

Article

Polyphenolic Profile and Dietary Fiber Content of Skins and Seeds from Unfermented and Fermented Grape Pomace

Massimo Guaita ^{1,*}, Alice Zocco ², Stefano Messina ¹, Silvia Motta ¹, Jean Daniel Coisson ² and Antonella Bosso ¹

¹ Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca Viticoltura ed Enologia, Via P. Micca 35, 14100 Asti, Italy; silvia.motta@crea.gov.it (S.M.); antonella.bosso@crea.gov.it (A.B.)

² Università degli Studi del Piemonte Orientale "A. Avogadro" - Dipartimento di Scienze del Farmaco, Largo Donegani 2, 28100 Novara, Italy; jeandaniel.coisson@uniupo.it (J.D.C.)

* Correspondence: massimo.guaita@crea.gov.it

Abstract

Due to the seasonality of its production and its polluting characteristics, the management and disposal of large amounts of grape pomace (GP) produced worldwide every year can pose a significant economic and environmental challenge. The research on the possible exploitation of GP for various purposes has been constantly growing during recent years, due to the increased general sensitivity to issues like the sustainability of agro-industrial production and the growing consumer demand for the use of natural versus synthetic compounds. This work concerned the determination of the polyphenolic profile and the dietary fiber content of skins and seeds from unfermented and fermented white and red grape pomace of different cultivars, sampled from local wineries in the Piedmont area (Italy) after winemaking. A double extraction was performed to maximize the extraction of polyphenols from grape pomace flours. The extractable polyphenols content (EPP) was determined in the extracts, while the non-extractable polyphenols (NEPP) linked to fiber were quantified as condensed tannins in the residue after extraction. The total dietary fiber (TDF) was determined for skins and seeds; limited to skins, the analysis was extended to the distinction between soluble and insoluble dietary fiber (SDF and IDF). The polyphenolic and dietary fiber content was significantly higher in seeds than in skins. However, from a nutritional point of view, the dietary fiber of skins may be more interesting due to the higher NEPP content than in seeds; moreover, the winemaking technique influenced the quantity and characteristics of skin fiber, which contained SDF, almost absent in seeds.

Keywords: winemaking byproducts; grape pomace; extraction; polyphenols; dietary fiber

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1. Introduction

The world production of grape pomace (GP) by the wine industry annually exceeds 10 Mt [1] (). Due to its seasonality and polluting characteristics, the management and disposal of large amounts of GP can pose a significant economic and environmental challenge; however, GP is one of the richest sources of natural phenolic compounds, which are widely studied, due to their effective antioxidant and free radical scavenging activities, as biologically active substances in the food industry (nutraceuticals, food preservatives), in medicine, pharmacology, and biomedicine, as well as for many other industrial applications (natural colorants, cosmetics, etc.).

The research on the possible exploitation of GP for various purposes has been constantly growing during recent years, due to the increased general sensitivity to issues like the sustainability of agro-industrial production and the growing consumer demand for the use of natural versus synthetic compounds. Limited to the food sector, GP is used to improve food quality and develop ingredients and products with high added value [2], to produce functional foods and food supplements (dietary fibers and polyphenols, pomace powder), and for food processing (biosurfactants, natural antioxidant, natural colorants) [3–5].

In addition to polyphenols, the nutritional value of GP is also due to the high content of dietary fiber (DF). The AACC (American Association of Cereal Chemists) defines DF as the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine [6]. DF includes polysaccharides, oligosaccharides, lignin and associated plant substances, and it has several beneficial effects for human health: It regulates intestinal transit, and prevents diabetes, hypertension, coronary heart disease, cardiovascular disease and colon cancer [7].

Based on its solubility in water, DF can be distinguished into:

- Insoluble dietary fiber (IDF), which includes insoluble hemicellulose, cellulose, resistant starch and lignin. Due to its ability to retain water, IDF promotes satiety and improves digestion.
- Soluble dietary fiber (SDF), which includes pectin, gums and mucilages. Due to its capacity to lower cholesterol levels and the glycemic index, SDF is associated with a reduction in the risk of cardiovascular disease.

Generally, fruit dietary fiber is of better quality than dietary fiber from cereals, due to its higher total and soluble fiber content, a better insoluble/soluble fiber ratio, water and oil holding capacity and colonic fermentability, as well as its lower phytic acid contents and caloric values [8]. In addition, fruit DF contains a significant amount of secondary compounds associated with it, such as polyphenols and terpenes. This type of fiber is called “antioxidant dietary fiber” (ADF), and the definition refers particularly to a concentrate of DF that contains high amounts of natural antioxidants associated with non-digestible compounds, generally defined as “non-extractable polyphenols” (NEPP) [9,10]. NEPP are a relevant fraction of dietary polyphenols exerting their main biological action in the colon, where they are extensively fermented by the action of microbiota, giving place to absorbable metabolites. NEPP have different potential health-related properties, particularly for gastrointestinal health, such as increases in antioxidant and antiproliferative capacities, reduction in intestinal tumorigenesis and modification of gene expression (down-regulation of genes associated with tumor development and proto-oncogenes, and up-regulation of tumor-suppressor genes) [11].

In view of the possible exploitation of GP as a food additive or dietary supplement in the food industry (nutraceuticals), this work is a follow-up to our previous work [12], focused on the determination of the polyphenolic profile and antioxidant activity of green extracts from GP skins and seeds of Italian cultivars. In the present work, the same GP samples were further characterized, for the first time to our knowledge, in terms of dietary fiber and NEPP content. To consider the compositional variability of GP, samples were analyzed from both the white winemaking process, in which the pomace was separated from the must before alcoholic fermentation (unfermented GP), and the red winemaking process, in which the pomace from red grape cultivars was separated from the wine after a period of contact (maceration) with the fermenting must (fermented GP). Seven grape cultivars, widely grown in the Piedmont area (Italy), were studied: Muscat blanc, Cortese, Arneis, Pinot noir, Barbera, Grignolino, Nebbiolo.

