

Review

Winery By-Products as Sustainable Sources of Proteins and Bioactive Peptides: Characterisation, Extraction and Potential Applications Under the EU Regulatory Framework

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Abstract

The global wine industry generates approximately 20 million tonnes of organic residues annually, representing a significant environmental and management challenge. While phenolic compounds from winery by-products have been extensively studied, protein and peptide fractions remain underutilised. This review provides a systematic overview of proteins derived from major winery side streams, including grapevine leaves, stems, pomace, seeds, and wine lees, with emphasis on their characterisation and recovery. Conventional and emerging extraction strategies are evaluated, with particular attention to green technologies such as ultrasound-assisted extraction (UAE), pulsed electric fields (PEF), and natural deep eutectic solvents (NADES) in the context of sustainable and resource-efficient processing. Enzymatic hydrolysis is discussed as a key approach for converting complex proteins into bioactive peptides with antioxidant, antimicrobial, and antihypertensive properties. Potential applications in agriculture, plant protection, animal nutrition, and food systems are considered, together with the implications of the EU circular economy regulatory framework. Overall, winery by-products are highlighted as promising nitrogen-rich secondary resources, and the review outlines valorisation pathways supporting nutrient recycling, waste reduction, and the development of a more sustainable agricultural bioeconomy.

Keywords: viticulture; winery by-products; protein extraction; bioactive peptides; regulatory framework; wine lees; applications



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1. Introduction

The winery industry represents one of the most significant agro-industrial sectors worldwide, ranking among the leading branches of global agriculture and exerting substantial economic, social, and cultural influence, particularly in traditional wine-producing regions [1,2]. According to the latest data from the International Organisation of Vine and Wine (OIV) [3], the global vineyard area reached approximately 7 million hectares in 2024. This surface supports an annual grape production of around 76 million tonnes, approximately half of which is directed toward winemaking, resulting in a global wine output of about 225 million hectolitres (Figure 1). As a direct consequence of this large-scale production, the wine sector generates an estimated 20 million tonnes of organic by-products

annually [4–6] along the entire production chain. This includes stages from vineyard management to fermentation, clarification, and ageing. These by-products—including grapevine leaves, stems, pomace, and wine lees—represent both an environmental challenge and a largely underutilised resource with considerable potential for integration into sustainable agricultural and circular economy strategies [5,7].

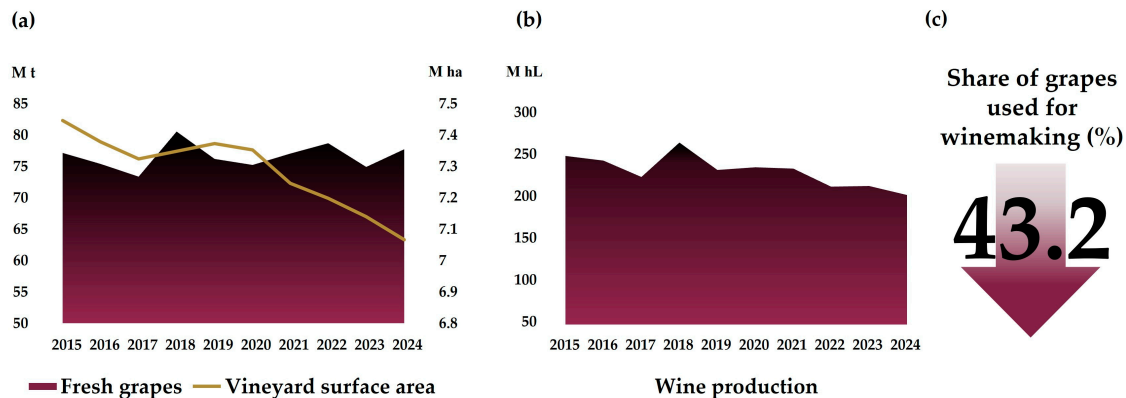


Figure 1. Global trends in (a) vineyard area and fresh grape production, (b) wine production, and (c) the proportion of grapes used for winemaking, 2015–2024 [3].

If not properly managed, these materials may impose a substantial environmental burden, contributing to soil degradation, water contamination, odour-related concerns, and greenhouse gas emissions [5]. In addition, the management of these by-products is further complicated by their seasonal generation during harvest periods, leading to temporary accumulation and increased pressure on storage and processing capacities. This is accompanied by significant logistical challenges and disposal costs, particularly in wine-producing regions where large volumes of biomass are concentrated within relatively short time frames [7]. From an agricultural standpoint, their accumulation may disrupt local nutrient cycles, particularly organic nitrogen and carbon flows within viticultural regions. In response, the European Union has established a comprehensive regulatory framework, primarily through the Waste Framework Directive (2008/98/EC), which prioritises waste prevention and the implementation of the waste hierarchy [8]. More recent initiatives, including the European Green Deal and the Circular Economy Action Plan, have further accelerated the transition toward sustainable valorisation pathways by promoting the conversion of waste streams into secondary resources that can be reintegrated into agricultural systems [9]. Valorisation approaches such as the production of natural additives, biofertilisers, soil amendments, and renewable energy have demonstrated potential to mitigate environmental impacts while generating additional economic value. Consequently, the adoption of resource-efficient technologies that optimise water use and facilitate side-stream recovery is increasingly recognised as a central component of sustainability in the wine sector and in broader agricultural systems [10,11]. Within this context, the regulatory status of winery by-products is particularly relevant for the recovery and utilisation of protein-rich fractions in food, feed, and fertiliser applications. While existing EU frameworks increasingly support their reclassification as secondary raw materials, thereby enabling valorisation, regulatory requirements related to safety, traceability, and intended use may also impose constraints, especially in applications involving protein-based ingredients. In this regard, understanding the composition and functional potential of winery by-products, particularly their protein fractions, becomes essential.

Winery by-products are characterised by a complex chemical composition rich in phenolic acids, tannins, anthocyanins, stilbenes, dietary fibres, minerals, lipids, carbohydrates, vitamins, and notably proteins and peptides, which together represent significant potential

for high-value utilisation and biotechnological applications [10,12–14]. Despite the extensive body of research on winery by-products, existing reviews have primarily focused on polyphenolic compounds or individual waste streams. However, an integrated perspective addressing protein and peptide fractions, extraction and processing strategies, and the EU regulatory framework remain limited. Therefore, this review aims to bridge these aspects by providing a comprehensive and systematic analysis within the context of sustainable agriculture and the circular bioeconomy. To date, research has predominantly focused on polyphenolic compounds, leaving protein and peptide fractions comparatively underexplored despite their recognised nutritional relevance and functional versatility [15]. In the context of the growing demand for sustainable alternative protein sources and increasing emphasis on nitrogen recovery within circular bioeconomy frameworks, plant-derived proteins from agro-industrial by-products have attracted increasing attention [16]. This trend is driven by the need for resource-efficient protein production systems and reduced environmental pressures associated with conventional protein sources. Winery by-products are particularly promising due to their abundance within existing agricultural systems and their largely underutilised protein potential. In contrast to many other agro-industrial by-products, winery by-products are generated within a well-established and centralised production system and often undergo fermentation processes that facilitate protein release and peptide formation, particularly in matrices such as wine lees.

Recent studies have begun to address this gap, as plant-derived agro-industrial side streams are increasingly recognised as promising sources of proteins and precursors of bioactive peptides [10,11]. Through targeted extraction, often employing emerging green technologies such as ultrasound-assisted extraction or pulsed electric fields, and subsequent enzymatic hydrolysis, structurally complex native proteins can be converted into peptides with enhanced solubility, bioavailability, and functional properties. The diverse protein profiles present in winery by-products, ranging from photosynthetic and metabolic proteins in leaves and lignin-associated proteins in stems to storage globulins in seeds and mannoproteins in wine lees, provide a broad spectrum of potential functionalities. These fractions have been reported to exhibit antioxidant, antimicrobial, antihypertensive, and immunomodulatory activities. In addition, their techno-functional properties, including emulsifying capacity, foaming behaviour, and colloidal stabilisation [12,17–20], support their potential use in feed, food, and oenological systems, as well as in agricultural applications related to nutrient recycling and bio-based input development. Despite these promising findings, a comprehensive and integrated understanding of these protein fractions remains limited.

Furthermore, recent studies indicate that a substantial proportion of grape-derived biomass remains within winery side streams generated during vinification, underscoring their recovery potential within circular viticulture models. Although growing interest has been directed toward individual matrices such as grape seeds, pomace, or wine lees, a comprehensive and systematic overview integrating protein fractions, processing strategies, and application potential remains limited.

Therefore, the aim of this review is to provide a critical and integrative synthesis of current knowledge on winery by-products as sustainable sources of proteins and bioactive peptides within the framework of sustainable agriculture and the circular bioeconomy. Particular emphasis is placed on the characterisation of major winery side streams, protein extraction strategies, green and emerging processing technologies, enzymatic hydrolysis approaches, and the resulting bioactivities and functional properties of derived peptides. Potential applications in agriculture, feed and food systems, and selected industrial sectors are also discussed. By integrating compositional, technological, and functional perspectives, this review seeks to highlight the underutilised protein potential of winery by-products and

to support the development of innovative valorisation pathways aligned with sustainable agricultural systems.

2. Winemaking By-Products: Characterisation and Chemical Composition

During wine production, various by-products are generated across multiple stages of the winemaking process. In addition to residues originating directly from vinification, this group also includes materials arising from vineyard management practices, such as leaves removed from the cluster zone, whose removal contributes to improved microclimatic conditions, light exposure, and aeration of grape clusters [21]. Figure 2 presents the approximate relative contribution of the principal vinification-derived by-products to total waste generated during winemaking, expressed on a mass basis, while grapevine leaves are not included, as their generation depends largely on vineyard canopy management practices and reliable global quantitative estimates are currently unavailable.

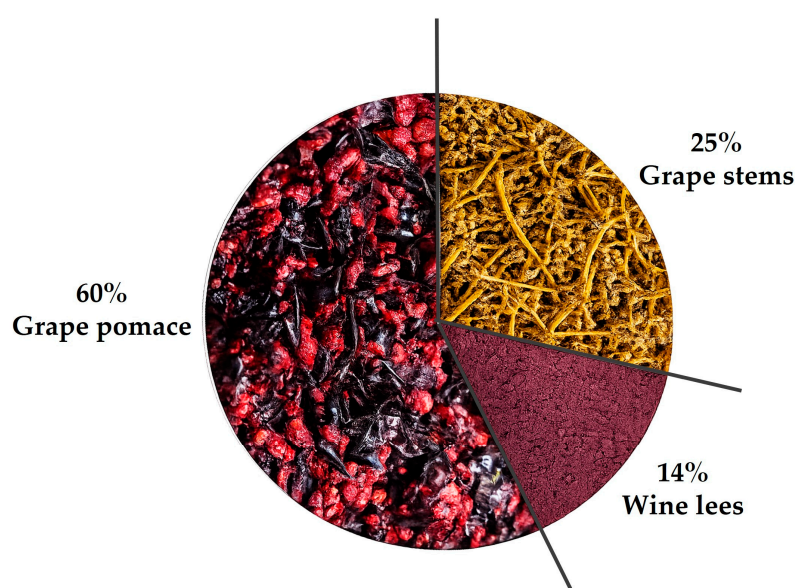


Figure 2. Approximate relative contribution of the principal vinification-derived by-products to total waste generated during winemaking, expressed on a mass basis.

