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Polyphenols in Sugar Beet Leaves (SBL): Composition, Variability and Valorization Opportunities

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Abstract

During mechanical harvesting, the above-ground biomass (sugar beet leaves) is routinely cut and left on the field as green fertilizer and represents a underuti-lisation of a seasonal stream of biomass with provable potential of bioactive compounds. The aim of this study was to show the distribution of polyphenol and protein content in the leaf blade and petioles in different sugar beet cultivars and different harvest times. Quantified total polyphenols and delineated the phenolic composition of SBL using complementary HPLC-DAD and LC-MS methods. In sugar beet leaf blades, protein content was from 19% to 29% and significantly affected by Individual cultivars and harvest date. Petioles showed a significantly lower protein content, typically ranging from 4.9% to 9.5%. The total polyphenol content (TPC) quantified using vitexin as a standard and was in the range of 7.8-11.0 mg/g DW for blades and 0.8 – 2.7 mg/g DW for petioles and it also depended on the harvest date for individual varieties. Leaf blades contained substantially higher concentration of vitexin derivatives (mean 7.4 ± 2.3 mg/g DW) than petioles (1.1 ± 0.6 mg/g DW). The percentage contribution of vitexin derivatives in to total polyphenol content in SBL blades and petioles was high across all samples (above 70%) and decreased with the delay in harvest time.

Keywords: sugar beet leaves; polyphenols; vitexin; proteins; HPLC-DAD; LC-MS

1. Introduction

Sugar beet (*Beta vulgaris L.* subsp. *vulgaris*) is a cornerstone crop for the European sugar industry. Poland is significant producer of sugar beets, in 2023/2024 according to Polish Sugar Association data the production volume was over 16 million tons [1]. Sugar beet root is the main source of sugar production in temperate climates. Approximately 75% of the beet's mass is water, and the main reserve substance, 15-20%, is sucrose. The remaining dry matter is mostly composed of building blocks: primarily pectin and pectin-forming compounds, and cellulose. Unlike the root, the above-ground part of the plant, the leaves, contain significantly more nutrients and bioactive substances because they function for synthesis, not storage. They contain antioxidant and antibacterial substances including: flavonols, saponins, quercetin, apigenin, folic acid, ferulic acid, omega-3 and -6 fatty acids, and others [2,3].

During mechanical harvesting, the above-ground biomass—sugar beet leaves (SBL)—is routinely cut and left on the field as green fertilizer. While this practice returns nutrients and organic matter to the soil, it also represents a substantial underutilisation of a consistent, seasonal stream of plant biomass with provable potential of high-value compounds in circular "waste-to-resource" strategies [2–4]. In that context, SBL should be increasingly viewed not as field residues but as a feedstock for integrated, low-footprint biorefineries positioned near the point of biomass generation [4].

Chemically, SBL comprise structural carbohydrates (cellulose, hemicelluloses, pectins), proteins (including RUBISCO-rich fractions), lipids, minerals and specialized metabolites. Reported dry-matter composition indicates substantial protein alongside carbohydrate and fiber fractions, with ranges shaped by cultivar, season and agronomy [4,5]. Within specialized metabolites, phenolics are prominent—mainly phenolic acids (e.g., caffeic/ferulic derivatives) and flavonoids (flavones, flavones)—and are readily resolved by contemporary LC-MS/MS) methods [6,7]. Beyond phenolic, SBL contain triterpenoid saponins typical of leafy vegetables. These components are important for plant function, their resistance as well as the potential for their valorization [8,9].

In sugar beet leaves, both polyphenol content and biological activity change over the course of development; high values are often observed around day ~60 (younger leaves), although some methods and post-processing steps yield later maxima (e.g., higher polyphenol content after dehydration at ~day 100). The relationship between total phenolic content and anti-inflammatory/antibacterial activity is generally positive, but depends on compound profile and assay conditions. [3,6]

The techno-economic rationale for SBL valorization is reinforced by the accordance of leaf protein recovery with phenolic extraction. On the protein side, leaf protein concentrates obtained via mild thermal or pH coagulation and membrane steps show favorable amino-acid profiles and techno-functional properties (solubility, foaming, emulsification), enabling co-products for food, feed or materials [10,11]. On the phenolic side, scalable routes span, aqueous/buffer extraction, enzyme-assisted disruption and membrane separations; more recently, green-solvent concepts such as natural deep eutectic solvents (NADES) have been explored to improve selectivity and sustainability [12,13].

Despite this promise, industrial uptake of SBL remains limited. Key bottlenecks include (i) heterogeneity in biomass composition driven by genotype, environment and harvest timing; (ii) co-extraction of chlorophylls, lipids and nucleic acids that can impair color, flavor and functionality; (iii) high endogenous polyphenol oxidase (PPO) activity that accelerates enzymatic browning and phenolic loss upon tissue disruption; and (iv) logistics of collecting and stabilizing a perishable, water-rich feedstock during a narrow sugar-campaign window [3,4]. These issues underscore the need for mild, selective process conditions and rapid stabilization to preserve phenolic integrity while meeting food/feed safety constraints.