2. Materials and Methods

GP was sampled from local wineries in the Piedmont area (Italy). In the white wine-making process, fresh GP is separated from the must after pressing, before the start of alcoholic fermentation: the resulting GP is therefore fresh unfermented GP (UGP). In the red winemaking process, the pomace is left in contact with the must during alcoholic fermentation (fermentative maceration) to allow the extraction of color (anthocyanins) and other polyphenolic compounds, then the fermented GP (FGP) is separated from the wine at the end of alcoholic fermentation, after racking off and pressing. Nine different types of GP were analyzed, that were sampled as winemaking byproducts of 7 grape cultivars: 3 white cultivars (Muscat blanc, Arneis, Cortese) and 4 red cultivars (Barbera, Grignolino, Pinot noir and Nebbiolo); Pinot noir was also sampled as UGP (from white winemaking), and Nebbiolo was sampled as FGP after macerations of different duration. The description of the material used is reported in Table 1. For each GP sample, the study focused on the composition of skins and seeds separately.

Table 1. Characteristics of the samples.

	Cultivar	Type of GP	Characteristics
white cultivars	Muscat blanc	UGP	unfermented
	Arneis	UGP	
	Cortese	UGP	
red cultivars	Barbera	FGP	fermented (10 days)
	Grignolino	FGP	fermented (10 days)
	Pinot noir 1	UGP	unfermented
	Pinot noir 2	FGP	fermented (10 days)
	Nebbiolo 1	FGP	fermented, short maceration (5 days)
	Nebbiolo 2	FGP	fermented, long maceration (15 days)

2.1. Sample Preparation

Before drying, UGP was washed with water to remove residual sugars. All GP samples were dried under controlled conditions: pre-drying for 48 h at room temperature in a ventilated and dehumidified room, followed by drying at 40 °C for 48 h, up to constant weight (residual humidity 5–10%). Once dried, the skins were separated from the seeds, and the 2 fractions were separately milled to obtain skin and seed flours.

An extraction method aimed at maximizing the extraction of the polyphenolic component from the flours was applied to determine the total polyphenolic content through the characterization of two different fractions: EPP (extractable polyphenols) and NEPP (non-extractable polyphenols). In parallel, the quantification of other components of dietary interest, such as dietary fiber and total proteins, was carried out.

2.2. Extraction of EPP

The polyphenolic extracts of skins and seed flours were obtained with a double solid/liquid extraction [13]: two successive extractions of 1 g of flour, first with 10 mL of methanol/water 50:50% *v/v* followed by 10 mL of acetone/water 70:30% *v/v* (extraction ratio 1:10 *w/v*). For each extraction, 15 min of ultrasound (50 W, 48 kHz ± 10%), 1 h of orbital shaker (ambient temperature), and 15 min of centrifuge (20 °C, 4000 rpm) were performed. All extractions were performed in duplicate. The supernatants were combined and dried under vacuum at 35 °C with a Genevac evaporator (EZ-2, Genevac®, Ipswich, UK) for the subsequent analysis of extractable polyphenols (EPP).

The residue after extraction was analyzed to determine the fraction of non-extractable polyphenols (NEPP) as condensed tannins (CT).

2.3. Polyphenolic Composition of EPP and NEPP

The content of total polyphenols (GAE), total flavonoids and total anthocyanins in the EPP fraction was determined with spectrophotometric methods [14] with a UV-Vis JASCO V-630 spectrophotometer (JASCO, USA). All analyses were performed in duplicate.

- Total anthocyanins and total flavonoids: The extract was diluted 50-fold with acidified ethanol (ethanol/H₂O/HCl 70:30:1) and the absorbance at 540 nm (total anthocyanins) and 280 nm (total flavonoids) was measured. The results were expressed, respectively, as malvidin and (+)-catechin equivalents.
- Total polyphenols: A total of 1 mL extract was diluted 20-fold with water, and 1 mL of diluted sample was added to 1 mL of Folin–Ciocalteu reagent, basified with 4 mL of sodium carbonate 10% w/v, and filled up to 20 mL. After 90 min, the absorbance at 750 nm was measured. The results were expressed as gallic acid equivalents (GAE).

The condensed tannins (CT) content was determined in the NEPP fraction with the method proposed by [10], based on the Bate–Smith reaction. Strong acidic conditions are needed to release NEPP from the dietary fiber (DF) matrix: The residue after the extraction is treated with 20 mL of 50% HCl in ethanol, containing Fe (II) as catalyst (300 mg/L of FeSO₄ × 7H₂O); blank reading at 532 nm; 30 min boiling with reflux tubes; final reading at 532 nm. The four points calibration curve of the reaction was calculated using the same procedure with 4 polyphenolic extracts with known concentrations of CT: 15, 30, 90 and 120 mg/L (determined with the phloroglucinolysis HPLC method—[12]). The same method with the same calibration curve was used to measure the CT content as EPP in the extracts.

2.4. Determination of Dietary Fiber

The percentage content of dietary fiber (total dietary fiber, TDF) of the flours was determined with the official enzymatic gravimetric method AOAC 985-29 (Total dietary fiber assay kit, Megazyme, Bray (Wicklow), Ireland; Foss Fibertech 1023, Foss Italia S.r.l., Padova, Italy). Briefly, samples were subjected to sequential enzymatic digestion by heat-stable α -amylase, protease and amyloglucosidase to simulate the digestion in the human body, thus separating and quantifying dietary fiber as indigestible residue, after correction for indigestible protein and ash content.