The winemaking process itself comprises a series of clearly defined technological steps, and the type and quantity of by-products generated depend on whether red or white wine is produced. Regardless of technological differences, both production routes begin with destemming, whereby grape stems constitute the first significant by-product of winemaking [22]. Following crushing and fermentation, grape skins and seeds are separated as the principal solid fractions of pomace, with their technological treatment depending on the vinification type, specifically the presence or absence of fermentation in contact with the solid berry components [23]. After the completion of alcoholic fermentation and subsequent sedimentation, wine lees are formed, defined as the sediment accumulating at the bottom of fermentation or ageing vessels, representing an additional by-product of wine production [24].

Considered collectively, these side streams constitute a heterogeneous yet chemically rich raw material base with substantial potential for further valorisation. An overview of the major winery by-products, including their composition and estimated availability, is summarised in Table 1, after which each by-product is discussed in greater detail in the following sections.

Table 1. Major winery by-products: estimated quantities, chemical composition and key bioactive components.

Winery By-Product	Estimated Quantity/Share (%)	Chemical Composition (% Dry Matter)	Key Bioactive Components	References
Grapevine leaves	No precise global estimate available	Lipids: 15, proteins: 2.6, carbohydrates: 8, dietary fibres: 5, total sugars: 3, minerals: Ca, K, Mg	Polyphenols (quercetin, resveratrol, caffeic, gallic acid, kaempferol)	[25–28]
Grape stems	25	Ash: 6–9, proteins: 6, cellulose: 20–30, hemicellulose: 3–20, lignin: 17–26, lipids: 0.6, insoluble fibre: 55, minerals: P, K, Mg, Ca, Fe	Polyphenols (flavan-3-ols, proanthocyanidins, hydroxycinnamic acids, flavonols, stilbenes), amino acids	[29–32]
Grape pomace	60	Whole grape pomace—ash: 4–8, proteins: 10, carbohydrates: 25, cellulose: 10, hemicellulose: 6, lignin 30–40, dietary fibres: 45, minerals: Fe, Cu, K, Ca, Mn.	Polyphenols (flavonoids and non-flavonoids)	[33–35]
		Grape skin—ash: 2–8, lipids: 2–5, proteins: 5–12, soluble sugars: 1–70, dietary fibres: 60	Polyphenols (anthocyanins, tannins, flavonoids)	[33,34,36,37]
		Grape seed—lipids: 15, proteins: 11, carbohydrates: 60–70, dietary fibres: 48	Polyphenols (catechins, procyanidins, phenolic acids, stilbenes, flavonols)	[33,34,38]
Wine lees	14	Red—ash: 0.04, lipids: 4.71, proteins: 45.98, carbohydrates: 19.5, dietary fibres: 20, minerals: K, P, Ca	Polyphenols (tannins), organic acids, β -glucans, mannoproteins, peptides	[17,18,39–42]
		White—ash: 0.03, lipids: 8–11, proteins: 30–40, carbohydrates: 20–25, dietary fibres: 20, minerals: K, P, Ca		

2.1. Grapevine Leaves

Grapevine leaves represent one of the least studied and least valorised by-products associated with viticultural and winemaking systems, despite being generated in substantial quantities during the vegetative period and after harvest [25,26]. In addition to residues directly linked to wine production, considerable amounts of leaf biomass are removed during grape ripening as part of standard canopy management practices. Leaf removal in the cluster zone is used to improve vine microclimate by increasing light penetration and temperature in the fruiting area, reducing canopy density and relative humidity, and lowering the incidence of pests and diseases. Balancing the proportion of older and younger, more photosynthetically active leaves may also improve grape composition and wine quality, including higher concentrations of polyphenolic and aromatic compounds and lower total acidity [43–45].

Despite this substantial biomass generation, grapevine leaves are still most commonly reincorporated into soil as organic amendments or fertilisers, while their potential as sources of protein-rich and bioactive fractions remains largely underexplored [25]. Their quantitative estimation is also complicated by the changing role of traditional defoliation practices under climate change conditions, which contributes to the lack of reliable global data on overall generation [45].

From a compositional perspective, grapevine leaves contain dietary fibres, proteins, essential minerals, and a notable proportion of polyunsaturated fatty acids (Table 1) [27,28]. They also contain a diverse range of phenolic constituents, including quercetin, resveratrol, caffeic acid, kaempferol, and gallic acid, which contribute to the antioxidant, antimicrobial,

and stress-protective properties of grapevine leaves and support their potential applications in both plant physiology and downstream functional processes [25,26].

Overall, grapevine leaves represent a compositionally valuable but quantitatively variable biomass stream whose potential for protein-related valorisation and peptide precursor applications remains insufficiently explored.

2.2. Grape Stems

Grape stems, also referred to as grape stalks, represent a significant winery by-product generated during destemming and account for approximately one quarter of the total side streams produced during wine production. Because they may contribute excessive astringency and negatively affect the organoleptic properties of wine, stems are typically removed prior to vinification, particularly before maceration, as part of standard quality-oriented processing [29].

Chemically, grape stems are characterised by a predominance of lignocellulosic structural polymers, together with a significant mineral fraction and minor lipid content (Table 1) [29,36,46,47]. They are increasingly recognised as underutilised sources of dietary fibres and bioactive compounds, especially antioxidants. Phenolic compounds are particularly prominent, with reported levels reaching approximately 5.8% of dry weight and including flavan-3-ols, hydroxycinnamic acids, monomeric and oligomeric flavonols, and stilbenes. In this context, catechins, proanthocyanidins (including B1–B3 dimers), quercetin, and its glycosylated derivatives further support the potential of grape stems as a source of functional phenolic constituents with potential high-value applications [30,31]. Depending on grape variety, stems may also provide essential nutrients, including amino acids such as tryptophan and selected minerals involved in physiological and antioxidant processes [32].

At present, grape stems are still most commonly managed through landfilling, composting, soil amendment, or use as animal feed. Nevertheless, their chemical composition indicates considerable potential for broader valorisation, including bioenergy production and biological processing routes aimed at recovering protein-rich and functional fractions for feed, soil management, and other agricultural applications [29,30]. However, their direct use as precursors of bioactive peptides remains less explored than that of grape pomace, grape seeds, or wine lees.

2.3. Grape Pomace

Grape pomace is the most important solid by-product of vinification and is generated after grape pressing, when juice (must) is separated from the remaining solid residue composed mainly of skins and seeds in an approximate 1:1 (*w/w*) ratio, together with residual pulp and minor stem fragments. In white winemaking, pomace is removed from the must immediately after pressing, whereas in red winemaking it remains in contact with the must during part of fermentation to enable the extraction of pigments and other berry constituents, after which it is removed as a fermented residue [33].

Typically, 20–30% of the processed grape mass is converted into pomace, making it the most abundant solid winery by-product, while it may account for up to 60% of the total solid winery waste [33,34]. Traditionally, it has been directed toward distillation, land spreading, or use as animal feed, although landfill disposal is still practiced in some regions [33].

The chemical composition of grape pomace is strongly influenced by grape cultivar, harvest timing, and vinification technology. Pomace from red wine production represents a fermented residue with reduced residual sugar content and partially extracted phenolic compounds, whereas white grape pomace remains unfermented and may retain more

polyphenols together with higher levels of residual sugars. Overall, pomace is a lignocellulosic matrix rich in structural polysaccharides, proteins, lipids, minerals and phenolic compounds (Table 1) [33,48]. These compounds originate mainly from skins and seeds and can be broadly classified into flavonoids and non-flavonoid constituents associated with numerous biological activities and potential health benefits [34,35]. In addition, whole pomace differs substantially from its individual fractions, especially skins and seeds, which justifies their separate consideration. Pomace and other berry components may also represent valuable sources of essential mineral elements [49,50]. Beyond its phenolic richness, grape pomace is also relevant as a heterogeneous protein-containing matrix, largely influenced by storage proteins derived from seeds, and has attracted increasing attention as a precursor of bioactive peptides obtained through enzymatic hydrolysis [12,19].

Because of its abundance and richness in bioactive constituents, grape pomace has considerable application potential in agricultural and food-related systems, including feed production, soil amendment, oenological uses, and functional ingredient development. In these contexts, it may serve as a source of natural colourants, antioxidants, antimicrobial compounds, and functional materials, including innovative packaging systems [34]. Its reuse in wine fining and bioenergy production further supports the economic and environmental sustainability of winemaking, while growing consumer interest in functional foods continues to stimulate the development of novel pomace-derived products [35,36].

Overall, grape pomace is the most abundant solid winery by-product and a highly promising substrate for multi-component valorisation.

2.3.1. Grape Skin

Grape skin is one of the most valuable fractions of grape pomace in terms of bioactive compound content, with approximately 70% of total grape phenolic compounds localised in this structure [36]. In addition, grape skin contains high levels of dietary fibres and sugars, with fibres content generally higher in red grape varieties than in white ones. Red grape skins also tend to contain higher amounts of crude protein, lipids, and minerals, while anthocyanins and tannins occur in substantial concentrations and play an important role in wine colour formation as well as in nutritional and functional properties [33,34].

Structurally, grape skin represents a fibre-dominated lignocellulosic matrix enriched with polysaccharides, proteins, minerals, and phenolic constituents (Table 1) [33]. Owing to this complex composition, both red and white grape skins have attracted increasing interest as promising sources of biologically active compounds [34]. Their nutritional and functional relevance is additionally supported by elemental analyses identifying iron, zinc, copper, and nickel among the predominant trace elements [37].

2.3.2. Grape Seed

Grape seeds constitute a relatively small proportion of berry mass, averaging about 5%, but they contain an exceptionally high share of grape phenolic compounds, accounting for approximately 60–70% of total grape polyphenols [38].

Chemically, grape seeds are characterised by a significant oil fraction dominated by unsaturated fatty acids, primarily oleic and linoleic acids, together with proteins, non-digestible carbohydrates, and a diverse phenolic profile. Among these, monomeric catechins and procyanidins predominate, while phenolic acids, stilbenes, flavonols, and tannins occur in lower amounts depending on cultivar and species [33].

Although grape seed oil is already commercially valuable and widely available, the broader valorisation of grape seeds remains only partially exploited, partly because the efficient separation of seeds from other pomace fractions is technologically demanding. Nevertheless, interest in grape seeds has increased considerably due to their potential as

sources of natural antioxidants, functional ingredients for food and agri-food systems, and raw materials for protein fraction isolation and bio-based material development [33].

Following oil extraction, defatted grape seed residue remains a fibre-rich lignocellulosic matrix containing proteins, residual lipids, ash, and structural polysaccharides (Table 1) [33,34]. Its nutritional relevance is further supported by the mineral composition of this fraction [50].