Current literature points to several knowledge gaps specific to SBL. First, reported phenolic profiles and total polyphenol contents vary widely across studies, reflecting differences in cultivars, growth stage, agronomy and analytical methods [4,6,10]. Second, systematic assessments of inter-seasonal and varietal variability in SBL phenolics remain sparse, limiting robust specifications for process design. Third, while integrated routes that co-recover phenolics and proteins are conceptually attractive, quantitative trade-offs between phenolic retention, antioxidant performance and protein concentrate purity/functionality are not yet fully resolved at pilot-relevant scales [10–14].

Analyzing characterization often involves spectrophotometric assays of total phenolics/antioxidant capacity (Folin-Ciocalteu; DPPH/ABTS/FRAP) and targeted/untargeted LC-MS/MS). Harmonizing analytical strategies that connect fast spectrophotometric screening with confirmatory LC-MS/MS) profiling would make it easier to compare across labs and seasons [6,7].

From a sustainability perspective, valorizing SBL could diversify revenues at the farm–factory interface. Appropriate logistics and application solutions enabling the extraction and production of natural bioactive substances based on SBL would result in beneficial changes in the form of effective and sustainable development of the sugar industry. Delivering such outcomes requires a combined view of composition, processing and function: i.e., understanding how mild operating variables (pH, temperature, residence time and oxygen exposure) govern phenolic recovery, stability and bioactivity, while yielding protein fractions with target techno-functional attributes[4,14].

Aim of the study. In this work we (i) quantify total polyphenols and delineate the phenolic composition of SBL using complementary HPLC-DAD and LC-MS methods; (ii) develop and compare extraction routes that co-recover a phenolic-rich extract and an protein from the same

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biomass; and (iii) we show the distribution of polyphenol and protein content in the leaf blade and petioles in different sugar beet cultivars and different harvest times.

2. Results and Discussion

2.1. Extraction Parameters

Before the entire research material was analyzed, one selected variety of beet leaves was subjected to preliminary extraction in order to select the extractant and its concentration. The JG variety was extracted using methanol (MtOH) and ethanol (EtOH) at various concentrations (50%, 60% and 70%). In all optimization experiments, the extraction was carried out under the conditions later applied in the main study (Section 3.2). Homogenized mixture of leaf blades or petioles was combined with solvent, sonicated in an ultrasonic bath for 15 min at room temperature.

The obtained results indicated that both the type of solvent and its concentration had a significant impact on the efficiency of polyphenol extraction in order to optimize the extraction stage.

The highest total polyphenol content was obtained using 70% methanol as the solvent. In the case of the leaf blade, the value reached 11.7 mg/g DW, while for the en-tire leaf it was 12.3 mg/g DW. These results were higher than those achieved with ethanol at comparable concentrations, demonstrating greater effectiveness of methanol as an extractant for polyphenols. In contrast, for the leaf petioles, the highest polyphenol content (0.6 mg/g DW) was recorded for extraction with 70 and 60% methanol and ethanol. This may result from differences in the chemical composition of individual leaf parts and the varying polarity of phenolic compounds present in the petioles. For both methanol and ethanol, an increase in solvent concentration up to 70% generally improved the extraction efficiency. However, in the case of ethanol, increasing the concentration above 60% did not lead to a further significant enhancement of extraction yield, unlike methanol.

Extraction using 70% MtOH as the extractant was used to evaluate the polyphenol content in all analyzed SBL samples (blades and petioles).

Table 1. The average content (± SD) of TPC in petioles and blades of the JG variety extracted using methanol (MtOH) and ethanol (EtOH) at various concentrations (50%, 60% and 70%).

	TPC mg/g DW					
	MtOH 70%	**MtOH 60%**	**MtOH 50%**	**EtOH 70%**	**EtOH 60%**	**EtOH 50%**
petioles	$11.7^{a} \pm 0.4$	$11.0^{a,b} \pm 0.1$	$10.4^{b} \pm 0.5$	$8.3^{c} \pm 0.2$	$8.9^{b,c} \pm 0.9$	$6.3^{d} \pm 0.5$
blades	$0.6^{a} \pm 0.07$	$0.6^{a} \pm 0.03$	$0.4^{b} \pm 0.03$	$0.6^{a} \pm 0.11$	$0.6^{a} \pm 0.18$	$0.4^{b} \pm 0.06$

^{a,b,c,d} – homogeneous groups.

2.2. Dray Weight Content

The analysis of dry weight (DW) content in sugar beet petioles and blades collected in August, September and October revealed statistically significant differences between plant organs, cultivars, and sampling periods. According to the dataset presented at Table 2, mean DW values for blades were 17.5% (range 14.5%–20.6%) in August, 19.8% (16.3%–23.9%) in September, and 20.2% (14.8%–19.7%) in October.

Table 2. The average content of dry weight (DW) (± SD) in parts of sugar beet leaves. Tables should be placed in the main text near to the first time they are cited.