The analysis was extended to the distinction between soluble and insoluble dietary fiber (SDF and IDF—AOAC 991.43) only for the skin samples, since SDF in the seeds is generally present at low concentrations: as grape seeds are not particularly rich in starch and other carbohydrates, the fibrous portion is largely represented by IDF [15,16].

Total nitrogen as indigestible protein fraction was determined in all DF fractions (TDF, IDF, SDF) applying the Kjeldahl method.

2.5. Statistical Analysis

The data were processed with univariate (one-way ANOVA, and Tukey's pairwise comparison test and correlation analysis) and multivariate (PCA and Cluster Analysis) analysis techniques (XLSTAT 2019, Data Analysis and Statistical Solution for Microsoft 250 Excel, Addinsoft, Paris, France, 2019).

3. Results and Discussion

3.1. Skins: Polyphenolic Composition

Table 2 reports the polyphenolic profile of the skin flours. The residual humidity of the flours ranged between 5.4 and 10.0%: All data reported in the table are corrected for humidity and refer to the dried flour weight (d.w.).

Table 2. Polyphenolic profile of the skin flours. The data are expressed in mg/g dry weight (d.w.) of skin flour.

Skins	GAE	Total Flavonoids	CTex	CTnex	CT TOT	Total Anthocyanins
Muscat blanc	23.8 b *	22.6 d	25.8 c	13.7 d	39.5 d	
Arneis	35.5 a	30.3 b	34.4 a	11.6 g	46.0 b	
Cortese	24.9 b	26.4 c	28.9 b	13.8 d	42.6 c	
Barbera	22.0 b	21.1 de	24.9 c	11.9 fg	36.8 e	3.1 b
Grignolino	21.5 b	20.0 de	25.8 c	14.5 c	40.3 cd	0.25 c
Pinot noir 1	34.6 a	34.5 a	35.5 a	12.1 f	47.6 b	6.2 a
Pinot noir 2	28.2 ab	29.0 bc	33.2 a	18.4 a	51.6 a	2.0 b
Nebbiolo 1	22.0 b	18.8 e	30.2 b	15.6 b	45.8 b	0.65 c
Nebbiolo 2	21.7 b	22.2 d	29.4 b	13.2 e	42.5 c	0.64 c
F	13.1	96.3	54.0	1137	90.0	114
Pr > F	0.000	<0.0001	<0.0001	<0.0001	0.000	<0.0001

* Different letters along the column discriminate the samples significantly different from one another ($p < 0.05$, Tukey's test). CTex = condensed tannins in the extractable polyphenolic fraction (EPP). CTnex = condensed tannins in the non-extractable polyphenolic fraction (NEPP). CT TOT = CTex + CTnex.

As regards EPP, the total polyphenol content (GAE) varied between 21.5 and 35.5 mg/g d.w. The highest GAE values were observed for Arneis and Pinot noir 1 (UGP) which were significantly different from all the others, but similar to each other. The only exception was Pinot noir 2, which had intermediate values and was not significantly different from all the others.

These values are in line with the findings of [13,17–19] for GP from French grape varieties, while on average were higher than the values reported by [20,21] for GP from Spanish and Greek grape varieties, ranging from 36.2 and 54.0 mg/g d.w. In any case, [13,19,22], considering documentary bibliographic values, highlight the generally higher polyphenolic content of GP compared to other fruit pomaces.

The CTex content varied between 24.9 and 35.5 mg/g d.w. (Table 2). The highest concentrations of CTex in the skins were found for Pinot noir (1 and 2) and Arneis, followed by a second group formed of Nebbiolo (1 and 2) and Cortese and, finally, a third group with the remaining samples (Muscat blanc, Barbera and Grignolino).

The content of CTnex varied between 11.6 and 18.4 mg/g d.w.: The highest concentration was found for Pinot noir 2 (FGP), followed, in descending order of concentration, by Nebbiolo 1 (short maceration), Grignolino, Cortese and Muscat blanc, Nebbiolo 2 (long maceration), Pinot noir 1 and Barbera and, finally, Arneis. This range of concentration is perfectly in line with the values found for the same raw material (GP skins) and with the same analytical method by [10,13,17,19]. Although significant differences were observed for Pinot noir between UGP (lower values) and FGP (higher values), the cultivar of origin appears to be the most discriminating factor between the GPs.

Considering total CT (CTex + CTnex), their content varied between 36.8 and 51.6 mg/g d.w.: The varietal differences are flattened, though Pinot noir (1 and 2) still maintains the highest values, followed by Arneis and Nebbiolo 1.

Only for red grape skins, total anthocyanins (red pigments) were quantified (Table 2): Their content varied between 0.25 and 6.2 mg/g d.w. The highest concentrations were observed for Pinot noir 1, followed by Barbera and Pinot noir 2, while the total anthocyanins content of the three remaining samples (Grignolino, Nebbiolo 1 and Nebbiolo 2) was considerably lower. The concentration of anthocyanins probably depends both on the original richness of the cultivar [23] and on the duration of the fermentative maceration (contact between skins and must).

3.2. Skins: Dietary Fiber Composition

The data relating to the fiber content of the skins are reported in Table 3.

Table 3. Percentage values (as d.w.) of total, insoluble and soluble fiber, and percentages of the residue of the nitrogenous component (expressed as proteins %) after enzymatic action in the respective fiber fractions.

