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ANTIDIABETIC POTENTIAL OF MICROWAVE-ASSISTED AQUEOUS GRAPE SKIN EXTRACTS FROM SELECTED WHITE TABLE GRAPE CULTIVARS

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Summary

Type 2 diabetes mellitus is one of the fastest-growing metabolic disorders worldwide, increasing the demand for naturally occurring compounds capable of improving postprandial glycaemic control. Grape skins represent an abundant source of biologically active phytochemicals, including polyphenols and other antioxidant constituents, which may contribute to antidiabetic activity. In the present study, the antioxidant capacity and inhibitory activities against *α*-amylase and *α*-glucosidase of aqueous microwave-assisted extracts obtained from the skins of ten white table grape cultivars were evaluated. Grape skin extracts were prepared using microwave-assisted extraction with acidified water as an environmentally friendly extraction solvent. Antioxidant activity was determined by the ferric reducing antioxidant power (FRAP) assay, whereas antidiabetic activity was assessed through inhibition of *α*-amylase and *α*-glucosidase. Significant differences among cultivars were observed for all analysed parameters. Sonja exhibited the highest ferric reducing antioxidant capacity, whereas the strongest inhibition of *α*-amylase and *α*-glucosidase was recorded for Fanny and Sonja, respectively. Interestingly, antioxidant activity did not correspond to enzyme inhibitory activity, indicating that the antidiabetic potential of grape skin extracts cannot be predicted solely from their overall reducing capacity. These findings suggest that the qualitative composition of phytochemicals rather than total antioxidant capacity plays a dominant role in digestive enzyme inhibition. Microwave-assisted aqueous extraction proved to be an efficient green extraction technique for obtaining biologically active grape skin extracts, supporting the utilisation of grape-processing by-products as valuable ingredients for functional foods and nutraceuticals.

Keywords: white table grape; grape skin; microwave-assisted extraction; antioxidant activity; FRAP; *α*-amylase; *α*-glucosidase; functional foods

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic metabolic disorders worldwide and represents a major public health challenge due to its continuously increasing incidence and numerous long-term complications. The disease is characterised by impaired glucose homeostasis resulting from insulin resistance and/or inadequate insulin secretion, leading to persistent hyperglycaemia. Poor glycaemic control substantially increases the risk of cardiovascular disease, nephropathy, retinopathy and neuropathy, making the prevention and management of postprandial hyperglycaemia an important therapeutic objective (Lu et al., 2024; Rastija et al., 2024).

One of the most widely accepted therapeutic approaches for controlling postprandial blood glucose levels is the inhibition of carbohydrate-hydrolysing enzymes in the gastrointestinal tract. *α*-Amylase initiates the hydrolysis of dietary starch into smaller oligosaccharides, whereas *α*-glucosidase catalyses the final conversion of oligosaccharides and disaccharides into absorbable glucose molecules. Inhibition of these enzymes delays carbohydrate digestion and glucose absorption, thereby attenuating postprandial glycaemic excursions. Clinically approved inhibitors such as acarbose, miglitol and voglibose effectively reduce postprandial blood glucose concentrations but are frequently associated with gastrointestinal adverse effects, including abdominal discomfort, flatulence and diarrhoea, limiting long-term patient compliance (Kalinovskii et al., 2023).

Consequently, increasing attention has been directed towards identifying naturally occurring enzyme inhibitors from plant-derived materials. Numerous studies have demonstrated that polyphenols, flavonoids, phenolic acids, tannins, terpenoids and other phytochemicals may inhibit *α*-amylase and *α*-glucosidase through different mechanisms, including competitive binding to catalytic sites, alteration of enzyme conformation and interaction with substrate-binding residues. Besides their inhibitory activity against digestive enzymes, many of these compounds exhibit pronounced antioxidant properties, reducing oxidative stress that contributes to the development and progression of diabetes and its complications (Sekhon-Loodu and Rupasinghe, 2019).