2.4. Wine Lees

Wine lees are a heterogeneous by-product that accumulates in vessels after fermentation, storage, treatment, filtration, or centrifugation of wine, in accordance with Council Regulation (EC) No. 1493/1999 [51]. They form as a sedimentary mass following must fermentation and other technological steps in wine production and may also arise during fining, racking, and decantation. Estimates indicate that wine lees account for approximately 14% of the total waste generated during vinification, corresponding to more than 1.26 million tonnes produced globally each year. Due to their high organic matter content and chemical oxygen demand, they may pose a significant environmental burden if not properly managed [17,39].

The quality and composition of wine lees depend on numerous factors, including grape variety and quality, fermentation temperature, yeast strain and type, storage duration, and agroecological and regional production conditions. Structurally, wine lees comprise both liquid and solid phases. The liquid phase mainly contains ethanol, lactic acid, and acetic acid, whereas the solid fraction includes microorganisms, predominantly yeasts, together with cellulosic and hemicellulosic materials, proteins, organic acids, phenolic compounds, and minerals [39,40]. The specific composition of red and white wine lees is summarised in Table 1.

Among the most valuable constituents of wine lees are β -glucans, which possess notable physicochemical and functional properties, and mannoproteins, whose extraction is considered one of the key strategies for lees valorisation because these macromolecules are major structural components of the yeast cell wall [17,18,40]. During autolysis after fermentation, yeast cells gradually degrade, releasing mannoproteins and other protein components together with a broad spectrum of peptides, many of which display antioxidant activity [52]. This highlights the relevance of wine lees as a source of proteins and bioactive peptides, as they contain abundant yeast-derived protein fractions and directly released peptide precursors formed during autolysis. Phenolic compounds adsorbed onto yeast cell walls further contribute to the antioxidant capacity of lees, with red wine lees generally containing higher total phenolic levels than white wine lees. Among these, tannins are particularly relevant because of their contribution to antioxidant properties and their potential significance in agri-food and feed-related applications [40–42].

Despite this considerable chemical and functional potential, wine lees remain under-utilised and are still most commonly discarded or used in limited applications such as ethanol production or soil fertilisation. These practices mainly reduce disposal costs rather than enabling systematic valorisation as a source of high-value compounds [40].

The principal winery by-products differ markedly in origin, abundance, matrix structure, and overall chemical composition, all of which directly influence their valorisation potential and processing requirements. These matrices exhibit distinct compositional profiles involving structural polysaccharides, lipids, minerals, phenolic compounds, and protein-rich fractions. Although this diversity broadens their potential applications, it also poses a challenge for industrial utilisation, as variability related to cultivar, agronomic conditions, harvest stage, and vinification practices may affect raw material consistency and the reproducibility of downstream processing.

3. EU Regulatory Framework for the Management and Valorisation of Winery By-Products

Following the identification and characterisation of the principal by-product streams generated during winemaking, their regulatory status must be examined not only from a waste management perspective but also in relation to agricultural production systems and rural development policies. Within the European Union, the Waste Framework Directive (WFD) establishes the fundamental legal basis governing the classification and management of waste, including recovery operations, for organic residues arising from agri-food activities, including viticulture [8].

Directive 2008/98/EC introduces the waste hierarchy, prioritising prevention, reuse, and recycling over energy recovery and disposal. In viticultural regions characterised by high seasonal biomass generation, the practical implementation of this hierarchy directly influences nutrient cycling, soil management practices, and the overall sustainability of agricultural landscapes [8]. The regulatory distinction between “waste” and “by-product” status is particularly significant, as it determines whether winery side streams can be reintegrated into agricultural value chains.

Under the European List of Waste, grape pomace, seeds, and wine lees are typically classified as non-hazardous organic waste when discarded. However, Article 5 of Directive 2008/98/EC provides a legal pathway for their classification as by-products if specific criteria are fulfilled, notably when further use is certain, no additional processing beyond normal industrial practice is required, and the substance is produced as an integral part of a production process, with its further use being lawful and not leading to overall adverse environmental or human health impacts [8,53]. This provision is highly relevant in agricultural contexts: residues reused as animal feed, soil amendments, composting substrates, or raw materials for further agro-industrial processing may avoid waste status and instead be recognised as non-waste materials or by-products. Such reclassification reduces administrative constraints and facilitates integration into circular nutrient management strategies at farm and regional levels. It may also improve marketability by allowing these materials to circulate as secondary raw materials rather than as waste. In practical terms, this can reduce logistical and administrative burdens associated with transport, storage, and further processing, thereby improving the economic feasibility of higher-value valorisation routes for producers.

Historically, EU wine policy has emphasised distillation as a major valorisation route for winemaking by-products [10]. Regulations limiting excessive pressing and prohibiting re-fermentation of pomace or lees for beverage alcohol production aim to preserve quality standards and prevent illicit alcohol markets. In several major wine-producing Member States, wineries exceeding defined production thresholds may be required to deliver by-products to authorised distilleries. Financial compensation is provided through instruments linked to the Common Agricultural Policy (CAP), which supports by-product distillation as part of sectoral and market-stabilisation measures [54,55]. While this framework ensures traceability and controlled processing, it predominantly channels biomass toward industrial alcohol production rather than agricultural reintegration.

In recent years, CAP reforms and rural development measures have increasingly aligned with circular bioeconomy objectives. Beyond traditional market-support instruments, CAP policies now emphasise resource efficiency, environmental sustainability, and innovation within agricultural systems. These orientations create opportunities for winery by-products to be valorised through alternative agricultural pathways, including composting, soil improvement strategies, feed production, and decentralised bio-based processing initiatives. Such approaches may contribute to improved soil organic matter manage-

ment, reduced dependency on imported feed proteins, and strengthened resilience of rural economies.

The European Green Deal and the Circular Economy Action Plan (2020) have further reinforced these objectives by promoting waste prevention, material recovery, and nutrient recycling across food systems [9]. Amendments introduced through Directive (EU) 2018/851 mandate the separate collection of bio-waste and establish enhanced recycling targets [56], encouraging Member States to develop infrastructure supporting the valorisation of agri-food residues. For viticultural regions, this evolving policy environment favours the development of integrated regional models in which winery side streams are processed into compost, feed ingredients, bio-based materials, or extracted functional fractions, including proteins and peptides.

From a downstream valorisation perspective, the applicable regulatory pathway depends on the intended end use of the recovered fraction. Winery-derived proteins or peptides intended for human consumption may fall under Regulation (EU) 2015/2283 on novel foods, depending on their history of consumption and classification, while feed applications are primarily governed by Regulation (EC) No 1831/2003. In agricultural contexts, Regulation (EU) 2019/1009 is particularly relevant, as it establishes a harmonised framework for EU fertilising products, including plant biostimulants, and may therefore provide a route for protein hydrolysates or related extracts. In addition, if such fractions are placed on the market as chemical substances or mixtures rather than as food, feed, or fertilising products, Regulation (EC) No 1907/2006 (REACH) may also become relevant if they meet the criteria for substances or mixtures placed on the market under REACH [57–60].

Beyond the applicable legal pathway, practical implementation also depends on the ability to satisfy safety and authorisation requirements. Where novel food or regulated feed uses are involved, this may require pre-market assessment supported by data on identity, composition, production process, contaminants, toxicological profile, allergenicity, and intended conditions of use. For winery-derived proteins and peptides, these requirements may become particularly challenging at scale because raw material variability can affect standardisation, safety assessment, and the reproducibility of downstream processing [61,62]. A practical example may help illustrate these distinctions. A fraction obtained from grape pomace and intended for animal nutrition would fall under the EU feed framework, whereas a protein or peptide fraction obtained from wine lees and intended for human consumption could fall under the novel food framework. This shows that regulatory classification depends on both the source material and the intended use of the recovered fraction.

Overall, the current EU legislative landscape—combining waste regulation, CAP instruments, and circular economy initiatives—provides a structured yet increasingly flexible framework for the agricultural valorisation of winery by-products. By clarifying the boundary between waste and secondary raw materials and linking environmental objectives with rural development funding, EU policy supports the transition toward circular viticulture. However, although this framework increasingly encourages circular bioeconomy approaches, its practical implementation remains more strongly aligned with established valorisation routes, such as distillation or conventional biomass use, than with the market entry of novel protein- and peptide-based ingredients. Within this evolving framework, protein- and peptide-rich fractions derived from winery residues may be positioned as functional components of sustainable agricultural systems rather than merely as waste streams.

4. Protein and Peptide Fractions: Bioactivity and Functional Potential

Grapes are generally characterised by low protein content, and efficient protein extraction is further hindered by the high concentrations of sugars and polyphenols present in grape berries [63]. Nevertheless, grape processing, including crushing, pressing, and fermentation, induces substantial physicochemical changes affecting the release, redistribution, and availability of proteins in the resulting by-products [64].

Within the overall chemical composition of winery by-products, proteins and peptides are present in lower proportions and remain relatively underutilised compared to major constituents such as polysaccharides and phenolic compounds. However, despite their comparatively lower abundance, these fractions are of particular interest due to their distinct bioactive and functional properties, which may confer high added value. For this reason, proteins and peptides are therefore considered here as a distinct class of components. This section provides an overview of protein and peptide fractions in selected winery by-products—grapevine leaves, stems, seeds, pomace, and wine lees—with particular emphasis on their bioactivity, as summarised in Table 2. In addition, a schematic overview of the main bioactivities and their distribution across winery by-products is presented in Figure 3.

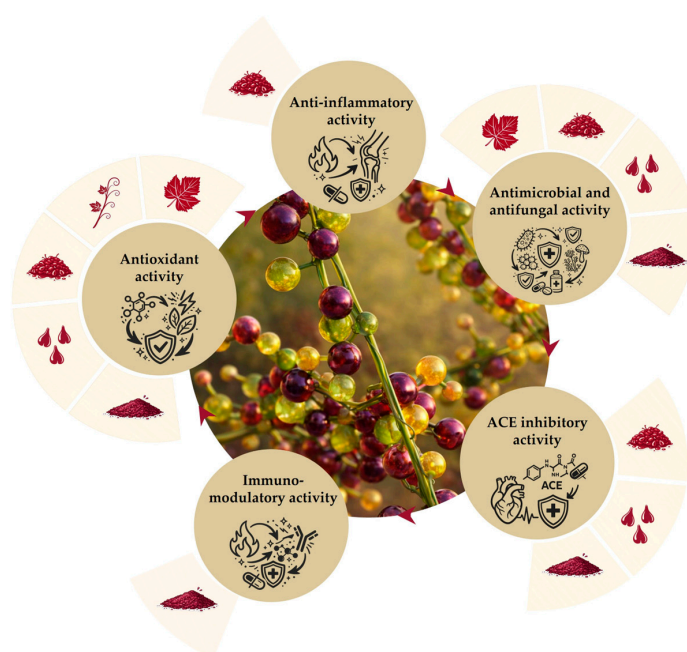


Figure 3. Schematic representation of the main bioactivities of protein and peptide fractions derived from winery by-products.

