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CHARACTERISTICS OF BIOMASS RESULTING FROM AGRO-INDUSTRIAL PROCESSES AND POSSIBILITIES OF ITS EVALUATION IN THE CONTEXT OF THE CIRCULAR BIOECONOMY

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Abstract. Waste management in the agro-industrial sector is a significant issue that demands a thoughtful and multifaceted approach, not only to prevent environmental contamination with harmful substances but also to produce value-added products. The selection of waste treatment technology should be based on the waste's nature, composition, and initial quantities, which are determined by the primary production cycle, raw materials, and applied conditions. This study focuses on exploring innovative methods to enhance the conversion rate and efficiency of organic waste biomass by incorporating small amounts of biologically active substances into the fermentation mix. The research also examines the impact of natural plant-based additives on various types of biomass within the agro-industrial sector. In agricultural areas where industries produce wine, spirits, beer, and juices, liquid waste is continuously produced in a state of ongoing digestion. This requires strict measures to prevent its direct disposal into landfills, water bodies, or other environmental compartments, as such actions could disrupt the natural balance of soil microorganisms, plants, and other organisms. Present-day methods for handling solid organic waste often include its application in agriculture, incineration, anaerobic digestion, composting, and related processes. Liquid waste from the agro-industrial sector can be treated through processes like sedimentation, settling, and anaerobic fermentation.

Keywords: waste, agro-industrial sector, management, value-added products, wine, spirits, beer.

Rezumat. Gestionarea deșeurilor din sectorul agroindustrial este o problemă importantă care necesită o abordare inteligentă și complexă, pentru a preveni poluarea mediului cu componente toxice, dar și pentru a obține produse cu valoare adăugată. Tehnologia de tratare a deșeurilor trebuie selectată în funcție de natura, compoziția și cantitățile inițiale ale deșeurilor, care depind de ciclul principal de producție, de materiile prime și de condițiile aplicate. Prezentul studiu se concentrează pe investigarea metodelor originale de creștere a ratei de conversie și a gradului de conversie a biomasei deșeurilor organice, utilizarea unor cantități mici de substanțe biologice active introduse în amestecul fermentat. S-a urmărit efectul unor aditivi de origine vegetală naturală, introduși în diferite tipuri de biomasă din sectorul agroindustrial. Concret, în regiunile agricole cu industrii producătoare de vin, băuturi

spirtoase, bere și sucuri, deșeurile lichide sunt generate în stare de digestie continuă, ceea ce înseamnă prevenirea strictă a deversărilor direct în gropile de gunoi, apă sau alte compartimente de mediu, deoarece pot încălca normele naturale. echilibrul microbiotei solului, al plantelor și al altor organisme vii. Metodele existente de gestionare a deșeurilor organice solide își asumă aplicarea în agricultură, ardere, digestie anaerobă, compostare etc. Deșeurile lichide din sectorul agroindustrial pot fi tratate prin sedimentare, decantare, fermentare anaerobă etc.

Cuvinte cheie: deșuri, sector agroindustrial, management, produse cu valoare adăugată, vin, băuturi spirtoase, bere.

1. Introduction

In recent years, the food industry has experienced unprecedented development, which is correlated with the rapid increase in the quantity of agricultural waste. Clearly, environmental issues and the negative impact of agricultural waste have become a major concern. Agricultural waste has considerable applicability due to its high resilience, low costs, availability, and ease of reuse. One of the primary environmental challenges facing today's society is the ongoing rise in the volume of organic waste. These alarming aspects have led to the necessity of designing sustainable development that suggests maintaining harmony and balance between humans and nature while promoting socio-economic progress. The circular bioeconomy for producing high-value products has attracted interest due to emerging policies focused on the reuse and sustainable recovery of underutilized local raw materials in various countries.

Beer, a fermented beverage with ancient origins, is currently the fifth most consumed drink worldwide. In 2018, global beer consumption across 170 major countries and regions was approximately 1.8879 billion hL [1]. According to reports [2], global beer production in the same year surpassed 1.94 billion hL, highlighting the significant economic impact of the beer manufacturing industry. Modern beer production is largely conducted on a large scale, yielding substantial amounts of beer and by-products.



Figure 1. Beer production process [3,4].

The larger red arrows indicate the steps where the main brewery by-products are removed.

The beer production process involves several sequential steps: grinding grains, mashing, filtering, boiling, fermenting, maturation, and packaging (Figure 1) [3,4]. The primary objective of this process is to convert starch from grains into simple sugars, extract these sugars, and ferment them using yeast to produce a lightly carbonated beverage with varying alcohol content. The first and most abundant by-product in the brewing process is generated after mashing. During this stage, spent grains are separated and removed once the liquid produced in mashing, known as wort, is extracted. Another type of waste is created after the wort boiling stage, where the thermal denaturation of proteins occurs, causing high molecular weight proteins to precipitate, forming a waste product known as hot trub. This hot trub, which contains spent hops, is separated and removed from the wort. Following this, yeast is added to initiate fermentation. Once fermentation is complete, most of the yeast is removed from the young beer, producing another by-product called spent beer yeast. Before the beer is packaged, it is typically filtered through diatomaceous earth or cellulose filters to eliminate any remaining yeast residues [3, 4].

2. Characteristics of biomass resulting from the brewing industry and reuse possibilities

Brewer's spent grain (BSG) is a low-value by-product of the brewing industry, generated in substantial quantities each year. BSG is the solid residue left from barley malt after wort production and represents around 85% of all residues produced by breweries [4].

This solid by-product contains water-insoluble proteins, along with the pericarp hull and seed coating from the original barley grain [5]. The dry matter of BSG consists of approximately 20% proteins and 70% fibers, with a negligible starch content. Due to its high protein content, BSG has potential applications similar to whey proteins, offering various health benefits to consumers. Additionally, BSG is rich in phenolic compounds, particularly ferulic acid and *p*-coumaric acid [6], as well as oligosaccharides and polysaccharides. Recent research suggests that dietary phenolic compounds may have anticancer, anti-inflammatory, and antioxidant properties [7,8], which has sparked significant interest in plant phenolics among the food industry, scientists and consumers.

BSG contains hydroxycinnamic acids such as ferulic acid, *p*-coumaric acid, and caffeic acid, all of which possess bioactive properties like antioxidant, anti-inflammatory, anti-atherogenic and anticancer effects [8]. Research shows that adding BSG to animal feed can boost milk production, increase milk fat content, and supply essential amino acids [9]. In human food applications, incorporating BSG into items like cakes and snacks has been shown to raise protein and fiber levels, although it can also significantly alter taste and texture [10]. BSG is increasingly recognized as a valuable source of fiber, protein and phenolic compounds like ferulic, *p*-coumaric, and caffeic acids [11].