Skins	Total Fiber (TDF)		Insoluble Fiber (IDF)		Soluble Fiber (SDF)	
	% TDF	% Protein	% IDF	% Protein	% SDF	% Protein
Muscat blanc	69.7 a *	15.7 cd	63.6 a	16.0 c	6.1 cd	12.9 bc
Arneis	63.0 bc	19.7 b	57.4 ab	19.9 b	5.6 cd	17.8 b
Cortese	67.8 ab	16.4 cd	62.3 a	16.3 c	5.5 d	18.3 ab
Barbera	52.8 d	17.0 c	41.3 d	19.1 b	11.6 b	9.6 c
Grignolino	63.6 b	15.3 d	47.1 cd	17.9 bc	16.5 a	7.8 c
Pinot noir 1	56.8 d	17.2 c	53.6 bc	16.9 c	3.1 e	24.4 a
Pinot noir 2	52.0 d	21.5 a	45.4 d	22.6 a	6.6 c	13.6 bc
Nebbiolo 1	55.0 d	17.3 c	43.1 d	19.2 b	11.9 b	10.5 c
Nebbiolo 2	57.3 cd	16.1 cd	45.9 d	17.9 bc	11.4 b	9.0 c
F	35.99	40.02	50.9	29.5	505	21.3
Pr > F	0.000	0.000	<0.0001	<0.0001	<0.0001	<0.0001

* Different letters along the column discriminate the samples significantly different from one another ($p < 0.05$, Tukey's test).

The total fiber content (TDF) varied from 52.1% of Pinot noir 2 to 69.7% of Muscat blanc; no significant differences were observed between the two samples of the same cultivar (52–57% for Pinot noir and 55–57% for Nebbiolo), subjected to different winemaking processes. Subsequently, the analysis was extended to the distinction between the insoluble fiber (IDF: cellulose, hemicelluloses with a higher polymerization degree, and lignin) and the soluble fiber (SDF: pectins and hemicelluloses, soluble in water): IDF prevailed on SDF in all samples.

Overall, white grape skins had on average higher concentrations of TDF and, above all, of IDF, compared to red grape skins. As regards the IDF content, two significantly different groups were distinguished: the first consisting of Muscat blanc, Cortese and Arneis (significantly higher values) and the second consisting of Pinot noir 2, Nebbiolo (1 and 2) and Barbera; finally, intermediate values were found for Pinot noir 1 (UGP) and Grignolino (FGP with short maceration).

The SDF content was on average lower in UGP samples, while the IDF content was lower in FGP samples. This trend suggests a structural modification of the fiber matrix during processing. Specifically, during the fermentative maceration of FGP, a fraction of the IDF may have undergone hydrolysis and subsequent release into the must. This phenomenon is likely driven by the synergistic action of the acidic environment (low pH) and enzymatic activity—both from endogenous grape hydrolases and potentially added oenological enzymes (e.g., pectinases and cellulases) used to enhance color extraction [24].

Furthermore, the dynamics of SDF in red winemaking are influenced by the evolving solvent polarity; the progressive increase in ethanol concentration during fermentation may have triggered the partial precipitation of soluble polysaccharides, such as pectins, which are then lost during the separation of the liquid phase from the pomace [25]. Regarding FGP, the significantly higher SDF concentrations found in Grignolino, Barbera, and Nebbiolo suggest that varietal characteristics and skin-to-pulp ratios may play a critical role in preserving the soluble fiber fraction, despite the maceration process.

According to [26], the high IDF content can be considered a positive result in view of possible uses of GP as a dietary supplement to increase the content of indigestible

insoluble compounds [27]. On the other hand, [28] reported that the low SDF content of GP limits its use for food application due to the healthier benefits for human nutrition of the SDF fraction. In this sense, our results show how red GP (Barbera, Grignolino and Nebbiolo) may be more interesting for the food industry than white GP.

Table 3 shows the percentage of nitrogenous compounds that were found in the residues at the end of the enzymatic process, mainly proteins indigestible by the proteases used in the hydrolysis step of the method AOAC 985-29. As regards IDF, the nitrogen residue represented on average about 18.5% of the collected residue and the values ranged from a minimum of 16.0% for Muscat blanc to a maximum of 22.6% for Pinot noir 2. As regards SDF, the indigestible protein residue was on average lower than in the IDF fraction, with an average value of 13.8%, but with a much wider range, from a minimum of 7.8% for Grignolino, to a maximum of 24.4% for Pinot noir 1. However, considering that the overall percentage of SDF was significantly lower than the IDF one, the mass quantities of the nitrogen residues in SDF were quite limited, and not enough to consider them as a source of proteins or amino acids (after hydrolysis). Conversely, it could be degraded by gut microorganisms and therefore represent a source of amino acids for the bacteria themselves.

3.3. Skins: Relationship Between the Analyzed Chemical Variables and Between the Studied Cultivars

A Pearson's correlation analysis was first conducted between all the parameters determined for the skins (Table 4).

Table 4. Pearson's correlation matrix between the parameters relating to dietary fiber and polyphenols in the skins.