Table 2. Protein content, dominant protein/peptide fractions, and key bioactivities of selected winery by-products.

Winery By-Product	Protein Content (% Dry Matter)	Dominant Protein/Peptide Fractions	Key Bioactivities	References
Grapevine leaves	9–20	Photosynthetic and metabolic proteins; PR and PR-1 proteins; dehydrins; VvprePIP peptide	Antioxidant, cryoprotective, chaperone-like, antifungal	[27,65–70]
Grape stems	5–11	Structural and lignin-associated proteins; stress-related proteins; tryptophan-rich protein fractions	Antioxidant	[29,32,71–73]

Table 2. Cont.

Winery By-Product	Protein Content (% Dry Matter)	Dominant Protein/Peptide Fractions	Key Bioactivities	References
Grape pomace	6–15	Seed-derived 11S legumin-like and 7S vicilin-like globulins; bioactive peptides (GPCKFYGGK, FSSFYGGK, YYGKF); proline-rich antimicrobial peptides	Antioxidant, antihypertensive, antimicrobial, anti-inflammatory	[12,19,64,74–76]
Grape seeds	11–18.9	11S globulins and 7S globulins; enzymatically generated bioactive peptides	Antioxidant, antihypertensive, antimicrobial	[19,74,77–79]
Wine lees	29–65	Mannoproteins and glycoproteins; diverse low-molecular-weight peptides; hydrolytic enzymes	Antioxidant, antihypertensive, antimicrobial, immunomodulatory	[12,20,39,42,80–83]

4.1. Grapevine Leaves

Grapevine leaves are most commonly investigated as a source of phenolic compounds, particularly flavonoids, and other antioxidant metabolites [25,65,84], whereas their protein and peptide fractions remain considerably less explored. Proteins, together with polyphenols and polysaccharides, represent key structural and functional components of plant tissues, contributing to cellular integrity, regulation of physiological processes, and defence responses against pathogens [85].

The protein content of grapevine leaves shows substantial variability, typically ranging from 9 to 20% of dry matter depending on cultivar, phenological stage, and agroecological conditions [27,65,86]. Protein accumulation is strongly influenced by environmental factors such as water availability, light exposure, and nutrient supply [87]. Abiotic stresses, particularly drought, induce adaptive changes in the leaf proteome, including increased synthesis of proteins involved in photosynthesis, carbohydrate metabolism, and oxidative stress defence [66]. Proteomic analyses have identified hundreds of proteins with diverse biological functions, predominantly associated with photosynthesis and primary metabolism, including RuBisCO, glutamine synthetase, malate dehydrogenase, and photosystem II components [67]. In addition, stress conditions modify the abundance of specific proteins, enabling the identification of potential biomarkers related to abiotic stress tolerance [66,87,88].

Beyond proteins involved in primary metabolic pathways, grapevine leaves also contain specific protein and peptide components with pronounced bioactivity, largely associated with plant defence responses. Pathogenesis-related proteins (27–38 kDa) and PR-1-like proteins (gPR-1a, gPR-1b, gPR-1c) contribute to resistance against pathogens [68]. In addition, dehydrins VviDHN2 and VviDHN4 have been identified, with VviDHN4 exhibiting multifunctional properties, including antioxidant activity, cryoprotective effects, molecular chaperone function, and antifungal activity [69]. Furthermore, the PAMP-induced secreted peptide precursor VvprePIP enhances resistance to *Plasmopara viticola* through BAK1-dependent receptor recognition and requires hydroxyproline modification for bioactivity [70].

Taken together, the available evidence suggests that protein and peptide fractions of grapevine leaves possess notable functional potential, primarily related to antioxidant protection, macromolecule stabilisation, and pathogen defence. Dehydrins appear particularly promising due to their combined molecular “shield”-like properties, protective roles under dehydration and low-temperature stress, and antimicrobial-related activity, suggesting the

possibility of obtaining multifunctional bioactive fractions [69]. Likewise, PR/PR-1 proteins and PAMP-induced peptide precursors further support the relevance of grapevine leaves as a source of functional proteins and peptides within by-product valorisation strategies, especially in the context of sustainable and bio-based applications [68,70].

4.2. Grape Stems

Grape stems represent one of the less investigated winery by-products in the context of protein and peptide fractions. Due to their lignocellulosic nature, stems are rich in structural components (mainly lignin and cellulose), which results in limited protein solubility and complicates protein extraction [29]. The protein content of grape stems is most commonly reported within the range of 5–11% on a dry matter basis [29,71,89].

A considerable proportion of the stem protein fraction consists of so-called “recalcitrant proteins”, which are strongly associated with the lignin structure and may account for up to 90% of total protein. This contributes to the underestimation of actual protein levels and limits their recovery using conventional extraction approaches [71]. Therefore, grape stems are increasingly recognised as a promising matrix for future studies aimed at the isolation and characterisation of lignin-protein complexes, as well as for the optimisation of biotechnological strategies (e.g., fermentation-based processes) to enhance the accessibility of protein fractions. In addition, strategic processing approaches, including alkaline hydrolysis and washing pre-treatments, have been shown to improve protein digestibility while maintaining antioxidant capacity [46].

The amino acid profile of stem proteins is characterised by the dominance of glutamic and aspartic acids and shows a remarkable consistency across cultivars [72]. Among essential amino acids, tryptophan deserves particular attention, as grape stems exhibited the higher levels than grape pomace and wine lees in the same study [73]. Moreover, the presence of minerals such as selenium, along with other bioactive components (proanthocyanidins, phenolic acids, flavonols and anthocyanins), contributes to the overall bioactive profile of grape stems, particularly in terms of antioxidant activity [32].

4.3. Grape Pomace

White grape pomace and grape skin represent distinct yet complementary sources of proteins and peptides with notable valorisation potential within the circular economy of winemaking. Their composition is strongly influenced by grape variety and agroecological conditions [90]. In grape skin, proteins constitute only a minor fraction of dry matter (2.30%) and are largely associated with the cell-wall matrix [78,91]. Proteomic analyses of fresh white grape skin, such as Sauvignon Blanc, identified 75 different proteins, with a predominance of pathogenesis-related (PR) protein families, including thaumatin-like proteins (TLPs, 18 kDa) and chitinases (37 kDa), which exhibit antifungal activity and increased expression during ripening, confirming their role in plant defence mechanisms [63,92].

In contrast, white grape pomace represents a complex residue composed of skins, seeds, and stem fragments in which the protein fraction is primarily derived from seed storage proteins, notably 11S legumin-like and 7S vicilin-like globulins. Although not inherently bioactive, these proteins can generate functionally relevant peptides after enzymatic hydrolysis using proteases such as Flavourzyme or Alcalase. Resulting hydrolysates have demonstrated antioxidant, angiotensin-converting enzyme (ACE) inhibitory, and antimicrobial activities, which are commonly associated with peptide size, hydrophobic amino acid residues, and specific sequence motifs [12,19,74].

Red grape skin is characterised by the predominance of structural and defence-related proteins, including extensins, proline-rich proteins, chitinases, and β -1,3-glucanases, whose abundance increases during ripening as part of the natural protective response of the

berry [63,90,93]. Nevertheless, studies specifically addressing the bioactivity of isolated skin proteins remain limited, as most application-oriented research focuses on whole pomace or seed fractions. Where evaluated, grape skin-enriched formulations have shown anti-inflammatory effects and favourable techno-functional properties, although many observed health-related benefits are primarily attributed to the polyphenolic fraction rather than proteins alone [94,95]. In addition, potential synergistic effects between peptides and co-extracted polyphenols cannot be excluded, which complicates the attribution of bioactivity to individual components. Therefore, further studies using purified protein and peptide fractions are needed to better elucidate their specific functional roles.

Red grape pomace, consisting of seeds, skins, and residual stems after vinification, represents a heterogeneous protein source with contents typically ranging from 6 to 15% of dry matter and an amino acid profile rich in glutamic and aspartic acids [19,64]. Enzymatically generated peptide fractions, particularly those enriched in proline residues, further support the antimicrobial and functional food potential of this matrix. In addition, pomace has been explored as a substrate for single-cell protein production and as a source of extracts with anti-inflammatory and cosmetically relevant biological activities, underscoring its broader applicability within sustainable by-product valorisation strategies [75,76,96].

4.4. Grape Seeds

Although grape skins and seeds together constitute structural components of grape pomace and are often considered jointly in compositional analyses, grape seeds are increasingly recognised as a distinct and particularly relevant matrix in studies focused on proteins and bioactive peptides.

Grape seeds represent the nutritionally most valuable fraction of pomace and contain approximately 11% protein on a dry matter basis, while in certain cultivars this content may reach up to 18.9% [77,78]. Regarding protein composition, storage proteins of the 11S globulin type predominate, accounting for up to 58.4% of the salt-soluble fraction, whereas 7S globulins represent secondary components [79]. These protein fractions are characterised by a molecular weight of approximately 43 kDa in monomeric form, with subunits ranging from 25.5 to 40.0 kDa, as well as a distinctive amino acid profile rich in glutamic and aspartic acids, glycine, and arginine, which together constitute about 59% of total amino acids [79,97,98]. Structural analyses indicate that grape seed 11S globulins possess defined secondary structures that enable interactions with wine polyphenols, contributing to anthocyanin stabilisation in wine systems [99].

Due to the high content of polyphenols and dietary fibres, protein isolation from grape seeds requires specific approaches aimed at the efficient removal of interfering components. For this reason, grape seeds are frequently used as a model matrix for optimising protein extraction and developing functional protein ingredients. Enzymatic hydrolysis using proteases such as Alcalase, Flavourzyme, and Novo-ProD leads to the formation of bioactive peptides with degrees of hydrolysis ranging from 22 to 52% [19,100]. Grape seed protein hydrolysates exhibit improved techno-functional properties compared with native proteins, including enhanced solubility and emulsifying capacity, high thermal stability (denaturation temperatures above 104 °C), and pronounced bioactivities—strong antioxidant capacity, ACE-inhibitory effects, and broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria [19,74]. These characteristics position grape seed proteins as valuable ingredients for wine fining and as effective allergen-free alternatives to conventional fining agents [101]. Their clarification efficiency has been reported to be comparable to that of potato protein, while alternative plant proteins, including grape seed proteins, may further modulate the phenolic composition and colour attributes of red wines [102,103].

4.5. Wine Lees

During yeast autolysis, proteins and peptides of different molecular weights are progressively released, making wine lees a significant source of biomolecules with potential nutritional and functional value [42]. Proteins constitute one of the dominant components of yeast biomass (29–65% of dry matter) and include all essential amino acids, while autolytic processes promote the release of free amino acids and peptides that may account for up to 40% of the total protein fraction. Consequently, wine lees are considered a potentially valuable source of essential amino acids, including lysine [39].