As a complex material made up of lignocellulosic biomass, BSG is rich in proteins (20-30%), fibers (30-70%), lipids, vitamins and minerals and contains around 12-28% lignin, 12-25% cellulose and 28% non-cellulosic polysaccharides, primarily arabinoxylans [12,13]. Previous studies have thoroughly reviewed and documented the chemical composition of BSG [9]. On average, around 14 kg of BSG is produced per hectoliter of wort, with a moisture content ranging between 75% and 90% [14]. The ash content in spent brewer's grains typically ranges from 2% to 7.9% [9]. BSG also contains vitamins, minerals, a variety of amino acids, oligo- and polysaccharides and a rich array of phenolic compounds [15]. Among the phenolic acids, BSG has particularly high levels of ferulic acid (1860-1948 mg/g) and *p*-coumaric acid (565-794 mg/g) [16], as well as sinapic, caffeic and syringic acids.

According to Mussatto et al. [10], BSG can be classified as a lignocellulosic material, composed of cellulose (a linear homopolymer of glucose units), hemicellulose, and lignin (a polyphenolic macromolecule), which together make up nearly 50% of the BSG by weight, as shown in Table 1. On a dry weight basis, BSG contains a considerable amount of monosaccharides, including significant quantities of glucose, xylose and arabinose. Hemicellulose, primarily composed of arabinoxylan (AX), is the dominant component of BSG, constituting up to 40% of its dry weight [16].

Table 1

Composition of BSG						
Components of BSG (g/kg dry mass)						
Proteins	Ash	Lignin	Cellulose (glucose)	Hemicellulose Xylose	Arabinose	Research conducted
153	46	278	168	199	85	[10]
240	24	119	254	-	-	[17]
246	12	217	219	206	90	[18]
-	46	169	253	-	-	[19]
247	42	194	217	136	56	[20]

Phenolic profile of BSG. Phenolic acids, primarily hydroxybenzoic acids and hydroxycinnamic acids, are secondary metabolites in plants, predominantly found in vegetables. These compounds have gained considerable research attention due to their anticancer, anti-inflammatory and antioxidant properties [10].

BSG is considered a significant source of phenolic acids, as the outer layers of barley grains contain substantial amounts of these compounds [10]. Notably, *p*-coumaric and ferulic acids (Figure 2) [23] are present in high concentrations in BSG. So, *p*-coumaric acid is present in both forms, with a free concentration of 0.48 ± 0.06 and a much higher bound concentration of 652.27 ± 160.5 and ferulic acid shows a minimal free form concentration of 0.072 ± 0.51 but exhibits a significantly high bound concentration of 3739.42 ± 270.80 [21, 23]. Research indicates that the ferulic acid content in BSG ranges from 1860 to 1948 $\mu\text{g/g}$, while *p*-coumaric acid content varies between 565 and 794 $\mu\text{g/g}$ [21]. The total phenolic content in BSG can also vary depending on the type of malt used [20, 22]. Recent studies have revealed that the majority of bioactive phenolic acids in BSG are found in bound form [23]. A different study also found that ferulic acid and *p*-coumaric acid were detected in elevated concentrations compared to AX and BSG includes substantial quantities of other bioactive compounds such as catechin, quinic acid and syringic acid [23].

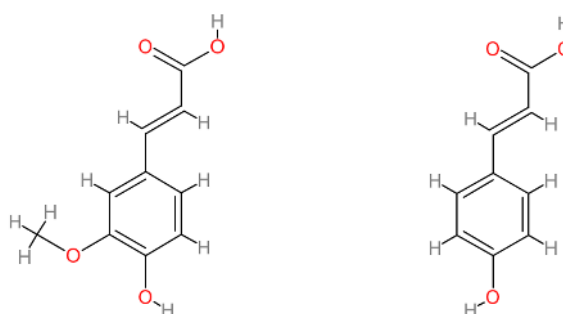


Figure 2. Structure of *p*-coumaric and ferulic acids ($\text{C}_{10}\text{H}_{10}\text{O}_4$).

Chen and Ho [24] demonstrated the antioxidant potential of ferulic acid using the DPPH and Rancimat methods. Their findings showed that although ferulic acid possesses antioxidant properties, it is less potent than caffeic acid and α -tocopherol [25]. Caffeic acid, in particular, has been identified as a powerful in vitro antioxidant and radical scavenger, effectively neutralizing DPPH and superoxide anions [25]. Furthermore, research using the DPPH assay has ranked several hydroxycinnamic acids by their antioxidant effectiveness in the following order: caffeic acid > sinapic acid = ferulic acid > ferulates > *p*-coumaric acid [26].

Both ferulic and caffeic acids exhibit strong antioxidant potential at low concentrations, with the ability to neutralize various free radicals. These phenolic acids scavenge reactive oxygen and nitrogen species, showing concentration-dependent activity against NO, superoxide, and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radicals. While caffeic acid was found to be more effective in scavenging DPPH radicals, ferulic acid performed better at scavenging ABTS and NO radicals [27].

In addition to their antioxidant properties, growing evidence suggests that phenolic acids may have anticancer effects. Caffeic acid, for instance, has shown antiproliferative activity against various cancer cell lines, including breast gland adenocarcinoma, lymphoblastic leukemia [28], and cervical cancer cells [28, 29]. The COX-2 (cyclooxygenase-2) assay has been used to assess the anticancer potential of these compounds. Overexpression of COX-2, which converts arachidonic acid to prostaglandins, serves a crucial role in inflammation and cancer development. Research has demonstrated that phenolic acids such as caffeic acid [30] and vanillic acid [31], along with polyphenols like epigallocatechin-3-gallate [32] and quercetin [33], inhibit COX-2 expression, potentially lowering cancer risk.

Incorporation of Brewer's Spent Grain (BSG) in Animal Feed and Food. As previously mentioned, BSG contains approximately 20% protein and 70% fiber, making it a valuable raw material or food ingredient [10]. It is especially beneficial in animal feed, particularly for ruminants. When paired with cost-effective nitrogen sources like urea, BSG can supply all essential amino acids required by ruminants [34]. This high nutritional value makes BSG a significant component in animal feed formulations.

Beyond its use in animal feed, BSG has been successfully incorporated into various human food products due to its low cost and rich nutritional profile. BSG is especially suitable for products like cookies and ready-to-eat snacks, where an increase in dietary fiber is desired [10]. In 1978, researchers explored the use of BSG in cookies by replacing flour with BSG at levels ranging from 5% to 60% [35]. It was found that adding 40% BSG significantly enhanced the cookies' physical qualities. This level of supplementation resulted in a 74% increase in nitrogen and a tenfold increase in crude fiber content. A study published in 2002 supported these findings, demonstrating that adding BSG (at 5-25%) to cookies significantly boosted the dietary fiber content [36].

However, it was concluded that 20% BSG was the optimal level for maintaining the sensory and structural properties of commercially available snacks. When BSG protein hydrolysates are incorporated into food, there may be concerns about the bitter taste of certain peptides, caused by their hydrophobic amino acid content [37].