Grapes (*Vitis vinifera* L.) are recognised as one of the richest dietary sources of bioactive phytochemicals. While grape seeds have traditionally attracted

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considerable scientific interest, grape skins also contain substantial quantities of biologically active compounds, including flavan-3-ols, flavonols, hydroxybenzoic and hydroxycinnamic acids, stilbenes, proanthocyanidins and numerous non-phenolic antioxidants. The concentration and composition of these compounds depend on genotype, environmental conditions, cultivation practices and the stage of berry ripening, resulting in considerable variation among grape varieties. These phytochemicals contribute not only to antioxidant capacity but also to a broad spectrum of biological activities, including anti-inflammatory, antimicrobial, cardioprotective and antidiabetic effects (Zhang et al., 2015; Ishmael et al., 2012; Hrnčić et al., 2019; Mendes et al., 2013).

Interest in grape by-products has markedly increased in recent years owing to the principles of sustainable agriculture and the circular bioeconomy. Grape pomace, consisting predominantly of skins and seeds, represents one of the largest by-products of the wine and grape-processing industries. Rather than being considered agricultural waste, grape skins are increasingly recognised as valuable raw materials for the production of functional foods, nutraceuticals and natural antioxidants because of their high content of health-promoting phytochemicals. The valorisation of these by-products simultaneously contributes to waste reduction and the development of value-added products (Rastija et al., 2024).

Croatia possesses a rich genetic diversity of indigenous grapevine varieties that constitute an important component of its agricultural heritage. Although these varieties are primarily valued for their contribution to regional viticulture and biodiversity conservation, information regarding the biological activity of their grape skins remains limited, particularly for white table grapevine varieties. Characterisation of their antioxidant capacity and enzyme inhibitory activity could therefore provide additional scientific evidence supporting their utilisation as natural sources of bioactive compounds with potential health-promoting applications (Pejić et al., 2010; Vuković et al. 2023).

Therefore, the aim of this study was to evaluate the antioxidant capacity of microwave-assisted aqueous grape skin extracts obtained from selected white table grape cultivars using the ferric reducing antioxidant power (FRAP) assay and to investigate their *in vitro* antidiabetic potential through inhibition of *α*-amylase and *α*-glucosidase. Furthermore, differences among grape varieties were compared in order to identify cultivars with the greatest potential as sources of natural antioxidant and antidiabetic compounds.

Materials and methodology

Preparation of grape skin extracts by microwave-assisted extraction

Grape clusters were harvested from the experimental vineyard of the Faculty of Agrobiotechnical Sciences Osijek located in Mandićevac, Croatia. The skins were manually separated from the pulp immediately after harvest.

Microwave-assisted extraction was performed using an ETHOS X microwave extraction system (Milestone, Sorisole, Italy). Fresh grape skins were extracted with water containing 1 % acetic acid at a solid-to-liquid ratio of 1:5 (1 g fresh skin to 5 mL extraction solvent). The extraction programme consisted of 500 W for 5 min followed by 250 W for 20 min. The obtained extracts were filtered, aliquoted and stored at -20 °C until further analyses.

Chemicals

α-Amylase from porcine pancreas (EC 3.2.1.1), *α*-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), acarbose, 4-methylumbelliferyl *α*-D-glucopyranoside, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride, ferrous sulphate, sodium phosphate salts, sodium carbonate, sodium hydroxide, soluble starch, 3,5-dinitrosalicylic acid (DNS), dimethyl sulfoxide (DMSO), acetic acid and all other analytical-grade reagents were obtained from commercial suppliers and used without further purification.

Determination of total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu spectrophotometric method according to Singleton et al. (1999), with minor modifications. Briefly, an appropriate aliquot of grape skin extract was mixed with distilled water, followed by the addition of 50 µL of 2 M Folin–Ciocalteu reagent. After vortex mixing, 200 µL of sodium carbonate solution (20 %, *w*/*v*) was added, and the reaction mixture was diluted to a final volume of 1 mL with distilled water. The samples were vortex-mixed after each addition of reagent and incubated in the dark for at least 30 min at room temperature. Absorbance was measured at 750 nm using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Gallic acid was used to construct the calibration curve (0–250 mg/L), and the results were expressed as mg gallic acid equivalents (GAE) per litre of extract. All measurements were performed in triplicate, while distilled water was used as the blank.