	DW, %					
Harvest	**August**		**September**		**October**	
Varieties	**blades**	**petioles**	**blades**	**petioles**	**blades**	**petioles**
JK	15.3 ± 0.3	13.1 ± 0.2	16.3 ± 0.3	13.3 ± 0.2	18.9 ± 0.2	11.7 ± 0.3

PA	15.6 ± 0.3	12.9 ± 0.3	18.5 ± 0.3	14.9 ± 0.2	19.7 ± 0.2	11.8 ± 0.2
GL	14.5 ± 0.2	12.4 ± 0.3	17.5 ± 0.3	18.2 ± 0.3	17.4 ± 0.2	13.1 ± 0.2
WO	15.1 ± 0.3	12.9 ± 0.2	17.6 ± 0.2	13.1 ± 0.2	16.2 ± 0.2	11.8 ± 0.2
JG	15.9 ± 0.7	12.3 ± 0.2	16.7 ± 0.8	12.6 ± 0.2	16. 9 ± 0.6	10.6 ± 0.3
JN	18.8 ± 0.2	21.7 ± 0.1	22.9 ± 0.2	14.3 ± 0.3	14.8 ± 0.2	15.8 ± 0.2
MR	20.6 ± 0.2	23.9 ± 0.2	23.9 ± 0.2	15.3 ± 0.3	15.2 ±0.2	16.9 ± 0.2
SL	20.1 ± 0.2	21.7 ± 0.3	21.0 ± 0.2	14.5 ± 0.2	15.1 ± 0.3	15.3 ± 0.2
OR	19.4 ± 0.3	22.2 ± 0.2	229 ± 0.3	15.1 ± 0.2	16.8 ± 0.3	16.1 ± 0.3
ZG	19.2 ± 0.4	22.2 ± 0.3	22.6 ± 0.3	14.8 ± 0.3	16.2 ± 0.2	16.1 ± 0.2

Petioles exhibited systematically lower dry matter contents of 13.8%, 15.1%, and 13. 9% for the same respective months. These results indicate that leaf blades generally accumulate more dry matter than petioles, and that the DW content increases from August to September, remaining stable thereafter. This trend is consistent with the physiological redistribution of assimilates during the later stages of sugar beet growth, when carbon allocation favors storage organs and older leaves undergo partial senescence accompanied by higher proportions of structural and phenolic compounds [3]. The obtained DW values for sugar beet blades are slightly higher than the lower range reported for fresh leaves in the literature, which typically spans 10–16% depending on genotype, growth stage, and environmental conditions. Vissers et al. [3] observed similar variability in sugar beet leaves, with older leaves showing increased metabolic and phenolic activity, potentially leading to elevated DW percentages. Values reported for fresh SBL biomass by Ebrahimi et al. [15] amounted about 18%.The dry matter of the fresh sugar beet reported by Dukić et al. [16] was 22.97 ± 0.32%.

Simultaneously, genotypic variability among cultivars could be also appears significant, which confirms the thesis that genetic background and phonological stage strongly affect dry matter deposition in leaves. Environmental factors—such as nitrogen fertilization, rainfall, and temperature—are likewise known to influence biomass composition and water relations in sugar beet foliage [15]. High nitrogen levels, for example, may increase soluble nitrogenous compounds but reduce carbohydrate deposition, thereby modulating DW outcomes.

2.3. Protein Content

Leaf blades exhibited protein content (Figure 1) ranging from approximately 19% to 29%. In sugar beet leaf blades, protein content was significantly affected by harvest date. Post hoc comparisons indicated three distinct homogeneous groups, with the lowest mean value in September (20.7%), a slightly higher level in October (21.2%), and the highest content in August (25.6%). Individual cultivars, such as Smart Latoria, exhibited exceptionally high statistical levels than others, reaching its maximum in October (29.6%), which may reflect genotype-specific accumulation or local environmental factors. Petioles showed a significantly lower protein content, typically ranging from 4.9% to 9.5%. Very small differences but statistically significant were observed between the different harvest months.

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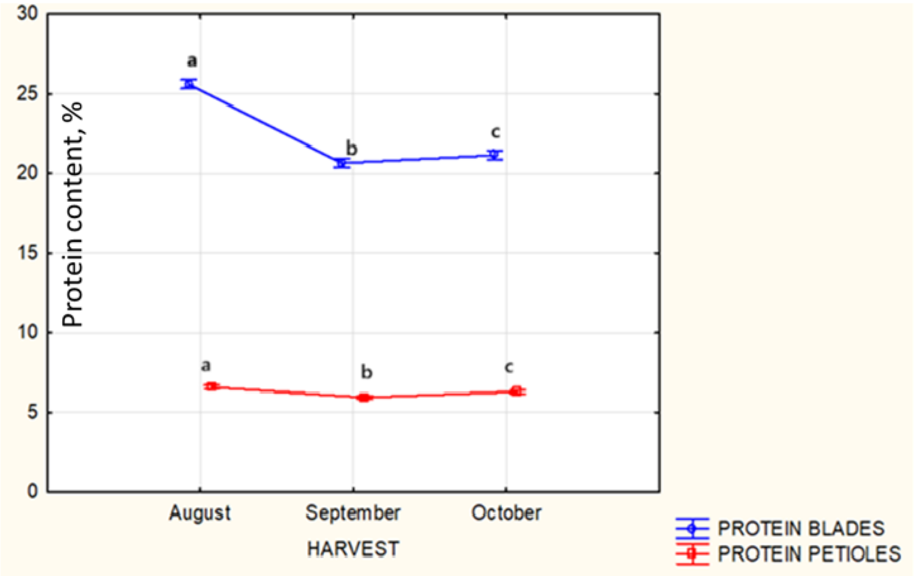

[a,b,c] – homogeneous groups

Figure 1. The content of protein in blades and petioles of SBL in % of DW.