Hot Trub. Another byproduct of beer production is hot trub, which refers to the sediments formed during wort boiling. The particle size of hot trub is between 30 and 80 μm [38, 39]. This insoluble precipitate is mainly made up of colloidal proteins that solidify during the boiling process, forming complexes with the polyphenols naturally found in the wort. Hot trub also includes complex carbohydrates, lipids, minerals, tannins, hop remnants, and

smaller malt particles [40]. These residues make up approximately 0.2-0.4% of the wort volume and are generally removed prior to fermentation. It's worth noting that hops, which contribute to the trub, are added and removed at various stages of brewing, with approximately 85% of hops used in beer production ending up as byproducts [41].

Hot trub contains a high level of moisture (80-90%), a dry matter content of about 15-20%, and a low ash content (2-5%) [41]. While its primary component is high molecular weight proteins, it also has a high carbon content due to the significant amount of reducing sugars (20%) present [39]. The protein content of hot trub can vary depending on the brewery but generally ranges from 40% to 70% [41, 42].

The formation of trub is an essential step in brewing, as removing polyphenols and soluble proteins is crucial to prevent the formation of insoluble complexes. These precipitates are undesirable in filtered pale beers, which are expected to be bright and clear [40].

Spent Brewer's Yeast (BSY). Residual brewer's yeast, also known as BSY, is the second largest byproduct in the beer manufacturing process, comprising up to 15% of total byproducts generated [42]. This yeast is recovered through sedimentation before beer maturation in the final stage of secondary fermentation [43]. Yeast can be reused up to six times in the brewing process. The *Saccharomyces cerevisiae* yeast, introduced at the start of fermentation, undergoes numerous divisions, resulting in a significant increase in yeast biomass. The growth rate of yeast depends on fermentation conditions in the brewery, with BSY contributing to beer losses ranging from 1.5% to 3% of the total beer volume produced [44]. On average, 0.6–0.8 lb/bbl (2.7 kg/m³) of yeast residue is generated from lager fermentation [41].

Yeast cells are rich in proteins (49%), carbohydrates (40%), minerals, vitamins (7%) and lipids (4%) [45]. BSY typically has a moisture content of 74%–86% and its dry matter content ranges from 10% to 16%, depending on the brewery [39]. The mineral residue (ash) content in spent yeast varies from 2% to 8.5%, with yeast richer in phosphate when it has been reused fewer times. Additionally, BSY is abundant in polyphenolic compounds and B vitamins, particularly B₁, B₂, B₃, B₆, and B₈ [46]. The carbon content in BSY is high, accounting for 45%–47% of the dry matter, and the carbon-to-nitrogen ratio of the residue is around 5.1–5.8 [39].

3. Characteristics of biomass resulting from the wine industry and reuse possibilities

Grapes are one of the most important fruit crops grown worldwide [47]. Grape production was estimated at approximately 77.8 million tons in 2018 [49]. According to FAO statistics, grapes are the most widespread fruit crop in the world [49].

Reports from the International Organization of Vine and Wine (OIV) show that 292 million L of wine were produced globally in 2018 [48]. The United States, Australia, Italy, Spain, France, and Germany are the main grape-producing countries [48]. Spain, China, Italy, Turkey, and France collectively contribute 50% of the total wine production worldwide [48].

In recent years, there has been a drastic shift in consumer demand. There is a growing preference for naturally processed products without additives and those that are safe [49]. Consumers prefer safer, tastier, and traditional products that are accepted as natural without other additives [49]. Therefore, replacing currently used synthetic food antioxidants (many of which are suspected of being carcinogenic) with natural ones is of interest to food technologists. Grape waste can be used to extract polyphenols for use in foods [50, 51]. Polyphenols not only exhibit antioxidant activity but also have other properties such as anticancer, antiallergic, antimutagenic, and anti-aging activities [52, 53].

Grape pomace, skins, stems, and seeds are among the major wastes generated by the wine industry. In addition, significant amounts of wastewater, wine yeast, shoots, and some filter residues generated by the wine industry are among the major causes of environmental degradation [54], as they lead to the emission of volatile organic compounds (VOCs), increased chemical oxygen demand infiltration of complex effluents with varied physicochemical properties into soils [55].

The toxicological effects of winemaking byproducts have been reported on terrestrial plants and aquatic organisms even at high dilutions, implying the need for proper treatment of wine industry waste [56]. Currently, wine industry waste is directed by producers either towards composting or disposal, but it could serve as a source for producing many bioproducts [57, 58, 59] (Figure 3).

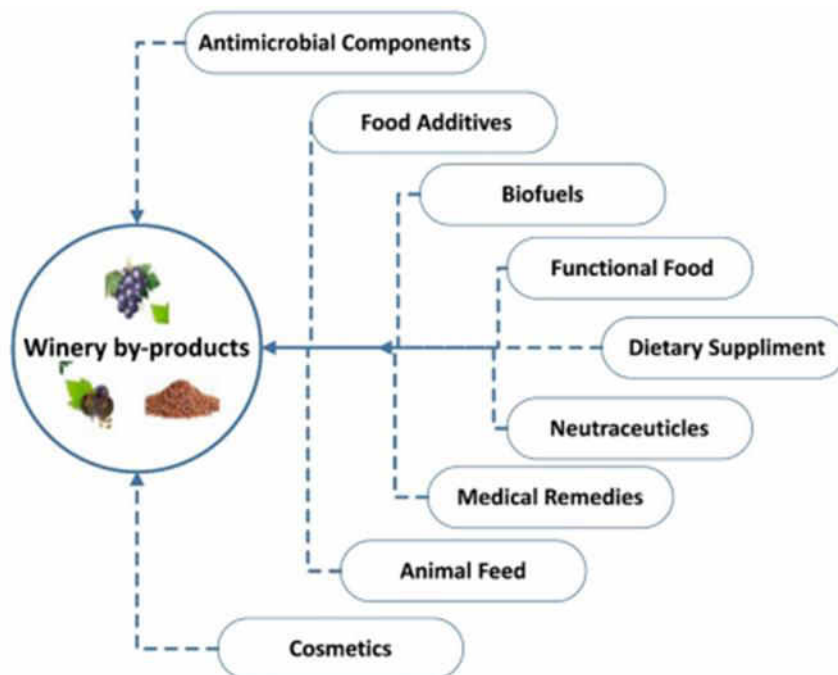


Figure 3. Valorization options for wine byproducts [57-59].

In addition to traditional uses limited to the fertilizer and biofuel industries, these byproducts could serve for obtaining food additives, including compounds with antimicrobial properties, preservatives, antioxidants for the production of functional foods, dietary supplements, nutraceuticals, medical remedies, and cosmetic products (Figure 3). The increasing need for energy and waste valorization through eco-friendly processes is pushing the transition from general practices to sustainable circular approaches [60].