Determination of antioxidant activity

Antioxidant capacity was determined using the ferric reducing antioxidant power (FRAP) assay based on the reduction of the Fe^{3+}–TPTZ complex to its ferrous form under acidic conditions, according to Benzie and Strain (1996), with minor modifications. The resulting blue Fe^{2+}–TPTZ complex was quantified spectrophotometrically at 593 nm.
Briefly, 20 µL of grape skin extract was mixed with 200 µL of freshly prepared FRAP reagent. Following incubation at 37 °C for 4 minutes, absorbance was measured at 593 nm. A calibration curve was prepared using ferrous sulphate standards, and antioxidant activity was expressed as µmol $FeSO_4$ equivalents per volume of extract. All analyses were performed in triplicate.

Determination of α-amylase inhibitory activity

The inhibitory activity against *α*-amylase was determined using a colorimetric assay based on the quantification of reducing sugars released during starch hydrolysis. DNS reagent reacts with reducing sugars under alkaline conditions, producing a coloured complex measurable at 540 nm.
Briefly, grape skin extracts were mixed with phosphate buffer and *α*-amylase solution, followed by pre-incubation at 37 °C for 15 minutes. Soluble starch was subsequently added as substrate and the reaction mixture was incubated for an additional 15 minutes. The reaction was terminated by adding DNS reagent, followed by heating for 10 min at temperatures above 90 °C. After cooling, absorbance was recorded at 540 nm. Each sample was analysed in triplicate, including its corresponding blank to correct for naturally occurring reducing sugars present in the extracts. Acarbose was used as the positive control.
The percentage inhibition of *α*-amylase activity was calculated according to Equation (1):

$$Inhibition\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \cdot 100 \quad (1)$$

Determination of α-glucosidase inhibitory activity

α-Glucosidase inhibitory activity was evaluated using a fluorimetric assay based on the hydrolysis of 4-methylumbelliferyl *α*-D-glucopyranoside. Following enzymatic cleavage, the released 4-methylumbelliferone was quantified fluorimetrically.
Briefly, *α*-glucosidase solution was mixed with diluted grape skin extracts and pre-incubated at 37 °C for 15 minutes. The reaction was initiated by adding the fluorogenic substrate and continued for 20 minutes.
Enzymatic activity was terminated with sodium carbonate solution, after which fluorescence intensity was measured using excitation and emission wavelengths of 360 and 450 nm, respectively. All measurements were performed in triplicate. Acarbose served as the positive control.
The percentage inhibition of *α*-glucosidase was calculated according to Equation (2):

$$Inhibition\ (\%) = \frac{FI_{control} - FI_{sample}}{FI_{control}} \cdot 100 \quad (2)$$

Results and discussion

Total phenolic content

Total phenolic content of grape skin extracts, determined by the Folin–Ciocalteu method and expressed as mg/L gallic acid equivalents (GAE), varied among the investigated varieties (Figure 1.). The highest value was determined for Sonja (343.51 mg/L GAE), which clearly separated this variety from all other samples. The second highest value was observed for Talisman (85.81 mg/L GAE), followed by Original (70.01 mg/L GAE), Fanny (56.13 mg/L GAE), Stefanie (49.90 mg/L GAE) and Palatina (46.95 mg/L GAE). Lower phenolic contents were recorded for Piroška (34.66 mg/L GAE), Primavera (33.62 mg/L GAE), Sophie (32.62 mg/L GAE) and Arkadia (28.87 mg/L GAE).
The pronounced dominance of Sonja indicates a markedly higher accumulation of Folin–Ciocalteu-reactive reducing compounds in its grape skin extract. Since the Folin–Ciocalteu assay is not fully specific for phenolic compounds, the obtained values should be interpreted as the overall reducing capacity of the extract expressed as gallic acid equivalents. Nevertheless, this assay remains widely used for the comparative estimation of phenolic-rich plant extracts and provides a useful first indication of their potential biological activity. Low standard deviation values recorded for all samples indicate good analytical repeatability.
These results confirm that grape skin extracts of white table grapevine varieties differ strongly in their phytochemical potential. Such variation may be attributed to genotype-dependent differences in phenolic biosynthesis, berry skin structure and

accumulation of reducing constituents. In comparison with the previously investigated indigenous Croatian white wine grapevine varieties, where Svetokriška belina showed the highest phenolic content (Rastija et al., 2024), the present results suggest that selected white table grape varieties, especially Sonja, may also represent valuable sources of phenolic and other reducing compounds.

