656–670. https://doi.org/10.1111/j.1753-4887.2010.00335.x

Wang, L., Huang, W. & Zhan, J. (2019). Grape Seed Proanthocyanidins Induce Autophagy and Modulate Survivin in HepG2 Cells and Inhibit Xenograft Tumor Growth in Vivo. *Nutrients,* 11. http:// doi:10.3390/nu11122983

Wang, L., Zhan, J. & Huang, W. (2020). Grape Seed Proanthocyanidins Induce Apoptosis and Cell Cycle Arrest of HepG2 Cells Accompanied by Induction of the MAPK Pathway and NAG-1. *Antioxidants (Basel),* 9. https://doi:10.3390/antiox9121200

Wijekoon, C., Netticadan, T., Siow, Y. L., Sabra, A., Yu, L., & Raj, P. (2022). *Associaçõ es potenciais entre Moléculas Bioativas , Atividade Antioxidante e Produção de Resveratrol em Vitis vinifera Frutas da América do Norte*. *27*(2).

Xia, L., Xu, C., Huang, K., Lu, J., & Zhang, Y. (2019). Evaluation of phenolic compounds, antioxidant and antiproliferative activities of 31 grape cultivars with different genotypes. *Journal of Food Biochemistry*, *43*(6), 1–13. https://doi.org/10.1111/jfbc.12626

Xu, Y., Simon, J. E., Welch, C., Wightman, J. D., Ferruzzi, M. G., Ho, L., Passinetti, G. M., & Wu, Q. (2011). Survey of polyphenol constituents in grapes and grape-derived products. *Journal of Agricultural and Food Chemistry*, *59*(19), 10586–10593. https://doi.org/10.1021/jf202438d

Xu, L., Tian, Z., Chen, H., Zhao, Y. & Yang, Y. (2021). Anthocyanins, Anthocyanin-Rich Berries, and Cardiovascular Risks: Systematic Review and Meta-Analysis of 44 Randomized Controlled Trials and 15 Prospective Cohort Studies. *Frontiers in Nutrition,* 8. http://doi: 10.3389/fnut.2021.747884

Yu, Y., Guo, D., Li, G., Yang, Y., Zhang, G., Li, S., & Liang, Z. (2019). The grapevine R2R3-type MYB transcription factor VdMYB1 positively regulates defense responses by activating the stilbene synthase gene 2 (VdSTS2). *BMC Plant Biology*, *19*(1). https://doi.org/10.1186/s12870-019-1993-6

Zecca, G., Labra, M., & Grassi, F. (2020). Untangling the Evolution of American Wild Grapes: Admixed Species and How to Find Them. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.01814

Zhai, K., Mazurakova, A., Koklesova, L., Kubatka, P. & Büsselberg, D. (2021). Flavonoids Synergistically Enhance the Anti-Glioblastoma Effects of Chemotherapeutic Drugs. *Biomolecules,* 11. https://doi.org/10.3390/ biom11121841

Zhang, H., Yu, D., Sun, J., Liu, X., Jiang, L., Guo, H., & Ren, F. (2014). Interaction of plant phenols with food macronutrients: Characterisation and nutritional-physiological consequences. *Nutrition Research Reviews*, *27*(1), 1–15. https://doi.org/10.1017/S095442241300019X

Zhang, L., Han, Z., & Granato, D. (2021). Polyphenols in foods: Classification, methods of identification, and nutritional aspects in human health. In *Advances in Food and Nutrition Research* (1st ed., Vol. 98). Elsevier Inc. https://doi.org/10.1016/bs.afnr.2021.02.004

Zhu, L., Li, X., Hu, X., Wu, X., Liu, Y., Yang, Y., Zang, Y., & Tang, H. (2021). *Características de Qualidade e Perfis de Antocianina de Diferentes Cultivares de Uva Vitis amurensis e híbridos do germoplasma chinês*. *26*(21). https://doi.org/10.3390/mol

CAPÍTULO III

Avaliação da capacidade antioxidante, composição volátil e fenólica conteúdo de variedades híbridas Vitis vinifera L. de *Sweet Sapphire* e S*weet Surprise.*

Evaluation of the antioxidant capacity, volatile composition and phenolic content of hybrid *Vitis vinifera L.* varieties *Sweet Sapphire* and *Sweet Surprise.*

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Abstract

The antioxidant components of extracts of hybrid *Vitis vinifera* L. varieties, *Sweet Sapphire* (SA) and *Sweet Surprise* (SU), were isolated and then characterized by high-performance liquid chromatography/Ultraviolet and electrospray ionization-tandem mass spectrometry (HPLC-UV-/ESI-MS-MS). As a result, 87 phenolic compounds were detected, among which the anthocyanin group must be detached. The antioxidant potentials of water and acetone extracts of these grape varieties were assessed by five different assays, including DPPH, ABTS, FRAP, ORAC and Folin–Ciocalteu assays. The Volatile Organic Compounds (VOCs) were isolated by two different methods and analyzed by GC/MS and GC/FID. The results obtained in this study showed significant differences among the varieties. SA acetone extract had the highest total phenolic content (200.75 mg GAE/100 g) and antioxidant potential measured by DPPH (1,393.19 μmol TEAC/g), FRAP (208.81 μmol Fe2SO4.g^{-1}) and ORAC (341.01 μmolar of Trolox eq./g). In ABTS analysis, SA water extract revealed the higher average value (549.37 μmol TEAC/g). SA samples showed high values of anthocyanins (23.04mg/100g) compared to SU samples (9.43mg/100g). Malvidin-3-O-glycoside (14.46mg/100g) and peonidin-3-O-glycoside (3.77mg/100g) were found as major compounds in SA. The volatile fraction of SU (42 components) was richer than that of SA (31 compounds). Fatty acids, alcohols and aldehydes were the major volatile compounds found in both grape cultivars. The hybrid grape cultivars are rich in anthocyanins and present high antioxidant capacities. They could be considered potential sources for the development of nutraceuticals and functional foods.

Keywords: Hybrid grapes varieties, antioxidant potential, phenolic and volatile compounds.

1. Introduction

Due to the beneficial effects on human health and its economic importance, grape is a fruit widely grown and eaten around the world. Historically, the production and export of grapes were controlled almost exclusively by traditional European countries. However, in recent years, South America has shown a significant rate of growth in production and export of grapes with two crops a year (Gutiérrez-Gamboa et al., 2020). Although in Brazil, the practice of viticulture is recent when compared to traditional European countries, there is an improvement in the quality of Brazilian grape cultivar composition due to the use of hybridization techniques (Olivati et al., 2019). In contrast to the almost exclusive growth of *V. vinifera* cultivars in traditional wine producing countries, hybrid grape cultivars represent more than 80% of the volume of grapes (1,399,262 tons) processed in Brazil (De Rosso et al., 2012; Nicolini et al., 2020).

Hybrid grapes are obtained from the crossing of two or more species of *Vitis* that allows the selection of characteristics of interest, such as high resistance to diseases and pathogens. As well, interspecific hybrids are characterized by different chemical compositions and are especially known to exhibit high content of phenolic compounds and specific profile of anthocyanins, being highlighted also to their potential to produce red quality wines (Samoticha et al., 2017). However, the hybrid varieties are currently little studied. Phenolic compounds have been extensively studied due to their potentially beneficial antioxidant, anti-inflammatory and anti-carcinogenic properties, which are spurring the interest of both industry and consumers for phenolic-rich foods (Gorzynik-Debicka et al., 2018; Kelly et al., 2018).

A wide range of biological activities have been attributed to these compounds, indicating that some fruit sources can provide more than nourishment. Natural foods of high nutritional quality play an important role in maintaining human health(Cory et al., 2018; Gülçin et al., 2002). As a result of this, much attention has been focused on the use of exogenous antioxidants, especially natural antioxidants to inhibit the oxidation of cellular components, thereby protecting from damage due to free radicals (S. Liu et al., 2019).

In the last decade there has been increasing interest in the determination of suitable dietary sources of antioxidant phenolic compounds (Delgado et al., 2019). However, there is little knowledge about the phenolic compounds of hybrid grape cultivars reported in literature: few reports describe only the anthocyanins for hybrid species (Fujita et al., 2020; Nixdorf & Hermosín-Gutiérrez, 2010).

Nowadays, liquid chromatography coupled high resolution tandem mass spectrometry

(LC-MS-MS) is considered the more effective tool for the structural characterization of these low molecular weight (MW) compounds (Santos et al., 2018). Metabolomics methods have high sensitivity, good resolution, and high-throughput capacity and should be able to reveal a great number of compounds in a single run (De Rosso et al., 2012; Gika et al., 2019).

Anthocyanins are phenol compounds present in grape skin and are responsible for the red color of both grapes and wines (R. N. Pereira et al., 2020; Shahab et al., 2020). These compounds have been widely studied in *V. vinifera* varieties because they play a key role in the organoleptic characteristics of grapes (Dumitru et al., 2019). They also have antioxidant, antimicrobial and anti-cancerogenic activities, showing a protective effect on the cardiovascular system (Demirbas et al., 2017; Qin et al., 2019). Anthocyanins are studied for grape variety characterization and represent an important resource for the natural colorant industry (Albuquerque et al., 2020; Chatham et al., 2020).

The chemistry of grape cultivars, especially varietal aroma, has a significant impact on the character of grapes, its sensory perception and its quality, impacting consumer acceptance. Varietal aroma can relate to a specific compound or to a small group of odoriferous molecules, but is usually attributable to the contribution of several volatile compounds occurring in grapes, in proportions that differ from one variety to another (Slegers et al., 2015). Those aromas comprise hundreds of volatile organic compounds (VOCs) made up of different chemical groups, including alcohols, esters, aldehydes, ketones, monoterpenoids and others. Methods for extracting VOCs often include liquid–liquid extraction, simultaneous distillation and extraction, headspace solid-phase microextraction (HS-SPME), and stir bar sorptive extraction techniques, among others (Lee et al., 2016).

Due to the large consumption of grapes in Brazil and its potential as an antioxidant source and functional food, this work aimed to characterize the phenolic and volatile compounds from new varieties of hybrids grapes (*Sweet Sapphire* (SA) and *Sweet Surprise* (SU)) by applying gas chromatographic and metabolomic techniques and probing their antioxidant properties.

This study is the first investigation to report the chemical composition and a comprehensive metabolic profiling of *Sweet Sapphire* (SA) and *Sweet Surprise* (SU).

2. Material and Methods

2.1.Grape Samples

Two grape cultivars, *Sweet Sapphire* (SA) and *Sweet Surprise* (SU), were provided by Labrunier farm located in Petrolina (Pernambuco, Brazil) and transported under refrigeration to the laboratory. The grape samples were then separated into 3 fractions: integral, peel and pulp and immediately frozen and stored at -80 °C in an ultrafreezer.

2.2. Colorimetric analysis

Colorimetric analysis was performed in grape samples to determine the color coordinates L* (lightness), a* (red/green, where +a indicates red and -a green) and b* (yellow/blue, where +b indicates yellow and -b blue) in a CM-5 (Konica Minolta, Japan) colorimeter (Westland et al., 2012).

2.3. Moisture, ash and total reducing sugar evaluation

The contents of moisture, ash and total reducing sugars were determined according to the standard methods (AOAC, 2000).

2.4. Sample preparation for the evaluation of its antioxidant capacity and phenolic composition

The samples (integral, peel and pulp) of the two varieties were extracted by means of 2 extractor solutions: (I) acetone 70% and (II) water. From 5 g of sample, 100 mL of extractor solution was added and followed by homogenization at room temperature (~ 20 °C) in a shaker (TE-420, Tecnal, Brazil) for 10 minutes in the absence of light. The samples were then centrifuged (Thermo Fisher Scientific, California, EUA) (5,000 *g*, 5 min, 20 °C) and filtered through analytical filter paper.

For antioxidant activity analysis, the extracts obtained were freeze-dried and after dilution, they were subjected to ultrasound bath (42 kHz) (Cristofoli, Brazil) for 30 minutes. The extracts obtained were filtered. Therefore, the following extracts were obtained: PESP-WE (*Sweet Sapphire* peel with water extractor), PESP-ACE (*Sweet Sapphire* peel with acetone extractor), PUSP-WE (*Sweet Sapphire* pulp with water extractor), PUSP-ACE (*Sweet Sapphire* pulp with acetone extractor), PESU-WE (*Sweet Surprise* peel with water extractor), PESU-ACE (*Sweet Surprise* peel with acetone extractor), PUSU-WE (*Sweet Surprise* pulp with water extractor), and PUSU-ACE (*Sweet Surprise* pulp with acetone extractor).

For UPLC-MS analysis, the extractor solutions were evaporated using a rotary

evaporator under vacuum (Savant, Thermo Scientific) at 40 °C and thereafter dissolved in methanol/acetonitrile/water (2:5:93; v/v). Stock solutions of 11 standards (caffeic acid, (+)-catechin, ellagic acid, (-)-epicatechin, gallic acid, gentisic acid, 4-hydroxybenzoic acid, myricetin, pyrogallol, quercetin and quercetin 3-O-glucoside) from Sigma-Aldrich (St-Louis, MO, USA) were prepared individually by dissolving accurately weighed amounts of standards in aqueous methanol. An aliquot of each stock solution was mixed to achieve a mixed standard solution with a final concentration of 10 ppm for each compound. Finally, extracts and standards were filtered through a 0.22 µm syringe filter and stored at -20 °C until UPLC-MS analysis.

2.5.Evaluation of the antioxidant activity and total phenolic content of grape extracts

2.5.1 DPPH Assay

Aliquots of 0.5 mL of the extracts were mixed with 2.5 mL DPPH (2,2-diphenyl-1-picrylhydrazyl) methanolic solution (60 µM) and allowed to react for 1 h in the dark. Measurements were performed in triplicates at 515 nm using a spectrophotometer (Turner 340, Germany). The percentage decline in the DPPH radical absorbance caused by the extracts was compared to a Trolox standard curve and the results were expressed as µmol of Trolox equivalents/g in wet basis (Brand-Williams et al., 1995).

2.5.2 ABTS Assay

In the method of ABTS (2.2 acid '-azino-bis 3-6 etilbenzotiazolin-sulfonic acid), three different concentrations of the extracts were used, and the readings were performed in triplicate. A standard curve with solutions of Trolox was produced. The results were expressed in Trolox equivalent antioxidant activity (Thaipong et al., 2006).

2.5.3 Ferric Reducing Antioxidant Power Assay (FRAP).

The antioxidant activity by FRAP assay according to Thaipong et al. (2006) with some modifications. Aliquots of 2.7 mL of TPTZ reagent (ferric 2,4,6-tripyridyl-S-triazine) were mixed with 0.5 mL of sample extract. After 30 min at 37 °C, the absorbance was read at 595 nm. The antioxidant capacity (FRAP) was expressed as Fe^{3+} equivalents (µmol Fe^{3+}/g in humid basis).

2.5.4 Oxygen-radical absorbance capacity assay (ORAC)

The ORAC procedure was performed using an automated plate reader (SpectraMax i3x,

Molecular Devices, USA) with 96 well plates. Experiments were conducted in phosphate buffer (pH 7.4) at 37 ºC. Peroxyl radical was generated using 2,2'-azobis(2-amidino-propane) dihydrochloride which was freshly prepared for each run. Fluorescein was used as the substrate. Fluorescence conditions were as follows: excitation at 485nm and emission at 520nm. The standard curve was linear between 0 and 50 mM Trolox and the results were expressed as μmolar Trolox equivalents/g fresh mass (Prior et al., 2003).

2.5.5. Total phenolic content Assay

Total phenolic content (TPC) analysis was performed using the Folin-Ciocalteu method. In brief, a 1 mL sample (1 mg/mL) was mixed with 1 mL of Folin-Ciocalteu's phenol reagent. After 5 min in room temperature, 10 mL of a 7% Na_2CO_3 solution was added to the mixture followed by the addition of 13 mL of deionized/distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23 °C, after which the absorbance was read at 750 nm. The TPC was determined from extrapolation of the calibration curve which was made by preparing gallic acid solution. The estimation of the total content of phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per 100g of humid sample (Saeed et al., 2012).

2.6. Identification of phenolic compounds by UPLC-MS^E

For UPLC-MS analyses, 2 μL of extracts and standards were injected in triplicate into an Acquity UPLC (Waters, Milford, MA) coupled to Xevo G2-S QTOF-MS/MS (Waters, Manchester, UK) system equipped with an electronspray ionization source (ESI) operating in negative ion mode. The column used was a UPLC HSS T3 C18 (100 mm x 2.1 mm, 1.8 μm) (Waters, Ireland). The column and autosampler were maintained at 30 °C and 8 °C, respectively. The flow rate was 0.6 mL/min and the mobile phase gradient elution consisted of acidified water (5 mM ammonium formate and 0.3% formic acid, v/v) (pump A) and acetonitrile containing 0.3% formic acid (pump B), as follows: 97% A at 0 min, 50% A at 6.8 min, 15% A at 7.4-8.5 min, followed by an additional equilibration step 97% A at 9.1-12 min. Data were acquired using a multiplexed MS/MS acquisition with alternating low and high energy acquisition (MS^E) on centroid mode, collecting data from *m/z* 50-1000. The capillary and cone voltage were set at 3.0 kV and 30 V, respectively. The desolvation gas (N_2) was set at 600 L/h at 450 °C, the cone gas was set at 50 L/h and the source temperature at 120 °C. MS/MS experiments were performed with collision energy ranging from 30-55 eV using ultra-high pure argon (Ar) as a collision gas. Data acquisition was performed by using MassLynx 4.1 (Waters

Corporation, Milford, MA). To ensure accuracy and reproducibility, all acquisitions were performed by infusing lock mass calibration with leucine-enkephaline (Waters Corporation, USA) (*m/z* 554.2615) at a concentration of 1.0 ng/L in acetonitrile: H_2O (50:50, v/v) with 0.1% (v/v) formic acid at a flow rate of 10 μL/min.

The raw data of all replicates were processed with Progenesis QI v2.1 (Nonlinear Dynamics, Waters Corporation, UK) with the following conditions: all runs, automatic limits, centroid data, resolution full-width at half maximum (FWHM) of 50,000, ionization negative ion mode, deprotonated molecule $[M - H]^-$. The identification of phenolic compounds was performed by searching for polyphenols with MetaScope, using a customized database of polyphenolic compounds from PubChemID by using the following parameters: precursor and fragment mass error tolerance (5 and 10 ppm, respectively) and retention time limit of 7.5 min. Target analysis was also applied for identification of the phenolic compounds by comparing the run parameters of phenolic standards such as the retention time, exact mass, mass error and the MS-MS spectra, besides the other above mentioned parameters. The processed data were exported to EZinfo, where Principal Components Analysis (PCA) was elaborated.

2.7. Anthocyanin Analysis

Integral samples were weighed (1 g) to extraction with methanol and formic acid solution in the ultrasound bath with subsequent centrifugation to discoloration of the solution. Then, an aliquot (1 mL) of the extract was dried with compressed air, being the same resuspended in methanol and formic acid. A high-performance liquid chromatography (HPLC) (Alliance 2695, Waters) equipped with photodiodes arrangement detector and a column C18 BDS (100 mm x 4.6 mm, 2.4 μm, ThermoScientific) applying gradient elution mode with acetonitrile and formic acid was used for the chromatography separation. The quantification of anthocyanins was made by external standardization, from isolated patterns. The results were expressed in mg/100 g (Lves et al., 2007).

2.8. Volatile fraction analysis

2.9. Identification of volatiles compounds by GC/MS

2.9.1. Solvent Extraction Method

This isolation process was based on a previous work (Radulovic *et al*., 2010). The whole intact fresh berries (150 g) were immersed into vessels with 100 mL of diethyl ether, containing

1 ppm of BHT as internal standard, in an ultrasonic bath for 15 min at room temperature. The obtained ether washings were gravity filtered through small columns packed with 1 g of Celite (Merck, Germany) in order to remove all the insoluble material and then concentrated to 0.5 mL at room temperature using a steam of N_2 before the chromatographic analyses. These analyses were performed with the aid of a gas chromatography system coupled to a mass spectrometer (GC/MS) or combined with a flame ionization detector (GC/FID).

The GC/MS analyses (two repetitions) were carried out using a Shimadzu GC-2010Plus/GCMS-QP2010 equipment containing a fused silica capillary column SPB-1 (100% dimethyl polysiloxane, 30 m x 0.25 mm i.d., film thickness 0.25 µm, Supelco, USA). The injector and interface were operated at 250ºC and 300ºC, respectively. Oven temperature was raised from 70–290ºC at a heating rate of 5ºC/min and then isothermally held for 30 min. As a carrier gas, He at 1.0 mL min^{-1} was used. The samples and external standards (1 µL) were injected in splitless mode. MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 35–500, scan time 0.32 s. Extract constituents were identified based on comparison of their mass spectra with those from NIST12.lib and NIST62.lib mass spectral libraries. Besides the mass spectral libraries, the identification was also carried out using reference substances and the comparison between the calculated linear retention indexes (relative to a C7-C40 alkane mixture - 1,000 µg mL^{-1} of each component in hexane) and those available in the scientific literature. Only the compounds identified using at least reference compounds and mass spectral data were identified. GC-FID analysis was carried out under the same experimental conditions using the same column as described for the GC/MS. The concentrations of the volatile compounds were estimated by the internal standardization method. Calibration curves were constructed by analyzing standard solutions at three different concentrations under identical experimental conditions. In the case of the tentatively identified compounds, the semi-quantification process was performed about the most structurally similar reference compounds available in our laboratory (see Table 3).