Several recent studies have highlighted these wine byproducts as a good opportunity for recovering antioxidant compounds, which could subsequently be used as nutraceuticals and ingredients for functional foods [61-64]. Polyphenol-rich extracts obtained from grape skins and seeds [65-68] can also be sourced from grape pomace for polysaccharides and fibers [55,69,70]. Similarly, grape stems, obtained after destemming, emerge as an important source of phenolic compounds [71-74], particularly stilbenes [55,75,76].

Several *in vivo* and *in vitro* studies with phenolic compounds present in wine and grape pomace have demonstrated significant health-promoting effects, such as neuroprotection for preventing cognitive and mental disorders [77, 78], prevention of cardiovascular diseases [80, 81], reduction of insulin resistance [82], and antiproliferative action against cancer cells [83].

Functional ingredients are obtained through aqueous or alcoholic solvent extraction processes [65,66,71,82,84], and more recently, through supercritical CO₂ extraction [85, 86]. These processes present technical difficulties for industrial scaling, especially due to the perishability of these products and waste logistics.

Considering the issue of winemaking waste, the search for a viable solution for utilizing byproducts to obtain ingredients with enhanced biological value necessitated conducting a study on processing winemaking waste to preserve their functionality through dehydration processes, which will allow for their further valorization. Additionally, it is necessary to develop efficient extraction techniques to achieve good recoveries of the compounds.

Chemical composition of grape pomace. Grape pomace represents the dehydrated byproduct from the pressing of grapes (*Vitis vinifera* L.) during the wine and grape juice production process. It contains the pulp, skins, seeds, peduncles, and possibly fragments of stems (the woody support of the grape cluster) [77]. Grape pomace contains a high concentration of phenolic compounds, as not all of these substances are fully extracted during winemaking [88]. These phenolic compounds, which are secondary plant metabolites, are known for their potential health benefits, including antioxidant, antimicrobial, antiviral, and anti-inflammatory properties [76]. Due to these attributes, grape pomace offers an affordable source of valuable phytochemicals, which can be utilized across various sectors such as pharmaceuticals, cosmetics, and the food industry [88]. With increased attention to the sustainability of agricultural practices, efforts have been made to utilize extracts from grape pomace in various industry fields. It has been demonstrated that spent grape pomace, after the extraction of bioactive compounds, can undergo procedures for the extraction of condensed tannins, recommended for adhesive production [87]. The elemental composition of grape pomace is presented in Figure 4.

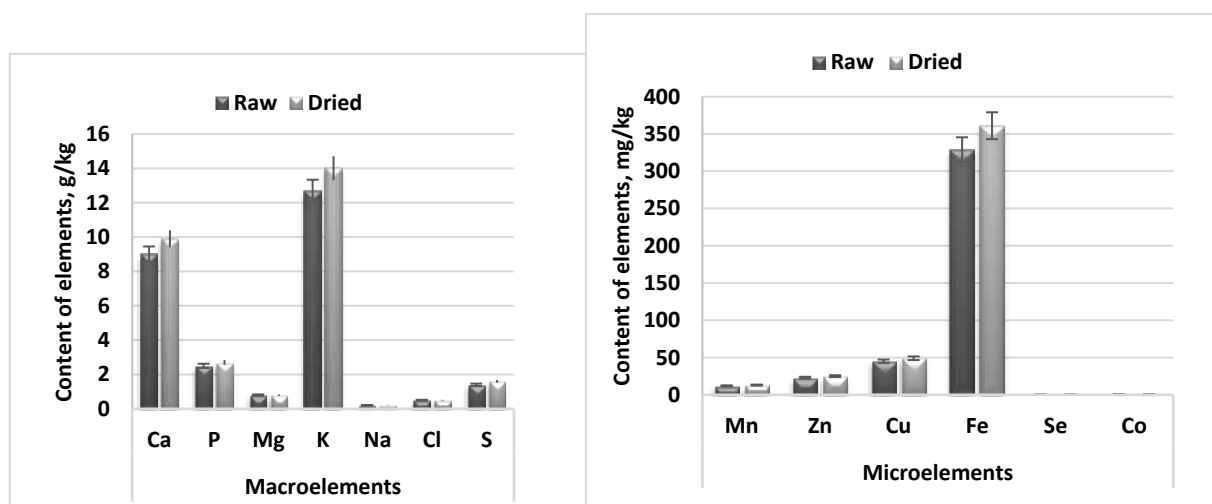


Figure 4. Elemental composition of grape pomace, macroelements and microelements.

*Composed by the author based on bibliographic sources [88,89].

As shown in Figure 4, grape pomace is an important source of iron, potassium, and manganese, as well as copper and zinc. It is low in elements such as sodium and calcium. Grape pomace can serve as a good source of essential minerals, as it does not accumulate elements that pose health risks.

The fatty acid composition of grape pomace is presented in Table 2 [90 - 94].

Table 2

Fatty Acid Composition of Grape Pomace*

Grape Pomace	Fatty acids, g/kg									Vitamine E, mg/kg
	Palmitic C16:0	Palmitoleic C16:1	Stearic C18:0	Oleic C18:1	Linoleic C18:2	Linolenic C18:3	Arachidic C20:0	Eicosenoic C20:1	Myristic C14:0	
Raw	2.9	0.06	1.8	6.6	29	0.2	0.07	0.07	0.02	4.4
Dried	3.2	0.07	1.9	7.2	31.8	0.2	0.08	0.08	0.03	4.9

*Composed by the author based on bibliographic sources [90-94]

According to the data presented in Table 2, grape pomace primarily contains linoleic acid (70.9%), oleic acid (16.1%), palmitic acid (7.1%), and stearic acid (4.3%) [92]. Erucic acid is absent. There is a significant amount of vitamin E (4.4 mg/kg in raw pomace, 4.9 mg/kg in dried pomace)[94]. It was found that the total fatty acid content in grape pomace constitutes 75% [90, 91].

The amino acid composition of grape pomace is presented in Table 3 [90-96].

Table 3

Amino acid composition of grape pomace

Grape Pomace	Lysine	Threonine	Cysteine	Isoleucine	Valine	Leucine	Phenylalanine	Tyrosine	Phenylalanine + Tyrosine	Arginine	Alanine	Aspartic Acid	Glutamic Acid	Glycine	Serine	Proline
Raw, g/kg	4.9	4.3	1.3	4.5	4.7	8.1	4.2	2.5	6.8	6.5	5.5	6.3	5.5	8	4.5	4.4
Dried, g/kg	5.4	4.7	1.4	4.9	5.2	8.9	4.6	2.8	7.4	7.1	6	6.9	21.4	8.7	4.9	4.8

*Composed by the author based on bibliographic sources [90-96]

Grape pomace has a significant content of glutamic acid (19.5 g/kg raw pomace, 21.4 g/kg dried pomace) and aspartic acid (6.3 g/kg and 6.9 g/kg, respectively) [92]. It also contains a notable amount of essential amino acids. However, most studies demonstrate that the nitrogen digestibility of grape pomace is moderate, varying from 16.3% in rabbits to 82.1% in fish [94]. In pigs, this indicator ranges from 47.7% to 68.1%, and for poultry, it is between 61% and 78% [91].