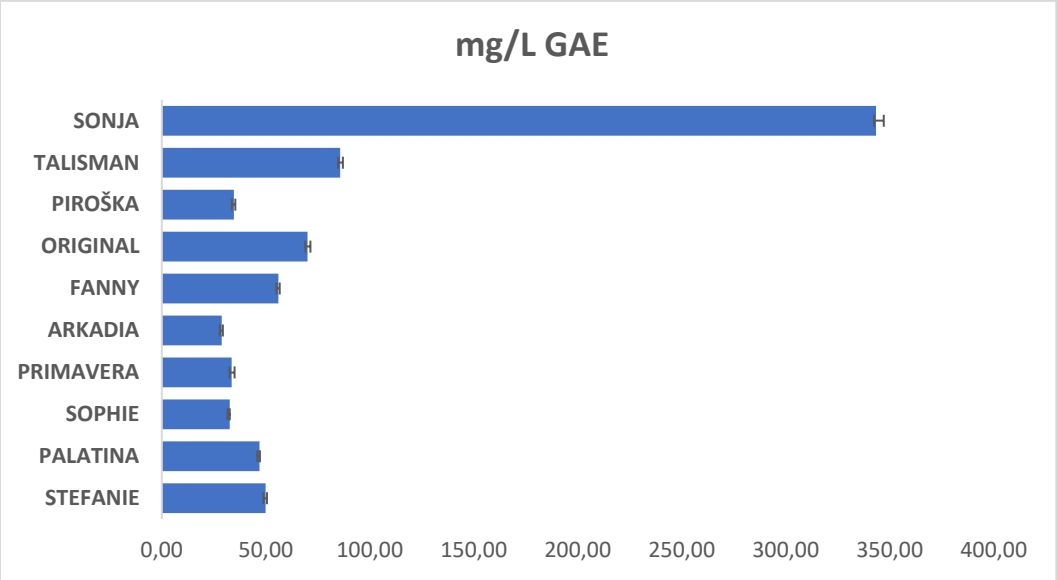

Figure 1. Total phenolic content of grape skin extracts expressed as mg gallic acid equivalents (GAE) per litre of extract. Values are presented as mean ± SD (n = 3)

Ferric reducing antioxidant power (FRAP)

The antioxidant capacity of grape skin extracts was evaluated using the ferric reducing antioxidant power (FRAP) assay, and the results are presented in Figure 2. Considerable differences were observed among the investigated grapevine varieties, indicating marked differences in the reducing capacity of the investigated cultivars.

Among the analysed samples, the Sonja variety exhibited by far the highest antioxidant capacity, reaching 281.15 ± 5.41 μmol Fe^{2+} equivalents g^{-1} fresh weight (FW). This value was approximately three times higher than those recorded for the remaining varieties and clearly distinguished Sonja as the variety with the greatest reducing potential. High FRAP values were also determined for Talisman (95.48 ± 0.30 μmol Fe^{2+} eq. g^{-1} FW) and Arkadia (95.17 ± 0.40 μmol Fe^{2+} eq. g^{-1} FW), followed by Original (87.31 ± 0.65 μmol Fe^{2+} eq. g^{-1} FW), Piroška (79.86 ± 1.61 μmol Fe^{2+} eq. g^{-1} FW), Fanny (74.75 ± 1.95 μmol Fe^{2+} eq. g^{-1} FW), Sophie (73.97 ± 0.58 μmol Fe^{2+} eq. g^{-1} FW), Stefanie (73.43 ± 0.81 μmol Fe^{2+} eq. g^{-1} FW) and Palatina (68.65 ± 1.11 μmol Fe^{2+} eq. g^{-1} FW). The lowest antioxidant capacity was determined for Primavera, with a FRAP value of 53.36 ± 0.97 μmol Fe^{2+} eq. g^{-1} FW.

The relatively low standard deviations obtained for all analysed extracts indicate good repeatability of the analytical procedure and confirm the reliability of the measured antioxidant capacities.

A comparison of the FRAP results with the total phenolic contents determined by the Folin–Ciocalteu assay revealed a similar ranking of grape varieties. The Sonja variety exhibited both the highest total phenolic content (343.51 mg L^{-1} GAE) and the highest ferric reducing antioxidant capacity, suggesting that phenolic compounds substantially contributed to its antioxidant activity.

However, the relationship between total phenolic content and FRAP values was not entirely proportional for all varieties.