Variables	TDF %	IDF%	SDF%	SDF/IDF Ratio	%N IDF	%N SDF	GAE	Total Flavonoids	CTex	CTnex	CT TOT
TDF%	1	0.867	−0.186	−0.345	−0.693	0.144	−0.011	−0.040	−0.293	−0.256	−0.371
IDF%	0.867	1	−0.650	−0.766	−0.593	0.547	0.357	0.386	0.087	−0.276	−0.053
SDF%	−0.186	−0.650	1	0.984	0.113	−0.858	−0.719	−0.822	−0.618	0.154	−0.461
SDF/IDF ratio	−0.345	−0.766	0.984	1	0.206	−0.846	−0.701	−0.796	−0.564	0.164	−0.410
% N IDF	−0.693	−0.593	0.113	0.206	1	−0.202	0.162	0.098	0.350	0.526	0.546
% N SDF	0.144	0.547	−0.858	−0.846	−0.202	1	0.779	0.873	0.708	−0.290	0.475
GAE	−0.011	0.357	−0.719	−0.701	0.162	0.779	1	0.893	0.798	−0.262	0.566
Total flavonoids	−0.040	0.386	−0.822	−0.796	0.098	0.873	0.893	1	0.817	−0.168	0.625
CTex	−0.293	0.087	−0.618	−0.564	0.350	0.708	0.798	0.817	1	0.052	0.886
CTnex	−0.256	−0.276	0.154	0.164	0.526	−0.290	−0.262	−0.168	0.052	1	0.510
CT TOT	−0.371	−0.053	−0.461	−0.410	0.546	0.475	0.566	0.625	0.886	0.510	1

Values in bold are different from 0 with a significance level $\alpha = 0.05$.

The trend of the total flavonoid content was similar to that of the GAE index: As reported in Table 4, the two variables were, in fact, significantly and positively correlated with each other ($r = 0.89$). Flavonoids are the most abundant class of molecules in the polyphenolic fraction, and therefore the major cause responsible for the modification of the GAE index. Also, the condensed tannins content (CTex) was significantly correlated with total polyphenols (GAE) and total flavonoids, although with lower r values (0.80 and 0.82, respectively).

No correlation was observed between the condensed tannin content of the NEPP fraction (CTnex) and that of the EPP fraction (CTex), nor between CTnex and GAE and total flavonoids. The concentration of CTnex varied with the raw material: The origin as UGP or FGP did not seem to have a discriminating effect, but the varietal differences prevailed.

A negative correlation was observed between SDF and IDF. This result confirms what was previously reported: The increase in SDF observed in FGP compared to UGP was always associated with a decrease in IDF, probably due to hydrolysis phenomena. On the

other hand, the correlation between TDF and IDF (which represents the largest share of TDF) was highly significant and positive, because, in addition to the reduction in IDF (hydrolysis from IDF to SDF), losses in dietary fiber also occurred during fermentation (FGP), probably due to precipitation.

Furthermore, the positive correlation observed between polyphenolic fractions and the nitrogen residue within the SDF fraction confirms the influence of the matrix in the application of the method for dietary fiber quantification [29]. In grape pomace, first, high concentrations of polyphenols may partially inhibit protease activity during the enzymatic stages of the AOAC method, leading to incomplete protein removal [30]. Second, the SDF fraction inherently includes “antioxidant dietary fiber” – polyphenols chemically bound to polysaccharides that can, in turn, sequester protein fractions, further inflating the gravimetric yield [20]. Conversely, the correlations between the SDF/IDF ratio and its individual components are considered artifacts of the mathematical calculation and do not imply a biological or chemical relationship.

Finally, a significant negative correlation was observed between the nitrogen residue of the SDF and the percentage of SDF; notably, this trend was not found for the IDF component. This relationship suggests a “dilution effect” within the soluble matrix: As the extraction yield of soluble polysaccharides increases, the relative proportion of co-precipitated nitrogenous compounds decreases, possibly due to a higher purity of the solubilized pectins [31].

Regarding the independence between fiber fractions (TDF, IDF, SDF) and condensed tannins (CTex, CTnex), this result can be explained by their distinct biological origins and locations within the grape berry. While dietary fiber constitutes the primary structural framework of the cell wall (cellulose, hemicellulose, and pectins), condensed tannins are secondary metabolites primarily stored in the vacuoles or associated with the cell wall through non-covalent bonds [32]. Their concentration in the resulting flour is therefore governed by different dynamics: The fiber content is a genetic trait related to the skin-to-pulp ratio and cell wall density, whereas the tannin content in the pomace is heavily modulated by the extraction kinetics during maceration and the specific winemaking conditions (e.g., ethanol concentration and temperature), which do not significantly alter the structural polysaccharide matrix [33].

The parameters “fiber content” (TDF, IDF and SDF) and “CTex” or “CTnex” were independent of each other, because they varied with the type of GP analyzed (grape cultivar, winemaking process).

All data (skin polyphenolic composition and fiber content) were subjected to Principal Component Analysis (PCA). Figure 1 shows the representation of the loadings (variables) and the scores (skin samples from different cultivars) in the space defined by the first two Principal Components, that overall describes 80% of the variability of the original data.

The first Principal Component is positively associated with the variables expressing the polyphenolic content of the skins (GAE, total flavonoids, CT TOT and CTex) and with the protein fraction of SDF, while it is negatively associated with SDF%. The second Principal Component is positively associated with the content of non-extractable condensed tannins (CTnex) and with the protein fraction of IDF, and negatively associated with IDF% and TDF%.