Polyphenol composition of grape pomace. Research suggests that the chemical makeup of grapes is shaped by both environmental conditions and grape varieties [55]. Various studies have explored the characteristics of carbohydrate polymers found in grape skins [95] and grape stems [96]. Grape pomace, known for its high polyphenol content, has been studied as a source of antioxidants. Since the "French paradox" was observed [97], numerous studies have emphasized the positive effects of grape or wine polyphenols on human health [98]. The general composition of several grape pomaces has also been

documented [95, 99]. Grape pomace contains components that inhibit the proliferation of Caco-2 and HT-29 cancer cells by inducing apoptosis, has potent free radical scavenging activity, and may provide protection against certain cancers [100].

Chemical characterization of grape pomace is necessary to evaluate their potential, determine extraction yields, and ensure quality control. Various phenolic compounds, representative of different structural types, have been identified. Profiles of phenolic compounds recovered from different winery wastes are dominated by gallic acid, catechin, and epicatechin. Additionally, hydroxytyrosol, tyrosol, cyanidin glycosides, and various phenolic acids such as caffeic, protocatechuic, syringic, vanillic, o-coumaric, and p-coumaric acids have been identified [101]. Different extraction systems quantitatively but not qualitatively alter the phenolic composition of grape pomace extracts.

Biological characterization involves antioxidant and antimicrobial tests for all extracts and seed oil, with the ability to inhibit α -glucosidase, α -amylase, α -tyrosinase, and ChE enzymes, along with anti-inflammatory activity and macrophage release stimulation [103].

The content of anthocyanins and flavan-3-ols in grape phenolic extracts varies depending on the grape variety and whether the extract comes from whole fruit or fermented pomace. However, all grape phenolic extracts significantly inhibit glucosyltransferases B and C (70-85% inhibition) at concentrations up to 62.5 $\mu\text{g/mL}$ ($P < 0.01$) [102]. Additionally, these extracts reduce the glycolytic pH drop caused by *Streptococcus mutans* without affecting bacterial viability, likely due to partial inhibition of F-ATPase activity (30-65% inhibition at 125 $\mu\text{g/mL}$; $P < 0.01$) [102]. Notably, fermented pomace extracts displayed similar or superior biological activity compared to whole fruit extracts [104]. These findings suggest that grape phenolic extracts, particularly from pomace, are highly effective against specific virulence traits of *S. mutans*, even with significant variations in their phenolic content.

Phenolic compounds are primarily synthesized from carbohydrates via the shikimic acid and acetate pathways. Shikimic acid leads to cinnamic acids and their derivatives through transamination and deamination. Acetates lead to polyketides or polyacetates (malonate). The structure of phenolic compounds ranges from a single aromatic nucleus with low molecular weight to complex tannins with very high molecular weight, depending on the nature of the carbon skeleton and the length of the aliphatic chain attached to the benzene nucleus [105]. Phenolic compounds are capable of conjugation with sugars or organic acids. Phenolic compounds can be divided into two major groups: flavonoids and non-flavonoids.

Flavonoids are a group of polyphenolic compounds consisting of 15 carbon atoms arranged in a C6-C3-C6 structure, where two aromatic rings are linked by a three-carbon bridge. They are the most abundant among all phenolic compounds. They are the most prevalent phenolic compounds and serve various functions in plants as secondary metabolites, including roles in UV protection, pigmentation, nitrogen fixation, and resistance to diseases. This C6-C3-C6 structure results from two synthetic pathways of phenolic compounds (Figure 5) [105]. The B ring and the three-carbon bridge form a phenylpropanoid unit, produced from phenylalanine through the shikimic acid pathway, while the A ring is derived from the condensation of three acetate units via the malonic acid pathway. The fusion of these two parts involves the condensation of a phenylpropanoid, 4-coumaroyl, with three malonyl-CoA, each contributing two carbon atoms. The reaction is catalyzed by chalcone synthase, thus generating tetrahydroxychalcone, which can subsequently generate all flavonoids [105].

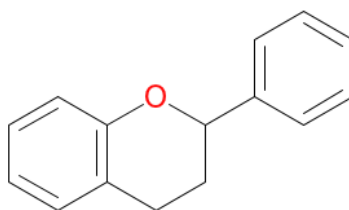
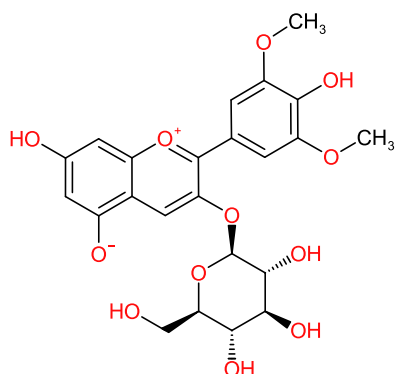


Figure 5. Flavonoid structure.

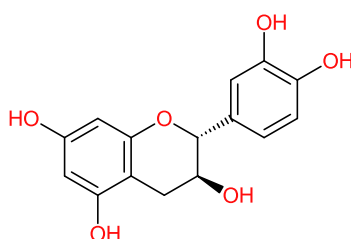
There are several groups of flavonoids, the main ones being flavones, flavonols, flavan-3-ols, isoflavones, flavanones, and anthocyanidins (Figure 6) [106]. Isoflavones are not present in grapes. The basic structure of flavonoids can undergo many substitutions, with hydroxyl groups typically at positions 4, 5, and 7. Most flavonoids exist as glycosides, varying greatly depending on the species and the nature of the sugars. Substitutions alter the solubility of flavonoids; hydroxylations and glycosylations generally make the compounds more hydrophilic, while other substituents, such as methylation, make them more lipophilic [106].

Flavonols are often highly widespread compounds. Flavonols such as myricetin, quercetin, and kaempferol are usually present as O-glycosides. The bond is most frequently at position 3 of the aromatic C ring, though substitutions at positions 5, 7, 4', 3', and 5' are possible [106]. The number of aglycones is limited, but there is a substantial number of derivatives. For example, kaempferol alone has over 200 conjugates with different osidic fragments. There is strong variability in flavonol concentration depending on the season and the grape variety considered. Their structure is planar. Four flavonols are predominantly present in grapes: kaempferol, quercetin (5-10 mg/kg), myricetin, and isorhamnetin. Quercetin derivatives are always predominant. The average maximum flavonol content in grapes is around 50 mg/kg but varies between 10 and 285 mg/kg [105].

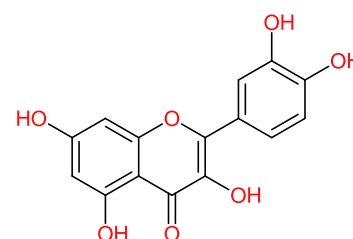
Flavanones are the first products of the flavonoid biosynthesis pathway. They are characterized by the absence of double bonding between C2 and C3 and by the presence of a chiral center at C2. Most naturally occurring flavanones have the B ring attached to the aromatic C ring. The structure of flavanones is highly reactive, leading to hydroxylation, O-methylation, and glycosylation reactions. Flavanones are present in grapes at concentrations of a few mg/kg [106].