For example, Arkadia displayed one of the highest FRAP values despite having the lowest total phenolic content among the investigated varieties, whereas Fanny exhibited relatively high phenolic content but only moderate reducing capacity. Such discrepancies indicate that antioxidant activity depends not only on the total concentration of phenolic compounds but also on their qualitative composition, molecular structure and reducing efficiency. Individual phenolic acids, flavonoids, flavan-3-ols and other reducing constituents differ in their electron-donating ability, meaning that extracts containing similar total phenolic contents may exhibit substantially different antioxidant capacities.

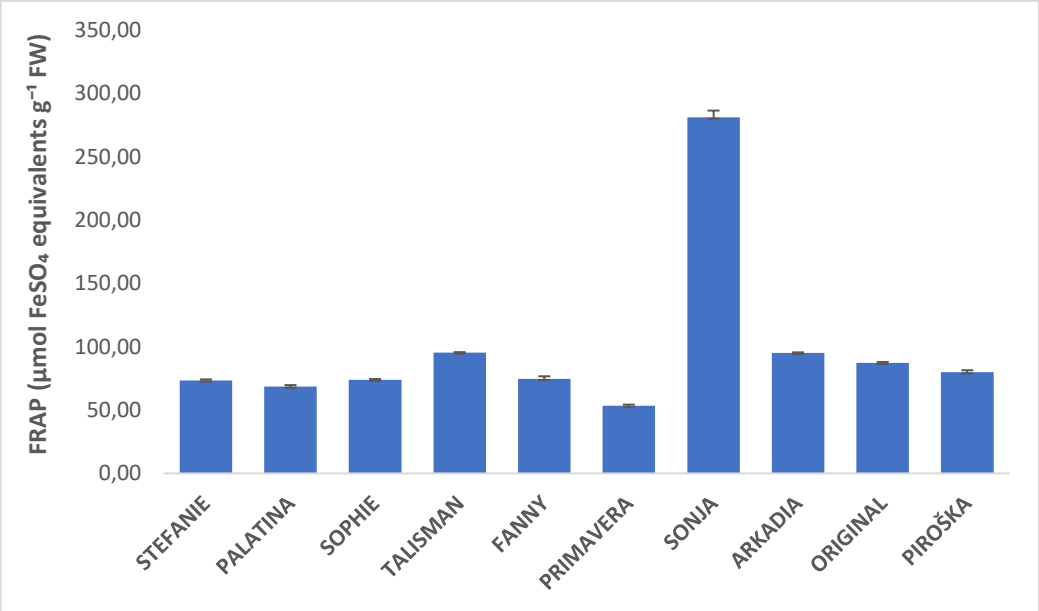

Figure 2. Ferric reducing antioxidant power (FRAP) of grape skin extracts. Values are presented as mean ± SD (n = 3)

α-Amylase inhibitory activity

The inhibitory activity of grape skin extracts against α-amylase is presented in Figure 3. Considerable differences were observed among the varieties, indicating pronounced variability in their ability to inhibit starch hydrolysis.

The highest inhibitory activity was recorded for Fanny, which inhibited α-amylase by 97.64 ± 2.05 %, demonstrating almost complete suppression of enzyme activity. Similarly, the extract of Sonja exhibited strong inhibition (89.17 ± 1.34 %), whereas Original showed moderate to high inhibitory activity (67.34 ± 2.49 %). Intermediate inhibition was observed for Arkadia (57.29 ± 3.52 %) and Palatina (46.29 ± 0.45 %), followed by Piroška (41.57 ± 2.72 %). Lower inhibitory effects were determined for Sophie (30.31 ± 0.45 %), Stefanie (29.43 ± 1.06 %), Talisman (29.52 ± 1.39 %) and Primavera (24.89 ± 2.12 %).

The inhibition of α-amylase by plant-derived polyphenols has attracted considerable attention because delaying starch digestion may reduce postprandial glucose excursions, representing one of the principal therapeutic strategies in the dietary management of type 2 diabetes mellitus.

Interestingly, the present results indicate that α-amylase inhibition was not directly proportional to either total phenolic content or antioxidant capacity. Although Sonja, possessing the highest phenolic content and FRAP value, also showed pronounced enzyme inhibition, the strongest inhibitory activity was observed for Fanny, whose total phenolic content and reducing capacity were moderate. Conversely, Talisman, despite exhibiting one of the highest FRAP values, demonstrated relatively weak inhibition of α-amylase.