2.9.2. Solid Phase Extraction Method

In this case, the volatile compounds were isolated according to a previous work of Araújo *et al*., 2014. The initial grape extract was obtained according to the following process: 140 g of the whole intact fresh berries of each grape cultivar were mixed with 250 mL distilled water and 30 g sodium chloride in a mixer. This material was transferred to a 500 mL volumetric balloon by vacuum filtration. Then, distilled water was used to complete the volume of the balloon. A solid-phase extraction technique allows the separation of the volatile compounds of

the grapes. Before the isolation of the aroma components, 100 μL of 3-methylbutanoic acid solution (2.5 μg μL^{-1} in absolute ethanol) were added to the samples (140 g) as an internal standard. A volume corresponding to 130 mL of the fruit aqueous extract was forced to pass through the column (flow rate = 1.5 mL min^{-1}) containing the adsorbent material [Porapak Q (50/80 mesh) - Supelco (Bellefonte, PA)]. After that, 20 mL of distilled water was passed through the column in the opposite direction. Finally, 50 mL of acetone was used to elute the volatile compounds adsorbed in the column and this material was concentrated to 50 μL. Each acetone extract was analysed by GC-FID and GC/MS techniques.

The same GC/MS system was used to evaluate the grape acetone extracts. In this case, the injector was operated at 230ºC. The temperature of the chromatographic oven was initially programmed to stay at 60ºC for 5 minutes. It was then increased in a 2ºC/minute rate to 120ºC, remaining in this last temperature for 10 minutes. Finally, it increased at a 5ºC/minute rate until it reaches 230ºC, where it was maintained for 30 minutes. Again, Helium was used as the carrier gas in a 1.0 mL min^{-1} flow. Injections of the samples and standards (1 μL) were also performed in splitless mode. The mass spectrometer operated at an ionization voltage of 70 eV, scanning the fragments in the range of 30 to 400 *m/z*, in cycles of 3 tenths of a second. The temperatures of the ion source and the GC interface were maintained at 240ºC. The identification and quantification of the volatile compounds were carried out as already mentioned.

2.10 Statistical analysis

Statistical analysis was performed to compare means by using GraphPad Prism (5.0) for statistical significance by one-way ANOVA (Tukey, $p<0.05$).

3. Results and discussion

3.1. Composition of grapes SA and SU

The composition of grapes SA and SU are presented in Table 1. Moisture, ash, total reducing sugar and colorimetric evaluation of SA and SU grapes. SA berry size ranged from 14 to 19 mm, whereas SU berry size ranged from 15-17 mm. The moisture contents of SA and SU were around 82-83%, very similar to the values (82%) found in pink Niagara grapes (Abe et al., 2007). The ash contents varied from 0.80±0.4 to 0.39±0.29 g/100g for SA and SU, respectively, being significantly smaller than Japanese grape that presented 2.16±0.04 g/100 g (Bampi et al., 2010) and Arinto (4.35 ± 0.03), Aragonês (5.53 ± 0.19), Merlot (6.98 ± 0.23), Talia (4.85 ± 0.31), Syrah (7.13 ± 0.06), Cabernet Sauvignon (8.36 ± 0.55), Trincadeira (6.26

± 0.37) (P. Pereira et al., 2020). In the same work, the reduction sugar mean content of the Japanese grapes was indicated as 12.57 ± 0.39 g/100g, compared with the samples of SA and SU, which present a smaller amount of reducing sugars (1.90 ± 0.04 and 1.70 ± 0.09 g/100ml, respectively).

The SA presented lower values of L* (7.71 and 16.23, respectively) than those found in the peel and pulp of SU (28.80 and 24.98, respectively), showing that SA is darker than SU. Crimson grape pulps presented similar L* (15.9) (Abe et al., 2007) values as SA. The values of a* of the peel and pulp of SU were higher (7.06 and 11.43, respectively) than those of SA (4.48 and 9.31, respectively), thus showing more shades of red. The values of b* of both samples were negative, evidencing blue tones in the barks (-0.47 and - 1.51 in SA and SU, respectively), although the values of b * of the pulps (1.97 and 4.90 on SA and SU, respectively) were positive, showing shades of yellow.

Table 1. Composition hybrid grapes cultivars *Sweet Sapphire* and *Sweet Surprise*.

Composition	*Sweet Sapphire*	*Sweet Surprise*
Berry (mm)	14-19	15-17
Moisture (%)	82.00±0.43	83.54±0.36
Ash (g / 100g)	0.80±0.40	0.39±0.29
Reducing sugar (g/ 100mL)	1.90±0.04	1.70±0.09
L* pulp	16.23	24.98
L* peel	7.71	28.8
a* pulp	9.31	11.43
a*peel	4.48	7.06
b* pulp	1.97	4.9
b*peel	-0.47	-1.51

3.2. Antioxidant activity of extracts

Interestingly, peel fractions of both samples showed greater antioxidant potential than pulp fruits, in the analysis of the antioxidant activity by the tested different methods. Generally, antioxidant measurements can be related either to the capacity of extracts to directly transfer hydrogen to a radical (DPPH or ABTS), to donate electrons (FRAP) or to act as competitors for peroxy radicals (ORAC test). Due to this, the antioxidant capacity of each extract cannot be determined by a single method. More than one type of measurement needs to be performed to take into account the various mode of action of antioxidants (Ky & Teissedre, 2015). Their results are shown in figure 1. Data from the literature of grape berries of Shiraz in two different regions, in Pune and Nasik, presented values near to 114.72 ± 11.65 μmol TEAC/g DW and

108.27 ± 11 µmol TEAC/g DW, approximately the same activity (Savalekar et al., 2019). Another study presented value of 410.79 µmol TEAC/g from skin of Grenache grape (Ky & Teissedre, 2015). Differently, hybrid grape peel showed values around 17 µmol TEAC/g fresh weight for the Uslo and Isabella varieties (Yilmaz et al., 2015). Tests were performed on duplicates of the extract. In the DPPH analysis, the PESP-ACE extract had the highest mean of 1,393.19 µmol TEAC / g fresh weight, followed by PESP-WE with 472.17 µmol TEAC / g fresh weight. There was no significant difference between the PUSP, PUSU and PESU extracts.

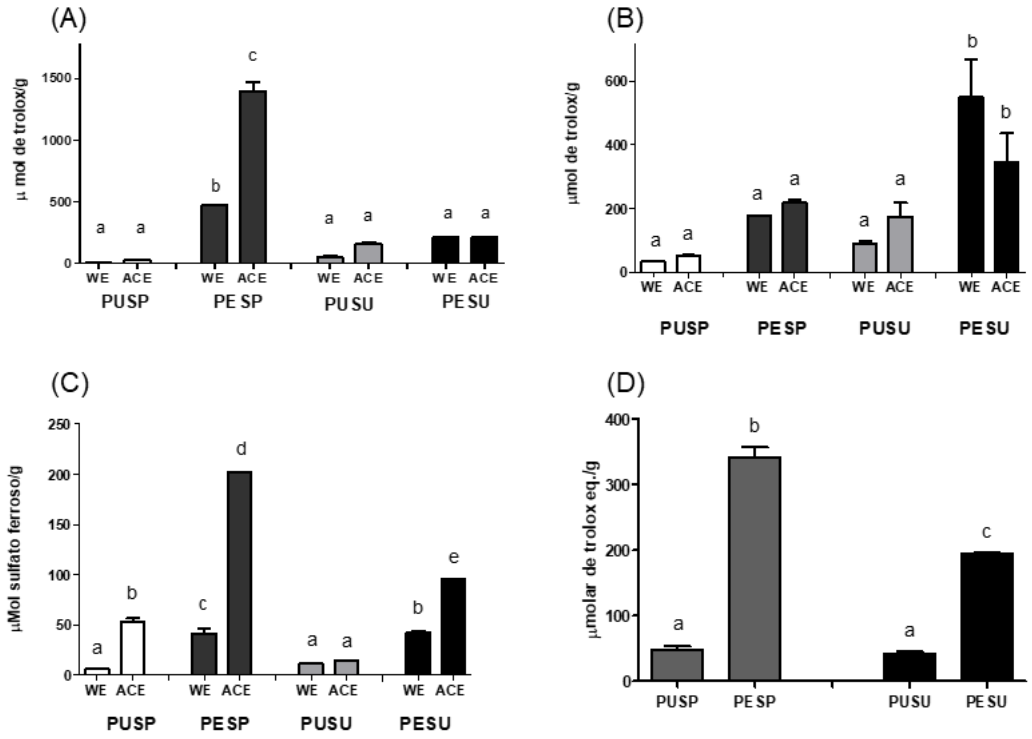

Figure 1. Antioxidant activity of the PUSP, PESP, PUSU and PESU samples by the DPPH (A), ABTS (B), FRAP (C) and ORAC (D) method.

Again, results from the ABTS and FRAP methods showed that the peel was the fraction with the highest antioxidant capacity. In the present work, in the ABTS analysis the SUPE-WE sample with a higher average value of 549.37 µmol TEAC/g fresh weight, which shows a high antioxidant activity. Also, in the ABTS analysis the PUSP, PESP and PUSU samples with both extractors, WE and ACE, showed no significant difference. Regarding the ABTS method, another work found in the literature with grape pomace had a value of 4.40 ± 0.15 µmol TEAC/g DW (Favati et al., 2020). In the work cited above with hybrid grapes, the Uslo variety had a

value of 4.78 μmol TEAC/g fresh weight and the Alfons variety obtained a value of 4.75 μmol TEAC/g fresh weight (Yilmaz et al., 2015).

In the FRAP analysis as well as in the DPPH analysis, the PESP-ACE sample had a higher average value of 201.81 μmol of ferrous sulphate/g fresh weight, followed by the PESU-ACE sample with a mean value of 95.3 μmol of ferrous sulphate/g fresh weight. (Ky & Teissedre, 2015) shows data near to 1.52 mM Fe2+/g DW in Syrah skin extract. According to a study carried out by Haas, 2015, with residue of grape juice processing, the antioxidant analysis by the FRAP method revealed values of 60 μmol TEAC/g fresh weight, being this value lower than those found in the present study. In the work done with the hybrid of the varieties Uslo, Alfons and Isabella, the following values were indicated: 17.22 μmol TEAC/g, 30.00 μmol TEAC/g and 56.55 μmol TEAC/g fresh weight, respectively (Yilmaz et al., 2015).

In the ORAC analysis, as well as in the DPPH and FRAP analyzes, the PESP sample presented a higher mean value of 341.01 μmolar of Trolox eq./g followed by the PESU sample with an average value of 193.99 μmolar of Trolox eq./g fresh weight. The PUSP and PUSU pulps presented no significant difference. In comparison, a work of the literature with the black seedless grape was found an average value of 23.58 μmolar of Trolox eq./g and in the grape Chardonnay an average value of 61.42 μmol of Trolox eq./g fresh weight (Instituto de Nutrición y Tecnología de los Alimentos (INTA), 2013). Other studies show values near to 25 μmol of Trolox eq./g in grape juice (Faria et al., 2016) and 62.75 μmol of Trolox eq./g in Syrah grape extract (de Camargo et al., 2019). The solutions used have different polarities demonstrating the presence of hydrophilic and lipophilic compounds with different characteristics depending on the fraction and fruit analyzed. Thus, a great potential of this variety is observed as source of antioxidant compounds mainly in the peel, part that is usually used.

3.3. Total phenolic compounds

In the determination of total phenolic compounds, the PESP-ACE sample had a higher mean value of 200.75 mg AGE/100 g fresh weight, followed by the PESU-ACE sample (Figure 2). In a study with Rosa Niagara grape cultivars, values from 208 to 214 mg AGE/100 g fresh weight were quantified, which shows values similar to those found in the present study (SOARES, M.; WELTER, L.; KUSKOSKI, E. M.; GONZAGA, L.; FETT, 2008).

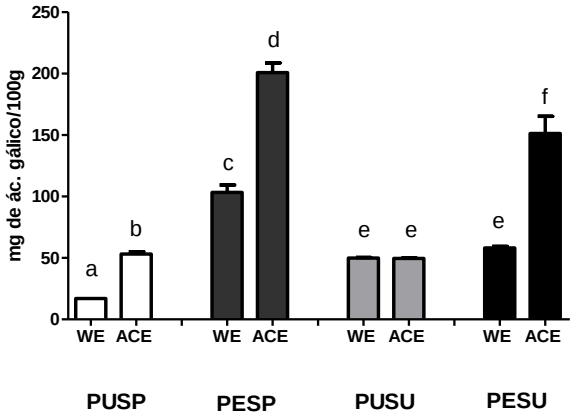

Figure 2. Total phenolic compounds of the PUSP, PESP, PUSU and PESU samples.

3.4. Phenolic composition by UPLC-MS^E

Metabolomics approach used in this work aimed to strength and show the diversity of phenolic compounds present in the samples, in addition to corroborate the analysis for determination of antioxidant capacity. Globally, a total of 87 phenolic compounds were tentatively identified, among them the main classes were flavonoids (Peixoto et al., 2018), phenolic acids (Chen et al., 2020), other polyphenols (Rasines-Perea & Teissedre, 2017) and lignans and stilbenes (Constantinou et al., 2017) (Table 4). The main subclass of identified compounds was hydroxybenzoic acids (Wang et al., 2016) followed by flavanols (H. Liu et al., 2019) and hydroxycoumarins (Zeitoun et al., 2020), belonging to phenolic acids, flavonoids and other polyphenols classes.

A targeted analysis taking account the data acquisition of the standard mix containing phenolic compounds were performed considering as parameters the exact mass, retention time and fragment spectra for confirmation. Among the reference standards, 11 compounds were identified in grape samples, being quercetin 3-O-glucoside, the most abundant compound, present in all extracts (water and acetone) and fractions analyzed (pulp and integral) of both grape varieties. The second most abundant compound was not present in the standard mix and so considered as tentatively identified as myricetin 3-O-glucoside. This compound was present in all samples except for pulp water extracts of both grapes (Table 3).

Some compounds presenting similar *m/z* but different retention times could also be identified, including for reference standards, showing the presence of isomeric forms of ferulic acid, vanillic acid, epigallocatechin, kaempferol and pyrogallol for instance. On the other hand, the identification of 26 compounds remained uncertain as indicated in Table 3, because the parameters such as retention time and mass spectra details (mass error, fragmentation score and isotope similarity) were similar between them and not sufficient to determine their identity.

Among them, the isomers of protocatechuic acid and dihydroxybenzoic acid can be cited.

The highest number of compounds were identified in the cultivar *Sweet Sapphire* integral with the acetone extractor, followed by the same sample with the water extractor. Indeed, the variety *Sweet Sapphire* showed the highest number of identifications and abundance of phenolic compounds, being the acetone the most efficient extractor, which can become evident with the principal component analysis (PCA) (Figure 3), corroborating the results of antioxidant assays.

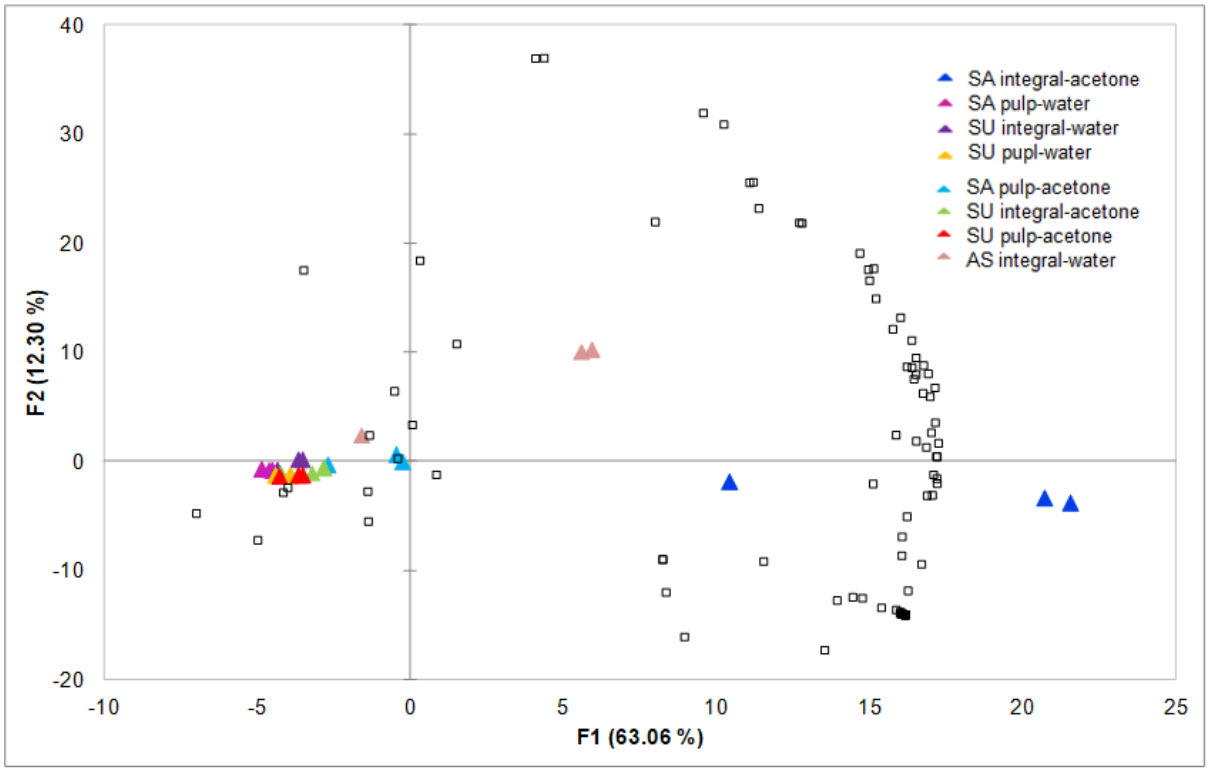

Figure 3. Principal Component Analysis - PCA, of the water extracts (WE) and acetone extracts (ACE) of the pulp and integral fractions of the cultivars *Sweet Surprise* and *Sweet Sapphire.*

The PCA (loadings bi-plot) can be used to assess the multivariations in perspective and to elucidate overall diferences between samples (Santos et al., 2019). In this study, the integral sample from *Sweet Sapphire* obtained by acetone extraction can be clearly discriminated from the others by principal component 1 (PC1), which explained 63% of the variation among the samples, due to the profile and relative abundance of the identified phenolic compounds, especially flavonoids.

Flavonoids showed the highest abundance, followed by phenolic acids, other polyphenols, stilbenes and lignans (Figure 4). As expected, integral samples showed a higher abundance of phenolic compounds than pulp. The extracts of Sapphire integral presented a total ion abundance of 6- and 3-folds superior to pulp, respectively for water and acetone. Whereas

the extracts of Surprise integral presented the double of total ion abundance than pulp, independently of extractor (Figure 4).

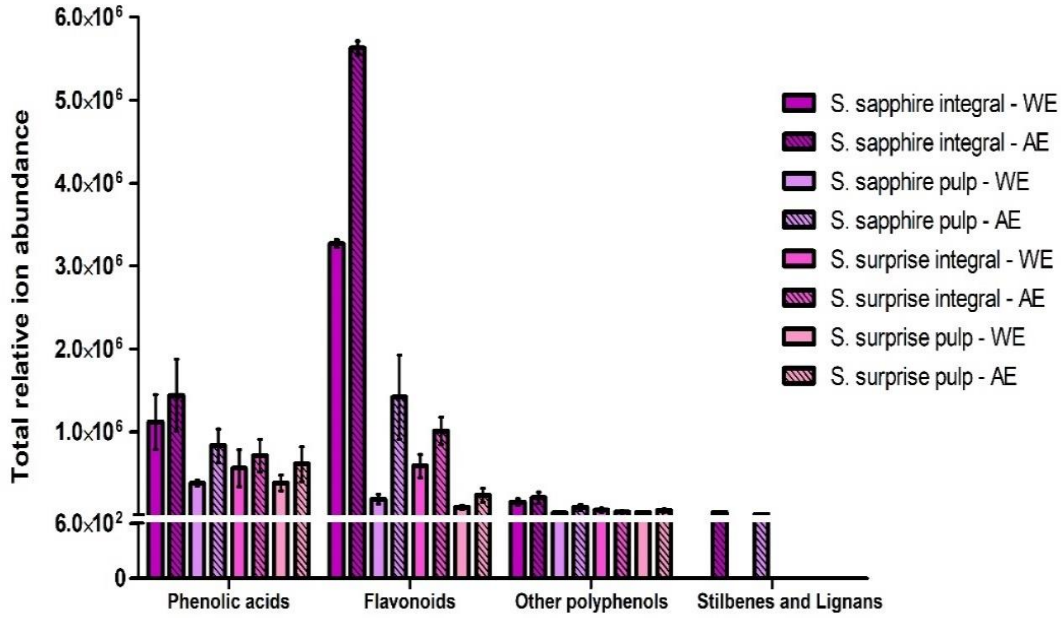

Figure 4. Total relative ion abundance.

Analyzing the difference between integral and pulp, it is possible to estimate the abundance in peels for each class of phenolic compounds. For Sapphire water extracts the phenolic compounds were mostly found in the peel: 94%, 85% and 66% respectively for flavonoids, other polyphenols and phenolic acids. Remarkably, stilbenes and lignans were only identified in acetone extracts, being the most part (86%) found in peels. Conversely, for Surprise water and acetone extracts respectively, phenolic acids were more abundant in the pulp (69% and 86%) than in integral samples, while flavonoids were mainly found in peel (84% and 76%). No differences were found for the other polyphenols class between peel and pulp. Stilbenes and lignans were not identified in these samples.

These results can explain and assign to these varieties the great antioxidant capacity. Indeed, flavonoid compounds have been pointed as effective compounds in the control of cellular cycle and apoptosis (Abotaleb et al., 2019; Coelho et al., 2016; Varela-Rodríguez et al., 2020). In this work, the main identified compounds comprised the flavonoid structures commonly reported for *Vitis vinifera* L., such as kaempferol, myricetin, quercetin and isorhamnetin and their monoglyceride derivatives (Lingua et al., 2016). The quercetin-3-O-glucuronide, found in all extracts, was previously appointed as a potential intervention for Alzheimer's disease and was demonstrated that has significantly reduced the generation of β-

amyloid (A) (Ho et al., 2013). Additionally, four proanthocyanins (also known as condensed tannins) found in the solid parts of berries were detected: procyanidin dimer B5, procyanidin B8 and procyanidin trimer C1 isomers. Remarkably, it was also detected the presence of nepetin and jaceosidin, compounds related to anti-inflammatory activity (Patel & Patel, 2016).