The total protein content in white wine lees averages approximately 205 mg/g dry matter, with a wide range of reported values (99–364 mg/g), indicating substantial variability depending on fermentation and vinification conditions [42]. However, the nutritional utilization of this protein fraction may be limited by the presence of protein–polyphenol complexes that can reduce protein digestibility [104]. The peptide profile of wine lees also undergoes pronounced dynamic changes during ageing: more than 2600 peptides have been identified immediately after fermentation, with their number increasing during the first 60 days and subsequently declining, reflecting continuous degradation and transformation of protein components, including mannoproteins, throughout wine storage [80].

From a bioactivity perspective, antihypertensive activity mediated through ACE-inhibition represents the most consistently characterised function of wine lees-derived peptides. Specific peptide sequences, such as GPCKFYGGK, FSSFYGGK, and YYGKF, have been identified as key contributors to ACE-inhibitory activity [12]. Yeast strain selection further influences the profile and abundance of bioactive peptides; notably, the non-*Saccharomyces* strain *Starmerella bacillaris* FRI751 generates substantially higher predicted antihypertensive potential than *Saccharomyces cerevisiae* EC1118 [20]. Computational analyses additionally suggest antimicrobial, immunomodulatory, and anti-obesity activities, although experimental validation of these effects remains limited [20]. Peptide bioactivity is also governed by molecular characteristics, particularly hydrophobicity and the frequency of short ACE-inhibitory motifs, with fractions below 3 kDa consistently showing the strongest ACE-inhibitory effects [12,81]. Collectively, these findings support the valorisation of wine lees as a source of bioactive peptides for functional food and nutraceutical applications, particularly in the development of products targeting cardiovascular health. In addition, their antimicrobial and immunomodulatory potential suggests possible applications as natural preservatives in food systems or as functional additives in feed formulations.

Compared with white lees, red wine lees represent a more complex matrix but likewise constitute an important source of bioactive proteins and peptides. Identified constituents include high-molecular-weight mannoproteins and glycoproteins (approximately 20 to >250 kDa) that exhibit notable stability in wine matrices [82,83,105]. In addition to peptide-related effects, enzymatic activities such as catalase and superoxide dismutase have been detected in lees extracts, together with enzymes involved in cell-wall degradation, including proteinases, pectinases, and glucanases [83]. Wine lees also demonstrate measurable antioxidant capacity, for example through inhibition of DPPH radicals, further confirming their broad functional profile as a matrix rich in bioactive components [83].

A comparative evaluation of winery by-products reveals distinct differences in protein content, extractability, and bioactive potential. Among the studied matrices, grape seeds represent the most promising source for protein recovery due to their relatively high protein content and well-characterised storage proteins, which readily generate bioactive peptides upon enzymatic hydrolysis. Grape pomace, although more heterogeneous, provides a versatile substrate for peptide production, particularly for antioxidant and ACE-inhibitory applications. Wine lees are distinguished by their richness in readily available peptides and

free amino acids, with strong evidence for ACE-inhibitory activity. In contrast, grapevine leaves contain moderate to high protein levels but remain underexplored in terms of peptide generation and downstream applications, while grape stems present challenges due to lignin-associated recalcitrant proteins that limit extraction efficiency.

Variability in reported protein content and bioactivity across studies can be attributed to differences in grape cultivar, environmental conditions, and processing methods, as well as to variations in extraction techniques and analytical approaches, which complicate direct comparisons. It should also be noted that most of the reported bioactivities are based on *in vitro* or *in silico* studies, and their translation to *in vivo* systems and industrial applications remains limited and insufficiently validated. Factors such as gastrointestinal digestion, peptide stability, and bioavailability, as well as potential safety aspects (e.g., allergenicity), may significantly influence their functional relevance under real conditions. Therefore, caution is required when interpreting application potential, and further *in vivo* validation and application-oriented studies are needed to confirm efficacy and safety.

5. Protein Extraction and Enzymatic Hydrolysis

The yield, composition, and techno-functional properties of protein and peptide extracts obtained from winery by-products are strongly influenced by the extraction method applied, as well as by the initial quality of the protein fraction, which depends on the technological treatment of the raw material. Given the variability in protein content, extractability, and bioactive potential among different winery by-products, the selection of appropriate extraction and processing strategies is of critical importance. Additionally, the functionality of the resulting products is shaped by complex interactions between proteins and other matrix components, including polysaccharides and polyphenols [106]. The following sections provide an overview of conventional, green, and advanced extraction techniques, together with enzymatic hydrolysis approaches, highlighting their applicability to different winery by-products, as illustrated in Figure 4 and summarised in Table 3.

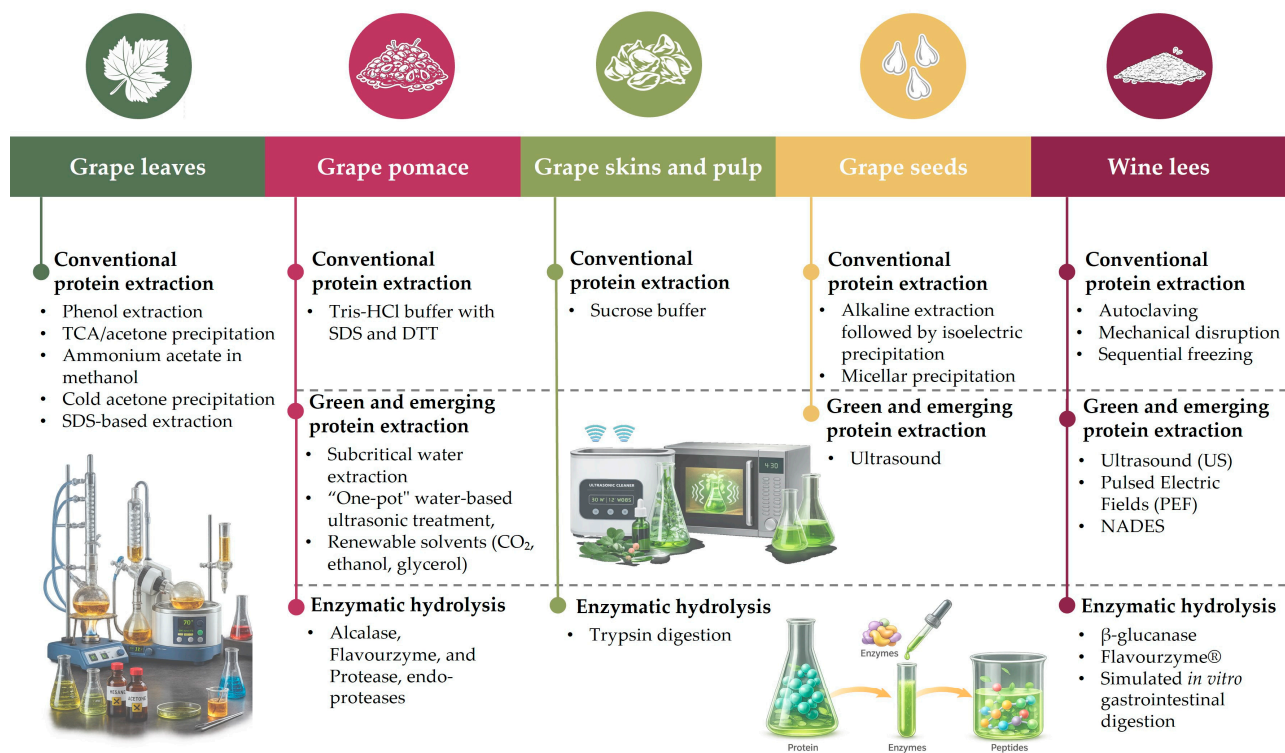


Figure 4. Schematic overview of conventional, green, and enzymatic approaches for protein extraction and hydrolysis from different winery by-products.

Table 3. Protein extraction and enzymatic hydrolysis strategies for winery by-products: functional outcomes and key limitations.

Winery By-Product	Extraction/Hydrolysis Approach	Key Outcome/Purpose	Challenges and Limitations	References
Grapevine leaves	Phenol extraction, TCA/acetone precipitation, ammonium acetate in methanol, SDS-based extraction	Proteomic-grade protein isolation; removal of interfering compounds; improved LC–MS/MS suitability	Low protein content; high polyphenol content; need for complex extraction protocols	[33,66,87,88,107,108]
Grape pomace	Tris-HCl buffer with SDS and DTT; enzymatic hydrolysis (Alcalase, Flavourzyme, Protease); subcritical water extraction; ultrasound-assisted one-pot extraction	Release of protein fractions and production of antimicrobial and ACE-inhibitory peptides; recovery of pectin-protein-polyphenol complexes	Complex heterogeneous matrix; co-extraction of polyphenols and polysaccharides; variable composition depending on processing conditions	[19,75,94,95,109,110]
Grape skins and pulp	Sucrose buffer + phenol extraction followed by precipitation and trypsin digestion	Efficient separation of proteins from polyphenols in phenolic-rich tissues	High polyphenol content; co-extraction of interfering compounds; complex and solvent-intensive protocols; potential protein modification during extraction	[92]
Grape seeds	Alkaline extraction-isoelectric precipitation (AE-IP); polyphenol removal; ultrasound pretreatment; salt extraction and micellar precipitation	Increased protein yield, purity, solubility, and ACE-inhibitory peptide potential	High polyphenol and fibre content; need for pre-treatment; protein–polyphenol interactions reducing extract purity; harsh alkaline conditions required	[97,98,111–113]
Wine lees	Autoclaving; Ultrasound extraction; pulsed electric fields β -glucanase; freezing-mechanical disruption-US; NADES extraction; enzymatic hydrolysis (β -glucanase, Flavourzyme); simulated digestion	Recovery of glycoproteins and mannoproteins; improved protein yield and functionality; production of ACE-inhibitory, antihypertensive, antimicrobial, and immunomodulatory peptides	Protein–polyphenol complexes reduce digestibility; variability related to fermentation conditions and yeast strain; energy-intensive extraction; complexity of matrix	[17,18,20,81,104,114–118]

5.1. Conventional Protein Extraction and Recovery Approaches

In grapevine leaves, protein extraction has been developed primarily for proteomic purposes. Unlike phenolic compounds, which can be efficiently extracted using green techniques such as ultrasound- and microwave-assisted extraction [119], the isolation of proteins and peptides still depends on traditional protocols. The most frequently employed workflows include phenol extraction, TCA/acetone precipitation, ammonium acetate in methanol, and, in some cases, cold acetone precipitation [66,87,88,107]. Buchs et al. [108] reported that standard protocols often fail to provide adequate yields or remove interfering compounds from leaf tissues, compromising LC–MS/MS analyses. They emphasised the need to tailor the protocol to tissue type and ensure removal of polymers and detergents. Among the evaluated procedures, SDS-based extraction yielded the most stable and purified protein extracts.

Similar challenges arise with grape skin and pulp, which are rich in polyphenols. Tian et al. [92] successfully applied sucrose buffer in combination with phenol extraction, enabling efficient separation of proteins from polyphenols and other interfering compounds. Following precipitation, the extracts were washed with methanol and acetone and subjected to trypsin digestion for proteomic analysis. This workflow exemplifies a conventional approach adapted to tissues with high phenolic content.