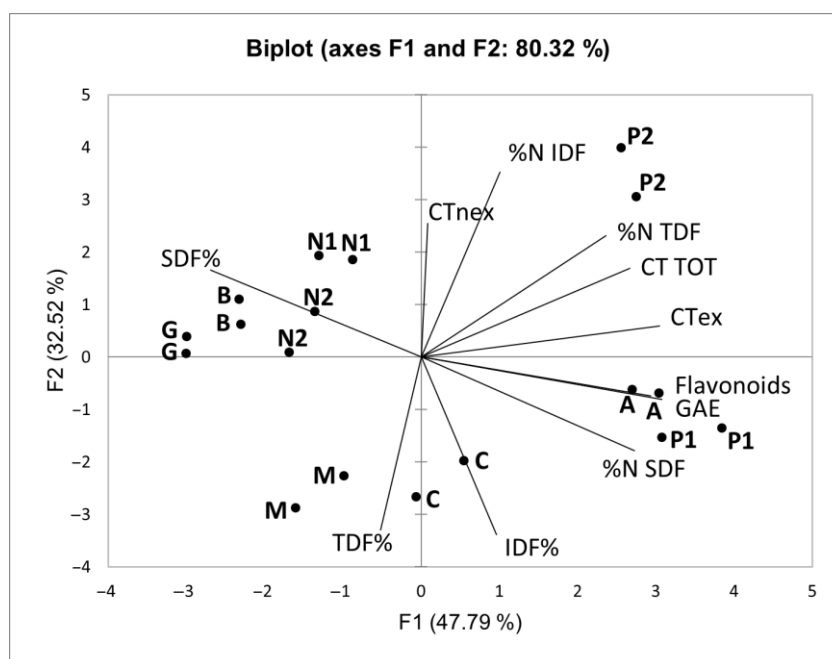


Figure 1. Graphical representation of the loadings (original variables: dietary fiber and polyphenolic parameters) and scores (skin samples, in bold) in the space described by the first two Principal Components (BiPlot) (Principal Component Analysis).

The first axis (PC1) discriminates the skin samples from each other based on their varietal origin and on the winemaking technique: In particular, Pinot noir (P1 and P2), Arneis and Cortese are distinguished from the others by the higher polyphenolic content and the lower SDF%. In general, the skins of red grape cultivars have a higher polyphenolic content than those of white grape cultivars, also due to the presence of high concentrations of anthocyanins, absent in white grapes. In red winemaking, during fermentation, significant losses of polyphenolic compounds (anthocyanins and tannins) from the skins are observed, due to extraction into the must as well as oxidation or precipitation [34]. The residual content of polyphenolic compounds in FGP at the end of fermentation/macération depends on many factors, including the duration of macération (duration of contact of the skins with the must) and the cultivar of origin (initial content of the grapes). From the distribution of the skin samples along the first axis (PC1), it can be observed that the skins of a red grape cultivar (Pinot noir 2), after fermentation/macération, can have an even higher polyphenolic content than the skins of white grape cultivars from non-fermented grapes (Moscato bianco and Cortese).

On the other hand, the second axis (PC2) discriminates FGPs from UGPs (winemaking technique: fermented vs. unfermented), due particularly to the higher content of non-extractable condensed tannins (CTnex) and to the lower IDF%. When considering the chemical composition data as a whole (Cluster Analysis with Ward's method), all FGPs, except Pinot noir 2, are distinguished from UGPs. Pinot noir 2 is classified in this second group mainly due to the higher polyphenolic content (extractable fraction) on the one hand, and to the lower SDF content on the other.

Overall, it is important to notice that the grape variety of origin influences above all the polyphenolic content of the extractable fraction (what is normally determined through chemical analysis), while the winemaking technique (fermented vs. unfermented) exerts its effect mainly on the IDF% content.

3.4. Seeds: Polyphenolic Composition

Table 5 shows the chemical composition of seed flours. The residual humidity of the flours ranged between 5.5 and 8.1% (data not reported): All data reported in the table are corrected for humidity and are referred to the dried flour weight (d.w.).

Table 5. Polyphenolic profile and TDF content of seed flours. The data are expressed as mg/g dry weight (d.w.) of seed flour.

Seeds	GAE	Total Flavonoids	CTex	CTnex	CT TOT	Total Anthocyanins	% TDF	% Protein
Muscat blanc	80.5 b *	159.2 a	77.4 c	4.8 a	82.2 c		72.7 ab	12.5 ab
Arneis	59.3 c	90.2 c	58.4 d	4.5 c	63.0 d		77.1 a	12.1 ab
Cortese	71.9 b	135.1 ab	81.8 ab	4.2 c	86.0 ab		69.5 ab	13.7 a
Barbera	38.6 de	55.6 de	37.8 f	3.4 d	41.2 f	0.23 b	70.5 ab	9.7 c
Grignolino	44.0 d	75.3 cd	59.3 d	4.9 a	64.3 d	0.27 b	68.8 ab	12.2 ab
Pinot noir 1	94.3 a	154.7 a	84.1 a	4.3 c	88.4 a	0.64 a	62.3 b	13.2 ab
Pinot noir 2	77.3 b	122.4 b	80.6 bc	4.4 bc	84.9 bc	0.44 ab	64.8 ab	12.8 ab
Nebbiolo 1	46.9 d	70.5 cd	46.3 e	3.6 d	49.9 e	0.24 b	71.9 ab	11.1 bc
Nebbiolo 2	28.7 e	35.1 e	29.9 g	4.3 bc	34.2 g	0.59 a	70.4 ab	9.1 c
F	140	65.0	1232	190	1365.5	14.9	3.1	14.5
Pr > F	<0.0001	<0.0001	<0.0001	<0.0001	0.000	0.003	0.1	0.0

* Different letters along the column discriminate the samples significantly different from one another ($p < 0.05$, Tukey's test). CTnex = condensed tannins in the extractable polyphenolic fraction (EPP). CTnex = condensed tannins in the non-extractable polyphenolic fraction (NEPP). CT TOT = CTex + CTnex.