In the class of phenolic acids, expected hydroxycinnamoyl tartaric acids, also known as caftaric (from caffeic acid), coumaric (from *p*-coumaric acid), and ferric acids (from ferulic acid), were found in grapes (Lingua et al., 2016). Recent research demonstrated that caffeic acid possesses a wide variety of pharmaceutical effects, including anti-inflammatory, anti-cancer, anti-thrombosis, anti-hypertensive, anti-fibrosis, and anti-viral activities. In addition, several studies supported the possible neuroprotective potential of caffeic acid in Aβ-induced cellular toxicity (Kim et al., 2015).

From the subclass of the hydroxybenzoic acids it was also detected the gentilic acid (dihydroxybenzoic acid), an active metabolite of salicylic acid, which has antioxidant, anti-inflammatory effects, as well as being able to improve cardiovascular problems related to aging, such as hypertension, atherosclerosis and dyslipidemia (Cavalcante et al., 2017). Recent research claims that gentilic acid inhibits oxidative stress and this may be one of the possible mechanisms for anti-Parkinson's (Kabra et al., 2014).

The piceatannol was the most abundant compound from the stilbenes class, this compound is a naturally occurring polyphenol found in various fruits and vegetables and has been reported to exhibit anticancer and anti-inflammatory properties. In addition, it was recently reported beneficial effects of piceatannol on hypercholesterolemia, atherosclerosis, and angiogenesis emphasizing its therapeutic potential in cardiovascular disease (Kershaw & Kim, 2017).

3.5. HPLC analysis for individual anthocyanins compounds

Among the phenolic compounds present in grapes, the main ones are anthocyanins. In grapes of *V. vinifera* L. there are five colored aglycons commonly occurring, namely cyanidin, peonidin, delphinidin, petunidin and malvidin (Rojas-Lema et al., 2021). Anthocyanins are located in the berry skin and are the main responsible compounds for the red color of grapes and wines (Lingua et al., 2016).

Our results showed that malvidin derivatives presented the highest content in both cultivars. Malvidin 3-O-glycoside with 14.46 mg/100 g in *Sweet Sapphire* and 2.76 mg/100 g in *Sweet Surprise*, in accordance with many authors (Albuquerque et al., 2020; Jiménez-Moreno et al., 2019; Ruta & Farcasanu, 2019). Cyanidin derivatives showed the lowest concentration

in SA with 0.63 mg/100 g, probably because this anthocyanin is the precursor of all others(Campayo et al., 2019). On the other hand, in SU, peonidin derivatives showed the lowest concentration with 0.77 mg/100 g. SS samples showed high values of total anthocyanins (23.04 mg/100 g) compared to the SU sample (9.43 mg/100 g) (Table 2).

Table 2. Total anthocyanins present in the cultivar's *Sweet Sapphire* and *Sweet Surprise*.

Anthocyanins	*Sweet Sapphire*	*Sweet Surprise*
Delphinidin-3-O-glucoside	1.98	1.22
Cyanidin-3-O-glucoside	0.63	1.09
Peonidin-3-O-glucoside	3.77	0.77
Petunidin-3-O-glucoside	2.2	nd
Malvidin-3-O-glucoside	14.46	2.76

3.6. Volatiles compounds

The results obtained from both extraction methods (see table 3) demonstrate that the volatile fraction of SU (42 components) was richer than that of SS (31 compounds). Two other compounds (bis(2-ethylhexyl)-phthalate and bis[2-(2-butoxyethoxy) ethyl] adipate) that appeared in both cultivars, must be considered food contaminants. This kind of contamination has been associated to foods marketed in R-PET boxes (Loos et al., 2020), as happen with our grape samples. Twenty-five compounds were found in both grape cultivars. The compounds heptadecanoic acid, nonadecane, 3,7-dimethyloctan-1-ol, octagonal, 3-hexene-2,5-dione and 4-hydroxy-5-methyl-2-hexanone were found only in the SS cultivar, while tetracosane, methyl hex decanoate, ethyl linoleate, ethyl arachidonate, ethyl decanoate, ethyl lignocaine, 1-tricosanol, manoyl oxide, pentagonal, 2,4-dimethyl-1,3-dioxolane-2-methanol, 4-methoxy-2,5-dimethyl-3(2H)-furanone, artemisia ketone, 2-phenoxyethanol, dimethylbenzylcarbinol acetate, diethyltoluamide, 2-p-tolylpyridine and benzophenone were found exclusively in the SU cultivar. The volatile compounds of SS could be divided in the following groups: fatty acids (7 members), hydrocarbons (9), esters (2), alcohols (3), terpene compounds (3), aldehydes (3) and miscellaneous compounds (4). On the other hand, the volatile compounds of SU could be classified as follows: fatty acids (6), hydrocarbons (9), esters (7), alcohols (4), terpene compounds (3), aldehydes (3) and miscellaneous compounds (10). According to data obtained from method 1, hexadecenoic acid (238.72 ± 327.87 ppb, 1-octacosanol (122.05 ± 25.80) ppb and 1-hexacosanol (105.84 ± 18.33) ppb were the major volatile compounds found in SS, while

1-hexacosanol (225.66 ± 30.90) ppb), 1-octacosanol (123.10 ± 29.20) ppb and hexagonal (65.56 ± 20.57) ppb) were the major ones in SU. The employment of method 2 allowed the characterization of linoleic acid (173.56 ± 103.44) ppb, hexadecenoic acid (147.46 ± 35.26) ppb and linolenic acid (23.51 ± 11.77) ppb as the major compounds of SS. The same happened with SU, but in this case hexadecenoic acid (138.20 ± 25.27) ppb changed position with linoleic acid (77.43 ± 24.49) ppb. Linoleic acid is the major compound found in grape seed oil (Lachman et al., 2015). Since SU and SS are classified as seedless grapes, the presence of this essential fatty acid in the volatile fraction of both cultivars (in a relatively high amount) was a surprise. Linoleic acid, as well as linolenic acid (another essential fatty acid), were only found by the employment of method 2 (solid phase extraction), in which the peel and pulp of grapes were mixed and analyzed together. These compounds were not found in the extracts obtained by method 1, in which only grape surface constituents were probably isolated. Some of the volatile compounds identified in the present study possess pharmacological actions. For instance, a previous study showed that palustrol is endowed with an anti-inflammatory activity (Baananou *et al*., 2015). The phenylethyl alcohol possess insect-attractant properties (Scognamiglio et al., 2012). Besides, this alcohol is also capable to exert inhibitory effect against the development of several Gram-negative microorganisms (Abi-Zaid, Riachi, de Maria, & Moreira,2015). Triterpenes are natural compounds showing a wide spectrum of biological effects. They proved to have anti-bacterial, anti-viral, anti-fungal, anti-oxidative, anti-inflammatory, anti-cancer and chemo preventive properties. Squalene and some of its related compounds (all classified as triterpenes) showed the ability to induce apoptosis in many neoplastic lines: leukemia, melanoma, colon cancer, prostate cancer, ovarian carcinoma, liver cancer, breast cancer, lung cancer and peripheral nervous system carcinoma (Chudzik et al., 2015). Their results are shown in table 4.

Table 3. Volatile compounds identified in *Sweet Surprise* (SU) and *Sweet Sapphire* (SA) cultivars by both methods.

Volatile compounds	LRI calculated	LRI literature	SU (method 1) (Avg ± SD) ppb	SU (method 2) (Avg ± SD) ppb	SS (method 1) (Avg ± SD) ppb	SS (method 2) (Avg ± SD) ppb
Fatty acids						
Tetradecanoic acid[b,c]	1746	1747[1]	nd	[(B)]2.98 ± 1.26	nd	[(B)]5.60 ± 4.10
Pentadecanoic acid	1844	1844[1]	nd	[(B)]1.82 ± 0.46	[(B)] 2.90 ± 1.76	nd
[*]Hexadecanoic acid[a,b,c]	1927	1950[3]	41.69 ± 21.52	138.20 ± 25.27	238.72 ± 327.87	147.46 ± 35.26
Heptadecanoic	2027	2022[3]	nd	nd	[(B)] 3.84 ± 2.34	nd

acid[b,c]						
*Linoleic acid[a,b,c]	2114	2113[2]	nd	77.43 ± 24.49	nd	173.56 ± 103.44
*Linolenic acid[a,b,c]	2117	2120[1]	nd	21.49 ± 2.97	nd	23.51 ± 11.77
Octadecanoic acid [b,c]	2126	2124[3]	(B) 17.85 ± 9.55	(B)5.18 ± 2.60	(B) 17.77 ± 18.89	(B)6.77 ± 5.28
Hydrocarbon						
1-Tetradecene[b,c]	1377	1389[3]	(I) 8.68 ± 8.77	nd	(I) 14.02 ± 9,88	nd
*Hexadecane[a,b,c]	1600	1600[3]	nd	4.41 ± 1.17	nd	8.82 ± 1.31
*Octadecane[a,b,c]	1800	1800[3]	nd	4.02 ± 1.33	nd	9.23 ± 4.06
*Nonadecane[a,b,c]	1900	1900[3]	nd	nd	nd	2.38 ± 1.19
*Tetracosane[a,b,c]	2400	2400[3]	6.73 ± 0.35	nd	nd	nd
*Pentacosane[a,b,c]	2500	2500[3]	33.33 ± 4.87	nd	17.56 ± 2.59	nd
*Hexacosane[a,b,c]	2600	2600[3]	15.19 ± 2.79	nd	9.20 ± 1.15	nd
*Heptacosane[a,b,c]	2700	2700[5]	54.70 ± 2.77	nd	66.57 ± 16.95	nd
*Octacosane[a,b,c]	2800	2800[5]	10.06 ± 2.07	nd	9.03 ± 3.46	nd
*Nonacosane[a,b,c]	2900	2900[7]	48.19 ± 1.33	nd	49.38 ± 6.81	nd
Esters						
Methyl hexadecanoate[b,c]	1908	1907[2]	nd	(E)0.94 ± 0.65	nd	nd
*Methyl linoleate[a,b,c]	2091	2092[3]	17.56 ± 16.54	nd	81.79 ± 113.54	nd
*Methyl linolenate [a,b,c]	2094	2098[3]	11.70 ± 4.76	2.35 ± 0.70	20.17 ± 23.98	nd
Ethyl linoleate [b,c]	2163	2173[5]	(E) 4.13 ± 0.06	nd	nd	nd
Ethyl arachinoate [b,c]	2361	2398[5]	(E) 12.19 ± 4.17	nd	nd	nd
Ethyl docosanoate[b,c]	2578	2597[5]	(E) 8.09 ± 3.97	nd	nd	nd
Ethyl lignocerate [b,c]	2775	2796[5]	(E) 2.18 ± 1.36	nd	nd	nd
Alcools						
1-Tricosanol[b,c]	2591	2632[6]	(D) 2.97 ± 0.22	nd	nd	nd
1-Tetracosanol[b,c]	2662	2658[7]	(D) 41.49 ± 902	nd	(D) 23.38 ± 2.94	nd
1-Hexacosanol[b,c]	2878	2954[6]	(D) 225.66 ± 30.90	nd	(D) 105.84 ± 18.33	nd
1-Octacosanol[b,c]	3112	3149[6]	(D) 123.10 ± 29.20	nd	(D) 122.05 ± 25.80	nd
Terpenic compounds						
Monoterpene						
3,7-dimethyloctan-1-ol[b,c]	1179	1196[3]	nd	nd	(M) 14.75 ± 9.37	nd
Sesquiterpene						
Palustrol[b,c]	1543	1557[3]	(A) 3.76 ± 0.87	nd	(A) 2.29 ± 0.13	nd
Oxygenated diterpene						
Manoyl oxide[b,c]	1990	1989[3]	(J) 18.95 ± 5.70	nd	nd	nd
Triterpene						
Squalene[b,c]	2812	2827[7]	(I) 20.31 ± 0.48	(I)14.31 ± 2.23	(I) 16.43 ± 3.73	nd
Aldehydes						
Tetracosanal[b,c]	2613	2635[5]	(C) 10.59 ± 2.00	nd	(C) 6.85 ± 0.57	nd
Pentacosanal[b,c]	2725	2733[3]	(C) 5.07 ± 2.16	nd	----	nd
Hexacosanal[b,c]	2817	2815[2]	(C) 65.56 ± 20.57	nd	(C) 55.67 ± 9.34	nd
Octacosanal[b,c]	2991	3032[3]	nd	nd	(C) 80.61 ± 24.36	nd
Miscellaneous compounds						
2,4-Dimethyl-1,3-dioxolane-2-methanol[b,c]	901	927[4]	nd	(H)14.54 ± 4.55	nd	nd

3-Hexene-2,5-dione[b]	929	955[4]	nd	nd	nd	[(K)]1.45 ± 1.93
4-Hydroxy-5-methyl-2-hexanone[b,c]	968	954[2]	nd	nd	nd	[(K)]2.54 ± 1.25
4-Methoxy-2,5-dimethyl-3(2H)-furanone[b,c]	1032	1031[1]	nd	[(K)]4.07 ± 3.01	nd	nd
*Phenylethyl Alcohol[a,b,c]	1081	1080[2]	15.42 ± 5.80	10.09 ± 5.69	nd	1.95 ± 0.59
Artemisia ketone[b,c]	1120	1070[2]	nd	[(K)]3.97 ± 1.20	nd	nd
2-Phenoxyethanol[b,c]	1179	1185[2]	[(H)] 9.33 ± 4.67	[(H)]6.97 ± 1.92	nd	nd
Dimethylbenzylcarbinol acetate[b,c]	1267	1298[3]	[(G)] 7.50 ± 7.31	nd	nd	nd
Triacetin[b,c]	1313	1313[2]	nd	[(G)]18.16 ± 4.62	nd	[(G)]9.28 ± 11.97
Diethyltoluamide[b,c]	1535	1571[1]	nd	[(L)]5.40 ± 1.89	nd	nd
2-p-Tolylpyridine[b,c]	1562	1557[4]	[(F)] 0.62 ± 0.02	nd	nd	nd
Benzophenone[b,c]	1571	1572[1]	nd	[(K)]0.76 ± 0.37	nd	nd
Contamination						
Bis(2-ethylhexyl)-phthalate[b,c]	2506	2507[3]	[(G)] 41.56 ± 22.85	[(G)]22.20 ± 14.48	[(G)] 52.92 ± 13.93	[(G)]37.69 ± 4.44
Bis[2-(2-butoxyethoxy)ethyl] adipate[b,c]	2756	2757[4]	[(E)] 24.39 ± 11.63	nd	[(E)] 12.67 ± 7.35	nd

[a]Identified by coelution with standard volatile compounds; [b]Identified by the mass spectra data; [c]Identified by comparin the calculated KI with the theoretical KI (literature); *compound considered definitely identified (identified at least b coelution with standard volatile compounds and mass spectra data); LRI - modified Kovats index calculated using C' C40 alkanes (Van den Dool and Kratz, 1963); Avg - average value; SD - standard deviation; nd – not-detecte compound; References: 1 – PubChem; 2- NIST; 3 – Pherobase; 4 – ChemSpider; 5 – Radulovic, Blagojevic and Pali 2010; 6 – Ahumada et al., 2001; 7 – Zito et al., 2010; (A) - concentration given in ppb farnesol equivalent; (B) concentration given in ppb hexadecanoic acid equivalent; (C) - concentration given in ppb decanal equivalent; (D) concentration given in ppb eicosanol equivalent; (E) - concentration given in ppb methyl linolenate; (F) - concentratic given in ppb pyridine equivalent; (G) - concentration given in ppb benzyl acetate equivalent; (H) - concentration give in ppb phenylethyl alcohol equivalent; (I) - concentration in given in ppb hexadecane equivalent; (J) - concentration given in ppb phenol equivalent; (K) - concentration given in ppb cyclohexen-1-one equivalent; (L) - concentration given in ppb toluene equivalent; (M) – concentration is given in ppb linallol equivalent; Method 1 – Solvent Extractic method; Method 2 – Solid Phase Extraction Method.

Table 4. List of tentative phenolic compounds and reference standard compounds identified in grape extracts by UPLC-MS^E.

Nº	Possible Identifications	*m/z* (exp.)	RT (min)	Molecular Formula	Score (%)	FS (%)	Mass Error (ppm)	IS	Extracts
PHENOLIC ACIDS									
1	3-Feruloylquinic acid isomer 1	367.1052	0.44	$C_{17}H_{20}O_9$	44.8	35.3	4.7	94.3	1-8
2	3-Caffeoylquinic acid *5-Caffeoylquinic acid isomer*	353.0894	0.44	$C_{16}H_{18}O_9$	45.2	44.1	4.4	87.1	1-8

3	4-p-Coumaroylquinic acid	337.0946	0.50	$C_{16}H_{18}O_8$	42.3	26.2	5.1	91.4	1-8
4	3-Feruloylquinic acid isomer 2	367.1052	0.62	$C_{17}H_{20}O_9$	35.6	0	4.8	83.5	4,7,8
5	3-Feruloylquinic acid isomer 3	367.1050	0.80	$C_{17}H_{20}O_9$	35.3	0	4.3	81.3	2,4
6	3-Feruloylquinic acid isomer 4	367.1052	0.96	$C_{17}H_{20}O_9$	49.3	70.5	4.7	81.3	2
7	**Gallic acid**	169.0139	1.21	$C_7H_6O_5$	46.6	36.4	-1.9	98.9	1-8
8	2,3-Dihydroxybenzoic acid Protocatechuic acid 2,4-Dihydroxybenzoic acid	153.0191	1.31	$C_7H_6O_4$	48	49.1	-1.3	92.5	1,2,4
9	3,4-dihydroxy-5-methoxybenzoic acid 4-O-Methylgallic acid	183.0296	1.41	$C_8H_8O_5$	38.3	0	-1.5	93.4	1,2,3
10	2,3,4-Trihydroxybenzoic acid isomer 1	169.0141	1.49	$C_7H_6O_5$	50	59.1	-0.6	91.6	2
11	2,3-Dihydroxybenzoic acid 2,6-Dihydroxybenzoic acid 3,5-Dihydroxybenzoic acid Protocatechuic acid 2,4-Dihydroxybenzoic acid	153.019	1.57	$C_7H_6O_4$	38.8	0	-2.2	96.5	1-8
12	Feruloyl tartaric acid isomer 1	325.0562	1.6	$C_{14}H_{14}O_9$	36.6	0	-1	84	2
13	2,3,4-Trihydroxybenzoic acid isomer 2	169.014	1.62	$C_7H_6O_5$	38.5	0	-1.6	94.3	1,2
14	2,3-Dihydroxybenzoic acid 2,6-Dihydroxybenzoic acid 3,5-Dihydroxybenzoic acid Protocatechuic acid 2,4-Dihydroxybenzoic acid	153.0191	1.78	$C_7H_6O_4$	38	0	-1.6	92.2	1,2,5,6,8
15	3-Hydroxybenzoic acid 2-Hydroxybenzoic acid	137.0242	2.1	$C_7H_6O_3$	40.2	8.2	-1.6	95	1-8
16	**Gentisic acid**	153.0188	2.15	$C_7H_6O_4$	54.1	82.6	-3.2	91.8	2
17	**4-Hydroxybenzoic acid**	137.0241	2.24	$C_7H_6O_3$	55.9	86.8	-2.4	95.4	1-8
18	p-Coumaric acid 4-O-glucoside isomer 4	325.0924	2.31	$C_{15}H_{18}O_8$	46.9	48	-1.5	88.1	1-8
19	m-Coumaric acid o-Coumaric acid	163.0398	2.31	$C_9H_8O_3$	56.9	96.2	-1.6	90.1	1,2,4,7
20	3,4-dihydroxy-5-methoxybenzoic acid 4-O-Methylgallic acid	183.0296	2.35	$C_8H_8O_5$	38.4	0	-1.7	93.9	1,2,5
21	*Vanillic acid isomer 2*	167.0348	2.41	$C_8H_8O_4$	46.3	40.1	-0.9	92.4	1,2
22	*Ferulic acid isomer*	193.0503	2.48	$C_{10}H_{10}O_4$	54.6	81.9	-1.7	93	2,3,5,6,7,8
23	Feruloyl tartaric acid	325.056	2.49	$C_{14}H_{14}O_9$	51.7	66	-1.5	94.3	1-8

	isomer 2								
24	p-Coumaric acid 4-O-glucoside isomer 3	325.0923	2.50	$C_{15}H_{18}O_8$	43.7	26.4	-1.7	94	1,2,4,5,6,7,8
25	Feruloyl glucose	355.1029	2.57	$C_{16}H_{20}O_9$	48.2	49.1	-1.5	93.5	1,2,4,5,6,7,8
26	p-Coumaric acid 4-O-glucoside isomer 1	325.0923	2.6	$C_{15}H_{18}O_8$	38.2	9.9	-1.7	83.3	5,6
27	**Caffeic acid**	179.0349	2.63	$C_9H_8O_4$	37.8	0	-0.7	89.9	1,2,3,4,5,6,8
28	p-Coumaric acid 4-O-glucoside isomer 2	325.0923	2.66	$C_{15}H_{18}O_8$	38.4	5.8	-1.7	88	1,2,4,5,6,7,8
29	*Vanillic acid isomer 2*	167.0346	2.85	$C_8H_8O_4$	51.9	68.1	-2.2	93.8	1,2
30	2,3,4-Trihydroxybenzoic acid isomer 3	169.0141	2.96	$C_7H_6O_5$	38.1	0	-0.7	91.6	1
31	2,3-Dihydroxybenzoic acid 2,6-Dihydroxybenzoic acid 3,5-Dihydroxybenzoic acid Protocatechuic acid 2,4-Dihydroxybenzoic acid	153.019	3.19	$C_7H_6O_4$	57.9	95.5	-2.3	96.6	1-8
32	**Ellagic acid**	300.9987	3.46	$C_{14}H_6O_8$	37.2	0	-1.1	87.4	1,2,4,6,8
33	2,3,4-Trihydroxybenzoic acid isomer 4	169.0142	3.73	$C_7H_6O_5$	38.9	0	-0.4	94.9	1,2
34	3,4Dihydroxyphenylacetic acid *Vanillic acid isomer 3*	167.0346	3.77	$C_8H_8O_4$	38.1	0	-2.4	93.3	1,2,4,5,7,8
FLAVONOIDS									
35	(+)-Gallocatechin *(-)-Epigallocatechin isomer*	305.0685	0.65	$C_{15}H_{14}O_7$	35.3	0	6.1	83.5	1,2,5,7
36	Procyanidin trimer C1 isomer 1	865.1976	1.46	$C_{45}H_{38}O_{18}$	46.1	49.5	-1.1	82.4	1,2
37	(+)-Gallocatechin *(-)-Epigallocatechin isomer*	305.0663	1.7	$C_{15}H_{14}O_7$	51.2	60.7	-1.3	96.7	1,2,4
38	Procyanidin dimer B5 Procyanidin dimer B8	577.1346	2.08	$C_{30}H_{26}O_{12}$	52.8	67.8	-1	97.3	1,2,4,6
39	(+)-Gallocatechin *(-)-Epigallocatechin isomer*	305.0662	2.15	$C_{15}H_{14}O_7$	37.7	0	-1.5	90.5	2
40	Quercetin 7,4'-O-diglucoside Quercetin 3,4'-O-diglucoside Quercetin 3-O-sophoroside 6,8-Dihydroxykaempferol 3-rutinoside	625.1395	2.29	$C_{27}H_{30}O_{17}$	35.8	0	-2.4	82.1	1,2
41	Procyanidin trimer C1 isomer 2	865.1974	2.35	$C_{45}H_{38}O_{18}$	36.5	0	-1.3	84.3	1,2,4
42	Ourateacatechin	319.0816	2.37	$C_{16}H_{16}O_7$	40.3	21.8	-2.2	82.6	1,2