In grape seeds, conventional approaches are more extensively developed. The majority of available studies address optimisation of extraction conditions, identification of major protein fractions in the endosperm, and potential varietal differentiation based on the protein profile [97]. Alkaline extraction followed by isoelectric precipitation (AE-IP) remains the standard method for isolating plant proteins. Baca-Bocanegra et al. [98] demonstrated that pH plays a pivotal role in extraction yield and purity, with pH–temperature interactions further influencing process efficiency. Removal of polyphenols from seed flour prior to extraction increased protein concentrate purity by 7.7%, underscoring the importance of eliminating phenolics that hinder protein solubility and recovery. Consistently, Cejudo-Bastante et al. [111] showed that highly alkaline conditions (around pH 10) are optimal for protein extraction from grape seeds, as increased negative charge enhances electrostatic repulsion and protein–water interactions, improving solubility and yield; removal of insoluble fibre further enhanced hydrolysate purity.

Conventional protein extraction from grape pomace is less common and is mainly used in peptidomic studies. Knuf et al. [19] extracted proteins using Tris-HCl buffer supplemented with SDS and DTT for denaturation and reduction in proteins within the lignocellulosic matrix. Although sonication was later used to accelerate extraction (see below), the core principle remains conventional buffer-assisted solubilisation.

For wine lees, De Iseppi et al. [17] applied autoclaving, ultrasound, and β -glucanase treatment to obtain extracts rich in glycoproteins and polysaccharides. Autoclaving produced fractions richest in glyco-compounds, while ultrasound- and enzyme-assisted extracts, though containing fewer polysaccharides, were more effective in improving protein stability in wine. Their subsequent study demonstrated that both red and white wine lees can serve as sources of mannoprotein-rich extracts with significant emulsifying and foaming properties [18], confirming that conventional thermal and enzymatic extraction schemes can generate functional protein–glycoprotein fractions suitable for food industry applications.

A critical comparison of conventional extraction approaches indicates that alkaline extraction followed by isoelectric precipitation (AE-IP) remains the most widely applied method due to its relatively high protein yield, simplicity, and scalability. However, its selectivity is often limited, as co-extraction of polysaccharides and phenolic compounds is common, particularly in matrices such as grape seeds and pomace. These interactions may lead to the formation of protein–polyphenol or protein–polysaccharide complexes, which

can enhance certain functional properties, but also reduce extract purity and complicate downstream processing. Phenol-based and TCA/acetone protocols provide higher purity, especially for proteomic applications, but are less suitable for food-related uses due to solvent toxicity and limited scalability. From an environmental and economic perspective, conventional methods are generally cost-effective but rely on chemical inputs and generate waste streams. Importantly, matrix-specific constraints, such as strong protein–polyphenol interactions and lignocellulosic barriers, remain major limiting factors affecting protein recovery. Proteins obtained through these methods typically retain their native or partially denatured structure, which may result in moderate digestibility that can be further improved by subsequent enzymatic hydrolysis. In this context, extraction conditions may influence protein structure and accessibility, thereby affecting hydrolysis efficiency, peptide release, and the resulting bioactivity.

5.2. Green and Emerging Extraction Technologies

Modern trends in the extraction of bioactive compounds from winery by-products aim to reduce the use of toxic solvents and energy inputs while maintaining or enhancing extraction yield and functionality. In this context, green solvents and physical techniques such as ultrasound (US), pulsed electric fields (PEF), and high-pressure processing have been increasingly explored [120].

In grape pomace, green extraction technologies were initially developed for polyphenol recovery but have also demonstrated potential for releasing protein fractions. Numerous studies employ water, CO₂, ethanol, and glycerol as low-toxicity, renewable solvents capable of extracting a broad spectrum of phenolic and associated macromolecular constituents [121]. Pedras et al. [109] further advanced this approach using subcritical water extraction, where increased temperature sequentially facilitates the removal of polyphenols, proteins, resins, starch, and soluble sugars, followed by partial solubilisation of hemicelluloses. In their study, a notable protein content (12.5%) was reported, with most protein extracted at the highest temperatures, although a fraction remained undetected due to tight binding within the lignocellulosic matrix.

Recent studies have expanded these strategies toward non-thermal, ultrasound-assisted processes. Gao et al. [110] described the extraction of a pectin-protein-polyphenol complex from white grape pomace using a one-pot, water-based ultrasonic treatment, demonstrating that acoustic cavitation can promote the simultaneous solubilisation of structural polysaccharides and associated protein fractions. Although primarily investigated in the context of functional ingredient recovery, this finding confirms the broader applicability of green physical techniques for releasing macromolecular assemblies containing proteins and peptides from winery side streams. Importantly, such processes may also induce structural modifications in proteins (e.g., partial unfolding or aggregation), which can directly influence enzyme accessibility during subsequent hydrolysis, thereby affecting peptide profiles and associated bioactivities.

Green and advanced techniques have been particularly emphasized for wine lees, where the goal is to accelerate yeast autolysis and release intracellular and cell-wall components. US, PEF, and their combination (PEF + US) have emerged as promising physical treatments. Cacciola et al. [114] showed that US markedly accelerates the release of colloidal particles (proteins, polysaccharides, glycoproteins) from wine lees, achieving effects comparable to prolonged lees ageing or enzymatic β -glucanase treatments. Ntourtoglou et al. [115] compared US, PEF, and PEF + US, finding that US produced the highest protein yield (5700 $\mu\text{g/mL}$ after 20 min), albeit with high energy consumption, whereas PEF + US resulted in a similar yield (5400 $\mu\text{g/mL}$) with more than 50% lower energy demand. Although PEF alone was the most energy-efficient for yeast inactivation,

it was far less effective for rapid protein release, especially under short treatments, making combined PEF + US the best compromise between yield and sustainability.

Beyond wave-based technologies, combined mechanical and thermal treatments have been evaluated. Cotârlet et al. [116] demonstrated that sequential freezing, mechanical disruption, and ultrasound treatment at elevated temperatures (≥ 70 °C) resulted in efficient yeast cell lysis and increased protein content in the extract by 67% compared with untreated samples. While effective, such approaches raise considerations regarding energy efficiency and industrial scalability relative to alternatives (high-pressure processing, bead milling, etc.).

Significant advancements have also been achieved using NADES for wine lees extraction. Teslić et al. [117] employed NADES combined with autoclaving to extract glycoproteins and polysaccharides. Acid–betaine-based systems (e.g., CA/BET) produced high yields and strong cell-wall destabilisation, generating low-molecular weight glycoproteins, whereas urea/choline chloride systems (UR/ChCl) generated higher molecular weight glycoproteins with superior emulsifying properties. These results demonstrate that careful selection of NADES composition and processing conditions enables targeted modulation of protein–glycoprotein structure and functionality.

In grape seeds, ultrasound is increasingly used as a pretreatment prior to enzymatic hydrolysis or conventional extraction. Ultrasound weakens the cell–matrix, enhances enzyme accessibility, and alters protein conformation, ultimately increasing the bioactivity of resulting peptides, including ACE-inhibitory activity [112]. Coşkun and Gülseren [113] further showed that simple aqueous techniques such as AE-IP, salt extraction, and micellar precipitation can significantly improve protein content in pumpkin, pomegranate, and grape seed concentrates while maintaining functionality and offering favourable industrial applicability.

Green and emerging extraction technologies offer clear advantages in terms of reduced solvent toxicity and improved sustainability profiles, particularly when water, ethanol, or NADES are employed. Physical techniques such as ultrasound and pulsed electric fields enhance mass transfer and cell disruption, often increasing extraction yield and facilitating the release of protein–polysaccharide complexes. However, their selectivity toward proteins remains limited, as co-extraction of other macromolecules is frequent. In addition, energy consumption, especially in ultrasound-assisted processes, and the cost of specialized equipment represent important constraints for large-scale implementation. Combined approaches (e.g., PEF + US or NADES-assisted extraction) show promise in balancing efficiency and sustainability, although their technology readiness level is still moderate. These co-extracted complexes may represent both an advantage and a limitation, depending on the intended application. While protein–polyphenol and protein–polysaccharide interactions can enhance functional properties such as emulsifying capacity and antioxidant activity, they also reduce extract purity, complicate downstream processing, and may affect protein digestibility and bioavailability, making the isolation of “pure” protein fractions challenging. Matrix-dependent effects, including tight binding of proteins within lignocellulosic structures or yeast cell walls, continue to limit complete protein recovery. Extracted proteins are often partially modified or associated with other biomolecules, which may influence their functional properties and bioavailability, requiring further processing for targeted applications. This highlights the trade-off between extract purity and functional performance. These structural changes may further impact downstream enzymatic hydrolysis efficiency and selectively promote the release of specific bioactive peptides, thereby linking extraction conditions to the functional potential of the final products.

5.3. Enzymatic Hydrolysis and Production of Bioactive Peptides

Enzymatic hydrolysis represents a key step in the valorisation of protein-rich winery by-products, enabling the transformation of less soluble, structurally complex pro-

teins into peptides and free amino acids with improved solubility, nutritional value, and potential bioactivity.

From a process perspective, enzymatic hydrolysis is governed by several key parameters, including enzyme specificity, degree of hydrolysis (DH), and reaction conditions such as pH, temperature, time, and enzyme-to-substrate ratio. Endopeptidases (e.g., Alcalase) preferentially cleave internal peptide bonds, generating a broad range of peptide fragments, whereas exopeptidases (e.g., Flavourzyme) further hydrolyse terminal amino acids, leading to shorter peptides and free amino acids. The degree of hydrolysis plays a central role in determining functional properties, as moderate DH values are often associated with enhanced bioactivity, while excessive hydrolysis may reduce functionality due to the formation of very short peptides or free amino acids. Reaction conditions directly influence enzyme activity and selectivity, thereby shaping peptide profiles and, consequently, the resulting functional and bioactive properties. Therefore, careful optimisation of hydrolysis parameters is essential to balance yield, functionality, and process efficiency. Furthermore, the presence of protein–polyphenol or protein–polysaccharide complexes can influence hydrolysis efficiency, as these interactions may limit enzyme accessibility or alter cleavage patterns [19].

In grape pomace, Knuf et al. [19] applied enzymatic hydrolysis (Alcalase, Flavourzyme, Protease, individually or combined) following protein extraction using Tris-HCl buffer with SDS and DTT. The resulting hydrolysates differed in peptide composition and bioactivity, with the most active fractions enriched in proline-containing sequences, a hallmark of many antimicrobial peptides. Rodríguez-Morgado et al. [75] developed an alternative approach by preparing a grape pomace enzymatic extract (GP-EE), in which pomace (20%) was suspended in water and hydrolysed with endoproteases at 60 °C and pH 8 under pH-stat control. Enzymatic treatment increased protein content (14%) and converted insoluble proteins into soluble peptides and free amino acids, significantly improving solubility across pH values and enhancing the nutritional and functional properties of the extract.