The highest concentration of total polyphenols (GAE) was observed for Pinot noir as UGP (Pinot noir 1), then, in decreasing order of concentration, Muscat blanc, similar to Pinot noir 2 (FGP), Cortese and Arneis. The other FGP samples with lower GAE concentrations ranked, in decreasing order of polyphenolic content, as follows: Nebbiolo 1 (short maceration), Grignolino, Barbera and Nebbiolo 2 (long maceration). A similar trend was observed for total flavonoids and CTex.

The condensed tannin content in the NEPP fraction (CTnex), despite the presence of significant differences (Table 5), varied little between the different samples and was independent from the polyphenol content of the extractable fraction (GAE, total flavonoids and CTex).

Considering total CT (CTex + CTnex), their content varied between 34.2 and 88.4 mg/g d.w.: The technological and varietal differences are noticeable, since high values were observed for all UGPs and, among FGPs, Pinot noir and Grignolino prevailed.

Finally, red grape seeds had a low content of total anthocyanins (Table 5), adsorbed on the surface of the seeds during the fermentative maceration. The concentration in total anthocyanins was low, with significant differences between the samples. The highest values were observed for Pinot noir 1 (UGP) and Nebbiolo 2 (FGP with long maceration): The total anthocyanin content, therefore, does not seem to depend on the duration of the contact between seeds and skins, probably because the adsorption phenomena of the coloring substance are very rapid, or they may also depend on the characteristics of the outermost cortical part of the seeds.

3.5. Seeds: Dietary Fiber Composition

The seeds were all very rich in dietary fiber, with concentrations ranging from 62.3% of Pinot noir 1 to 77.1% of Arneis (Table 5). It is interesting to notice that, in the case of the two cultivars of which two different samples have been characterized (Pinot noir and Nebbiolo), the measured DF contents were very close, independently of the winemaking technique (70–71% for Nebbiolo and 62–64% for Pinot noir). In decreasing order of DF

content, the samples ranked as follows: Arneis with the highest value (77%), followed by Barbera, Nebbiolo and Muscat blanc with values ranging between 70 and 72%, then Grignolino and Cortese (values below 70%), and finally Pinot noir with the lowest DF content.

The percentage content of the nitrogen residue (expressed as proteins %) in TDF varied from 9.1 to 13.7% (Table 5): Cortese seeds (highest content) were significantly different from those of Barbera and Nebbiolo (1 and 2) (lowest values). In this regard, procedures for the extraction of proteins from grape seeds are currently under study [16], and some works [35] have verified interesting results with their use as protein clarifiers in wines.

3.6. Seeds: Relationship Between the Analyzed Chemical Variables and Between the Studied Cultivars

The correlation between variables and the multivariate analysis were also performed for the seed samples. Table 6 shows the correlation matrix between the chemical parameters that describe the polyphenolic content of seed flours: Highly significant correlation coefficients between GAE, total flavonoids and C_{Tex} were observed ($r = 0.964$ between GAE and total flavonoids, $r = 0.938$ between GAE and C_{Tex} and $r = 0.940$ between total flavonoids and C_{Tex}), higher than those reported for the skins. The close correlation between these analytical parameters depends, as already highlighted in a previous work [36], on the fact that seeds contain essentially a single class of polyphenols, namely condensed tannins, which show a homogeneous response to the different analytical methods.

Table 6. Pearson's correlation between the parameters relating to dietary fiber and polyphenols in the seeds.

Variables	TDF%	%N TDF	GAE	Total Flavonoids	C _{Tex}	C _{Tnex}	CT TOT
TDF%	1	−0.344	−0.355	−0.280	−0.374	0.012	−0.370
% N TDF	−0.344	1	0.814	0.817	0.922	0.417	0.922
GAE	−0.355	0.814	1	0.964	0.938	0.297	0.935
Total flavonoids	−0.280	0.817	0.964	1	0.940	0.370	0.940
C _{Tex}	−0.374	0.922	0.938	0.940	1	0.416	1.000
C _{Tnex}	0.012	0.417	0.297	0.370	0.416	1	0.436
CT TOT	−0.370	0.922	0.935	0.940	1.000	0.436	1

Values in bold are different from 0 with a significance level $\alpha = 0.05$.

Besides the expected correlations between the three main polyphenolic indexes (GAE, flavonoids and C_{Tex}) reported in Table 6, it is also interesting to notice the correlation between these indexes and the nitrogen residue ($r = 0.814$ between %N and GAE, $r = 0.817$ between %N and total flavonoids, and $r = 0.922$ between %N and C_{Tex}). Also, in this case, it can be assumed that a higher polyphenol content may inhibit the action of proteases during the hydrolysis process in the fiber extraction method, or that part of this fraction remains bound to polyphenols.

The results of the multivariate analysis with PCA, calculated with the parameters relating to the polyphenolic and TDF content of the seed samples, are reported in Figure 2.

The first Principal Component is positively associated with the extractable polyphenolic components of the seeds (GAE, total flavonoids, C_{Tex}, and CT TOT). The seed samples from unfermented GP (UGP) are positioned on the positive side of the first Principal Component. The lower polyphenolic content of FGP samples is probably due to losses that occur during the fermentative maceration of the grapes. Pinot noir is an exception: Its high natural polyphenolic content in the seeds [12,37] has influenced the high polyphenolic concentration of FGP samples, which are close to UGP samples.