43	**(+)-Catechin**	289.0714	2.37	$C_{15}H_{14}O_6$	42.5	20.6	-1.3	93.7	1,2,4,6
44	Hesperetin Homoeriodictyol	301.0709	2.4	$C_{16}H_{14}O_6$	55	95.7	-2.9	82.9	2
45	Myricetin isomer 1	317.0298	2.43	$C_{15}H_{10}O_8$	36.3	0	-1.5	83.4	2
46	**(-)-Epicatechin**	289.0714	2.76	$C_{15}H_{14}O_6$	37.1	0	-1.3	86.9	1,2
47	Quercetin 3-O sophoroside 6,8-Dihydroxykaempferol 3-rutinoside	625.14	2.9	$C_{27}H_{30}O_{17}$	55.9	98.9	-1.6	82.5	2,4
48	Jaceosidin	329.0662	3.06	$C_{17}H_{14}O_7$	45.6	48.1	-1.4	81.8	1,2
49	6-Hydroxyluteolin	301.0349	3.08	$C_{15}H_{10}O_7$	51	70.6	-1.5	86.3	2
50	Myricetin isomer 3	317.0274	3.09	$C_{15}H_{10}O_8$	52.5	89.2	-9.1	83.4	1,2
51	Myricetin 3-O-glucoside	479.0826	3.09	$C_{21}H_{20}O_{13}$	56.6	86.7	-1.1	97.6	1,2,4,5,6
52	2-(3,4-dihydroxyphenyl)-2- hydroxypropanoic acid	197.0455	3.19	$C_9H_{10}O_5$	57.2	96.7	-0.3	89.6	1,2
53	Nepetin Isorhamnetin Rhamnetin	315.0507	3.28	$C_{16}H_{12}O_7$	55.9	97.8	-0.9	82.7	2
54	Quercetin 3-O-glucuronide	477.0668	3.46	$C_{21}H_{18}O_{13}$	45.5	32.3	-1.4	97	1,2,4,5,6,7,8
55	6-Hydroxyluteolin	301.0345	3.46	$C_{15}H_{10}O_7$	52.3	72.3	-3	92.6	1,2,4,5,6,8
56	**Quercetin 3-O-glucoside**	463.0877	3.48	$C_{21}H_{20}O_{12}$	55.8	82.2	-1.1	98	1-8
57	Dihydroquercetin	303.0508	3.58	$C_{15}H_{12}O_7$	53.9	86.6	-0.8	83.6	2
58	Miscanthoside Eriodictyol 7-O-glucoside	449.1084	3.58	$C_{21}H_{22}O_{11}$	53.6	71.1	-1.2	98.4	1,2,4
59	*Kaempferol isomer*	285.0401	3.58	$C_{15}H_{10}O_6$	51.7	68.9	-1.1	91.1	1,2
60	Dihydroquercetin 3-O-rhamnoside	449.1071	3.85	$C_{21}H_{22}O_{11}$	45.2	45.2	-4	85.5	2
61	Luteolin 7-O-glucoside Luteolin 6-C-glucoside	447.0928	3.85	$C_{21}H_{20}O_{11}$	56.7	93.7	-1.1	90.9	1,2,4
62	Isorhamnetin 3-O-glucoside Isorhamnetin 3-O-galactoside	477.1033	3.88	$C_{22}H_{22}O_{12}$	49.5	54.8	-1.2	94	1-8
63	Myricetin isomer 2	317.0298	3.93	$C_{15}H_{10}O_8$	54.7	81.9	-1.5	93.4	1,2,4,6
64	Naringin 4'-O-glucoside	433.1135	3.98	$C_{21}H_{22}O_{10}$	52.3	67.6	-1.2	95.2	1,2,4
65	Apigenin 7-O-glucoside	419.1344	4.05	$C_{21}H_{24}O_9$	42.2	29.5	-0.8	82.5	1,2
66	*Quercetin 3-O-glucoside* Quercetin 4'-O-glucoside Quercetin 3-O-galactoside Myricetin 3-O-rhamnoside	463.0872	4.13	$C_{21}H_{20}O_{12}$	56.7	98.5	-2.2	87.7	2,4
67	**Myricetin**	317.0298	4.18	$C_{15}H_{10}O_8$	56.8	91.5	-1.5	94.4	1,2,4,6
68	6-Hydroxyluteolin Morin isomer 1	301.0347	4.28	$C_{15}H_{10}O_7$	36.2	0	-2.4	83.6	2,4
69	Morin isomer 2	301.0348	4.74	$C_{15}H_{10}O_7$	55.6	94.7	-1.9	85.6	2,6,8
70	**Quercetin** Rhamnetin	301.0353	4.96	$C_{15}H_{10}O_7$	36.7	0	-0.2	83.6	2,4,6
STILBENES AND LIGNANS									
72	Syringaresinol	417.1524	2.57	$C_{22}H_{26}O_8$	35.7	0	-7.4	86.6	2

73	Piceatannol	243.066	3.82	$C_{14}H_{12}O_4$	37.4	0	-1.1	88.3	2,4
OTHER POLYPHENOLS									
74	Pyrogallol Isomer 1	125.0242	0.44	$C_6H_6O_3$	43.9	28.4	-2	93.3	1-8
75	Pyrogallol Isomer 2	125.0241	0.5	$C_6H_6O_3$	38.7	0	-2.4	96.5	1,2,4,7,8
76	Pyrogallol Isomer 3	125.0243	0.89	$C_6H_6O_3$	38.8	0	-1.2	95.5	1-8
77	Pyrogallol Isomer 4	125.0241	1.1	$C_6H_6O_3$	38	0	-2.6	93	1,2,4,5
78	**Pyrogallol**	125.0242	1.21	$C_6H_6O_3$	39	0	-2	97.4	1-8
79	Hydroxytyrosol	153.0554	1.66	$C_8H_{10}O_3$	53.4	78.4	-2.2	91	1,2,4,6
80	Umbelliferone	161.024	1.98	$C_9H_6O_3$	55.4	88.7	-2.3	91.3	1,2,5,6
81	Oleoside dimethylester	417.1397	2.23	$C_{18}H_{26}O_{11}$	35.7	0	-1.3	80	1,2
82	Esculetin isomer 1	177.0189	2.33	$C_9H_6O_4$	38.4	0	-2.6	95.1	2,3,4
83	Esculetin isomer 2	177.019	2.59	$C_9H_6O_4$	51.2	67.2	-2.1	91.5	1,2,4,5,6,7,8
84	Scopoletin	191.0344	2.85	$C_{10}H_8O_4$	52.3	76.2	-2.9	88.9	2
85	4-Hydroxybenzaldehyde	121.0292	2.86	$C_7H_6O_2$	44.9	33.9	-2.2	93.5	1,2,3,5,7,8
86	Bergapten Xanthotoxin Mellein	215.0336	3.08	$C_{12}H_8O_4$	46	50.3	-6.5	87	1,2

m/z: mass/charge; RT: retention time; FS: fragmentation score; IS: isotopic similarity; Bold: compounds confirmed by reference standards. Italic: isomers of reference compounds identified in other RT. Compounds within the same number indicated more than one possibility of identification with the same *m/z* and similar parameters. The numbers of the column "extracts" refer to the samples where the compound was identified: 1- S. sapphire integral - water extracts, 2- S. sapphire integral - acetone extracts, 3- S. sapphire pulp - water extracts, 4- S. sapphire pulp - acetone extracts, 5- S. surprise integral - water extracts, 6- S. surprise integral - acetone extracts, 7- S. surprise pulp - water extracts, 8- S. surprise pulp - acetone extracts.

4. Conclusions

This work highlights the significantly higher concentration of phenolic compounds and antioxidant properties of hybrid *Vitis vinifera* L. varieties, SA and SU. By using a combination of UPLC-MS^E, HPLC-PDA, GC-FID and GC–MS techniques, we characterized the phenolic and volatile compounds in different extracts of these hybrid varieties. A total of 87 phenolic compounds were identified by comparison with standards and by fragmentation patterns. The volatile fraction of SU (42 components) was richer than that of SA (31 compounds). Fatty acids, alcohols and aldehydes were the major volatile compounds found in both grape cultivars. The DPPH, ABTS, FRAP and ORAC assays revealed that the acetone extract of the SA peel presents a higher antioxidant activity as well as moderate anthocyanin values. Its consumption presents high bioactive potential, being its efficient use in the prevention of pathologies.

To our knowledge, the present study is the first one to characterize the hybrid *Vitis vinifera* L. varieties and the results of this work will contribute to guide new studies and to evaluate its economic potential as a natural food source from the Northeast region of Brazil.

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Notes

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

5. References

Abe, L. T., Mota, R. V. da, Lajolo, F. M., & Genovese, M. I. (2007). Compostos fenólicos e capacidade antioxidante de cultivares de uvas Vitis labrusca L. e Vitis vinifera L. *Ciência e Tecnologia de Alimentos*, *27*(2), 394–400. https://doi.org/10.1590/S0101-20612007000200032

Abotaleb, M., Samuel, S. M., Varghese, E., Varghese, S., Kubatka, P., Liskova, A., & Büsselberg, D. (2019). Flavonoids in cancer and apoptosis. *Cancers*, *11*(1). https://doi.org/10.3390/cancers11010028

Albuquerque, B. R., Pinela, J., Barros, L., Oliveira, M. B. P. P., & Ferreira, I. C. F. R. (2020). Anthocyanin-rich extract of jabuticaba epicarp as a natural colorant: Optimization of heat- and ultrasound-assisted extractions and application in a bakery product. *Food Chemistry*, *316*(February), 126364. https://doi.org/10.1016/j.foodchem.2020.126364

AOAC. (2000). Official Methods of Analysis of AOAC International. *Association of Official Analysis Chemists International*, Method ce 2-66.

https://doi.org/10.3109/15563657608988149

Baananou, S., Bagdonaite, E., Marongiu, B., Piras, A., Porcedda, S., Falconieri, D., & Boughattas, N. A. (2015). Supercritical CO_2 extract and essential oil of aerial part of Ledum palustre L.--Chemical composition and anti-inflammatory activity. *Natural Product Research*, *29*(11), 999–1005. https://doi.org/10.1080/14786419.2014.965167

Bampi, M., Bicudo, M. O. P., Fontoura, P. S. G., & Ribani, R. H. (2010). Composição centesimal do fruto, extrato concentrado e da farinha da uva-do-japão. *Ciência Rural*, *40*(11), 2361–2367. https://doi.org/10.1590/S0103-84782010001100018

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Campayo, A., Serrano de la Hoz, K., García-Martínez, M. M., Sánchez-Martínez, J. F., Salinas, M. R., & Alonso, G. L. (2019). Spraying ozonated water on Bobal grapevines: Effect on grape quality. *Food Research International*, *125*(July). https://doi.org/10.1016/j.foodres.2019.108540

Cavalcante, F. M. L., Almeida, I. V., Düsman, E., Mantovani, M. S., & Vicentini, V. E. P. (2017). Cytotoxicity, mutagenicity, and antimutagenicity of the gentisic acid on HTC cells. *Drug and Chemical Toxicology*, *0545*(May). https://doi.org/10.1080/01480545.2017.1322606

Chatham, L. A., Howard, J. E., & Juvik, J. A. (2020). A natural colorant system from corn: Flavone-anthocyanin copigmentation for altered hues and improved shelf life. *Food Chemistry*, *310*(November 2019), 125734. https://doi.org/10.1016/j.foodchem.2019.125734

Chen, Y., Hong, Y., Yang, D., He, Z., Lin, X., Wang, G., & Yu, W. (2020). Simultaneous determination of phenolic metabolites in Chinese citrus and grape cultivars. *PeerJ*, *2020*(6), 1–16. https://doi.org/10.7717/peerj.9083

Chudzik, M., Korzonek-Szlacheta, I., & Król, W. (2015). Triterpenes as potentially cytotoxic compounds. *Molecules*, *20*(1), 1610–1625. https://doi.org/10.3390/molecules20011610

Coelho, P. L. C., de Freitas, S. R. V. B., Pitanga, B. P. S., da Silva, V. D. A., Oliveira, M. N., Grangeiro, M. S., Souza, C. dos S., El-Bachá, R. dos S., Costa, M. de F. D., Barbosa, P. R., Nascimento, I. L. de O., & Costa, S. L. (2016). Flavonoids from the Brazilian plant croton betulaster inhibit the growth of human glioblastoma cells and induce apoptosis. *Revista Brasileira de Farmacognosia*, *26*(1), 34–43. https://doi.org/10.1016/j.bjp.2015.05.013

Constantinou, S., Gómez-Caravaca, A. M., Goulas, V., Segura-Carretero, A., & Manganaris, G. A. (2017). Metabolic fingerprinting of must obtained from sun-dried grapes of two indigenous Cypriot cultivars destined for the production of 'Commandaria': A protected destignation of origin product. *Food Research International*, *100*, 469–476. https://doi.org/10.1016/j.foodres.2016.11.015

Cory, H., Passarelli, S., Szeto, J., Tamez, M., & Mattei, J. (2018). The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. *Frontiers in Nutrition*, *5*(September), 1–9. https://doi.org/10.3389/fnut.2018.00087

de Camargo, A. C., Biasoto, A. C. T., Schwember, A. R., Granato, D., Rasera, G. B., Franchin, M., Rosalen, P. L., Alencar, S. M., & Shahidi, F. (2019). Should we ban total phenolics and antioxidant screening methods? The link between antioxidant potential and activation of NF-κB using phenolic compounds from grape by-products. *Food Chemistry*, *290*, 229–238. https://doi.org/10.1016/j.foodchem.2019.03.145

De Rosso, M., Tonidandel, L., Larcher, R., Nicolini, G., Ruggeri, V., Dalla Vedova, A., De Marchi, F., Gardiman, M., & Flamini, R. (2012). Study of anthocyanic profiles of twenty-one hybrid grape varieties by liquid chromatography and precursor-ion mass spectrometry. *Analytica Chimica Acta*, *732*, 120–129. https://doi.org/10.1016/j.aca.2011.10.045

Delgado, A. M., Issaoui, M., & Chammem, N. (2019). Analysis of main and healthy Phenolic compounds in foods. *Journal of AOAC International*, *102*(5), 1356–1364. https://doi.org/10.5740/jaoacint.19-0128

Demirbas, A., Yilmaz, V., Ildiz, N., Baldemir, A., & Ocsoy, I. (2017). Anthocyanins-rich berry extracts directed formation of Ag NPs with the investigation of their antioxidant and antimicrobial activities. *Journal of Molecular Liquids*, *248*, 1044–1049. https://doi.org/10.1016/j.molliq.2017.10.130

Dumitru, B. F., Bunea, C. I., & Donici, A. (2019). Phenolic, Anthocyanin Composition and Color Measurement At Red Wines From Dealu Bujorului Vineyard. *Agricultura*, *109*(1–2), 14–28. https://doi.org/10.15835/agrisp.v109i1-2.13256

Faria, S. B. A., Rosse, V., Dias, J. F., Moreira Xavier, N., & Azeredo, V. B. (2016). Effect of grape juice consumption on antioxidant activity and interleukin-6 concentration in lactating rats. *Nutrición Hospitalaria*, *33*(6), 1418–1423. https://doi.org/10.20960/nh.804

Favati, F., Pasini, G., Vignale, F., Tolve, R., & Simonato, B. (2020). *foods E ff ect of the Refining Process on Total Hydroxytyrosol ,*. 1–11.

Fujita, R., Hayasaka, T., Jin, S., Hui, S. P., & Hoshino, Y. (2020). Comparison of anthocyanin

distribution in berries of Haskap (Lonicera caerulea subsp. edulis (Turcz. ex. Herder) Hultén), Miyama-uguisu-kagura (Lonicera gracilipes Miq.), and their interspecific hybrid using imaging mass spectrometry. *Plant Science*, *300*(August), 110633. https://doi.org/10.1016/j.plantsci.2020.110633

Gika, H., Virgiliou, C., Theodoridis, G., Plumb, R. S., & Wilson, I. D. (2019). Untargeted LC/MS-based metabolic phenotyping (metabonomics/metabolomics): The state of the art. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *1117*(April), 136–147. https://doi.org/10.1016/j.jchromb.2019.04.009

Gorzynik-Debicka, M., Przychodzen, P., Cappello, F., Kuban-Jankowska, A., Gammazza, A. M., Knap, N., Wozniak, M., & Gorska-Ponikowska, M. (2018). Potential health benefits of olive oil and plant polyphenols. *International Journal of Molecular Sciences*, *19*(3). https://doi.org/10.3390/ijms19030686

Gülçin, I., Buyukokuroglu, M. E., Oktay, M., & Kufrevioglu, O. I. (2002). On the in vitro antioxidative properties of melatonin. *J Pineal Res.*, *33*(3), 167–171. https://doi.org/20920 [pii]

Gutiérrez-Gamboa, G., Liu, S. Y., & Pszczólkowski, P. (2020). Resurgence of minority and autochthonous grapevine varieties in South America: a review of their oenological potential. *Journal of the Science of Food and Agriculture*, *100*(2), 465–482. https://doi.org/10.1002/jsfa.10003

Haas, I. (2015). Resíduo obtido do processamento do suco de uva: Caracterização e cinética de secagem. *Statewide Agricultural Land Use Baseline 2015*, *1*, 1–124. https://doi.org/10.1017/CBO9781107415324.004

Ho, L., Ferruzzi, M. G., Janle, E. M., Wang, J., Gong, B., Chen, T. Y., Lobo, J., Cooper, B., Wu, Q. L., Talcott, S. T., Percival, S. S., Simon, J. E., & Pasinetti, G. M. (2013). Identification of brain-targeted bioactive dietary quercetin-3-O- glucuronide as a novel intervention for Alzheimer's disease. *FASEB Journal*, *27*(2), 769–781. https://doi.org/10.1096/fj.12-212118

Instituto de Nutrición y Tecnología de los Alimentos (INTA). (2013). *Base de dados de Atividade Antioxidantes*.

Jiménez-Moreno, N., Volpe, F., Moler, J. A., Esparza, I., & Ancín-Azpilicueta, C. (2019). Impact of extraction conditions on the phenolic composition and antioxidant capacity of grape stem extracts. In *Antioxidants* (Vol. 8, Issue 12). https://doi.org/10.3390/antiox8120597

Kabra, M., Bhandari, S., Sharma, A., & Gupta, R. (2014). Evaluation of anti-parkinson's

activity of gentisic acid in different animal models. *Journal of Acute Disease*, *3*(2), 141–144. https://doi.org/10.1016/S2221-6189(14)60031-7

Kelly, E., Vyas, P., & Weber, J. T. (2018). Biochemical properties and neuroprotective effects of compounds in various species of berries. *Molecules*, *23*(1). https://doi.org/10.3390/molecules23010026

Kershaw, J., & Kim, K.-H. (2017). The Therapeutic Potential of Piceatannol, a Natural Stilbene, in Metabolic Diseases: A Review. *Journal of Medicinal Food*, *20*(5), 427–438. https://doi.org/10.1089/jmf.2017.3916

Kim, J. H., Wang, Q., Choi, J. M., Lee, S., & Cho, E. J. (2015). Protective role of caffeic acid in an Aβ25-35-induced Alzheimer's disease model. *Nutrition Research and Practice*, *9*(5), 480–488. https://doi.org/10.4162/nrp.2015.9.5.480

Ky, I., & Teissedre, P. L. (2015). Characterisation of Mediterranean grape pomace seed and skin extracts: Polyphenolic content and antioxidant activity. *Molecules*, *20*(2), 2190–2207. https://doi.org/10.3390/molecules20022190

Lachman, J., Hejtmánková, A., Táborský, J., Kotíková, Z., Pivec, V., Střalková, R., Vollmannová, A., Bojňanská, T., & Dědina, M. (2015). Evaluation of oil content and fatty acid composition in the seed of grapevine varieties. *LWT - Food Science and Technology*, *63*(1), 620–625. https://doi.org/10.1016/j.lwt.2015.03.044

Lee, B., Lin, P. ching, Cha, H. soo, Luo, J., & Chen, F. (2016). Characterization of volatile compounds in Cowart muscadine grape (Vitis rotundifolia) during ripening stages using GC-MS combined with principal component analysis. *Food Science and Biotechnology*, *25*(5), 1319–1326. https://doi.org/10.1007/s10068-016-0207-3

Lingua, M. S., Fabani, M. P., Wunderlin, D. A., & Baroni, M. V. (2016). From grape to wine: Changes in phenolic composition and its influence on antioxidant activity. *Food Chemistry*, *208*, 228–238. https://doi.org/10.1016/j.foodchem.2016.04.009

Liu, H., Su, B., Zhang, H., Gong, J., Zhang, B., Liu, Y., & Du, L. (2019). Identification and functional analysis of a flavonol synthase gene from grape hyacinth. *Molecules*, *24*(8), 1–15. https://doi.org/10.3390/molecules24081579

Liu, S., Sui, Q., Zou, J., Zhao, Y., & Chang, X. (2019). Protective effects of hawthorn (Crataegus pinnatifida) polyphenol extract against UVB-induced skin damage by modulating the p53 mitochondrial pathway in vitro and in vivo. *Journal of Food Biochemistry*, *43*(2), 1–12. https://doi.org/10.1111/jfbc.12708

Loos, K., Zhang, R., Pereira, I., Agostinho, B., Hu, H., Maniar, D., Sbirrazzuoli, N., Silvestre, A. J. D., Guigo, N., & Sousa, A. F. (2020). A Perspective on PEF Synthesis, Properties,

and End-Life. *Frontiers in Chemistry*, *8*(July), 1–18. https://doi.org/10.3389/fchem.2020.00585

Lves, R. I. E. L. A., Arkeet, C. O. C., Levidence, B. E. A. C., & Ovotny, J. A. A. N. (2007). *Anthocyanins Present in Selected Tropical Fruits : Acerola , Jambolão , Jussara , and Guajiru*. 9389–9394.