In grape seeds, efforts have increasingly focused on producing peptides with targeted bioactivities. Enzymatic hydrolysis of seed protein concentrates, particularly when combined with ultrasound pretreatment, increases enzyme susceptibility and yields hydrolysates with stronger ACE-inhibitory activity [112], positioning grape seed proteins as promising precursors for functional food ingredient development within food and feed systems.

Wine lees represent the most extensively investigated matrix for enzymatic hydrolysis. Rowe et al. [118] identified 219 protein species released into wine during a nine-month lees ageing model, with long-lasting proteins consisting predominantly of mannoproteins and selected glycolytic enzymes, suggesting their stability and potential contribution to colloidal and sensory wine properties. Subsequent studies have explored targeted hydrolysis of lees proteins.

Bravo et al. [81] obtained wine lees hydrolysates using Flavourzyme®, producing peptide-rich fractions with strong ACE-inhibitory activity. Ultrafiltration, RP-HPLC, and nano-HPLC–MS/MS identified six ACEi peptides, several of which (e.g., FK-TTDQQTRTTVA, NPKLVTIV, TVTNPARIA, LDSPSEGRAPG, LDSPSEGRAPGAD) demonstrated significant antihypertensive effects in vivo in spontaneously hypertensive rats. This study provided the first direct evidence that wine lees can serve as a source of ACE-inhibitory and antihypertensive peptides with potential relevance for functional ingredient development.

Moreira et al. [20] expanded these findings by applying in vitro gastrointestinal digestion to wine lees obtained from fermentations with different yeast strains (*Starmerella bacillaris* FRI751, *Saccharomyces cerevisiae* EC1118, and their sequential fermentations). LC–MS/MS identified 243 unique peptide sequences, and in silico analyses revealed strong predicted antihypertensive activity, particularly for *S. bacillaris* extracts, as well as antimicrobial, immunomodulatory, and putative anti-obesity bioactivities. These results confirm that

the protein-peptide fraction of wine lees, especially after targeted enzymatic or simulated digestion, is a rich source of bioactive peptides with broad potential applications.

Overall, the combination of conventional and green extraction techniques with targeted enzymatic hydrolysis enables not only more efficient utilisation of protein fractions from grapevine leaves, stems, skins, seeds, pomace, and wine lees, but also the production of peptides with distinct nutritional and functional properties. In this context, the selection of enzyme type and control of hydrolysis conditions is critical for directing peptide composition and maximising targeted bioactivities. This integrated approach supports the development of novel protein- and peptide-based ingredients for feed and food systems, while simultaneously contributing to nutrient recycling, waste reduction, and the advancement of sustainable viticulture.

6. Applications of Winery By-Product-Derived Proteins and Peptides

In recent years, the protein and peptide fractions of winery by-products have attracted considerable attention as sources of bioactive and techno-functional biomolecules with potential applications across agricultural and agri-food systems. These components, isolated from grapevine leaves, stems, pomace, seeds, and wine lees, exhibit a broad spectrum of biological activities—including antioxidant, antimicrobial, antihypertensive, and immunomodulatory properties—as well as the ability to stabilize colloidal systems, act as emulsifiers, and form foams, making them relevant candidates for nutrient recycling strategies, food and feed development, plant protection approaches, agricultural product stabilisation, and broader industrial applications [12,18,20].

A structured summary of the principal application pathways, sources, and functional roles of protein and peptide fractions derived from winery by-products is provided in Table 4.

While the potential of proteins and peptides derived from winery by-products is increasingly recognised, it is important to distinguish between different levels of technological maturity. Many reported bioactivities are still predominantly supported by *in vitro* and *in silico* evidence. In contrast, pilot-scale and industrial applications remain limited and are mainly associated with oenological uses, feed applications, and selected functional ingredients. Therefore, further validation, including *in vivo* studies, process optimisation, and regulatory approval, is required for broader implementation. Accordingly, the applications outlined in this section should be interpreted in the context of their respective levels of technological readiness, as also reflected in the structured overview presented below (Figure 5).



Figure 5. Application pathways of protein and peptide fractions derived from winery by-products within a circular viticulture framework.

Table 4. Application domains, functional roles, and implementation status of protein and peptide fractions derived from winery by-products.

Winery By-Product	Application Domain	Functional Role and Intended Outcome	Implementation Level	References
Grapevine leaves	Crop production/ Biostimulants	Activation of plant defence pathways (PR proteins, PAMP-related peptides); stress protection; enhanced pathogen resistance and abiotic stress tolerance; reduced reliance on synthetic agrochemicals	Research stage	[68–70,122,123]
Grape stems	Animal nutrition/ Nutrient recycling	Solid-state fermentation–assisted valorisation; conversion of lignocellulosic biomass into protein-enriched substrates; production of single-cell protein; improved nitrogen utilisation	Experimental–pilot	[71–73,124]
Grape pomace	Feed/ Functional ingredients	Substrate for microbial protein production; source of bioactive peptides (antimicrobial, ACE-inhibitory); development of value-added feed materials and functional ingredients	Experimental–pilot	[12,19,96]
Grape seeds	Oenology/ Food systems	Techno-functional proteins and hydrolysates (emulsifying, stabilising); application as allergen-free fining agents; improvement of food formulation properties and wine quality	Partly applied (oenology); emerging (food systems)	[74,79,100,101,106]
Wine lees	Oenology/ Nutraceuticals	Mannoproteins for colloidal and colour stabilisation; source of bioactive peptides (ACE-inhibitory, antimicrobial); applications in functional foods and nutraceuticals	Applied (oenology); emerging (nutraceuticals)	[12,18,42,80,81,105,117,125]

6.1. Crop Production and Plant Protection

Within contemporary crop production systems, particularly in viticulture, protein and peptide fractions derived from winery by-products should not be regarded solely as bioactive molecules of downstream industrial interest, but as biologically active constituents intrinsically linked to plant physiology and defence. Their recovery and targeted utilisation therefore represent an opportunity to reconnect post-processing biomass streams with primary production systems.

Proteins isolated from grapevine leaves are closely associated with endogenous defence pathways and stress adaptation mechanisms. Pathogenesis-related (PR) proteins and PR-1-like proteins, together with PAMP-induced signalling peptides, are involved in pathogen recognition and activation of immune responses, indicating their relevance in the development of bio-based plant protection strategies [68,70]. Dehydrins further strengthen this perspective. Their accumulation under low-temperature and drought stress, combined with demonstrated antioxidant, cryoprotective, and antifungal properties, suggests their potential role in protective formulations designed to enhance stress resilience in crops [69,123]. In parallel, systems-level studies identifying subtilisin-like serine proteases as components of grapevine resistance networks support the concept that specific protein fractions from viticultural residues may serve either as molecular markers in breeding and stress-monitoring strategies or as functional constituents of biostimulant formulations [122].

From a crop-production standpoint, this repositioning of winery by-products is particularly relevant in regions characterised by intensive seasonal biomass generation. Instead of representing a discontinuity in nutrient and nitrogen flows, these fractions may contribute to circular viticulture models in which biologically active macromolecules are reintegrated into plant production systems, thereby reducing reliance on synthetic inputs and reinforcing agroecological approaches to disease management and stress mitigation.

6.2. Animal Nutrition

The utilisation of protein-containing fractions derived from winery by-products in animal nutrition represents a complementary and strategically important dimension of agricultural valorisation. In this context, the emphasis shifts from direct plant defence functions toward nutrient recovery and the generation of alternative protein sources within livestock systems.

Grape stems, despite their lignocellulosic structure and limited inherent protein digestibility, have demonstrated significant potential following biotechnological upgrading. Solid-state fermentation has been shown to enhance protein accessibility and nutritional value, enabling the production of protein-enriched biomass and single-cell protein suitable for use in ruminant feed [71,72]. Such processes facilitate the conversion of structurally complex residues into nutritionally relevant feed ingredients, contributing to improved nitrogen utilisation efficiency and supporting more sustainable livestock production models.

The amino acid composition of stem-derived fractions, including relatively high tryptophan content and its association with antioxidant capacity, further indicates functional relevance beyond basic nutrition [73]. These characteristics may contribute to oxidative stability and overall animal health when incorporated into feed formulations. Additionally, regulatory proteins such as VISRT1, although primarily studied in plant stress responses, further highlight the biological complexity of stem-derived fractions and their broader agro-biotechnological relevance [124].

Grape pomace has likewise been explored as a substrate for microbial protein production, offering a route toward value-added feed ingredients while simultaneously reducing organic waste burdens [96]. In this framework, winery by-products serve not only as

supplementary feed materials but as platforms for bioconversion, reinforcing integrated crop–livestock production systems and circular nutrient flows.

6.3. Food and Oenology

Among all valorisation routes, applications in food systems and oenology remain among the most technologically advanced and partially implemented. Here, protein and peptide fractions contribute through complementary structural and functional mechanisms.

These applications are closely associated with the specific functional properties of the derived proteins and peptides. For example, emulsifying and foaming capacities enable their use in food formulations and stabilisation processes, while enzyme-inhibitory activities, such as ACE-inhibition, support their incorporation into functional foods targeting cardiovascular health. Similarly, antioxidant and antimicrobial properties facilitate applications in food preservation, active packaging systems, and agricultural formulations.

In white grape pomace, identified glycoproteins influence protein stability and allergenic reactivity within wine matrices [126]. Ultrasound-assisted extraction of pectin–protein–polyphenol complexes has further demonstrated the possibility of recovering multifunctional macromolecular assemblies exhibiting emulsifying and antioxidant properties suitable for functional ingredient development [110]. In red grape pomace, enzymatic hydrolysis generates peptide fractions with antimicrobial and ACE-inhibitory activities, supporting their potential as natural preservatives or antihypertensive nutraceutical ingredients [12,19].

Grape seeds represent a particularly relevant matrix in this domain. Defatted grape seed flour exhibits techno-functional properties comparable to established plant proteins [106], while seed protein extracts have been successfully applied as allergen-free fining agents in winemaking, improving sensory attributes without introducing conventional animal-derived proteins [79,101–103]. Moreover, grape seed protein hydrolysates contribute to colour stabilisation and antioxidant capacity in red wines, illustrating a direct functional link between protein structure and oenological performance [74,100].

Wine lees constitute an established source of mannoproteins that enhance colour stability, colloidal balance, and mouthfeel in wines [105]. Beyond these traditional uses, peptide fractions isolated from lees display antihypertensive, antimicrobial, and immunomodulatory activities [12,80,81]. Lees-derived extracts have also been explored as emulsifiers and stabilisers in food systems [18,42], while advanced extraction strategies such as NADES enable targeted recovery of glycoprotein fractions with tailored functional properties [117]. Collectively, these findings demonstrate that winery-derived proteins are not merely secondary constituents but structurally and functionally active components capable of enhancing product quality and functional performance.

6.4. Emerging Industrial Uses

Beyond agriculture and food systems, protein and peptide fractions derived from winery by-products exhibit broader biotechnological relevance. Although many of these applications remain at experimental or preclinical stages, their multifunctional properties suggest significant cross-sector potential.