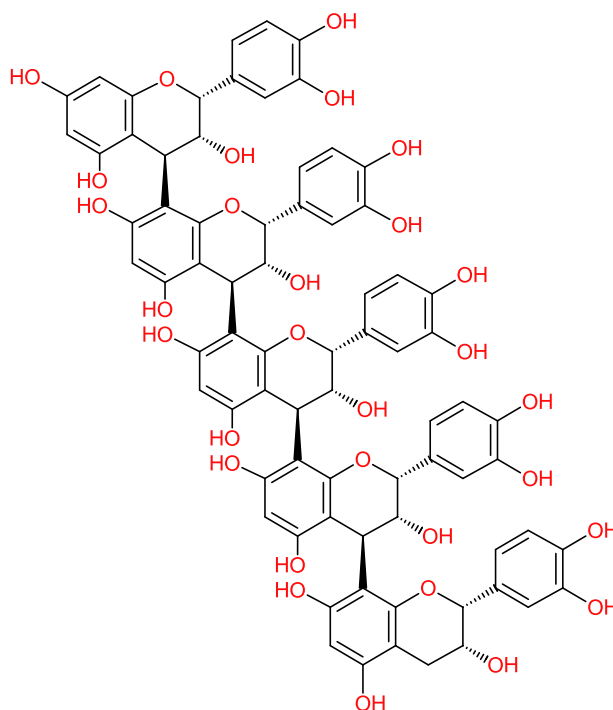
Anthocyanin glycoside
(Malvidin 3-O-beta-D-glucoside)



Flavan-3-ol
(+)-Catechin



Flavonol
(Quercetin)



Polymeric polyphenol (Condensed tannin, n = no. Of monomeric units)

Figure 6. Main classes of flavonoids.

Flavan-3-ols are the most structurally complex group of flavonoids. They include simple monomers like (+)-catechin and its isomer (-)-epicatechin, as well as larger oligomers and polymers known as proanthocyanidins [107]. Proanthocyanidins are formed from catechin and epicatechin with oxidative couplings between C4 positions of the heterocycle and C6 or C8 of the adjacent monomer. Procyanidin oligomers consist of 2 to 5 catechin or epicatechin units, while polymers contain 6 or more units. Additionally, flavan-3-ols can be esterified with gallic acid or, alternatively, hydroxylated to form gallo-catechins and gallotannins. Flavan-3-ols in grapes are primarily found as polymers. Seed tannins are composed of proanthocyanidins (polymers of catechin and epicatechin), partially galloylated, while those in grape skins also contain prodelfinidins (polymers of gallo-catechin and epigallocatechin) [108]. The average number of monomer units, defined as the average degree of polymerization (D_{Pm}), can reach up to 18 in a seed-derived fraction and approaches 30 units in a grape skin extract [108].

Flavones are structurally very similar to flavonols, with the difference being the absence of a hydroxyl group at C3. There are also many possible substitutions for flavones, such as hydroxylation, methylation, O- and C-alkylation, and glycosylation. Flavones are mainly present as glycosides. Grapes contain very small amounts of flavones [106, 107].

Anthocyanidins are widely present in the plant kingdom, primarily as glycosides, and are found exclusively in the skins of black grapes (absent in white grapes) [109]. They are responsible for red, blue, and purple colors depending on the pH of the environment [110]. These compounds are involved in protecting plants against excessive sunlight. The most common anthocyanidins are pelargonidin, cyanidin, delphinidin, peonidin, and malvidin, but these compounds are present only as glycosylated conjugates, anthocyanins. Anthocyanidins can form conjugates with hydroxycinnamic acids and organic acids (malic acid and acetic acid) [111]. Unlike other species (hybrids) with high levels of diglycosylated anthocyanins at C-3' and C-5', *Vitis vinifera* contains only traces of these compounds. This is due to the

predominant presence of 3-monoglucoside anthocyanidins, especially malvidin 3-O-glucoside and its acyl derivatives [112]. Anthocyanins are present in grapes with average contents ranging from 500 to 3,000 mg/kg, but can reach up to 5,000 mg/kg [110].

Non-flavonoids. The main non-flavonoids of nutritional importance are phenolic acids, hydroxycinnamic acids, and stilbenes (Figure 7) [113].

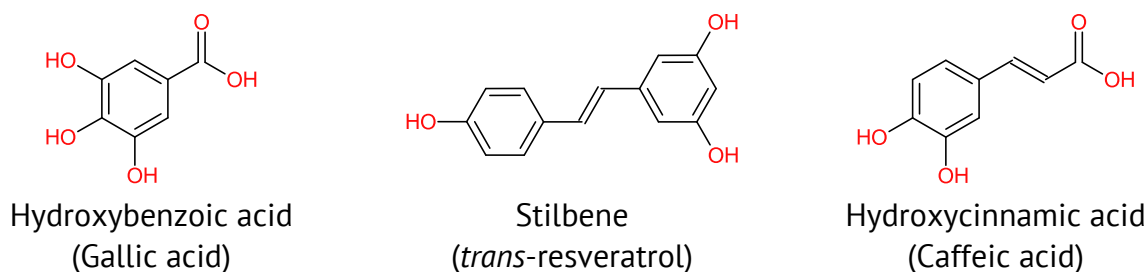


Figure 7. Structure of main non-flavonoids.

Phenolic acids. Hydroxybenzoic acids have a C6-C1 structure, consisting of a benzene ring attached to an aliphatic chain. These include vanillic acid, syringic acid, gentisic acid, and gallic acid [114]. The primary compound is gallic acid, which is found in grapes between 100 and 230 mg/kg [105, 109].

Hydroxycinnamic acids. Cinnamic acid is a C6-C3 compound formed through the deamination of phenylalanine, a process catalyzed by phenylalanine ammonia-lyase. This reaction produces p-coumaric acid through the hydroxylation of cinnamic acid. Both cinnamic acid and hydroxycinnamic acids are commonly referred to as phenylpropanoids. Their basic structure consists of a benzene ring attached to a 3-carbon aliphatic chain, often featuring one or more hydroxyl groups that may be esterified with an aliphatic alcohol [115]. The most common hydroxycinnamic acids include caffeic, p-coumaric, ferulic, and sinapic acids. These compounds are produced through a series of hydroxylation and methylation reactions. They often accumulate in plants as esters of tartaric acid, forming compounds such as coumaric (an ester of p-coumaric acid), caftaric (an ester of caffeic acid), and fertaric acid (an ester of ferulic acid). These esters play significant roles in plant metabolism and contribute to the antioxidant properties of various foods and beverages [115]. These constituents are primarily found in the pulp of grape berries. The major hydroxycinnamic acid in grapes is caftaric acid (caffeoyl tartaric ester), which can be found at levels of approximately 200 mg/kg [116].