The obtained results showed a distinct differentiation of protein content between leaf fractions and across sampling periods. The higher protein content in leaf blades compared to petioles confirming that mesophyll tissues are the primary reservoir of soluble proteins, while petioles contain mainly structural and carbohydrate-rich tissues with low nitrogen content. The seasonal trend—a gradual decline from August to September—suggests a redistribution of nitrogen compounds from the leaves to the storage root during the later growth stages, consistent with the physiological transition toward carbohydrate accumulation.

The protein contents were comparable to the range of literature data reported for sugar beet leaves and other green plant tissues. Recent studies report protein levels in dried sugar beet leaves typically between 18% and 29% (DW basis), depending on genotype and agronomic factors [17–20]. Higher values, exceeding 30%, have been occasionally reported for concentrated protein fractions or enzymatically extracted preparations [15,21]. Seasonal decline in total protein also reflects well-known nitrogen remobilization patterns, as previously observed in sugar beet and related species [22].

Sugar beet leaves constitute a protein-rich biomass, particularly in the blade fraction and underline the potential of sugar beet leaf material as a valuable protein source for biorefinery or feed applications [14].

2.4. Polyphenols in Sugar Beet Leaves

2.4.1. Characterization of Polyphenolic Compounds Identified in SBL by LC–MS

The LC/MS analyses of methanolic extracts of lyophilized sugar beet leaves revealed a profile of polyphenolic constituents, primarily composed of flavonoid glycosides and phenolic acids. Fifteen compounds were tentatively identified based on their retention times, UV–VIS spectra, molecular ions $[M{-}H]^{-}$, and diagnostic fragment ions obtained in MS/MS mode.

Among the detected metabolites, flavones and flavonols were the predominant groups (Table 3).

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Table 3. The list of metabolites identified in sugar beet leaves extract via LC /MS technique.

No	t_R (min)	Tentative identification	nominal mass (Da)	MS data (*m/z*)	MS/MS data (*m/z*)	UV-VIS max (nm)
1	27.1	apigenin dihexoside	594	$[593]^-$	473, 413, 293	269, 339
2	28.1	apigenin hexopentoside	564	$[563]^-$	413, 293	269, 328
3	28.7	(iso)rhamnetin hexopentoside	610	$[609]^-$	285	270, 336
4	28.8	vitexin	432	$[431]^-$	341, 311, 283	269, 338
5	29.0	flavonol dihexoside	636	$[635]^-$	473, 413, 311, 293	268, 336
6	29.3	(iso)rhamnetin dihexoside	640	$[639]^-$	315	270, 352
7	29.7	flavonol dihexoside	636	$[635]^-$	593, 575, 515, 473, 413, 311, 293	268, 335
8	30.4	(iso)rhamnetin hexopentoside	610	$[609]^-$	315	254, 363
9	30.5	flavonol dihexoside	636	$[635]^-$	575, 473, 455, 329, 311, 293	270, 335
10	30.9	acylated apigenin hexopentoside	606	$[605]^-$	563, 545, 455, 433, 413, 395, 353, 311, 293	269, 333
11	31.8	acylated apigenin hexopentoside	606	$[605]^-$	545, 455, 443, 311, 293	270, 335
12	34.1	diferulic acid	388	$[387]^-$ $[193]^{-2}$	309, 289, 96 289, 193, 96	270, 326

Several apigenin derivatives were identified, including apigenin dihexoside (*m/z* 593), apigenin hexopentoside (*m/z* 563), and two acylated apigenin hexopentosides (*m/z* 605). The fragmentation ions at *m/z* 413 and 293 and UV–VIS absorption maxima near 269–339 nm confirmed the presence of apigenin as the aglycone moiety. The acylated derivatives showed additional fragments consistent with ferulic or p-coumaric residues, suggesting the presence of acyl substituents typical for plant defense-related modifications. In addition, vitexin (apigenin-8-C-glucoside, *m/z* 431) was detected, indicating the coexistence of both O- and C-glycosylated apigenin forms in the leaf tissue.

The flavonol fraction was dominated by (iso)rhamnetin-based glycosides, such as rhamnetin dihexoside (*m/z* 639) and rhamnetin hexopentoside (*m/z* 609), together with several unidentified flavonol dihexosides (*m/z* 635). Fragment ions at *m/z* 315 and UV absorption maxima between 335–352 nm correspond to rhamnetin aglycone, a methylated derivative of quercetin commonly reported in *Beta species*. The occurrence of multiple isomers differing in retention time implies structural diversity related to sugar composition and linkage position. Such structural heterogeneity of flavonol glycosides is often associated with differences in bioavailability and biological activity, including antioxidant and anti-inflammatory properties.

Besides flavonoids, one phenolic acid derivative, diferulic acid (*m/z* 387), was also identified. Its fragmentation ions at *m/z* 289 and 193, together with a UV maximum near 326 nm, are characteristic

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of cross-linked ferulate dimers. Diferulic acid participates in the formation of covalent linkages in plant cell walls, contributing to mechanical resistance and oxidative stress protection.