These findings suggest that enzyme inhibition depends primarily on the qualitative composition of phenolic constituents rather than their total concentration.

Overall, the results indicate that several indigenous varieties possess considerable α-amylase inhibitory activity, with Fanny and Sonja emerging as particularly promising sources of naturally occurring enzyme inhibitors.

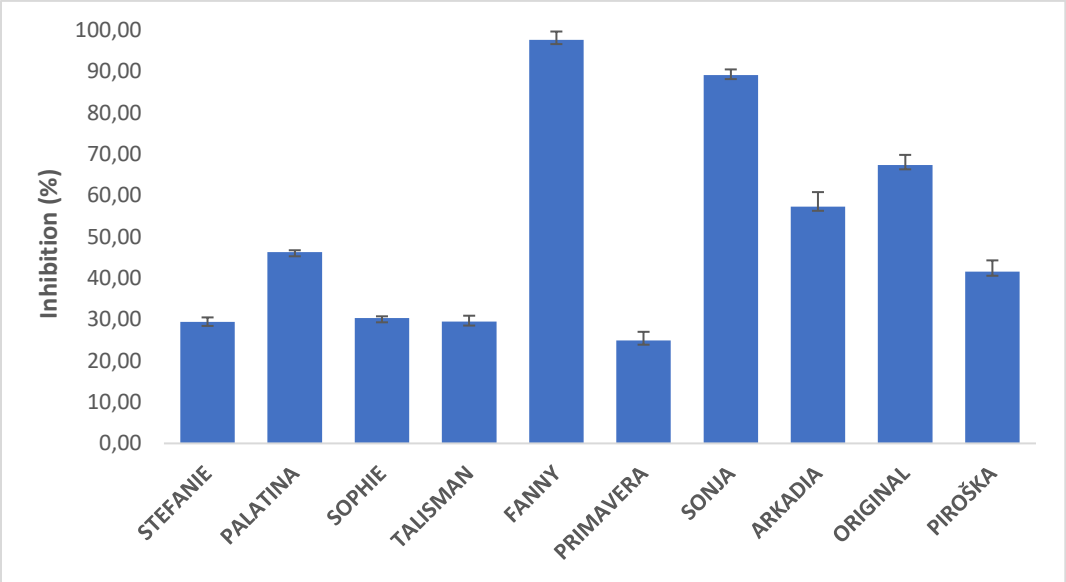

Figure 3. Inhibitory activity of grape skin extracts against α-amylase.
Values are presented as mean ± SD (n = 3)

α-Glucosidase inhibitory activity

The inhibitory activity of grape skin extracts against α-glucosidase is presented in Figure 4. The highest inhibitory activity was recorded for Sonja, which almost completely inhibited α-glucosidase, reaching 100.06 ± 0.12 % inhibition. High inhibitory activities were also observed for Talisman (88.40 ± 0.95 %) and Original (80.32 ± 0.55 %). Moderate inhibition was exhibited by Stefanie (72.93 ± 2.24 %), Primavera (70.52 ± 0.73 %) and Piroška (68.00 ± 0.91 %). Slightly lower activities were recorded for Sophie (63.34 ± 0.90 %), Fanny (61.16 ± 1.61 %) and Arkadia (61.00 ± 0.71 %), whereas Palatina showed the lowest inhibitory activity (58.12 ± 0.60 %).

Despite these differences, all investigated extracts inhibited α-glucosidase by more than 58%, indicating a promising *in vitro* antidiabetic potential of grape skin extracts.

Unlike the results obtained for α-amylase, the ranking of grape varieties according to α-glucosidase inhibition corresponded more closely with their antioxidant characteristics. The Sonja variety, which exhibited the highest total phenolic content and the greatest ferric reducing antioxidant power, also demonstrated the strongest α-glucosidase inhibitory activity. Likewise, Talisman and Original, characterised by relatively high FRAP values, ranked among the most effective enzyme inhibitors. These observations suggest that reducing compounds, particularly phenolic constituents, substantially contribute to α-glucosidase inhibition.