Peptides derived from grape pomace and wine lees have demonstrated antioxidant, antihypertensive, and antimicrobial effects *in vitro*, supporting their candidacy for incorporation into nutraceutical formulations and functional product systems [12,20]. Preparations enriched in mannoproteins with enzymatic and radical-scavenging activity further extend potential utility toward cosmetic and biotechnological applications, where stabilisation, emulsification, and bioactivity are of interest [83].

Protein fractions from grapevine leaves and stems, currently explored mainly within agro-biotechnological research, may also possess broader industrial relevance following adequate safety validation and functional characterisation. Their stress-protective and stabilising properties suggest possible roles in biomolecule protection systems or as components of innovative bio-based materials.

The suitability of specific winery by-products for these applications is largely determined by their protein composition. For instance, seed-derived storage proteins and lees-derived mannoproteins exhibit distinct structural and functional characteristics that make them particularly suitable for applications in nutraceutical, cosmetic, and stabilisation systems.

In addition to these applications, winery-derived proteins and peptides show promising potential in the development of bio-based materials, including biodegradable films, coatings, and active packaging systems. Their film-forming ability, combined with antioxidant and antimicrobial properties, supports their use in active packaging applications aimed at extending food shelf life and reducing reliance on synthetic materials. Furthermore, these fractions can be utilised in encapsulation and delivery systems for bioactive compounds, where their structural and interfacial properties enable controlled release and protection of sensitive molecules. Such functionalities also highlight their potential relevance in pharmaceutical and nutraceutical applications, although most of these applications remain at an early stage of research and require further validation [127].

Overall, the diversification of utilisation pathways underscores a central premise of this review: winery by-products constitute not simply waste streams, but structured biological resources whose protein and peptide fractions can be strategically directed toward multiple sectors. Their effective integration, however, requires continued emphasis on scalability, regulatory clarity, and system-level alignment with sustainable agri-food production models.

Despite the promising application potential, several limitations must be considered for the practical implementation of winery by-product-derived proteins and peptides. The compositional variability of raw materials, influenced by grape variety, cultivation conditions, and processing methods, may affect reproducibility and consistency. In addition, the standardisation of peptide mixtures remains challenging due to their structural complexity. Stability and shelf-life issues, particularly related to protein degradation and oxidation, may further limit their applicability. Furthermore, economic feasibility, cost competitiveness, and scale-up challenges represent critical factors for successful industrial implementation.

7. Challenges and Future Perspectives

The key challenges associated with the valorisation of winery-derived proteins and peptides can be systematically categorised into raw material variability, technological and process limitations, biological and functional validation, regulatory barriers, and economic and market challenges.

Although the growing body of evidence highlights the nutritional, functional, and biotechnological relevance of proteins and peptides derived from winery by-products, several structural challenges continue to limit their integration into agricultural and agri-food systems at scale. Within the context of sustainable viticulture, one of the principal constraints remains the pronounced heterogeneity of winery residues. Their composition is strongly influenced by grape cultivar, pedoclimatic conditions, vineyard management practices, harvest timing, and vinification technologies. Such variability directly affects protein yield, peptide profile, and functional properties, thereby complicating standardisation, reproducibility, and quality assurance of derived fractions intended for agricultural or feed applications [7,10]. The absence of harmonised analytical protocols and certified reference

materials further constrains cross-study comparability and slows technological transfer to practice.

From a technological perspective, efficient recovery of protein fractions remains challenged by the intrinsic complexity of plant and yeast matrices and by strong molecular interactions between proteins, polyphenols, polysaccharides, and lignocellulosic structures. In agricultural contexts, where economic feasibility and environmental compatibility are decisive, extraction processes must be robust, scalable, and adaptable to seasonal biomass availability. Conventional extraction strategies may rely on harsh chemical conditions that are not fully aligned with sustainability goals, while emerging green technologies—such as ultrasound-assisted extraction, pulsed electric fields, and natural deep eutectic solvents—although promising, still require optimisation in terms of energy efficiency, process continuity, and compatibility with existing winery infrastructures [17,19,115,117]. In particular, scaling up these technologies and integrating them into existing winery operations remain critical challenges, especially in the context of seasonal biomass availability and supply chain constraints. Future research should therefore prioritise integrated processing platforms capable of operating at semi-industrial scale and directly connected to winery production lines, enabling on-site or regional valorisation of protein-rich side streams.

A further limitation concerns the translational gap between reported bioactivities and field-level validation. While numerous *in vitro* and *in silico* studies describe antioxidant, antimicrobial, antihypertensive, or immunomodulatory effects of winery-derived peptides, significantly fewer investigations address their performance under real agricultural conditions. For applications in crop production, plant protection, or animal nutrition, controlled greenhouse and field trials are required to confirm efficacy, stability, dose–response relationships, and long-term environmental impact. Similarly, in feed and food systems, comprehensive safety assessments, allergenicity testing, and bioavailability studies remain essential for regulatory approval and practical adoption. Moreover, safety considerations require greater attention, particularly regarding potential contaminants such as pesticide residues, heavy metals, and mycotoxins, as well as batch-to-batch variability. The development of standardised quality control protocols will be essential to ensure product safety and regulatory compliance [12,20].

Regulatory and legislative considerations also shape future implementation pathways. The classification of winery residues as waste or by-products continues to influence permissible valorisation routes, especially when reintegration into agricultural systems is intended. Although the European regulatory framework increasingly promotes circular bioeconomy principles, clearer guidance addressing protein and peptide fractions from agricultural and food-processing side streams would facilitate their recognition as secondary raw materials suitable for feed, soil, or plant-related applications [5,10]. Harmonisation across Member States would further support cross-border innovation and industrial uptake.

In addition to regulatory considerations, economic and market factors play a critical role in determining the feasibility of large-scale implementation. Large-scale implementation of winery-derived protein valorisation ultimately depends on its cost competitiveness relative to conventional protein sources, such as soybean or whey proteins. While growing demand for plant-based and alternative proteins provides a favourable market context, the economic viability of these systems is strongly influenced by processing costs, logistics, and seasonal availability of raw materials. Furthermore, the development of efficient value chains requires investment in infrastructure for collection, storage, and processing of winery residues. The establishment of regional or decentralised biorefinery models may therefore represent a key strategy to ensure consistent supply, reduce transportation costs, and improve overall economic sustainability [128].

Consumer acceptance represents an additional factor influencing the successful market integration of winery-derived protein products. The perception of materials originating from agro-industrial by-products or “waste” streams may pose a barrier, particularly in food and nutraceutical applications. Transparent communication, appropriate labelling, and clear demonstration of safety and functionality will be essential to build consumer trust. Concurrently, increasing demand for sustainable and functional foods may support the acceptance of such products within targeted market segments [129].

Looking ahead, the advancement of winery-derived protein and peptide valorisation will depend on multidisciplinary integration. Coupling proteomics and metabolomics with process engineering, agronomic testing, and life-cycle assessment can provide a systems-level understanding of environmental and economic performance. In viticultural regions, regional biorefinery models that integrate residue collection, protein extraction, fermentation, and downstream applications may represent a viable pathway toward circular nutrient management. Strengthened collaboration among research institutions, wineries, agricultural producers, and regulatory authorities will be essential to translate laboratory findings into operational strategies that enhance resource efficiency, reduce environmental burdens, and reinforce the sustainability of viticulture and agricultural production systems.

Future research should focus on the optimisation of protein extraction and purification strategies, including selective peptide isolation, as well as on the *in vivo* validation of bioactive effects. Additionally, the development of standardised and scalable processing protocols will be essential to ensure reproducibility and facilitate industrial implementation. Particular attention should be given to the integration of these processes into biorefinery-based systems, enabling efficient and sustainable valorisation of winery by-products.

8. Conclusions

Winery side streams, including grapevine leaves, stems, pomace, seeds, and wine lees, represent a structurally diverse and chemically rich biomass resource directly embedded within agricultural production systems. On a global scale, the wine industry generates approximately 20 million tonnes of such by-products annually. Within these matrices, protein content varies substantially, ranging from approximately 5–11% in grape stems and 6–15% in grape pomace to 11–18.9% in grape seeds, 9–20% in grapevine leaves, and up to 29–65% in wine lees, where proteins and peptides are present in more accessible and functionally relevant forms. Rather than being regarded merely as low-value processing residues, these materials should be recognised as recyclable, protein-containing fractions capable of contributing to circular nutrient flows and more sustainable viticultural practices.

The evidence synthesised in this review demonstrates that protein and peptide fractions from winery by-products constitute a scientifically substantiated yet underexploited component of agro-industrial biomass. Beyond their biochemical characterisation, these fractions exhibit functional properties relevant to crop production, plant protection, animal nutrition, and oenological stabilisation. In this context, winery-derived proteins emerge as multifunctional agents positioned at the interface between primary agricultural production and downstream agri-food processing. A key contribution of this review lies in the integration of compositional, technological, and regulatory perspectives, thereby providing a unified framework for understanding winery by-products not only as sources of bioactive compounds but as functional elements within circular bioeconomy systems.

Advances in extraction technologies, including conventional, green, and hybrid approaches, together with targeted enzymatic hydrolysis, have expanded the feasibility of recovering and tailoring these fractions for practical use. When integrated into winery-level or regional valorisation models, such approaches align with circular bioeconomy principles by enabling nutrient recycling, reducing organic waste burdens, and

generating value-added inputs within the same agricultural landscape from which the biomass originates.

Nevertheless, the transition from laboratory-scale studies to operational implementation requires coordinated progress in several priority areas. First, the standardisation of extraction and hydrolysis protocols is essential to ensure methodological consistency and reproducibility across different raw materials and processing conditions. Second, the development of scalable and economically viable biorefinery models is needed to support industrial-level implementation and integration into existing winery infrastructures. Third, systematic *in vivo* validation across agricultural, feed, and food-related applications is required to confirm functional efficacy, bioavailability, and safety under real conditions. Finally, regulatory harmonisation and clearer classification frameworks for winery by-products as secondary raw materials remain critical for enabling market access and broader adoption.

Overall, the valorisation of winery-derived proteins and peptides should be viewed not solely as a technological innovation, but as a strategic component of sustainable viticulture and integrated agricultural systems. By reconnecting post-processing biomass with agricultural functionality, these underutilised fractions have the potential to enhance nutrient efficiency, reduce environmental pressure, and strengthen the long-term resilience of wine-producing regions.

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Abbreviations

The following abbreviations are used in this manuscript:

ACE	Angiotensin-Converting Enzyme
AE-IP	Alkaline Extraction-Isoelectric Precipitation
CA/BET	Citric Acid/Betaine
CAP	Common Agricultural Policy
DH	Degree Of Hydrolysis
DTT	Dithiothreitol
EU	European Union
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry

NADES	Natural Deep Eutectic Solvents
OIV	International Organisation Of Vine And Wine
PEF	Pulsed Electric Fields
PR proteins	Pathogenesis-Related Proteins
SDS	Sodium Dodecyl Sulfate
TCA	Trichloroacetic Acid
UAE	Ultrasound-Assisted Extraction
UR/ChCl	Urea/Choline Chloride
WFD	Waste Framework Directive

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