The results demonstrate that sugar beet leaves are a rich source of polyphenolic compounds, particularly glycosylated flavones and flavonols, with apigenin and isorhamnetin as dominant aglycones, including C-glycosylated derivatives such as vitexin. The presence of acylated and mixed glycosides suggests complex secondary metabolism pathways related to photoprotection and stress adaptation.

According to recent studies [4,23] on sugar beet leaves, the flavone C-glycoside vitexin is the quantitatively dominant individual phenolic compound, although phenolic acids (e.g. ferulic and gallic acids) and other flavonoids also substantially contribute to the overall polyphenol pool.

These findings align with previous reports on *Beta vulgaris* phenolic composition and confirm the high antioxidant potential of the leaf fraction, which may represent a valuable by-product for nutraceutical and functional food applications.

2.4.2. Total Polyphenol Content in SBL

Since vitexin was identified as the predominant individual phenolic compound in sugar beet leaves, the total polyphenol content (TPC) — sum of all phenolic peaks was quantified using vitexin as an external standard. The average TPC (Table 4) in SBL blades was 9.7 mg/g DW (SD 2.67). The highest TPC was found in the WO, JK, MR, and SL varieties (approx. 10–11 mg/g DW). The lowest values were found in the JN and JG varieties (below 9 mg/g DW).

Table 4. The average total polyphenol content (± SD) in SBL blades and petioles of various varieties.

VARIETY	TPC, mg/g DW BLADES	TPC, mg/g DW PETIOLES
JK	10.3 ± 4.2	0.8 ±0.3
PA	9.7 ± 3.2	1.0 ±0.4
GL	9.7 ± 2.8	1.4 ±0.5
WO	11.0 ± 3.6	0.8 ±0.3
JG	8.7 ± 2.9	0.8 ±0.3
JN	7.8 ± 2.2	1.7 ±0.3
MR	10.2 ± 14	2.2 ±0.3
SL	10.2 ± 1.9	2.7 ±0.4
OR	9.1 ± 1.5	1.8 ±0.3
ZG	10.2 ± 1.0	1.7 ±0.5

Statistically significant differences were found between the varieties, and the WO and JK varieties may have higher leaf antioxidant potential. SBL petioles had a significantly lower mean polyphenol content compared to blades, amounting to 1.5 mg/g DW, with a standard deviation of 0.73. The highest concentrations were found in the SL and MR varieties (2.7 mg/g DW and 2.2 mg/g DW, respectively), and the lowest in the JK, WO, and JG varieties (approx. 0.8 mg/g DW).

The highest polyphenol content in blades was recorded during the August harvest, decreasing with harvest date (Table 5). This suggests that early harvests promote greater polyphenol accumulation, and their content decreased as the plants matured. Unlike blades, petioles show less variability and a slightly different trend – the maximum content was recorded in September, which may be due to a shift in phenolic metabolism between plant parts. Polyphenol content in blades strongly depends on harvest date, while in petioles this factor is less affected, but there are clear differences between cultivars.

Table 5. The average total polyphenol content (± SD) in SBL blades and petioles at different harvest times.

HARVEST	Average TPC, mg/g DW blades	Average TPC, mg/g DW petioles
August	12.4[a] ± 1.7	1.6[c] ± 0.7
September	9.6[b] ± 1.2	1.7[c] ± 0.5
October	7.2[b] ± 1.8	1.2[d] ± 0.8

[a,b,c,d] – homogeneous groups.

Significant genotypic variability was observed among the tested varieties, with polyphenol contents in whole leaves ranging from 9.48 mg/g DW (JG) to 12.93 mg/g DW (SL) (Figure 2).

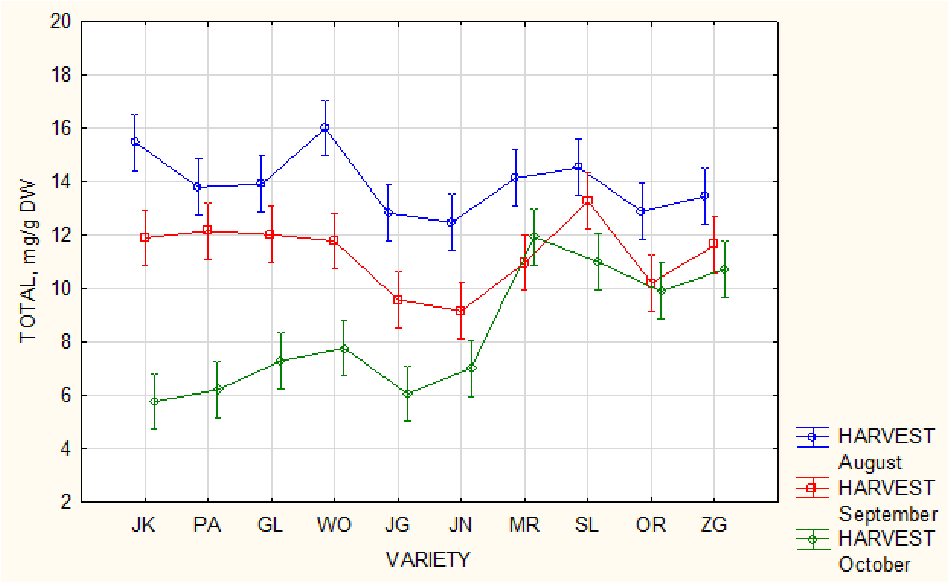

Figure 2. The content of total pholyphenols in SBL.