Nicolini, G., Roman, T., Flamini, R., Tonidandel, L., Gardiman, M., & Larcher, R. (2020). Thiol precursors in Vitis mould-tolerant hybrid varieties. *Journal of the Science of Food and Agriculture*, *100*(7), 3262–3268. https://doi.org/10.1002/jsfa.10344

Nixdorf, S. L., & Hermosín-Gutiérrez, I. (2010). Brazilian red wines made from the hybrid grape cultivar Isabel: Phenolic composition and antioxidant capacity. *Analytica Chimica Acta*, *659*(1–2), 208–215. https://doi.org/10.1016/j.aca.2009.11.058

Olivati, C., de Oliveira Nishiyama, Y. P., de Souza, R. T., Janzantti, N. S., Mauro, M. A., Gomes, E., Hermosín-Gutiérrez, I., da Silva, R., & Lago-Vanzela, E. S. (2019). Effect of the pre-treatment and the drying process on the phenolic composition of raisins produced with a seedless Brazilian grape cultivar. *Food Research International*, *116*(April 2018), 190–199. https://doi.org/10.1016/j.foodres.2018.08.012

Patel, K., & Patel, D. K. (2016). Medicinal importance, pharmacological activities, and analytical aspects of hispidulin: A concise report. *Journal of Traditional and Complementary Medicine*, *7*(3), 360–366. https://doi.org/10.1016/j.jtcme.2016.11.003

Peixoto, C. M., Dias, M. I., Alves, M. J., Calhelha, R. C., Barros, L., Pinho, S. P., & Ferreira, I. C. F. R. (2018). Grape pomace as a source of phenolic compounds and diverse bioactive properties. *Food Chemistry*, *253*(January), 132–138. https://doi.org/10.1016/j.foodchem.2018.01.163

Pereira, P., Palma, C., Ferreira-Pêgo, C., Amaral, O., Amaral, A., Rijo, P., Gregório, J., Palma, L., & Nicolai, M. (2020). Grape Pomace: A Potential Ingredient for the Human Diet. *Foods*, *9*(12), 1772. https://doi.org/10.3390/foods9121772

Pereira, R. N., Coelho, M. I., Genisheva, Z., Fernandes, J. M., Vicente, A. A., Pintado, M. E., & Teixeira, e. J. A. (2020). Using Ohmic Heating effect on grape skins as a pretreatment for anthocyanins extraction. *Food and Bioproducts Processing*, *124*, 320–328. https://doi.org/10.1016/j.fbp.2020.09.009

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., & Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry*, *51*(11), 3273–3279.

https://doi.org/10.1021/jf0262256

Qin, F., Yao, L., Lu, C., Li, C., Zhou, Y., Su, C., Chen, B., & Shen, Y. (2019). Phenolic composition, antioxidant and antibacterial properties, and in vitro anti-HepG2 cell activities of wild apricot (Armeniaca Sibirica L. Lam)kernel skins. *Food and Chemical Toxicology*, *129*(May), 354–364. https://doi.org/10.1016/j.fct.2019.05.007

Rasines-Perea, Z., & Teissedre, P. L. (2017). Grape Polyphenols' effects in human cardiovascular diseases and diabetes. *Molecules*, *22*(1), 1–19. https://doi.org/10.3390/molecules22010068

Rojas-Lema, S., Torres-Giner, S., Quiles-Carrillo, L., Gomez-Caturla, J., Garcia-Garcia, D., & Balart, R. (2021). On the use of phenolic compounds present in citrus fruits and grapes as natural antioxidants for thermo-compressed bio-based high-density polyethylene films. *Antioxidants*, *10*(1), 1–23. https://doi.org/10.3390/antiox10010014

Ruta, L. L., & Farcasanu, I. C. (2019). Anthocyanins and anthocyanin-derived products in yeast-fermented beverages. *Antioxidants*, *8*(6). https://doi.org/10.3390/antiox8060182

Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. *BMC Complementary and Alternative Medicine*, *12*(1), 1174. https://doi.org/10.1186/1472-6882-12-221

Samoticha, J., Wojdyło, A., & Golis, T. (2017). Phenolic composition, physicochemical properties and antioxidant activity of interspecific hybrids of grapes growing in Poland. *Food Chemistry*, *215*, 263–273. https://doi.org/10.1016/j.foodchem.2016.07.147

Santos, R. D. O., Gorgulho, B. M., Castro, M. A. De, Fisberg, R. M., Marchioni, D. M., & Baltar, V. T. (2019). Principal component analysis and factor analysis: Differences and similarities in nutritional epidemiology application. *Revista Brasileira de Epidemiologia*, *22*, 1–14. https://doi.org/10.1590/1980-549720190041

Savalekar, K., Ahammed Shabeer, T. P., Khan, Z., Oulkar, D., Jain, P., Patil, C., & Banerjee, K. (2019). Targeted phenolic profiling of Sauvignon blanc and Shiraz grapes grown in two regions of India by liquid chromatography-tandem mass spectrometry. *Journal of Food Science and Technology*, *56*(7), 3300–3312. https://doi.org/10.1007/s13197-019-03802-w

Scognamiglio, J., Jones, L., Letizia, C. S., & Api, A. M. (2012). Fragrance material review on phenylethyl alcohol. *Food and Chemical Toxicology*, *50*(SUPPL. 2), S224–S239. https://doi.org/10.1016/j.fct.2011.10.028

Shahab, M., Roberto, S. R., Ahmed, S., Colombo, R. C., Silvestre, J. P., Koyama, R., & de Souza, R. T. (2020). Relationship between anthocyanins and skin color of table grapes

treated with abscisic acid at different stages of berry ripening. *Scientia Horticulturae*, *259*(September 2019), 108859. https://doi.org/10.1016/j.scienta.2019.108859

Slegers, A., Angers, P., Ouellet, É., Truchon, T., & Pedneault, K. (2015). Volatile compounds from grape skin, juice and wine from five interspecific hybrid grape cultivars grown in Quebec (Canada) for wine production. *Molecules*, *20*, 10980–11016. https://doi.org/10.3390/molecules200610980

SOARES, M.; WELTER, L.; KUSKOSKI, E. M.; GONZAGA, L.; FETT, R. (2008). Compostos Fenólicos E Atividade Antioxidante Da Casca De Uvas Niágara E Isabel. *Rev. Bras. Frutic*, *30*(1), 59–64. https://doi.org/10.1590/S0100-29452008000100013

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, *19*(6–7), 669–675. https://doi.org/10.1016/j.jfca.2006.01.003

Varela-Rodríguez, L., Sánchez-Ramírez, B., Hernández-Ramírez, V. I., Varela-Rodríguez, H., Castellanos-Mijangos, R. D., González-Horta, C., Chávez-Munguía, B., & Talamás-Rohana, P. (2020). Effect of gallic acid and myricetin on ovarian cancer models: A possible alternative antitumoral treatment. *BMC Complementary Medicine and Therapies*, *20*(1), 1–16. https://doi.org/10.1186/s12906-020-02900-z

Wang, S., Mateos, R., Goya, L., Amigo-Benavent, M., Sarriá, B., & Bravo, L. (2016). A phenolic extract from grape by-products and its main hydroxybenzoic acids protect Caco-2 cells against pro-oxidant induced toxicity. *Food and Chemical Toxicology*, *88*, 65–74. https://doi.org/10.1016/j.fct.2015.12.005

Westland, S., Ripamonti, C., & Cheung, V. (2012). CIELAB and Colour Difference. *Computational Colour Science Using MATLAB®*, 49–74. https://doi.org/10.1002/9780470710890.ch5

Yilmaz, Y., Göksel, Z., Erdoğan, S. S., Öztürk, A., Atak, A., & Özer, C. (2015). Antioxidant Activity and Phenolic Content of Seed, Skin and Pulp Parts of 22 Grape (Vitis viniferaL.) Cultivars (4 Common and 18 Registered or Candidate for Registration). *Journal of Food Processing and Preservation*, *39*, 1682–1691. https://doi.org/10.1111/jfpp.12399

Zeitoun, H., Khan, Z., Banerjee, K., & Salameh, D. (2020). *Ultrasound Assisted Extraction Optimization and Profiling of Associated Predominant Metabolites*.

CAPÍTULO IV

Atividade antiproliferativa e efeito antioxidante in vitro das variedades híbridas de *Vitis vinifera L*. *Sweet Sapphire* and *Sweet Surprise* em células de câncer de próstata humano utilizando abordagens in vitro e in sílico.

Antiproliferative and apoptosis effects of hybrid varieties of *Vitis vinifera* L. *Sweet Sapphire* and *Sweet Surprise* on human prostate cancer cells using in vitro and *in silico approaches.*

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Abstract

Objective: Grape hybrids are characterized by different chemical compositions; often with high Grape hybrids are characterized by different chemical compositions, often with a high phenolic compound content and a specific profile of anthocyanins. The aim of this study was to characterize and compare the constituents of hybrid *Vitis vinifera* L. varieties *Sweet Sapphire* (SA) and *Sweet Surprise* (SU) extracts and their influence on apoptosis induction and antiproliferative effects on human prostate cancer cells.

Methods: We used the MTT assay to evaluate the cytotoxic effect of different concentrations of extracts of SA and SU, on the Prostate adenocarcinoma cell lines PC-3 and DU-145 at 24, 48, and 72 hours. To analyze the inhibiting impact by flow cytometry, use 24 and 48 hours. The anthocyanins were submitted in *silico* where the concentration of each anthocyanin was estimated based on the maximum recommended dose and intestinal absorption rate.

Result: Our results showed that malvidin derivatives present the highest content in both cultivars. In the main anthocyanin identified, we recorded 14.46mg/100g malvidin-3-O-glycoside in SA and 2.76 mg/100 g in SU. A reduction in cell viability of DU-145 (45 and 65%) and PC-3 (63 and 67%) cells after 48h treatment with SA and SU, respectively, was found via MTT assay. Flow cytometry showed that the treatment with anthocyanin-rich extracts from SA and SU had an inhibitory impact on cell development due to G_2/M arrest and caused a rise in apoptotic cells in relation to the control group. In silico none of the compounds were predicted as hepatotoxic.

Conclusion: The findings of this study highlight the potential of hybrid *Vitis vinifera* L. varieties as an important source of natural antioxidants and their protective effect against prostate cancer cells.

Keywords: Cancer – Antioxidant – Grape - Hybrid – Anthocyanins

Introduction

Due to the beneficial effects on human health and its economic importance, grape is a fruit widely grown and eaten around the world. Historically, the production and export of grapes were controlled almost exclusively by traditional European countries. However, in recent years, South America has shown a significant rate of growth in production and export of grapes with two crops a year (Gutiérrez-Gamboa and Pszczólkowski, 2020). Although the practice of viticulture in Brazil is recent when compared to traditional European countries, there is an improvement in the quality of Brazilian grape cultivar composition due to the use of hybridization techniques (Olivati et al., 2019). In contrast to the almost exclusive growth of *V. vinifera* cultivars in traditional wine producing countries, hybrid grape cultivars represent more than 80% of the volume of grapes (1,399,262 tons) processed in Brazil (De Rosso et al., 2012; Nicolini et al., 2020).

Hybrid grapes are obtained from the crossing of two or more species of *Vitis* that allows the selection of characteristics of interest, such as high resistance to diseases and pathogens. Interspecific hybrids are also characterized by different chemical compositions, are especially known to exhibit high content of phenolic compounds and a specific profile of anthocyanins and are highlighted for their potential in the production of quality red wines (Samoticha et al., 2017). However, the hybrid varieties are currently scarcely studied. Phenolic compounds have been extensively studied due to their potentially beneficial antioxidant, anti-inflammatory, and anti-carcinogenic properties, which are spurring the interest of both industry and consumers for phenolic-rich foods (Gorzynik-Debicka et al., 2018).

A wide range of biological activities have been attributed to these compounds, indicating that some fruit sources can provide more than nourishment (Kelly et al., 2018). Natural foods of high nutritional quality play an important role in maintaining human health (Gülçin et al., 2002; Cory et al, 2018). As a result, much attention has been focused on the use of exogenous antioxidants, especially natural antioxidants to inhibit the oxidation of cellular components, thereby protecting from damage caused by free radicals (Liu et al., 2019).

The administration of phytochemicals into diets or the possibility of applying chemo preventive agents has been demonstrated in the literature to be effective against allergies, hypertension, viruses, inflammations, arthritis, mutations, and carcinogenesis (Hamza et al., 2018). Cancer is a group of diseases characterized by the growth and multiplication of abnormal cells, and if not controlled, can easily lead to death. Among the types of cancer affecting the world's male population, prostate cancer is the second most common (De Martel et al., 2020). The number of incident cases of prostate cancer has increased more than any other malignancy, regardless

of development status (Russo et al., 2017). Animal studies have shown that phenolic compounds can prevent and/or delay the progression of initiation of different types of cancer (Basli et al., 2017), prostate cancer among them (Singh et al, 2004; Darweesh et al., 2020).

In the last decade there has been increasing interest in the determination of suitable dietary sources of antioxidant phenolic compounds (Delgado et al., 2019). However, there is little knowledge about the phenolic compounds of hybrid grape cultivars reported in literature: few reports describe only the anthocyanins for hybrid species (Nixdorf et al., 2010; Fujita et al., 2020).

Anthocyanins are phenol compounds present in grape skin and are responsible for the red color of both grapes and wines (Shahab et al., 2020; Pereira et al., 2020). These compounds have been widely studied in *V. vinifera* varieties because they play a key role in the organoleptic characteristics of grapes (Dumitru et al., 2019). They also have antioxidant, antimicrobial and anti-cancerogenic activities, showing a protective effect on the cardiovascular system (Demirbas et al., 2017; Qin et al, 2019). Anthocyanins are studied for grape variety characterization and represent an important resource for the natural food colorant industry (Albuquerque et al., 2020; Chatham et al., 2020).

The chemistry of grape cultivars, especially varietal aroma, has a significant impact on the character of grapes, their sensory perception, and quality, influencing consumer acceptance. Varietal aroma can relate to a specific compound or to a small group of odoriferous molecules but is usually attributable to the contribution of several volatile compounds occurring in grapes, in proportions that differ from one variety to another (Slegers et al., 2015). Those aromas comprise hundreds of volatile organic compounds (VOCs) made up of different chemical groups, including alcohols, esters, aldehydes, ketones, monoterpenoids, and others. Methods for extracting VOCs often include liquid–liquid extraction, simultaneous distillation and extraction, headspace solid-phase microextraction (HS-SPME), and stir bar sorptive extraction techniques, among others (Lee et al., 2016).

Hence, the aim of this study was to identify and compare the extracts of two Hybrid grapes fruits obtained by water extracts. These extracts were additionally investigated for their antiproliferative effect and apoptotic induction in prostate (DU-145 and PC-3) cancer cells. The differential of the article was to address issues not yet discussed and identified the main phenolic compounds (anthocyanins) in two-selected species of hybrid grapes and their interaction between the antioxidant properties and antiproliferative activity in prostate cancer.

Materials and methods

Samples

Two grape cultivars, *Sweet Sapphire* (SA) and *Sweet Surprise* (SU), were provided by Labrunier farm, located in Petrolina (Pernambuco, Brazil). These new hybrid cultivars are classified as table grapes. The grapes were harvested ripe and after the fruits were completely developed and then were transported under refrigeration to the laboratory. Initially, the grapes were then separated into three types of samples - whole, skin and pulp - and immediately frozen and stored at -80°C in an ultrafreezer without undergoing any type of mechanical action, extraction, or homogenization.

For the preparation of extracts of *Sweet Sapphire* (SA) and *Sweet Surprise* (SU), the fruits were cleaned, and the peel was manually isolated from the pulp. Approximately 250 g of pulp of SA and SU was extracted with 80 mL of distilled water and then shaken for 2 h. After the pulp maceration period, the aqueous SA and SU extracts were filtered through Whatman #1 filter paper. The extracts were then frozen at -80°C in an ultra-freezer and lyophilized (Terroni® LD 300, São Carlos, SP, Brazil) for 24 h. After this process, extracts were frozen at -20°C until use in the experiments (Vizzotto et al., 2011).

Total phenolic content assays

Total phenolic content (TPC) analysis was performed using the Folin–Ciocalteu method. A 1 mL of sample (1 mg/mL) was mixed with 1 mL of Folin–Ciocalteu's phenol reagent. After 5 min at room temperature, 10 mL of a 7% Na_2CO_3 solution was added to the mixture followed by the addition of 13 mL of distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. The TPC was determined by extrapolation from the calibration curve, which was made by preparing gallic acid solution (1 mg/ml). The estimation of the total content of phenolic compounds was performed in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (Saedd et al., 2012).

Anthocyanin analysis

Whole grape samples were cut into pieces with a scalpel and weighed until 1 g on an analytical scale. The grape was macerated with 1 mL of methanolic / formic acid (90:10; v/v) in a mortar and then placed in the ultrasound bath and subsequently subject to centrifugation until discoloration of the solution was achieved. A high-performance liquid chromatography (HPLC) (Alliance 2695, Waters) equipped with photodiodes arrangement detector and a column C18 BDS (100 mm × 4.6 mm, 2.4 μm, ThermoScientific) utilizing a gradient elution mode with

acetonitrile and formic acid was used for the chromatography separation. The quantification of anthocyanins was made by external standardization using isolated patterns. The results were expressed in mg of cyanidin-3-O-glucoside equivalents /100 g of fresh weight (Lves et al., 2007).

Cell assays

Prostate adenocarcinoma cell lines PC-3 and DU-145 were kindly provided by the Cell Interactions Laboratory, Federal University of Rio de Janeiro (UFRJ). PC-3 is a prostate cancer bone metastasis cell line, while DU-145 is a prostate cancer brain metastasis cell line (Sobel et al., 2005). The prostate cell lines were cultured in RPMI medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 1% amphotericin, pH 7.4, under 5% CO_2 atmosphere and 37°C temperature. Controls used in cell assays were the cell lines in the same medium without any extract.

MTT cell viability assay

Cell viability was determined using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay (Mosmann, 1983). The cells were plated in 96-well microplates with 1.0×10^4 cells/well. After incubation with the extract, the culture medium is removed and added 200 µL of MTT. After incubation for 4 h, the MTT was removed and 200 µL of dimethyl sulfoxide (DMSO) were added to solubilize the formazan. Samples were read in an ELISA reader (Bio-Rad iMARK) at 570 nm. Cell viability was calculated in comparison with the control (100%).

Cell cycle analysis

Cells were plated in 6-well microplates with 5.0×10^5 cells/well. Prostate cancer cells incubated for 48 h in the presence and in absence of the four samples at different concentrations (500 µg/mL and 1,000 µg/mL) were detached using trypsin solution at 25°C. The cell suspension was analyzed for DNA content by a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA). Vindlov's reagent was used to read 30,000 cells for each replicate (Pozarowawski and Darzynkiewicz, 2004). Cells with lower DNA content than G_1 in the cell cycle distribution were considered hypodiploid cells (subG1). Relative proportions of diploid G_0/G_1 (2n), S (>2n, but < 4n), and G_2/M (4n) indicative of DNA content were acquired using Cell Quest iPro. The percentage of cell population in each specific phase was estimated with FlowJo v 10.0.6 software and compared to the control.

Apoptosis assays

Phosphatidylserine externalization was observed through the Annexin-V assay using the flow cytometry technique (Van Engeland et al., 1998) to indicate the percentage of cells that were

likely viable in apoptosis or nonapoptotic cell death. Prostate adenocarcinoma cells were incubated in a 6-well microplate using 5.0×10^5 cells/well with the extracts for 48 h. The cells were detached using a trypsin solution, and subsequently the propidium iodide and annexin markers were added. Detection was carried out with a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA) using Cell Quest iPro software to count 30,000 cells units/replicate. The cell populations analyzed were recognized by their forward scatter (FSC)/side scatter (SSC) properties. Fluorescein isothiocyanate (FITC) green fluorescence was measured at 530 ± 30 nm (FL1 detector) and propidium iodide red fluorescence was measured at 585 ± 42 nm (FL2). The percentage of viable cells and cells in early or late apoptosis or non-apoptotic death was calculated using FlowJo v 10.0.6 software.