Nevertheless, the relationship was not entirely linear. For example, Arkadia displayed one of the highest FRAP values but only moderate α-glucosidase inhibition, whereas Primavera, despite possessing the lowest antioxidant capacity, exhibited stronger inhibition than several varieties with higher FRAP values. Such observations indicate that enzyme inhibition depends not only on the total concentration of phenolic compounds but also on their structural diversity, molecular interactions and possible synergistic effects among individual phytochemicals.

Comparison of the inhibitory activities against α-amylase and α-glucosidase further demonstrated that different grape varieties preferentially inhibited individual digestive enzymes. While Fanny exhibited the strongest α-amylase inhibition, Sonja proved to be the most potent α-glucosidase inhibitor. These findings suggest that different classes of grape phytochemicals may possess different affinities towards the two digestive enzymes, emphasising the complexity of the antidiabetic activity of grape skin extracts.

The obtained results indicate that grape skin extracts of indigenous Croatian white table grapevine varieties possess considerable potential as natural α-glucosidase inhibitors. In particular, Sonja, Talisman and Original demonstrated pronounced inhibitory activities and therefore represent promising candidates for further phytochemical profiling and identification of the compounds responsible for their antidiabetic effects.

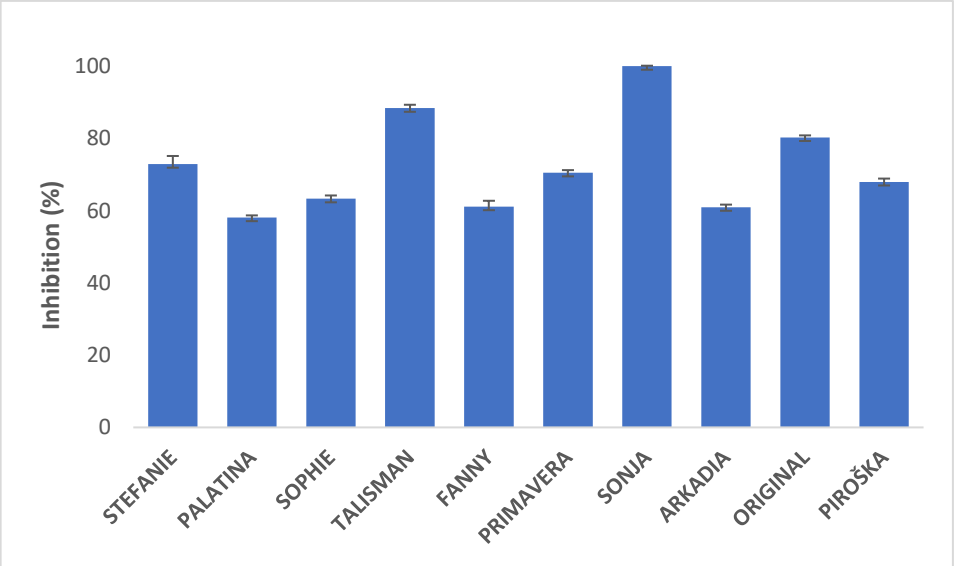

Figure 4. Inhibitory activity of grape skin extracts against α-glucosidase. Values are presented as mean ± SD (n = 3)

Conclusion

The present study demonstrated that grape skin extracts obtained from selected white table grapevine varieties possess considerable antioxidant and *in vitro* antidiabetic potential, although significant differences were observed among the investigated cultivars. The highest total phenolic content, ferric reducing antioxidant capacity and α-glucosidase inhibitory activity were determined for the Sonja variety, indicating that this cultivar represents a particularly rich source of bioactive compounds with potential health-promoting properties. In contrast, Fanny exhibited the strongest α-amylase inhibitory activity, suggesting that the inhibition of carbohydrate-hydrolysing enzymes is influenced not only by the total concentration of phenolic compounds but also by their qualitative composition and possible synergistic interactions among phytochemicals.

The comparison of antioxidant capacity and enzyme inhibitory activities revealed that reducing power alone could not fully explain the observed antidiabetic effects, highlighting the complexity of the phytochemical composition of grape skin extracts. These findings indicate that different grapevine varieties may contain distinct profiles of bioactive compounds that selectively contribute to antioxidant activity and inhibition of digestive enzymes.

These findings support the utilisation of white table grape skins as promising raw materials for functional foods and nutraceuticals with antioxidant and antidiabetic potential while also contributing to the sustainable valorisation of grape-processing by-products.

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