Stilbenes are polyphenolic compounds with a C6-C2-C6 structure, two benzene rings linked by a methylene bridge. They are produced by plants in response to fungal, bacterial, or viral attacks, as demonstrated for trans-resveratrol [117]. Resveratrol is synthesized by condensing 4-coumaroyl with 3 malonyl CoA, each providing 2 carbon atoms. The reaction is catalyzed by stilbene synthase, with the products being the same as for flavonoid synthesis, the only difference being the enzyme that catalyzes the reaction. Resveratrol in its cis- and trans- forms is found in plant tissues, mainly as trans-resveratrol-3-O-glucoside [118]. Oligomeric forms of stilbenes, such as pallidol and viniferin, have also been identified in grapes, and more recently, a tetramer of resveratrol, hopeaphenol [119].

The content of catechins and proanthocyanidins (catechin oligomers) varies depending on the type of grape. In table grapes, the content of these compounds ranges from 243 to 1,108 mg/kg, with over 89% generally located in the seeds [120]. Table 4 presents the distribution of polyphenolic compounds according to the different parts of red grape berries.

Table 4

Distribution of polyphenolic compounds in grapes (mg/kg)

Compounds	Pulp	Skin	Seeds
Tannins	Traces	100-500	1000-6000
Anthocyanins	-	500-3000	-
Phenolic Acids	20-170	50-200	-

*Composed by the author based on bibliographic sources [120-123]

This distribution confirms that the grape pomace resulting from the winemaking process is an extremely concentrated source of bioactive compounds.

Tannins in grape pomace are primarily composed of proanthocyanidins or catechin tannins. These compounds are made up of monomeric or polymeric units of flavan-3-ols [61].

Proanthocyanidins, also known as condensed tannins, are synthesized as secondary polyphenolic metabolites through the flavonoid biosynthetic pathway. Discovered in 1947 by Jacques Masquelier, who developed and patented techniques for extracting oligomeric procyanidins from pine bark and grape seeds, proanthocyanidins continue to attract attention due to their biological and physiological properties [121]. They are composed of procyanidin and prodelfinidin units linked together by a C4-C8 bond (Figure 8) [122,123].

However, the high polyphenol content is a disadvantage for using pomace as animal feed and may pose potential pollution issues when used as soil fertilizer.

Antioxidant and microbiostatic properties of CBA from grape pomace. Numerous studies demonstrate the effectiveness of CBA extracted from grape pomace on health, particularly due to their antioxidant, anti-inflammatory, and microbiostatic effects related to the significant CBA content [124]. The antioxidant activity of grape pomace extracts was evaluated in obese male mice with diet-induced obesity (ODI) by measuring their oxygen radical absorption capacity (ORAC) [125]. Male ODI mice were randomly assigned to one of three treatment groups (n = 12): a normal diet group (DN), a high-fat diet group (Gr), and a high-fat diet group supplemented with grape pomace extracts (GrTS). After 12 weeks of treatment, the mice in the high-fat diet groups gained 29% more weight compared to those in the DN group. Supplementation with grape pomace extracts, estimated at 250 mg/kg/day (GrTS group), reduced plasma levels of C-reactive protein by 15.5% in mice fed a high-fat diet, suggesting a potential anti-inflammatory effect [125]. Grape pomace extracts (GPE) demonstrate anti-inflammatory effects in diet-induced obesity, largely due to their high content of polyphenolic compounds and anthocyanins, measured at 475.4 mg of gallic acid equivalent/g and 156.9 mg of cyanidin 3-glucoside equivalent/g, respectively [124, 125]. GPE also contains catechin (28.6 mg/g) and epicatechin (24.5 mg/g), as well as other antioxidants, including quercetin (1.6 mg/g), trans-resveratrol (60 µg/g), gallic acid (867.2 µg/g), coumaric acid (511.8 µg/g), p-hydroxybenzoic acid (408.3 µg/g), and protocatechuic acid (371.5 µg/g). The antioxidant activity, as measured by the ORAC assay, was 4133 µmol TE/g [125].

A recent study examined the effectiveness of antioxidant supplements in reducing oxidative stress [126], particularly focusing on the impact of grape beverages and extracts on oxidative stress markers in athletes. The study detailed the polyphenolic doses, participant demographics, and exercise protocols used [126].

Grape pomace extracts were also tested for their antibacterial properties against several strains, including *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus*