The highest concentrations were found in varieties SL, MR, and ZG, whereas the lowest values occurred in JG and JN. Such variation may reflect differences in metabolic activity and secondary metabolite pathways among genotypes of plants.

When compared with literature data, the total phenolic content determined in this study aligns with or slightly exceeds the values reported for sugar beet leaves extracted under similar conditions. Ebrahimi et al. [4] reported average total phenolic content of 6.8 – 17.2 mg/g DW for various extraction techniques and up to 69.4 mg/g DW using ultrasound-assisted extraction, depending on solvent composition and extraction parameters. Similarly, Maravić et al. [23] found 4.5–17.2 mg/g DW in dried leaves using ethanol extraction, Dukić et al. [16] from 13-18 mg/g DW, whereas El-Gengaihi et al. [23] and observed lower concentrations 1.6–16.1 mg/g DW.

The predominant phenolic compound class in sugar beet leaves, as highlighted by Ebrahimi et al. [4] and Maravić et al. [23], includes flavonoids such as vitexin, isovitexin, and catechin derivatives, alongside phenolic acids like ferulic and p-coumaric acid. These compounds contribute to strong antioxidant properties and have been linked to hepatoprotective and anti-inflammatory activities. Variations in polyphenol content across studies may arise from genotype-dependent expression of biosynthetic enzymes, environmental stress conditions, and growth stage at harvest [22].

Overall, the results indicate that the sugar beet leaves analyzed in this study represent a valuable source of polyphenolic antioxidants, with levels comparable than those reported in recent literature. These findings support the growing evidence that sugar beet leaves—traditionally considered an

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agricultural by-product—can be valorized as a promising raw material for obtaining high-value phenolic compounds, aligning with current biorefinery and sustainability concepts.

A pronounced seasonal effect was also evident. Mean polyphenol levels declined progressively from August (13.94 mg/g DW) to October (8.37 mg/g DW). This trend suggests that early-harvest leaves are richer in phenolic compounds, likely due to more active biosynthesis and lower oxidative degradation in younger tissues.

Seasonal changes in foliar phenolics have been reported for sugar beet leaves, with plant age affecting total phenolic content and browning behaviour [3]. The interaction between variety and harvest time further supports this pattern: early-harvest samples of varieties such as WO and JK exhibited the highest phenolic concentrations (up to 15.9 mg/g DW), while late-harvest samples from the same varieties showed substantial decreases.

When compared with published data, the present values (8–15 mg/g DW, vitexin equivalents) fall within or slightly above the upper range reported for *Beta vulgaris* leaves. The differences in polyphenol values obtained here likely reflect the use of different extraction methodologies, different of method analysis and a different reference compound (vitexin instead of gallic acid), which typically yields more conservative but chemically specific quantification [4]. Moreover, discrepancies may also arise from whether entire leaves are included in the analysis or only the leaf blades, with petioles omitted, as these plant parts differ in their polyphenolic composition. Such methodological differences highlight the importance of comparing results within the same analytical framework.

2.4.2. Vitexin and Vitexin Derivatives Content in SBL

Vitexin (apigenin-8-C-glucoside) is a widely distributed flavone C-glycoside occurring in numerous medicinal and food plants. High levels of vitexin and its derivatives have been reported in passionflower (Passiflora incarnata), hawthorn (Crataegus spp.), chaste tree (Vitex agnus-castus) and bamboo leaves, as well as in several cereals and pseudocereals such as millet and buckwheat. Recent studies also indicate that sugar beet (*Beta vulgaris*), particularly its leaves, may represent an additional, underutilised source of vitexin for futher utilization for example in the human diet [26]. Due to this multifunctional bioactivity, vitexin is increasingly regarded as a promising compound for nutraceutical and functional food applications, as well as a lead structure in the development of phytopharmaceuticals aimed at supporting cardiovascular health, neuroprotection and metabolic homeostasis [27–29].

Presented in study leaf blades contained substantially higher concentration of vitexin (mean 7.4 ± 2.3 mg/g DW) than petioles (1.1 ± 0.6 mg/g DW) (Figure 3). This is conditioned by consistent with the structural and physiological specialization of leaf tissues, leaf blades accumulate higher concentrations of photoprotective flavonoids. Comparable tissue-dependent phenolic distributions in sugar beet have been documented by Vissers et al. [3]. The SL variety exhibited the highest mean vitexin concentration (10.6 mg/g DW), while JN showed the lowest (6.2 mg/d DW). These study indicate that genetic factors influence vitexin accumulation, but effects may be moderated by environmental or physiological variability.