In silico approaches

The anthocyanins tentatively identified were submitted to *in silico* approaches as proposed by Galvão (2021). Molecular structure was obtained with the SMILES strings at PubChem (https://pubchem.ncbi.nlm.nih.gov/). For their recommended maximum dose (MRD, expressed as mg/kg of body weight/day) the QSAR framework algorithm was applied (Lazar *in silico* toxicology, https://lazar.in-silico.ch/predict) based on similarity structural alerts, The pkCSM algorithm (http://bleoberis.bioc.cam.ac.uk/pkcsm/prediction) was used for intestinal absorption rate (IAR) and hepatotoxicity. The blood brain barrier (BBB) permeability was estimated with the SwissADME algorithm (http://www.swissadme.ch/). Additionally, the blood concentration (BC, expressed as mg/kg of body weight) of each anthocyanin was estimated based on the maximum recommended dose and the intestinal absorption rate.

Statistical analyses

All assays were performed in triplicate and the results are expressed by mean ± standard deviation. Data were analyzed using GraphPad Prism (version 5.04, GraphPad Software, San Diego, CA, USA). Results were compared by one-way analysis of variance (ANOVA) together with the Tukey post-test with a confidence level of 95%. Cell cycle and apoptosis assays tested 99% and 99.9% confidence levels.

Results

The composition of the extracts used in this work was previously presented and globally, a total of 87 phenolic compounds were tentatively identified, and among these the main classes were flavonoids, phenolic acids. Flavonoids presented the highest abundance, followed by the phenolics acids, other polyphenols, stilbenes and lignans.

Cyanidin derivatives showed the lowest concentration in SA with 0.63 mg/100 g. In SU,

peonidin derivatives showed the lowest concentration with 0.77 mg/100 g. The SA sample showed high values of total anthocyanins (23.04 mg/100 g) compared to the SU sample (9.43 mg/100 g). In the determination of total phenolic compounds, the SU extract sample had the highest mean value of 55.67 mg AGE/100 g fresh weight, followed by the SA sample (Figure 1).

In the cell culture assays (cell viability, cell cycle, and apoptosis) the results are discussed based on the most used tests — extract of hybrid grapes in liquid and secondary metabolites alone or accompanied by grape extract. According to Camby et al. (Camby et al.,1994), PC-3 cell line appears to maintain a higher degree of differentiation than the DU-145 cell line, both are insensitive to androgens, and growth is similar because they grow as colonies. The two cell lines are similar in that they present themselves in the form of islands containing polygonal cells predominantly adorned in monolayers with central elements stacking on top of each other. The PC-3 cell line is more differentiated than the DU-145 cell line (Cristofani et al., 2018)

In this study, the cell proliferation was evaluated by the MTT (3-(4,5 dimethylthiazol2-yl) -2,5-diphenyl tetrazolium bromide) assay, which is one of the most used methods to access the action and interaction of natural products such as fruits and plants on cell proliferation, viability, and cytotoxicity. This assay is based on the reduction of a tetrazolium salt to a purple insoluble formazan by metabolically active cells. The absorbance of the solubilized formazan is taken as a measure of the number of living cells. To evaluate the effect of SA and SU on the viability of the two prostate cancer cell lines, DU-145 and PC-3, they were treated with 10-1000 µg/mL of each extract for 24h and 48h. A reduction in cell viability of DU-145 (45 and 65%) and PC-3 (63 and 67%) cells after 48h treatment with SA and SU, respectively, was found via MTT assay. These results have been supported by epidemiological studies in which the consumption of grape was associated with decreased risk of several types of cancer, including lung, prostate, and colon cancer.

Through the cell viability assessment with the MTT assay it was possible to analyze the cytotoxic effect of *Sweet Sapphire* and *Sweet Surprise* as shown in figure 2 referring to the DU-145 cells and figure 3 for the PC-3 cells at incubation times of 24h and 48h. Both grape extracts showed viable DU-145 and PC-3 cell reduction. The extracts began to show a decrease in this viability after 24h of treatment in low concentrations, presenting a better effect on the concentration of 1000 µg/ml. On the other hand, for the PC-3 cell, no significant difference was seen between the concentrations of 500 and 1000 µg/ml.

Regarding the incubation period of 24 and 48 h, no significant difference was seen in the reduction capacity obtained at the different concentrations for DU-145. However, for PC-3, the

action was seen to take place more optimally in the period of 24 hours because in 48 hours the number of viable cells increases, leading to suggest that the cells have a mechanism for proliferating. Based on the MTT results, two of the six tested concentrations were selected for the cell cycle and apoptosis exams.

The cell cycle analyses showed a decrease in the two lines of the G_0/G_1 phase, as well as in the S and G_2/M phases, regardless of treatment. This result shows that the treatments with the extracts of SA and SU showed positive results, since there was a decrease in proliferation in the different phases, but for both, there was no statistically significant difference (Figures 4 and 5). The concentrations of 500 μg/mL and 1000 μg/mL were tested at 48h of incubation time to visualize the moment when cell viability decreased during cell growth, followed by the evaluation of the percentage of viable cells in the different phases of the cell cycle.

Table 1 shows the decrease in the percentage of cells in phase G_0/G_1, and cell decrease in phase S and G_2/M, observed in comparison with the control at the concentration of 1000 and 500 μg/mL for DU-145, both for the extracts of SA and SU. For the PC-3 cell line, the same cellular behavior as the DU-145 scan was observed. The treatment showed a positive result for both, demonstrating to affect their proliferation.

For both concentrations, no evidence was found to prove statistical difference between the two. In the cell cycle and apoptosis experiments, in a study with DU-145 in treatments of 24 and 48 hours, the extract induced the phase G_0/G_1 stop and cell death (subG_1 phase), and these effects occurred in a time-dependent manner. Proteins that had increased were associated with the stop cycle cell in phase G_0/G_1 (table 1). The extract also significantly induced cell death by apoptosis leading to morphological and biochemical marks, such as cell shrinkage, membrane bubbles and condensation of nuclear chromatin.

In the apoptosis assay, the type of cell death caused by the bioactive compounds of the grape was evaluated by flow cytometry, and whether it would be able to trigger the apoptotic process without necrosis. For the cell line of brain metastasis (DU-145), we noticed that both concentrations (500 and 1000 μg/mL) showed statistical difference in relation to their control with a higher percentage of initial and late apoptosis (Table 2 and Figure 4). For the PC-3 cell line, the difference between treated cells and control is noticeably clear due to having several cells concentrated in the regions of early and late apoptosis, while the control is concentrated in viable cells (Table 3 and Figure 5).

In the apoptosis assay, the type of cell death caused by the bioactive compounds of the grape was evaluated by flow cytometry, and whether it would be able to trigger the apoptotic process without necrosis. For the cerebral metastasis cell line (DU-145), we noticed that both

concentrations (1000 and 500 µg/mL) showed statistical difference ($p<0.05$) in relation to their control with a higher percentage of initial and late apoptosis (table 2 and 3).

Different algorithms are used in this approach, which contributes to increase the range of studied parameters. In this study, the recommended maximum dose, the intestinal absorption rate, hepatotoxicity, and BBB permeability were predicted.

The Table 4 presents the results of the *in-silico* approach. The compounds with the least recommended maximum dose were peonidin 3-O-glucoside and petunidin 3-O-glucoside, with 59.5 and 60.2 mg/kg of body weight/day, respectively. However, these compounds had the higher absorption rate (50% for peonidin and 42% for petunidin). Cyanidin 3-O-glucoside, delphinidin 3-O-glucoside and malvidin 3-O-glucoside both had around 30% of intestinal absorption rate. However, the compound with the higher estimated blood concentration after intestinal absorption was delphinidin 3-O-glucoside due to the higher maximum recommended dose between the five compounds. None of the compounds were predicted as hepatotoxic. On the other hand, none of the compounds were predicted to pass the blood brain barrier.

Discussion

In a study of Rosa Niagara grape cultivars, values from 208 to 214 mg AGE/100 g fresh weight were quantified, which shows values high to those found in the present study (Soares et al., 2008). In this study, we used only pulp, which may explain the difference for other studies that study the whole grape. Grape seeds are richer sources of total phenolic content than peel and pulp and peel was found to be richer than pulp for all nine Karaerik grape clones (Xia et al., 2018; Kupe et al., 2021;).

Among the phenolic compounds present in grapes, the main ones are anthocyanins (Xia et al., 2018). In grapes of *V. vinifera* L., five coloured aglycons commonly occur, namely cyanidin, peonidin, delphinidin, petunidin and malvidin (Abe et al, 2007). Anthocyanins are in the berry skin and are the compounds mainly responsible for the red colour of grapes and wines (Lingua et al., 2016; Spada et al., 2022). Our results showed that malvidin derivatives present the highest content in both cultivars. We recorded 14.46 mg/100 g malvidin 3-O glycoside in *Sweet Sapphire* and 2.76 mg/100 g in *Sweet Surprise,* in accordance with Balik (2013) in more recent years.

Phenolic compounds were found in the extracts 94%, 85% and 66%, respectively, for flavonoids, other polyphenols, and phenolic acids (Pascoal et al., 2022).

The extract also significantly induced cell death by apoptosis leading to morphological and biochemical marks, such as cell shrinkage, membrane bubbles and condensation of nuclear

chromatin being time-dependent effects (Lin et al., 2018).

Regarding cell viability and cell cycle results, the lack of cell cycle regulation is a fundamental aspect in cancer development. Normal cells only proliferate in response to cell development or signals that occur during mitosis (Oshima and Campisi, 1991). The cell cycle consists of distinct phases of events that occur in a cell in preparation for cell division: Phase G_0 is seen as an extended G_1, (gap 1, or G_1, stage), copies its DNA (synthesis, or S, stage), prepares to divide (gap 2, or G_2, stage), and divides (mitosis, or M, stage). Steps G_1, S and G_2 make up an interphase, which explains the gap between cell division (Prakash et al., 2001). Each phase of the cell cycle is heavily regulated and there are checkpoints to detect potential DNA damage and allow it to be repaired before a cell split. If the damage cannot be repaired, a cell becomes the target of apoptosis, which is a programmed cell death process that occurs in multicellular organisms in different circumstances and involves different stages (Obermuller-jevic Uc et al., 2003).

The morphological and biochemical marks presented in the induction, by extract, of cell death by apoptosis may have been time-dependent effects (Lin et al., 2018).

Other studies have shown that the extract of grape seed powder negatively regulated Cyclin D1 and caused a slight positive regulation of PTEN (a tumor suppressor that modulates apoptosis, cell cycle, and migration) in DU-145 cells. The pAkt levels, which promote cell survival and prevent apoptosis, were affected by treatment with the extract in PC-3 cells. The p21 expression was induced in a dose-dependent manner after treatment with extract in PC-3 (Agarwal et al., 2000; Kumar et al., 2018).

Assays in an isolated polyphenolic fraction of procyanidin-rich grape seeds tested in the DU-145 cells presented results suggestive that this treatment possibly involves the modulation of mitogenic signaling and cell cycle 52 regulators and G_1 stop induction, cell growth inhibition, and apoptotic death (Agarwal et al., 2000). The differential activity in selective targeting of cancer cells, preserving normal cells acting as pro-oxidants, occurs by inducing and degrading DNA in the presence of metal ions such as copper. This mechanism for polyphenols that involves the mobilization of chromatin-bound copper and the consequent pro-oxidant action that leads to cell death has anticancer activity and apoptosis inducers. As it is known that copper levels in tissues and cells are significantly elevated in several malignant diseases. Cancer cells are more subject to redox cycles between copper ions and polyphenols to generate reactive oxygen species (ROS) responsible for DNA breakdown. In other types of cancer, such as colorectal (Ravindranathan et al., 2019) the use of oligomeric proanthocyanins from grape seed extracts demonstrated cell cycle stoppage, double tape breaks and p53 protein accumulation in

cells. In lung cancer (Mao et al., 2016), the procyanidin extract inhibited dose-dependent proliferation, induced apoptosis, and negatively regulated microRNA known as oncomirs that mediate pro or antitumor effects. Grape extract containing a mixture of bioactive compounds may have pharmacokinetics and pharmacological potency superior to isolated metabolites, presenting higher potential as a drug for natural products (Kumar et al., 2018).

The previous metabolomics results corroborate with our antioxidant analysis results and we can suggest the tentatively identified phenolic compounds play this role according to the literature, depending on the quantity found in each food matrix. Indeed, flavonoid compounds have been pinpointed as effective compounds in the control of cell cycle and apoptosis (Pascoal et al., 2022).

In cancer research, it is extremely relevant to ensure the safety of the compounds that are studied, even if these were from edible fruits as grapes. The in-silico approaches are recently being used as a tool for the study of safety, as well as a predictor of compounds toxicity (Galvão et al., 2021).

The in-silico studies were conducted, considering that, since it may be metastatic cerebral prostate cancer (Sobel and Sadar, 2005), passing the BBB is relevant for the exposure of metastatic cells to compounds that are capable of deterred their cell cycle and/or lead to apoptosis).

Even though cancer is among the most studied human diseases in a systemic way, its significant challenges remain close to the great potential of cancer biology in silico, preventing full realization (Edelman et al., 2010).

In conclusion, the results of this study, both extracts of hybrid grapes, *Sweet sapphire* and *Sweet surprise,* were able to reduce the viability of prostate cancer cells in the two cell lines presented, as they were able to cause a cytotoxic effect, as demonstrated in the results of cellular viability. Their compounds inhibited cell growth and proliferation in PC-3 and DU-145 as well as inhibited growth and apoptosis death by cell parade in phase S. In addition, the extract was considered non-toxic due to absence of hepatotoxicity and the maximum recommended dose for further possible assays was established.

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Data Availability: The data used to support findings of this study are included within the article.

References

Abe LT, Da Mota RV, Lajolo FM, Genovese MI (2007). Phenolic compounds and antioxidant capacity of grape cultivars *Vitis labrusca* L. and *Vitis vinifera* L. *Food Science and Technology*, **27**, 394–400.

Agarwal C, Sharma Y, Agarwal R (2000). Anticarcinogenic Effect of a Polyphenolic Fraction Isolated from Grape Seeds in Human Prostate Carcinoma DU145 Cells: Modulation of Mitogenic Signaling and Cell-Cycle Regulators and Induction of G1 Arrest and Apoptosis. *Molecular Carcinogenesis*, **28**, 129–38.

Albuquerque BR, Pinela J, Barros L, Oliveira MBPP, Ferreira ICFR (2020). Anthocyanin-rich extract of jabuticaba epicarp as a natural colorant: Optimization of heat- and ultrasound-assisted extractions and application in a bakery product. *Food Chemistry*, **30**, 316:126364.

Balík J, Kumšta M, Rop O (2013). Comparison of anthocyanins present in grapes of L. varieties and interspecific hybrids grown in the Czech Republic. *Chemical Papers*, **67**(10), 1285–92.

Basli A, Belkacem N, Amrani I (2017). Health benefits of phenolic compounds against cancers. Phenolic compounds–Biological activity. London, UK: IntechOpen, 193-210.

Brito ES, Araújo MCP,Alves REA et al (2007). Anthocyanins Present in Selected Tropical Fruits: Acerola, Jambolão, Jussara, and Guajiru. *J. Agric. Food Chemistry*, **55,** 9389–94.

Camby I, Etievant C, Petein M, et al (1994). Influence of culture media on the morphological differentiation of the PC-3 and DUI45 prostatic neoplastic cell lines. *The Prostate*, **24**, 187–96.

Chatham LA, Howard J E, Juvik JA (2020). A natural colorant system from corn: Flavone-anthocyanin copigmentation for altered hues and improved shelf life. *Food Chemistry*, **310**, 125734.

Cory H, Passarelli S, Szeto J, Tamez M, Mattei J (2018). The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. *Frontiers in Nutrition*, **5**, 1–9.

Cristofani R, Montagnani MM, Cicardi ME, et al (2018). Dual role of autophagy in sensitivity to docetaxel in prostate cancer cells. *Cell Death and Dis*ea*se*, **9,** 889.

Darweesh RS, El-Elimat T, Zayed A, et al (2020). The effect of grape seed and green tea extracts on the pharm acokinetics of imatinib and its main metabolite, N-desmethyl imatinib, in rats. *BMC Pharmacology and Toxicology*, **21**, 1-15.

De Martel C, Georges D, Bray F, Ferlay J, Clifford GM (2020). Global cancer burden attributable to infections in 2018: a worldwide incidence analysis. *Lancet Glob Health*. **8,** 180-90.

De Rosso M, Tonidandel L, Larcher R, et al (2012). Study of anthocyanic profiles of twenty-one hybrid grape varieties by liquid chromatography and precursor-ion mass spectrometry. *Analytica Chimica Acta*, **732**, 120–29.

Delgado AM, Issaoui M, Chammem N (2019). Analysis of main and healthy Phenolic compounds in foods. *Journal of AOAC International*, **102**, 1356–64.

Demirbas A, Yilmaz V, Ildiz N, Baldemir A, Ocsoy I (2017). Anthocyanins-rich berry extracts directed formation of Ag NPs with the investigation of their antioxidant and antimicrobial activities. *Journal of Molecular Liquids*, **248**, 1044–9.

Dohadwala MM, Vita JA (2009). Grapes and cardiovascular diseases. *J Nutr*.,**139**, 1788-93.

Dumitru BF, Bunea CI, Donici A (2019). Phenolic, Anthocyanin Composition and Color Measurement at Red Wines from Dealu Bujorului Vineyard. *Agriculture*, **109**, 14–28.

Edelman LB, Eddy JA, Price ND (2010). In silico cancer models. *Wiley Interdiscip Rev Syst Biol Med.*, **2**, 438-59.

Fujita R, Hayasaka T, Jin S, Hui S P, Hoshino Y (2020). Comparison of anthocyanin distribution in berries of Haskap (Lonicera caerulea subsp. edulis (Turcz. ex. Herder) Hultén), Miyama-uguisu-kagura (Lonicera gracilipes Miq.), and their interspecific hybrid using imaging mass spectrometry. *Plant Science*, **300**, 110633.

Galvão BVD, Araujo-Lima CF, Santos MCP, et al (2021). Plinia cauliflora (Mart.) Kausel (Jaboticaba) leaf extract: In vitro anti-Trypanosoma cruzi activity, toxicity assessment and phenolic-targeted UPLC-MSE metabolomic analysis. *Journal of Ethnopharmacology*, **277,** 114217.

Gorzynik-Debicka M, Przychodzen P, Cappello F, et al (2018). Potential health benefits of olive oil and plant polyphenols. *International Journal of Molecular Sciences*, **19**, 547.

Gülçin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI (2002). On the in vitro antioxidative properties of melatonin. *J Pineal Res*., **33**, 167–71.

Gutiérrez-Gamboa G, Liu SY, Pszczólkowski P (2020). Resurgence of minority and autochthonous grapevine varieties in South America: a review of their oenological potential. *Journal of the Science of Food and Agriculture*, **100**, 465–82.

Hamza AA, Heeba GH, Elwy HM, et al (2018). Molecular characterization of the grape seeds extract's effect against chemically induced liver cancer: In vivo and in vitro analyses. *Scientific reports*, **8**, 1-16.

Kelly E, Vyas P, Weber JT (2018). Biochemical properties and neuroprotective effects of compounds in various species of berries. *Molecules*, **23**, 26.

Kumar A, D'silva M, Dholakia K, Levenson AS (2018). In vitro anticancer properties of table grape powder extract (GPE) in prostate cancer. *Nutrients*, **10,** 1804.

Kupe M, Karatas N, Unal MS, et al (2021). Phenolic Composition and Antioxidant Activity of Peel, Pulp and Seed Extracts of Different Clones of the Turkish Grape Cultivar 'Karaerik'. *Plants*, 10, 2154.

Lee B, Lin PC, Cha HS, Luo J, Chen F (2016). Characterization of volatile compounds in Cowart muscadine grape (Vitis rotundifolia) during ripening stages using GC-MS combined with principal component analysis. *Food Science and Biotechnology*, **25**, 1319–26.

Lin CH, Chan HS, Tsay HS, et al (2018). Ethyl acetate fraction from methanol extraction of Vitis thunbergii var. taiwaniana induced G0/G1 phase arrest via inhibition of cyclins D and E and induction of apoptosis through caspase-dependent and -independent pathways

in human prostate carcinoma DU1. *Environmental Toxicology*, **0**, 1–33.

Lingua MS, Fabani MP, Wunderlin DA, Baroni MV (2016). From grape to wine: Changes in phenolic composition and its influence on antioxidant activity. *Food Chemistry*, **208**, 228-38.

Liu S, Sui Q, Zou J, Zhao, Y, Chang X (2019). Protective effects of hawthorn (Crataegus pinnatifida) polyphenol extract against UVB-induced skin damage by modulating the p53 mitochondrial pathway in vitro and in vivo. *Journal of Food Biochemistry*, **43**, 1–12.

Mao JT, Smoake J, Park HK, Lu QY, Xue B (2016). Grape seed procyanidin extract mediates antineoplastic effects against lung cancer via modulations of prostacyclin and 15-hete eicosanoid pathways. *Cancer Prevention Research*, **9**, 925–32.

Mosmann T (1983). Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *Journal Immunological Methods*, **65**, 55-63.

Nicolini G, Roman T, Flamini R, et al (2020). Thiol precursors in Vitis mould-tolerant hybrid varieties. *Journal of the Science of Food and Agriculture*, **100**, 3262–68.

Nixdorf SL, Hermosín-Gutiérrez I (2010). Brazilian red wines made from the hybrid grape cultivar Isabel: Phenolic composition and antioxidant capacity. *Analytica Chimica Acta*, **659**, 208–15.

Obermuller-Jevic UC, Olano-Martin E, Corbacho AM, et al (2003). Lycopene inhibits the growth of normal human prostate epithelial cells in vitro. *J Nutr*, **133**, 3356-60.

Olivati C, Nishiyama YPO, Souza RT, et al (2019). Effect of the pre-treatment and the drying process on the phenolic composition of raisins produced with a seedless Brazilian grape cultivar. *Food Research International*, **116**, 190–9.

Oshima J, Campisi J (1991). Fundamentals of cell proliferation: cell cycle control. *J Dairy Sci,* **74**, 2778-87.

Pascoal GFL, Cruz MAAS, Abreu JPA, et al (2022). Evaluation of antioxidant capacity, volatile composition, and phenolic content of hybrid varieties *Vitis vinifera* L. *Sweet Sapphire* and *Sweet Surprise*. *Food Chem*. ,**336**, 130644.