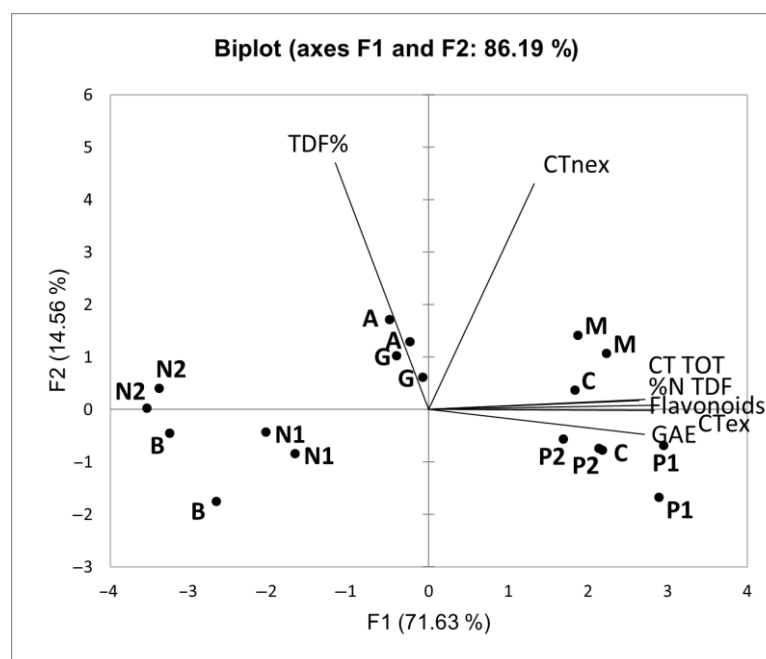


Figure 2. Graphical representation of the loadings (original variables: dietary fiber and polyphenolic parameters) and scores (seed samples, in bold) in the space described by the first two Principal Components (BiPlot) (Principal Component Analysis).

The second Principal Component, positively associated with the TDF and CTnex parameters, does not allow for discriminating seed samples either by the winemaking technique or by the cultivar of origin. It should be noted, however, that the variability described by the second Principal Component is only 14.56% of the total variability (about five times lower than that of the first PCA). The data were then subjected to Cluster Analysis (Ward's method) which, as for the skin samples, classified the seed samples into two groups: one consisting of the UGP samples and Pinot noir as FGP (Pinot noir 2), and the other consisting of the remaining FGP samples. Excluding Arneis, the same distribution is observed along the first Principal Component.

3.7. Comparison Between Skins and Seeds Composition

Significant differences in polyphenolic content were observed between skins and seeds: For all samples, the skins had much lower GAE values than the corresponding seeds, from -70% for Muscat blanc to -25% for Nebbiolo 2. The GAE index was highly and positively correlated with the condensed tannins content, which was higher in the seeds, especially in those deriving from UGP, with significant differences between cultivars.

The NEPP fraction (CTnex) was higher in the skins, from 2.6 (Arneis) to 4.4 times (Nebbiolo 1), than in the respective seeds. This difference may be explained by the different lignin content of skins and seeds (higher in seeds, lower in skins), as reported by [26]. As lignin is synthesized by the same route of flavonoids, lower lignin contents may correspond to higher NEPP concentrations. Despite the presence of significant differences, the NEPP content was more homogeneous among the seeds from different cultivars (3.4–4.9 mg/g d.w.) compared to the skins (11.6–18.4 mg/g d.w.).

These dietary polyphenols linked to the dietary fiber matrix can be considered as prebiotics, since they represent a fermentable substrate for gut bacterial microflora in addition to indigestible carbohydrates and proteins [10].

In accordance with documentary bibliographic values, grape pomace represents a superior source of dietary fiber compared to other fruit and vegetable matrices [10,38–41]. Our results confirm that TDF content is significantly higher in seeds than in skins. The

lack of correlation ($r = 0.38$) between skins and seeds underscores their distinct physiological roles and structural compositions; seeds are characterized by a highly lignified endocarp designed for embryo protection, whereas skins consist of parenchymal cells with a different pectin–cellulose balance [42,43]. Limited to skins, it is interesting to notice how the management of the winemaking process has influenced the quantity and characteristics of the skin fiber: The TDF and IDF content was higher in UGP skins, while the opposite was observed for the SDF fraction.

Limited to skins, it is interesting to notice how the management of the winemaking process has influenced the quantity and characteristics of the skin fiber: The TDF and IDF content was higher in UGP skins, while the opposite was observed for the SDF fraction.

These results are original: To our knowledge, the determination of skins and seeds DF has never been carried out before on GP from Piedmont grapes. Considering the data as a whole, our results (TDF%: 52.1–77.1; IDF%: 41.3–63.6; SDF%: 3.14–16.5) are in line with the findings of [13,17,26,42,44,45], obtained for whole grape pomace (skins + seeds), while [19] obtained much lower values and greater difference between white (lower values) and red (higher values) grape varieties.

4. Conclusions

A partial characterization of different kinds of grape pomace sampled after white and red winemaking was carried out, focusing on two macroclasses of compounds that are interesting for the functionality of this byproduct: the polyphenolic fraction and the dietary fiber fraction.

The content of both classes of molecules was significantly higher in seeds than in skins. However, when considering the nutritional value of GP flours as a possible food ingredient (e.g., as a dietary supplement), it is interesting to notice that the polyphenolic fraction linked to the fiber (NEPP), considered as prebiotic, was on average more than three times higher in skins than in the respective seeds (13.9% by weight for skins, 4.3% for seeds, on average). Moreover, the fiber obtained from skins may also be more interesting from a dietary point of view due to the presence of SDF (3.1–16.5% by weight, on average 8.7%), almost absent in grape seeds.

These results are of practical interest, when the use of flour is aimed at enriching food in polyphenolic compounds and dietary fiber. Therefore, depending on the origin of GP, its use could be directed to different areas.

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