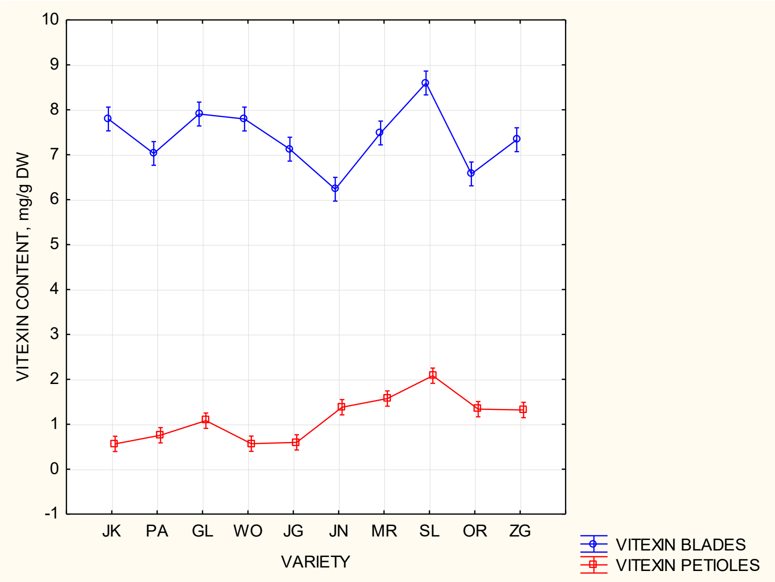
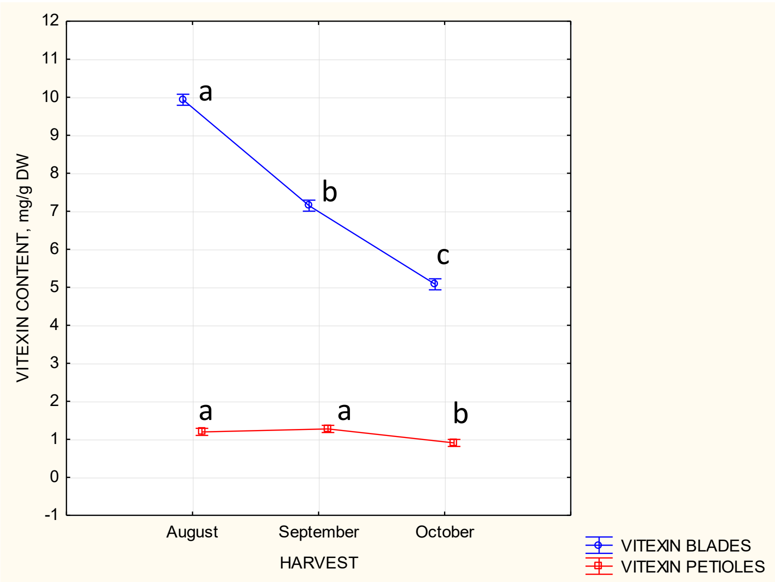


Figure 3. The content of vitexin in different various SBL.

Harvest timing markedly affected vitexin accumulation, the highest concentrations measured in August (9.9 mg/g DW), the lowest in October (5.1 mg/g DW) (Figure 4). This trend reflects leaf maturation and reduced flavonoid biosynthesis later in the season, as also reported in other studies examining seasonal or developmental changes in plant phenolics [30,31]. The significant interaction between harvest time and part of leaves demonstrates that leaf blades exhibited a sharper seasonal decrease than petioles.



[a,b,c] – homogeneous groups

Figure 4. Variation in vitexin content depending on harvest time.

The percentage contribution of vitexin derivatives to total polyphenol content (TPC) was high across all samples (above 70%) and decreased from August (80.1%) to October (72.5%). No significant variation in percentage contribution of vitexin derivatives to TPC was observed between blades and petioles, that although the total phenolic content is much higher in blades, the relative composition of phenolic remains stable between tissues.

The results demonstrate that leaf blades and early-season harvests of sugar beet leaves are optimal for maximizing vitexin content, which is consistent with biochemical studies describing the

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regulation of C-glycosyl flavonoid biosynthesis in leaves and emphasize the potential for optimizing harvest and processing strategies for vitexin-rich plant material.

3. Materials and Methods

3.1. Plant Material

Research material included sugar beet leaves from ten sugar beet varieties: collected in 2021 in three different harvested dates - 1st of August, 3rd of September and 13th of October and collected in 2022 at 1st of August, 7th of September and 1st of October. Sugar beet leaves varieties collected in 2021: Jagienka JK (Kutno Sugar Beet Breading Farm, KHBC, Poland), Pacyfik PA (Maribo®, Denmark), Gladiata GL (KWS, Germany), Wojownik WO (SESVanderHave, France) and Jagiellon JG (Wielkopolska Sugar Beet Farm WHBC, Poland) and in 2022: Jantar JN (KHBC, Poland), Mariza MR (Maribo®, Denmark), Smart Latoria SL (KWS, Germany), Orlik OR (SESVanderHave, France) and Zagłoba ZG (WHBC, Poland). The leaves were harvested from the experimental field in Poland. The research material, in the laboratory, was divided into sugar beet leaf blades and petioles. To preserve the material, it was ground using liquid nitrogen and then freeze-dried under reduced pressure (0,1 bar) at−50 °C (CHRIST Alpha 1-2 LDplus lyophilizer (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). The resulting powder constituted the homogeneous research material and stored in a polypropylene container with a desiccant. Examples photos of leaves from different dates of harvested of JG variety are presented on Figure 5.

Figure 5. Photos of sugar beet leaves JG varieties harvested at the 1st of September (A), the 3rd of September (B) and the 13th of October (C).