Pereira R N, Coelho MI, Genisheva Z, et al (2020). Using Ohmic Heating effect on grape skins as a pretreatment for anthocyanins extraction. *Food and Bioproducts Processing*, **124**, 320–28.

Prakash P, Russel RM & Krinsky NI (2001). In vitro inhibition of proliferation of estrogen-dependent and estrogenindependent human breast cancer cells treated with carotenoids or retinoids. *J Nutr,* 131, 1574-1580.

Pozarowski P, Darzynkiewicz Z (2004). Analysis of cell cycle by flow cytometry. *Methods in*

Molecular Biology (Clifton, N.J.), **281**, 301–12.

Qin F, Yao L, Lu C, et al (2019). Phenolic composition, antioxidant, and antibacterial properties, and in vitro anti-HepG2 cell activities of wild apricot (*Armeniaca Sibirica* L. Lam) kernel skins. *Food and Chemical Toxicology*, **129**, 354–64.

Ravindranathan P, Pasham D, Goel A (2019). Oligomeric proanthocyanidins (OPCs) from grape seed extract suppress the activity of ABC transporters in overcoming chemoresistance in colorectal cancer cells. *Carcinogenesis*, **40**, 412–21.

Russo GI, Campisi D, Di Mauro M, et al (2017). Dietary consumption of phenolic acids and prostate cancer: A case-control study in Sicily, Southern Italy. *Molecules*, **22**, 2159.

Saeed N, Khan MR, Shabbir M (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complementary and Alternative Medicine. *Sensores (Basileia),* **12**, 5702-14.

Samoticha J, Wojdyło A, Golis T (2017). Phenolic composition, physicochemical properties, and antioxidant activity of interspecific hybrids of grapes growing in Poland. *Food Chemistry*, **215**, 263–73.

Shahab M, Roberto SR, Ahmed S, et al (2020). Relationship between anthocyanins and skin color of table grapes treated with abscisic acid at different stages of berry ripening. *Scientia Horticulture*, **259**, 108859.

Singh RP, Tyagi AK, Dhanalakshmi S, Agarwal R, Agarwal C (2004). Grape seed extract inhibits advanced human prostate tumor growth and angiogenesis and upregulates insulin-like growth factor binding protein-3. *International journal of cancer*, **108**, 733-40.

Slegers A, Angers P, Ouellet É, Truchon T, Pedneault K (2015). Volatile compounds from grape skin, juice, and wine from five interspecific hybrid grape cultivars grown in Quebec (Canada) for wine production. *Molecules*, **20**, 10980–1016.

Soares M, Welter L, Kuskoski EM, Gonzaga L, Fett R (2008). Phenolic Compounds and Antioxidant of Niagara Grape Bark and Isabel. *Brazilian Fruit. J.*, **30,** 59–64.

Sobel RE, Sadar MD (2005). Cell lines used in prostate cancer research: A compendium of old and new lines - Part 1. *Journal of Urology*, **173**, 342–59.

Spada V, Di Stasio L, Ferranti P, Addeo F, Mamone G, et al. (2022). Expression of Differential Protein in Red Grape Blackberry Skin with Varied Hybrid Character. *Int. J. Mol. Sci.*, **23**, 1051.

Van Engeland M, Nieland LJW, Ramaekers FCS, Schutte B, Reutelingsperger CPM (1998). Annexin V-Affinity assay: A review on an apoptosis detection system based on

phosphatidylserine exposure. *Cytometry*, **31**, 1–9.

Vizzotto M, Pereira MC (2011). Blackberry (Rubus sp.): Optimization of the extraction process for determination of antioxidant phenolic compounds. Rev. *Brazilian Journal of Fruit Culture*, **33**, 1209–14.

Xia L, Xu C, Huang K, Lu J, Zhang Y (2018). Evaluation of phenolic compounds, antioxidant and antiproliferative activities of 31 grape cultivars with different genotypes. *J Food Biochem*., **43**,12626.

Table1. Effect of SA and SU extracts (500-1000 µg/ml) on cell cycle progression in DU-145 and PC-3 cells after 48 hours.

DU-145				PC-3			
	G_0/G_1	S	G_2/M		G0/G1	S	G2/M
Control	72.69±1.25	11.12±2.57	13.92±1.28	**Control**	83.84+4.06	7.00+2.65	15.42+2.61
SA 500 µg/ml	63.00±1.23*	21.35±1.20*	12.60±1.95	**SA 500 µg/ml**	56.86+14.79*	17.92+7.85*	10.07+4.08*
SA 1000 µg/ml	65.57±2.11*	19.42±3.64*	10.02±3.35	**SA1000 µg/ml**	63.09+11.63*	11.05+3.55*	10.94+3.46*
Control	72.69±1.25	11.12±2.57	13.92±1.28	**Control**	83.84+4.06	7.00+2.65	15.42+2.61
SU 500 µg/ml	65.36±0.89*	21.53±1.07*	10.65±2.08	**SU 500 µg/ml**	72.88+1.51*	11.91+3.18*	13.34+1.77
SU 1000 µg/ml	64.58±0.62*	22.81±1.70*	10.21±2.78	**SU 1000 µg/ml**	68.67+3.91**	9.71+1.10*	14.80+0.96

Results are expressed as a percentage of total cells. Significant differences between untreated cells (Control) and cells treated with grape extracts were compared ($p < 0.05$; ** $p < 0.01$;). Abbreviations: SA-Sweet Sapphire; SU- Sweet Surprise.*

Table 2. Apoptosis rate of the DU- 145 cell line treated with SA (500-1000 µg/ml) and SU (500-1000 µg/ml) extracts.

Treatment	Extract concentration	Viable cell DU-145	Initial apoptosis	Late apoptosis	Necrosis
Control	--	89.72±0.49	1.01±0.38	1.29±0.22	7.75±0.11
SA	500 µg/ml	89.22±1.97	3.83±1.30*	2.18±0.34	6.51±0.62
	1000 µg/ml	84.00±2.33*	3.83±1.30*	5.63±0.63*	4.36±0.54*
SU	500 µg/ml	87.02±2.41	4.59±1.86*	3.73±0.73	4.62±0.19
	1000 µg/ml	81.60±0.91*	8.14±1.00**	6.91±0.82*	3.32±0.60

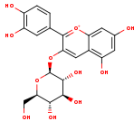
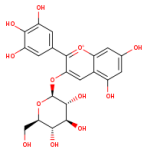
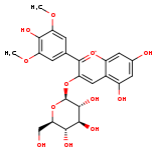
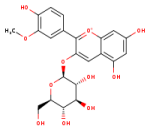
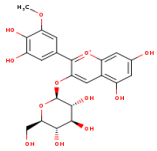
Results are expressed as a percentage of total cells. Significant differences between untreated cells (Control) and cells treated with grape extracts were compared ($p < 0.05$; ** $p < 0.01$;). Abbreviations: SA-Sweet Sapphire; SU- Sweet Surprise.*

Table 3. Apoptosis rate of the PC-3 cell line treated with SA (500-1000 µg/ml) and SU (500-1000 µg/ml) extracts.

Treatment	Extract concentration	Viable cells PC-3	Initial apoptosis	Late apoptosis	Necrosis
Control	--	98.66±0.68	0.26±0.13	0.36±0.034	0.06±0.013
SA	500 µg/ml	92.92±3.39*	0.34±0.11	2.12±0.13*	6.49±0.79**
	1000 µg/ml	93.15±1.17*	0.35±0.07	2.22±0.45*	4.49±1.20**
SU	500 µg/ml	86.77±4.32**	10.2±0.70**	4.36±0.41**	3.72±1.32**
	1000 µg/ml	88.72±1.23**	1.45±0.54**	3.85±0.50**	5.72±1.26**

Results are expressed as a percentage of total cells. Significant differences between untreated cells (Control) and cells treated with grape extracts were compared ($p < 0.05$; ** $p < 0.01$;). Abbreviations: SA-Sweet Sapphire; SU- Sweet Surprise.*

Table 4. Results of the in-silico approach of anthocyanins identified in the extracts studied.

Anthocyanin	PUBChem		LAZAR	pkCSM		SwissADME	BC
	Molecular structure	Molecular formula weight	MRD	IAR (%)	Hepatotoxicity	BBB permeability	
Cyanidin 3-O-glucoside		$C_{21}H_{21}ClO_{11}$ 484.84 g/mol	99.9	29.927	No	No	29,9
Delphinidin 3-O-glucoside		$C_{21}H_{21}O_{12}^{+}$ 465.38 g/mol	129.0	32.504	No	No	41.9
Malvidin 3-O-glucoside		$C_{23}H_{25}ClO_{12}$ 528.89 g/mol	92.0	31.346	No	No	28.8
Peonidin 3-O-glucoside		$C_{22}H_{23}O_{11}^{+}$ 463.41 g/mol	59.5	50.098	No	No	30.3
Petunidin 3-O-glucoside		$C_{22}H_{23}O_{12}^{+}$ 479.41 g/mol	60.2	42.394	No	No	25.5

Abbreviations: MRD – maximum recommended dose in mg/kg of body weight/kg; IAR – Intestinal absorption rate; BBB – blood brain barrier; BC – blood concentration after intestinal absorption expressed as mg/kg of body weight.

FIGURE LEGENDS

Figure 1. Total phenolic compounds and total anthocyanins present in the cultivar's *Sweet Sapphire* and *Sweet Surprise*.

Figure 2. Effect of treatment of *Sweet Sapphire* (SA) and *Sweet Surprise* (SU) extracts under the viability of DU-145 prostate cancer cells after 24h (A., B. and E) and 48h (C., D and F.) of incubation. ($p < 0.05$). CT – control.

Figure 3. Effect of treatment of *Sweet Saphire* (SA) and *Sweet surprise* (SU) extracts under the viability of PC-3 prostate cancer cells after 24h (A., B and E.) and 48h (C., D and F.) of incubation. ($p < 0.05$). CT – control.

Figure 4. Apoptosis induction in 48h in DU-145. Control Cells (CT) and cells treated with grape extracts of SA (*Sweet Sapphire*) e SU (*Sweet Surprise*) (500 µg/mL) and (1000 µg/mL). The top-down quadrants clockwise: initial apoptosis (Annexin V+ PI-), late apoptosis (Annexin V+ PI+), non-apoptotic death (Annexin V- PI+) and viable cells (Annexin- PI-).

Figure 5. Apoptosis induction in 48h in PC-3. Control Cells (CT) and cells treated with grape extracts of SA (*Sweet Sapphire*) and SU (*Sweet Surprise*), (500 µg/mL and 1000 µg/mL). The top-down quadrants clockwise: initial apoptosis (Annexin V+ PI-), late apoptosis (Annexin V+ PI+), non-apoptotic death (Annexin V- PI+) and viable cells (Annexin- PI-).

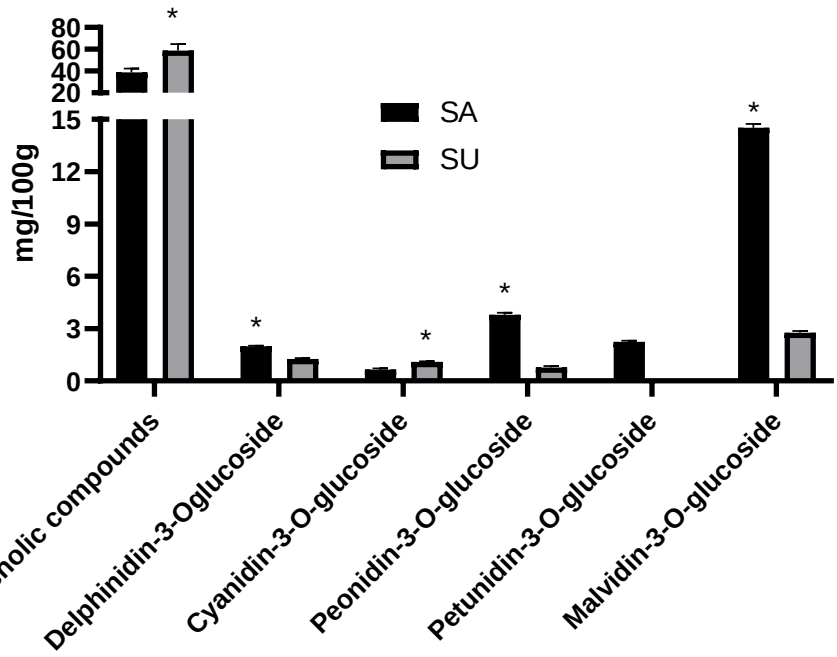


Figure 1. Total phenolic compounds and total anthocyanins present in the cultivars *Sweet Sapphire* (SA) and *Sweet Surprise* (SU).

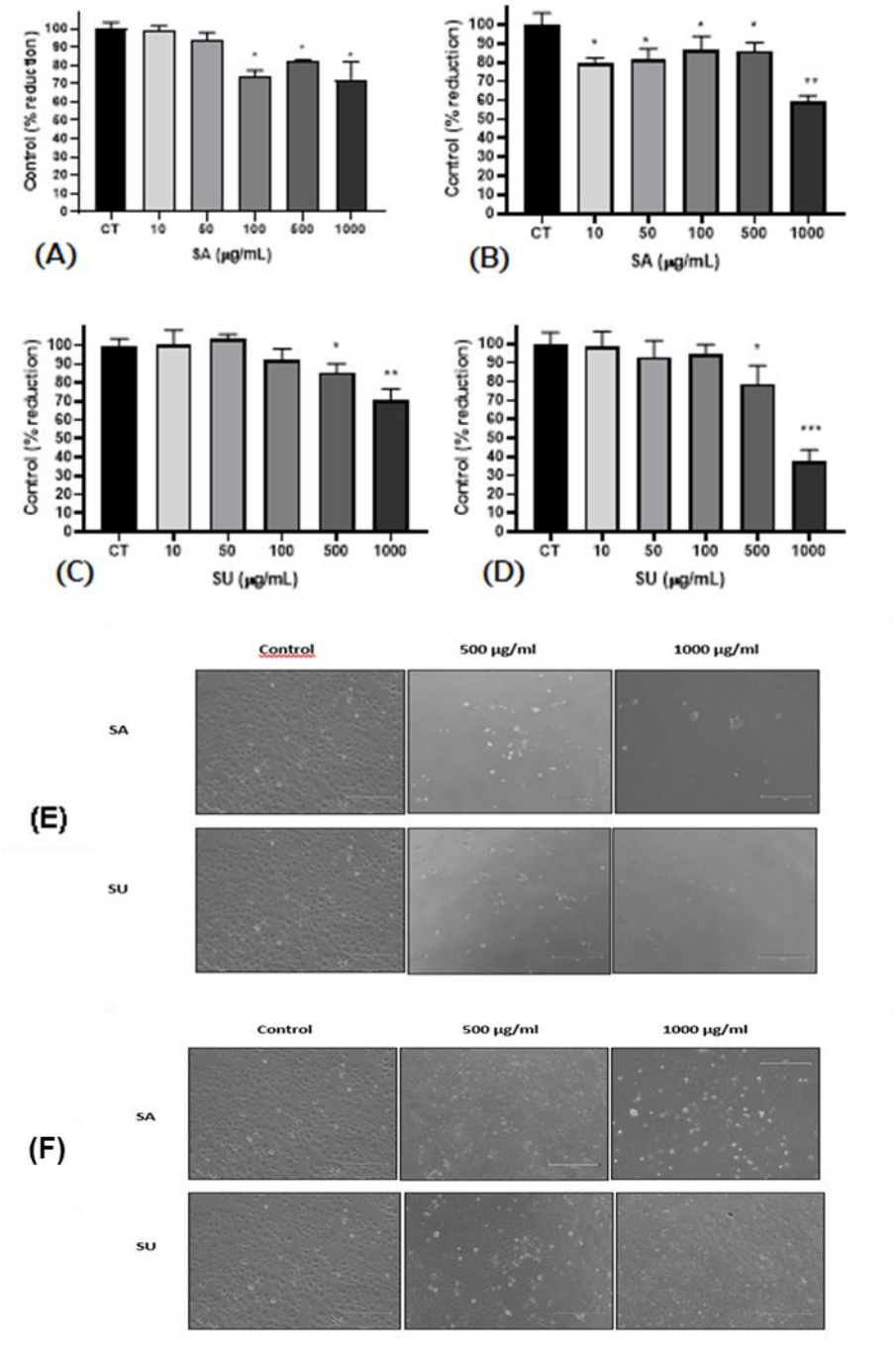


Figure 2. Effect of treatment of *Sweet Sapphire* (SA) and *Sweet Surprise* (SU) extracts under the viability of DU-145 prostate cancer cells after 24h ((A), (B), and (E)) and 48h ((C)., (D) and (F)) of incubation. ($p< 0.05$). CT – control.

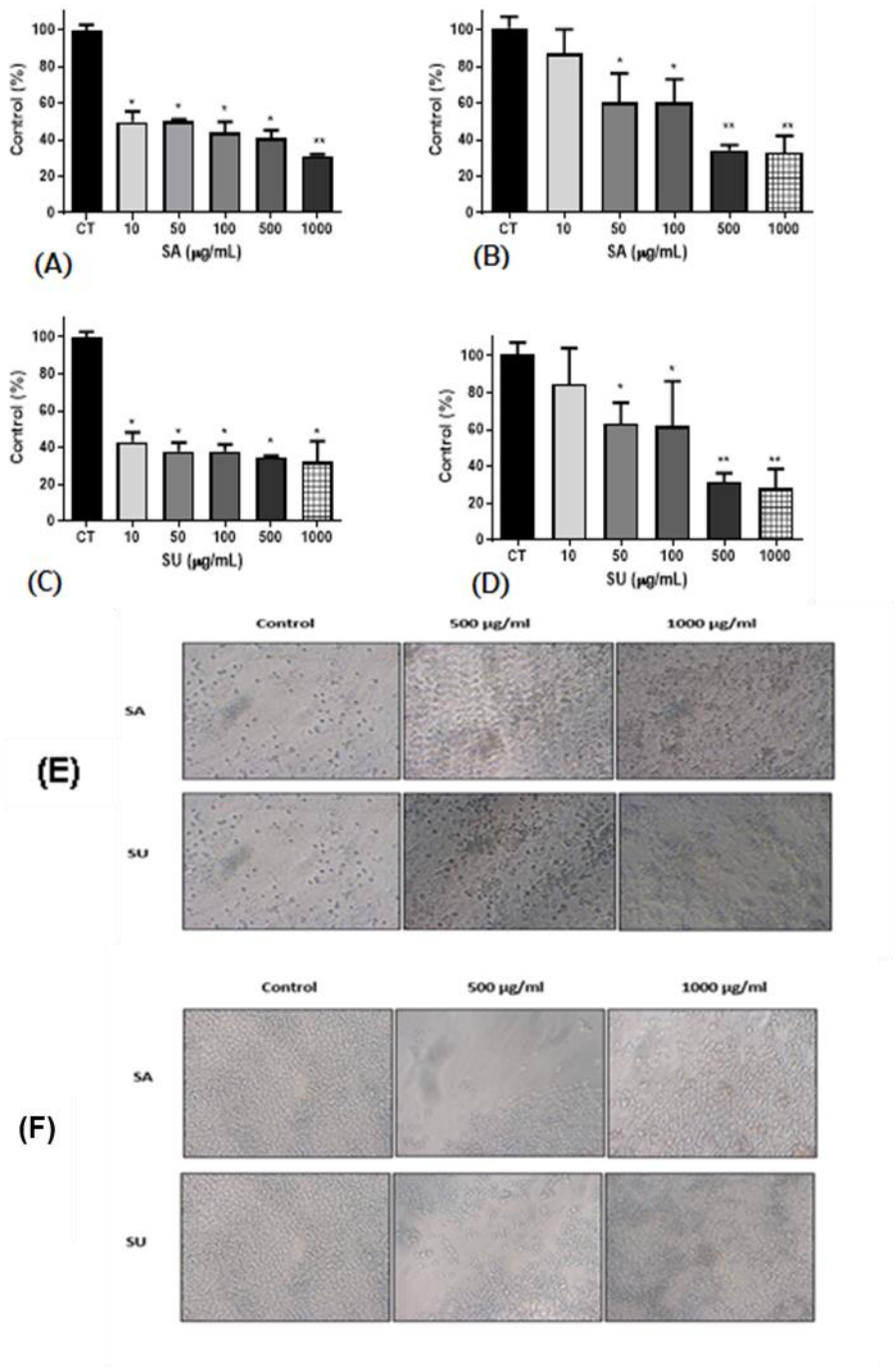


Figure 3. Effect of treatment of *Sweet Sapphire* (SA) and *Sweet Surprise* (SU) extracts under the viability of PC-3 prostate cancer cells after 24h ((A), (B), and (E) and 48h ((C)., (D) and (F)) of incubation. ($p< 0.05$). CT – control.