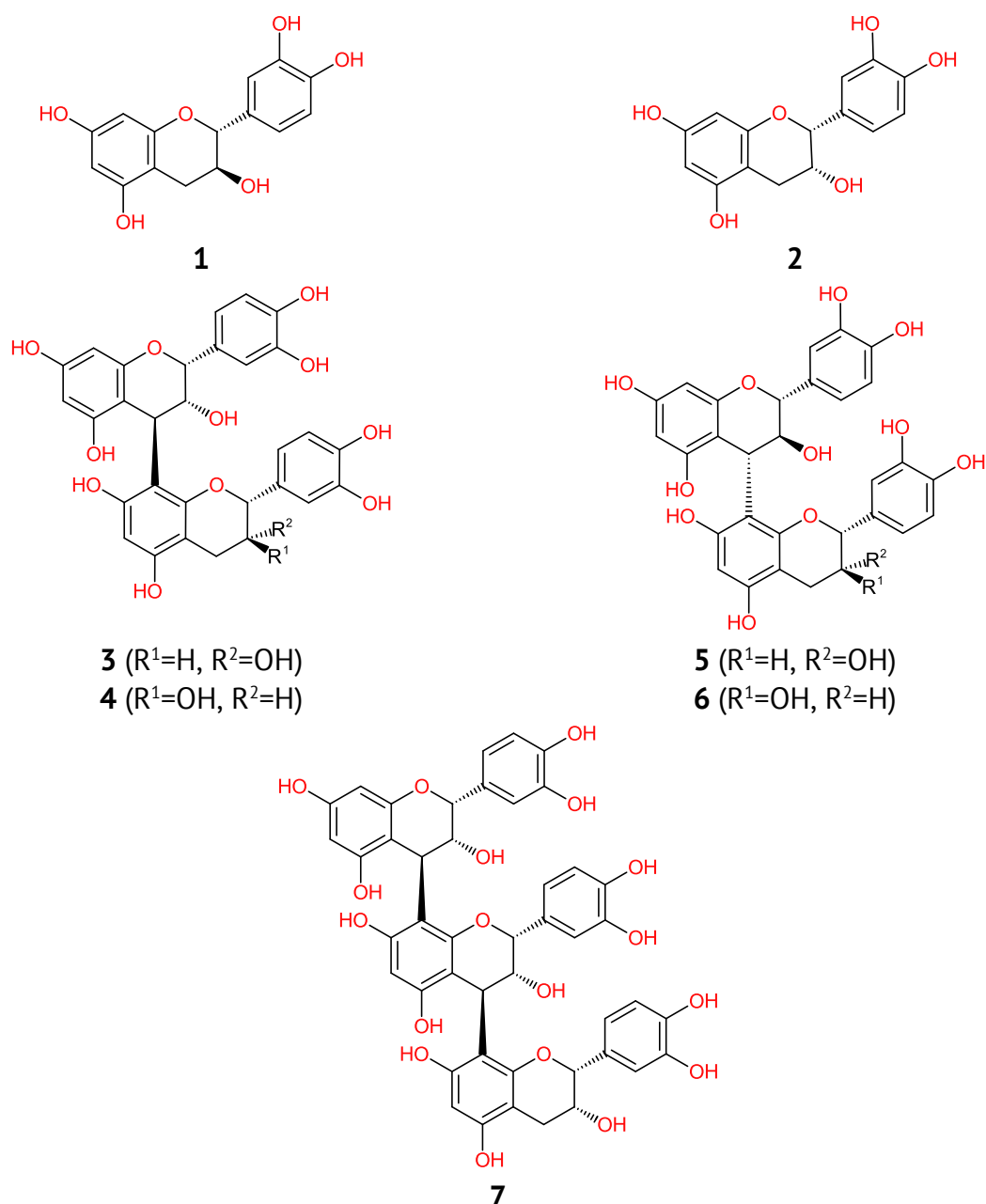


Figure 8. Structures of flavan-3-ols and proanthocyanidins – main constituents of grape seed extract: (+)-catechin (1), (-)-epicatechin (2), proanthocyanidin B1 (3), B2 (4), B3 (5), B4 (6), and C1 (7)

aureus, strains of *Escherichia coli* and *Pseudomonas aeruginosa* [53]. The results showed that Gram-positive bacteria were inhibited at concentrations of 850-1000 ppm, while Gram-negative bacteria required 1250-1500 ppm for inhibition [52].

Additionally, since a significant amount of polyphenols are not absorbed in the small intestine, their interaction with colonic microbiota was studied. The influence of polyphenolic extracts on the growth of *Lactobacillus acidophilus* CECT 903 was investigated in vitro through agar diffusion tests and liquid medium cultures. Grape phenolic extracts and some standard phenolic compounds (caffeic, gallic, tannic acids, catechin, epicatechin, and quercetin) were tested. None of the tested phenolic compounds exerted an inhibitory effect on *Lactobacillus acidophilus* growth at a maximum concentration of 5000 $\mu\text{g}/\text{disk}$ diffusion tests in agar. It was found that the phenolic extract from grape pomace (1 mg/mL) induced a significant increase in *Lactobacillus acidophilus* biomass in liquid culture media [127].

Phenolic compounds in grape pomace undoubtedly have therapeutic properties, particularly for certain chronic conditions such as atherosclerosis, diabetes, hypertension, and some types of cancer [128]. Among the mechanisms of action of phenolic compounds involved in the prevention of chronic pathologies are:

1. Grape pomace demonstrates significant antioxidant activity, as measured by ABTS• and DPPH• assays, as well as H₂O₂ scavenging tests. This high level of antioxidant activity is strongly associated with the presence of flavan-3-ols, phenolic acids, and ethyl gallate. Additionally, grape skin has been shown to exert cellular antioxidant effects on adenocarcinoma cells, with an EC₅₀ value of 56.4 mg total phenolic content (TPC)/mL, which is closely linked to the presence of flavonols and anthocyanins [129].

2. A saving effect on endogenous antioxidants (vitamin E, vitamin C, β-carotene, etc.) [130].

3. A saving effect on antioxidant enzymes (SOD - superoxide dismutase, catalase, SeGSHPx – glutathione peroxidase) [131].

4. A significant effect on reducing cholesterol and rebalancing blood lipids high density lipoproteins (HDL) and low – density lipoproteins (LDL) [132].

5. Chelation effect on oxidation cofactors, fatty acids, and certain metal ions (Fe²⁺, Cu²⁺) [133].

6. An inhibitory effect on oxidative enzymes such as cyclooxygenases and lipoxygenases [102].

7. Effect on NO synthesis in endothelial cells of the arterial wall, leading to vasorelaxation and membrane hyperpolarization through extracellular potassium release [134].

8. An inhibitory effect on the genesis of NADPH oxidase production in vascular wall cells (thoracic aorta and heart), resulting in a reduction in free radical production [135].

9. A significant effect is the presence of trans-resveratrol. Trans-resveratrol has been shown to have beneficial effects on diseases related to oxidative and/or inflammatory processes and extends lifespan. A study aimed at estimating the dietary intake of four stilbenes in the Spanish adult population allowed for the mediation of intake and their sources [136]. Among the four stilbenes studied, trans-piceid was the most abundant, accounting for 53.6% of the total, followed by trans-resveratrol at 20.9%, cis-piceid at 19.3%, and cis-resveratrol at 6.2%. The majority of the research and development on these compounds focused on wines (98.4%), with only 1.6% attributed to grapes and grape juices, while contributions from sources like nuts, pistachios, and berries were negligible, making up less than 0.01% [136].

As a natural food ingredient, resveratrol possesses significant antioxidant potential, antitumor activity, and is considered a potential candidate for the prevention and treatment of various types of cancer [137]. The anticancer properties of resveratrol have been confirmed in numerous *in vitro* and *in vivo* studies, demonstrating its ability to inhibit all stages of carcinogenesis, including initiation, promotion, and progression. In addition to its anticancer effects, resveratrol exhibits a wide range of bioactive properties, such as anti-inflammatory, cardioprotective, vasorelaxant, phytoestrogenic, and neuroprotective effects. Despite these promising benefits, the pharmaceutical application of resveratrol faces challenges due to its poor solubility, low bioavailability, and potential adverse effects. As a result, numerous studies have focused on estimating the resveratrol content in wines and grape pomace from various sources in an effort to improve its therapeutic potential [138-141]. The general conclusion is that this component accumulates depending on plant metabolism, agroclimatic conditions, and other difficult-to-predict factors.

4. Conclusions

Managing waste in the agro-industrial sector is critical for preventing environmental pollution while simultaneously capitalizing on opportunities to obtain value-added products. Waste from industries like wine, spirits, beer, and juice production can have harmful effects on ecosystems if not properly managed. For example, improper disposal of liquid waste can damage soil chemical composition, disrupt the balance of microorganisms, and negatively affect plants and other living organisms. Solid organic waste is often managed through agricultural application, anaerobic digestion, composting, and incineration—though incineration is costly and linked to air emissions. Liquid waste, on the other hand, is treated via methods like sedimentation, decantation in stabilization ponds, and anaerobic fermentation to reduce its environmental impact. The key challenge is that agro-industrial waste often contains toxic components harmful to plants and ecosystems, making direct soil disposal unsuitable. However, this waste can be repurposed as a renewable source of value-added products, offering potential for sustainable development within the agro-industrial sector.

Conflict of Interest: The author declares no conflict of interest.

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