3.2. Extraction of Polyphenols

Based on the optimization experiments described in Section 2.1, all sugar beet leaf samples were extracted using 70% methanol (v/v)containing 0.1% formic acid (v/v).

The extraction process took place in an ultrasonic bath (Polsonic/Sonic-10) at room temperature. 0.5 g of leave blades or leave petioles were mixed with 3 ml of extractant for 15 minutes. The extraction process was repeated three times. After each extraction, the mixture was centrifuged using centrifuge (MPW 260H, Poland) for 10 minutes at 12 000 g and the supernatant was decanted. The collected supernatant was transferred quantitatively to 10 ml graduated flask and topped upwith extractant.

3.3. Chromatographic Analysis

The extracts were analysed using high-performance liquid chromatography with a diode-array spectrophotometric detector (HPLC – DAD, Dionex) and using high-performance liquid chromatography with a mass spectrophotometer (HPLC-MS, LCQ DECA, Thermo-Finnigan), equipped qith an ESI source in the negative mode, to identify polyphenolic compounds. Separation and identification of polyphenolics was conducted with the Phenomenex Luna 5 μm C18 column (250 × 4.6 mm). The extracts were filtered through membrane filters with a pore diameter of 0.45 mm before injection.

The separation was conducted in the following conditions: flow - 0.5 ml min^{-1}, column temperature - 30°C, injection volume – 20 μL. Mobile phase A consisted of 1% formic acid in water,

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and mobile phase B was 0.5% formic acid in 80 % acetonitrile (80:19.5:0.5, ACN:H2O:HCOOH, v/v/v). The separation gradient was as follows: 0–6 min, 4% (v/v) B; 6.5 – 12.5 min, 4-12% (v/v) B; 12,5–44 min, 12-36% (v/v) B; 44-45 min, 36-60% (v/v) B; 45–50 min, 60% (v/v) B, 50-52 min, 60-4% (v/v) B and 52–65 min, 4% (v/v) B.

The DAD detector recorded spectra simultaneously in the range of 200–600 nm, and the mass spectrometer recorded spectra in negative mode. The ion source parameters were set as follows: vaporizer temperature, 500 °C; ion spray voltage, 4 kV; and capillary temperature, 400 °C; the sheath gas flow rates were 75 arbitraryunits, respectively.The MS/MS data was generated using helium gas to fragment precursor ions. Data were collected using the Chromeleon® softwareversion 6.70 (Dionex, USA) and the Xcalibur software version 1.2 (Thermo-Finnigan).

The sum of all identified polyphenolic compounds in the analyzed material determined by the chromatographic method was converted into the vitexin standard.

3.4. Dry Weight

Dry weight (DW) content was assessed by drying the samples at 105 °C until a constant mass was achieved. This widely used gravimetric method relies on the removal of water through evaporation from a precisely weighed portion of the sample. The dry matter value is obtained from the change in sample mass before and after the drying process.

3.5. Protein

The total protein content was determined using the Kjeldahl method (AOAC 2001.11). Measured the nitrogen in samples and estimated the total protein. The nitrogen-to-protein conversion factor was set at 6.25 [32].

3.6. Statistical Analysis

All the analyses of sugar beet leaves were performed thrice; the tables provide the average values and standard deviation (SD). Data were subjected to basic statistics of two-factor ANOVA analysis and of variance with post hoc NIR Fishera test for identification of differences between the groups. Statistical analyses were carried out using STATISTICA data analysis software system, with level of confidence $p < 0.05$ [33].

4. Conclusions

In this work, quantification of total polyphenols and detailed profiling of the phenolic composition of sugar beet leaves (SBL) confirmed that SBL are a rich source of polyphenolic compounds, particularly glycosylated flavones and flavonols, with apigenin and isorhamnetin as dominant aglycones and vitexin as the quantitatively dominant flavone C-glycoside. The presence of acylated and mixed glycosides points to complex secondary metabolism related to photoprotection and stress adaptation. These findings are consistent with previous reports on *Beta vulgaris* phenolics and confirm the high antioxidant potential of the leaf fraction, supporting its possible use as a by-product for nutraceutical and functional applications.

Mapping the distribution of polyphenol and protein contents between leaf blades and petioles across cultivars and harvest times, we showed that tissue type, genotypic background and phenological stage strongly shape the functional profile of SBL. Leaf blades consistently exhibited higher TPC than petioles, whereas protein accumulation was strongly genotype- and harvest-dependent. Simultaneously, the variability observed between cultivars and sampling dates, together with literature evidence on the impact of nitrogen fertilisation, rainfall and temperature, indicates that both genetic and environmental factors modulate dry matter deposition and biomass composition in sugar beet foliage. Vitexin derivatives accounted for more than 70% of TPC in all samples, with their relative contribution decreasing from August to October but remaining similar between blades and petioles.

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In general, sugar beet leaves constitute a protein-rich biomass, particularly in the blade fraction, and a concentrated source of bioactive phenolics, notably vitexin and related flavonoids. Optimizing cultivar selection, harvest time, and the proportion of blades versus petioles included in the feedstock, in combination with integrated extraction strategies, offers a realistic route to incorporate SBL into food, feed, nutraceutical and cosmetic applications. In this way, the valorization of sugar beet leaves can contribute to circular bioeconomy and zero-waste concepts in the sugar industry.

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