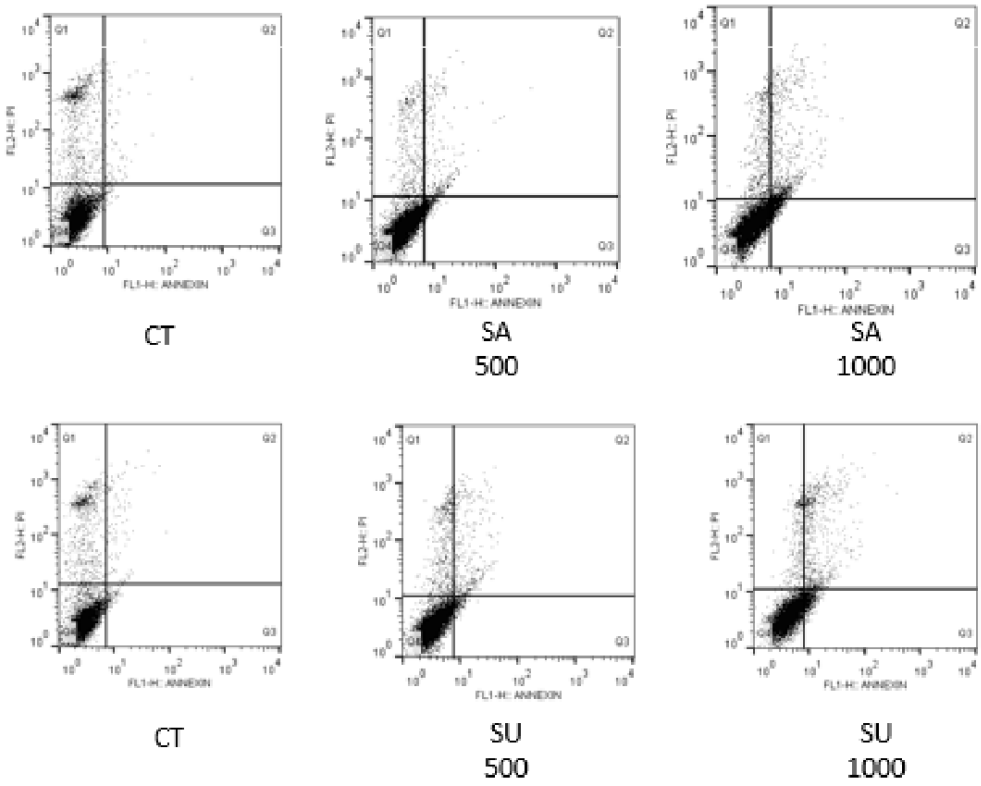


Figure 4. Apoptosis induction in 48h in DU-145. Control Cells (CT) and cells treated with grape extracts of SA *(Sweet Saphire*) e SU (*Sweet Surprise*) (500 µg/mL) and (1000 µg/mL). The top-down quadrants clockwise: initial apoptosis (Annexin V+ PI-), late apoptosis (Annexin V+ PI+), non-apoptotic death (Annexin V- PI+) and viable cells (Annexin- PI-).

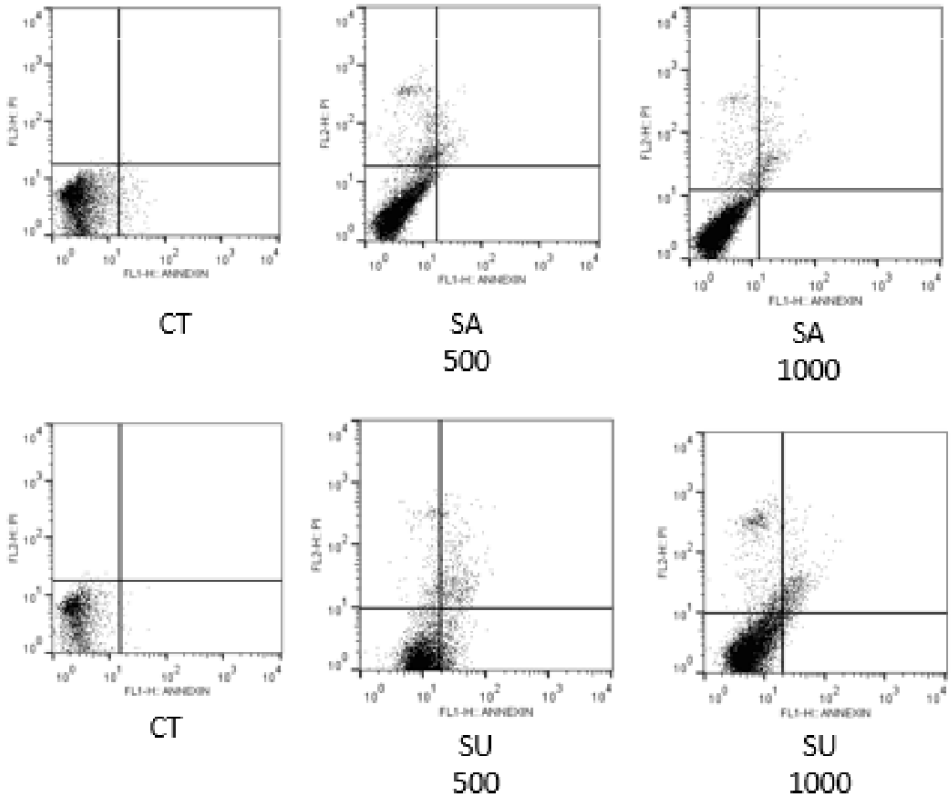


Figure 5. Apoptosis induction in 48h in PC3. Control Cells (CT) and cells treated with grape extracts of SA (*Sweet Saphire*) and SU (*Sweet Surprise*), (500 µg/mL and 1000 µg/mL). The top-down quadrants clockwise: initial apoptosis (Annexin V+ PI-), late apoptosis (Annexin V+ PI+), non-apoptotic death (Annexin V- PI+) and viable cells (Annexin- PI-).

CONSIDERAÇÕES FINAIS

Os ensaios realizados para este estudo, destacaram a concentração significativamente maior de compostos fenólicos e propriedades antioxidantes das variedades híbridas *Vitis vinífera L*., SA e SU, que acentuam seus efeitos anticancerígenos em células de câncer de próstata. Utilizando uma combinação de técnicas UPLC-MSE, HPLC-PDA, GC-FID e GC-MS, caracterizamos os compostos fenólicos e voláteis em diferentes extratos dessas variedades híbridas. Um total de 87 compostos fenólicos foram identificados em comparação com os padrões e por padrões de fragmentação.

A fração volátil de SU (42 componentes) era mais rica que a de SA (31 compostos). Ácidos graxos, álcoois e aldeídos foram os principais compostos voláteis encontrados em ambas as cultivares de uva. Os ensaios DPPH, ABTS, FRAP e ORAC revelaram que o extrato de acetona da casca SA apresenta maior atividade antioxidante, bem como valores moderados de antocianina. Seu consumo apresenta alto potencial bioativo, sendo seu uso eficiente na prevenção de patologias.

Mediante aos dados apresentados, sugere-se que as amostras dos extratos de SA e SU, podem desempenhar importante papel na redução da viabilidade celular, na modulação do ciclo celular e, no controle da progressão tumoral, via indução de apoptose, já que foram capazes de reduzir a viabilidade celular, de modo que seus compostos inibiram o crescimento e a proliferação celular em linhagens PC-3 e DU-145.

Como perspectivas futuras, outros estudos com esses compostos, através de ensaios clínicos em animais e humanos, serão importantes para melhor compreensão e confirmação dos achados obtidos no presente estudo. Assim como, também estudos que informem a composição química das variedades existentes, já que sabemos da existência de variedades ainda não catalogadas, e de outras ainda desconhecidas, que poderiam ser úteis para a saúde da população, tanto para alimentação quanto para prevenção e tratamento de doenças.

REFERÊNCIAS BIBLIOGRÁFICAS

Aires, M. V. L., Modesto, R. M. G., & Santos, J. S. (2021). Os benefícios da uva na saúde humana: uma revisão. **Research, Society and Development**, 10(14), e281101421825. https://doi.org/10.33448/rsd-v10i14.21825

Bassanesi, G., Andrade Touguinha, L. B., & Salvador, M. (2020). Capacidade Antioxidante De Sucos E Néctares De Uvas Do Rio Grande Do Sul. **Revista Internacional de Ciências**, 10(2), 55–67. https://doi.org/10.12957/ric.2020.48081

de Martel, C., Georges, D., Bray, F., Ferlay, J., & Clifford, G. M. (2020). Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. **The Lancet Global Health**, 8(2), e180–e190. https://doi.org/10.1016/S2214-109X(19)30488-7

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. **Antioxidants and Redox Signaling**, 18(14), 1818–1892. https://doi.org/10.1089/ars.2012.4581

Gomez, H. A. G., Marques, M. O. M., Borges, C. V., Minatel, I. O., Monteiro, G. C., Ritschel, P. S., Zanus, M. C., Diamante, M. S., Kluge, R. A., & Lima, G. P. P. (2020). Biogenic Amines and the Antioxidant Capacity of Juice and Wine from Brazilian Hybrid Grapevines**. Plant Foods for Human Nutrition**, 75(2), 258–264. https://doi.org/10.1007/s11130-020-00811-5

Gonçalves, D., Maria, G., Andrade, I. De, & Fernandes, A. (2022). Potencial biológico e aplicações tecnológicas de frutas vermelhas: uma visão geral. 1. **Food Chemistry Advances**, 1. https://doi.org/10.1016/j.focha.2022.100014

Instituto Nacional de Câncer (INCA). Estimativa 2022 de Incidência de Câncer no Brasil. Rio de Janeiro, RJ: [s.n.]. Disponível em: < http://www1.inca.gov.br/rbc/n_64/v01/pdf/15-resenha-estimativa-2022-incidencia-de-cancer-no-brasil.pdf >. nov. 2022.

Izadfar, F., Belyani, S., Pormohammadi, M., Alizadeh, S., Hashempor, M., Emadi, E., Sangsefidi, Z. S., Jalilvand, M. R., Abdollahi, S., & Toupchian, O. (2023). The effects of grapes and their products on immune system: a review. **Immunological Medicine**, 0(0), 1–5. https://doi.org/10.1080/25785826.2023.2207896

Kowalczyk, B.; Bieniasz, M.; Kostecka-Gugała, A. The Content of Selected Bioactive Compounds in Wines Produced from Dehydrated Grapes of the Hybrid Variety ‘Hibernal’ as a Factor Determining the Method of Producing Straw Wines. **Foods.** 2022, 11, 1027. https:// doi.org/10.3390/foods11071027

Kucera, R., Pecen, L., Topolcan, O., Dahal, A. R., & Costigliola, V. (2020). Gerenciamento do câncer de próstata: crenças de longo prazo, desenvolvimentos epidêmicos no início do século XXI e soluções dimensionais 3PM Abstrair. 11(3), 399–418. https://doi.org/10.1007/s13167-020-00214-1

Lin, C. H., Chan, H. S., Tsay, H. S., Funayama, S., Kuo, C. L., & Chung, J. G. (2018). Ethyl acetate fraction from methanol extraction of Vitis thunbergii var. taiwaniana induced

G0/G1 phase arrest via inhibition of cyclins D and E and induction of apoptosis through caspase-dependent and -independent pathways in human prostate carcinoma DU1. **Environmental Toxicology**, 33(1), 41–51. https://doi.org/10.1002/tox.22491

Nicolini, G., Roman, T., Flamini, R., Tonidandel, L., Gardiman, M., & Larcher, R. (2020). Thiol precursors in Vitis mould-tolerant hybrid varieties. **Journal of the Science of Food and Agriculture,** 100(7), 3262–3268. https://doi.org/10.1002/jsfa.10344

Olędzki, R., Lutosławski, K., Nowicka, P., Wojdyło, A., & Harasym, J. (2022). Non-Commercial Grapevines Hybrids Fruits as a Novel Food of High Antioxidant Activity. **Foods**, 11(15), 2216. https://doi.org/10.3390/foods11152216

Silva, T. S. J. d., Soares, A. A., Rocha, T. M., Pimenta, A. T. Á., Miron, D., Silva, R. J. F., Viana, G. S. B., & Leal, L. K. A. M. (Kalyne. (2020). Spondias mombin: Quality control and anti-inflammatory activity in human neutrophils. **Journal of Herbal Medicine**, 24(August). https://doi.org/10.1016/j.hermed.2020.100393

Qin, L., Xie, H., Xiang, N., Wang, M., Han, S., Pan, M., Guo, X., & Zhang, W. (2022). Dynamic Changes in Anthocyanin Accumulation and Cellular Antioxidant Activities in Two Varieties of Grape Berries during Fruit Maturation under Different Climates. **Molecules**, 27(2). https://doi.org/10.3390/molecules27020384

Rotili, M. C. C., Villa, F., da Silva, D. F., Rosanelli, S., Braga, G. C., & Ritter, G. (2022). Bioactive compounds, bromatological and mineral characterization of blackberries in a subtropical region. **Revista Ceres**, 69(1), 13–21. https://doi.org/10.1590/0034-737X202269010003

Samoticha, J., Wojdyło, A., & Golis, T. (2016). Phenolic composition, physicochemical properties and antioxidant activity of interspecific hybrids of grapes growing in Poland. Food Chemistry. https://doi.org/10.1016/j.foodchem.2016.07.147

Wei, Z., Liu, X., Huang, Y., Lu, J., & Zhang, Y. (2019). Volatile aroma compounds in wines from Chinese wild/hybrid species. **Journal of Food Biochemistry**, 43(10). https://doi.org/10.1111/jfbc.12684

Zhu, L., Li, X., Hu, X., Wu, X., Liu, Y., Yang, Y., Zang, Y., & Tang, H. (2021). Características de Qualidade e Perfis de Antocianina de Diferentes Cultivares de Uva Vitis amurensis e híbridos do germoplasma chinês. 26(21). https://doi.org/10.3390/mol.

APÊNDICES

APÊNDICE A – Artigo referente ao capítulo III, publicado

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Evaluation of the antioxidant capacity, volatile composition and phenolic content of hybrid *Vitis vinifera* L. varieties sweet sapphire and sweet surprise

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ABSTRACT

Bioactive compounds were extracted using two different extraction solvents (acetone and water) from pulp and whole grape berries derived from hybrid *Vitis vinifera* L. varieties Sweet sapphire (SP) and Sweet surprise (SU) and were characterized based on a comprehensive metabolomic approach by chromatography coupled with mass spectrometry (UPLC-QTOF-MSE and GC-FID/MS). GC-FID/MS analysis was performed with two different extraction methods (solvent extraction method and solid-phase extraction). Anthocyanins were characterized and quantified by HPLC-UV. The antioxidant potential was assessed by different assays. SP acetone-extract from grape skin had the highest mean to DPPH, FRAP, ORAC and phenolic content SP samples, also showed higher anthocyanin content. Globally, 87 phenolic compounds were identified. The relative quantification by UPLC-MS^E showed flavonoids the most abundant class. Forty two compounds were found in the volatile fraction of SU, while only thirty one volatile compounds were found in the SP samples.

1. Introduction

The grape is a fruit widely grown and eaten around the world, due to its beneficial effects on human health. Historically, the production and export of grapes were controlled almost exclusively by European countries in which the grape was traditionally grown. However, in recent years South America has seen a significant rate of growth in the production and export of grapes, and the region is able to produce two crops a year (Gutierrez-Gamboa, Liu, & Pszczolkowski, 2020). In Brazil, the practice of viticulture is recent when compared to the activities of traditional producers in Europe, but in the last decade there has been an improvement in the quality of Brazilian grape cultivar composition due to the use of hybridization techniques (Oliveri et al., 2019). In contrast to the almost exclusive growth of *V. vinifera* cultivars in traditional wine-producing countries, hybrid grape cultivars represent more than 80% of the volume of grapes (1,399,262 tons) processed in Brazil (Nicolini et al., 2020).

Hybrid grapes are obtained by crossing two or more species of *Vitis* and selecting characteristics of interest, such as high resistance to diseases and pathogens, being an advantage to increase productivity (Amaral, 2019). Originating in California (USA), two new hybrid varieties, Sweet sapphire (SP) and Sweet surprise (SU), are currently

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APÊNDICE B – Artigo, referente ao capítulo IV, Já adequado conforme revisão dos editores da revista e enviado para publicação.

Aguardando publicação.

Asian Pacific Journal of Cancer Prevention
Official publication of the Asian Pacific Organization for Cancer Prevention

Reference Number: APJCP-2303-8877 **Date:10/09/2023**

Dear Dr. Anderson Junger Teodoro,

The APJCP editorial board is glad to inform you that the manuscript titled **"Antiproliferative and Apoptosis Effects of Hybrid Varieties of Vitis vinifera L. Sweet Sapphire and Sweet Surprise on Human Prostate Cancer Cells Using In Vitro and In Silico Approaches"** has been accepted for publication in the Asian Pacific Journal of Cancer Prevention. The Manuscript will be published in our upcoming issue with the following authorship information:

Corresponding author: Anderson Junger Teodoro
First Author: Marta Angela de Almeida Sousa Cruz
Listed Co-Authors: *Marta Angela de Almeida Sousa Cruz, Gabriela de Freitas Laiber Pascoal, Maria Eduarda De Souza Jacintho, Maria Luisa Barambo Wagner, Pedro Paulo Saldanha Coimbra, Carlos Fernando de Araujo-Lima, Antonio Palumbo Junior, Anderson Junger Teodoro*

Our production team will soon send you the manuscript's galley proof for your final evaluation.

Thank you for your interest in publishing in APJCP.

SA Mosavi Jarrahi, MSPH, Ph.D.
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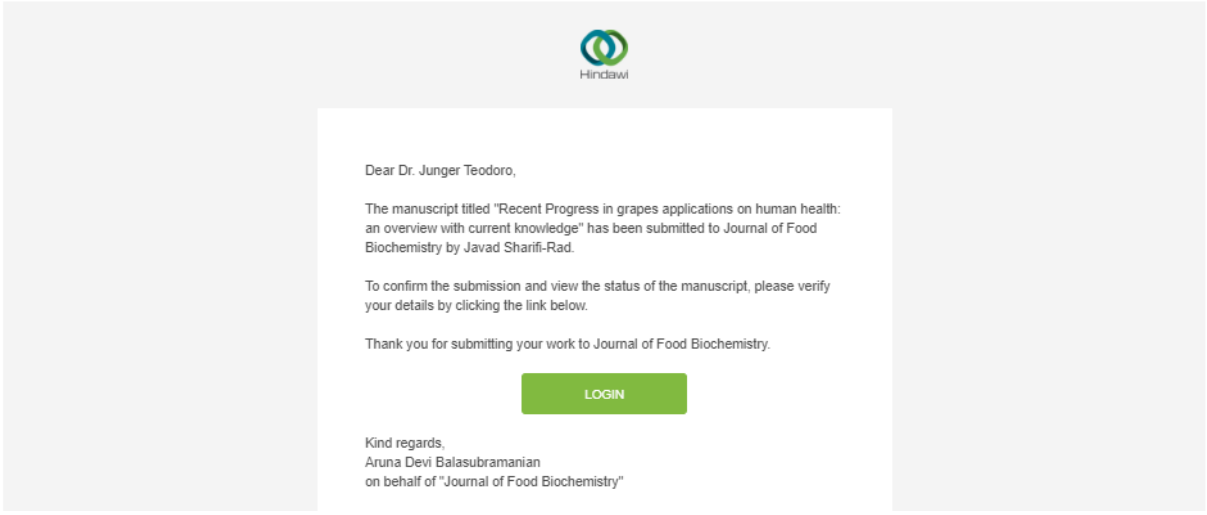
Asian Pacific Journal of Cancer Prevention

APÊNDICE C – Artigo, referente ao capítulo I, submetido para publicação.

---------- Forwarded message ---------
De: **Journal of Food Biochemistry** <jfbc@hindawi.com>
Date: sex., 8 de set. de 2023 às 10:56
Subject: Manuscript submitted to Journal of Food Biochemistry
To: Anderson Junger Teodoro <atteodoro@gmail.com>



Hindawi

Dear Dr. Junger Teodoro,

The manuscript titled "Recent Progress in grapes applications on human health: an overview with current knowledge" has been submitted to Journal of Food Biochemistry by Javad Sharifi-Rad.

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Thank you for submitting your work to Journal of Food Biochemistry.

LOGIN

Kind regards,
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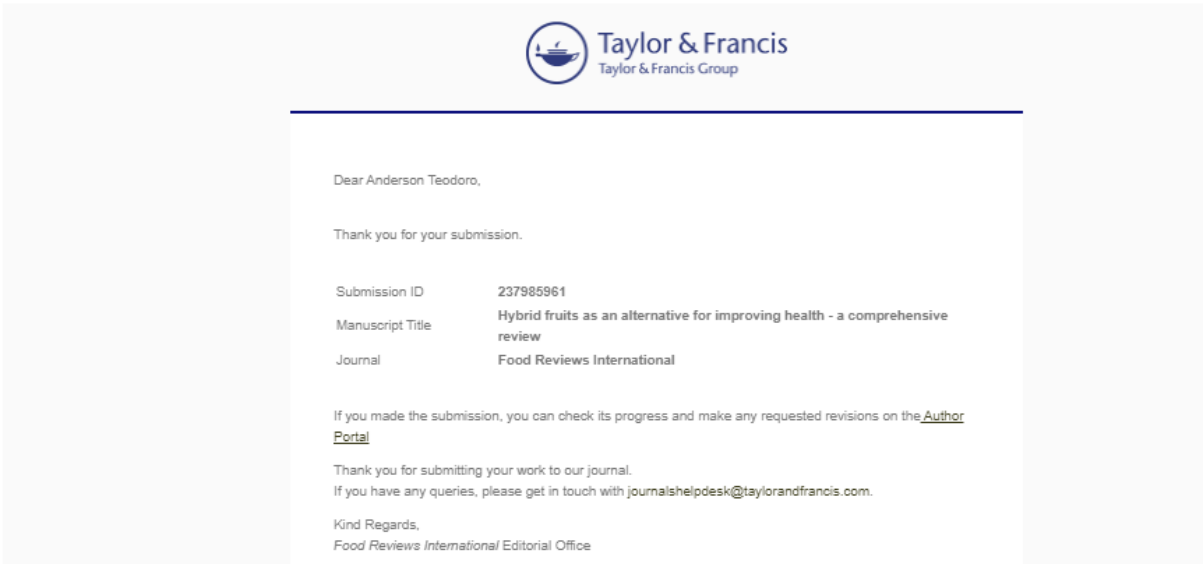
APÊNDICE D – Artigo, referente ao artigo II, submetido para publicação.

---------- Forwarded message ---------
De: <journalshelpdesk@taylorandfrancis.com>
Date: qua., 13 de set. de 2023 às 15:42
Subject: Submission received for Food Reviews International (Submission ID: 237985961)
To: <atteodoro@gmail.com>



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Dear Anderson Teodoro,

Thank you for your submission.

Submission ID	237985961
Manuscript Title	**Hybrid fruits as an alternative for improving health - a comprehensive review**
Journal	**Food Reviews International**

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Thank you for submitting your work to our journal.
If you have any queries, please get in touch with journalshelpdesk@taylorandfrancis.com.

Kind Regards,
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