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In vitro biological activity and nutritional evaluation of purple potato (*Solanum tuberosum* L. var. Vitelotte)

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Abstract

This study aimed to produce functional flour from purple potatoes, which are relatively less known and have limited usage. Purple potatoes (*Solanum tuberosum L.* var. Vitelotte) and yellow potatoes (*Solanum tuberosum L.*) used in this study were cultivated in Türkiye. The purple potato flour (PPF) displayed higher values across various parameters, including total phenolic compounds, total flavonoids, total anthocyanins, antioxidant capacity, phenolic content, as well as *in-vitro* biological activities such as bioaccessibility, anti-diabetic activities, and anti-inflammatory properties, compared to the yellow potato flour (YPF). The mineral content ranking for both flour samples was K > P > Na > M g > Ca > Fe > Zn > Mn > Cu > Se. The predominant phenolic compounds in PPF were chlorogenic acid, D-(+) malic acid, ferulic acid, and succinic acid. The compounds from anthocyanins found in purple vegetables and fruits, including malvidin chloride, cyanidin chloride, and cyanin chloride, were identified in PPF. PPF exhibited an average *in-vitro* bioaccessibility of 60%, whereas YPF demonstrated a slightly lower value of 48%. Regarding their *in-vitro* anti-diabetic activities, PPF showcased an α-amylase inhibition rate averaging 51.67% and an α-glucosidase inhibition rate at 36.22%. As a result of the study, it was observed that purple potato flour was a rich source of total phenolic content, dietary fiber, minerals, antioxidants, and anthocyanins. Considering its gluten-free nature, this functional flour is considered a potential alternative flour source for individuals with celiac disease, opening up new possibilities for various applications in our daily nutrition.

Keywords Purple potato (*Solanum tuberosum L.* var. Vitelotte) flour, Functional properties, In-vitro bioaccessibility, In-vitro anti, Diabetic activities, In-vitro anti, Inflammatory

Introduction

Gluten, a protein found primarily in wheat, rye, and barley, acts like a glue, providing dough with its characteristic elasticity. However, despite its significant effect on baked goods, it also has several negative effects on human health. Conditions such as gluten sensitivity and celiac disease are common health issues that affect approximately 1% of the population worldwide [1, 2]. Celiac disease is a chronic intestinal malabsorption disorder characterized by an immune-mediated sensitivity to gluten, a protein found in wheat, barley, and rye, in individuals with a genetic predisposition. Affected individuals cannot consume gluten-containing cannot consume gluten-containing foods because they cannot tolerate gluten and gluten-like proteins (avenin, secalin, hordein, zein) in grains and grain products. Apart from celiac patients, people with gluten allergy or

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gluten sensitivity also need gluten-free foods. [2]. The use of grain-based flour, such as wheat, corn, and rice, is widespread worldwide. However, with changing dietary habits in modern times, there is a growing trend towards alternative flour sources [3]. The increasing interest in healthy nutrition, coupled with the rising prevalence of conditions, such as celiac disease and wheat allergies, contributes to the preference for functional flours [4].

Primary examples of functional flours include legume flours (chickpea, lentil flour, etc.), grain flours (sorghum, buckwheat, etc.), seed flours (amaranth, flaxseed, chia, etc.), and root vegetable flours (potato, turnip, red beet, carrot, etc.). Common characteristics of these flours include gluten-free composition, high nutritional values, low carbohydrate content, and suitability for a healthy diet [5, 6].

The potato plant (*Solanum tuberosum* L.) ranks fourth in global consumption after corn, wheat, and rice, primarily due to its ease of cultivation and adaptability to various geographical regions [5]. Potatoes are characterized by high levels of carbohydrates, protein, dietary fiber, vitamins (C, B3, and B6), and minerals (magnesium, potassium, phosphorus, iron, and zinc). Additionally, potatoes serve as a valuable antioxidant source [8–12].

Traditionally known for their yellow color, potatoes can now be produced in various colors due to advancements in technology and increased biodiversity. The presence of carotenoids, a group of color pigment compounds, allows potatoes to exhibit colors such as orange and red, while anthocyanins contribute to colors such as blue and purple [13, 14]. These potatoes, which have higher levels of polyphenol compounds than yellow potatoes, demonstrate significantly stronger antioxidative effects [15–17]. A study conducted with red and purple potatoes reported that the levels of potassium and phosphorus levels were significantly higher compared to yellow potatoes [18]. A similar study found that purple potato flours obtained through freeze-drying had six times higher flavonoid content than those made from yellow potatoes, and that the anthocyanin content of the flours was also significantly higher [19].

Anthocyanins, in addition to their robust antioxidant activity, also exhibit anti-hypertensive, anti-mutagenic, anti-microbial, anti-diabetic, and anti-carcinogenic properties. As a result, anthocyanins, along with other polyphenols, play a crucial role in protecting against various health issues such as cardiovascular diseases, oxidative stress, inflammation, obesity, and diabetes. Besides their health benefits, polyphenolic compounds, which are rich in biological activity, have been observed to participate in numerous biological reactions in the body [20–23]. However, after ingestion, the food matrix undergoes extensive transformation in the body, reducing the bioavailability and bioaccessibility of the polyphenols it contains. This complicates our understanding of the true effects of polyphenol-rich foods on our health. Therefore, it is crucial to conduct various biological activity studies such as in vitro bioaccessibility, in vitro anti-diabetic activities, and in vitro anti-inflammatory studies to assess the effects and stability after in vitro digestion and to accurately understand the effects of foods on our bodies [23].

Before being consumed, potatoes can be prepared in various ways such as fried potato strips, chips, crackers, crisps, french fries, and starch, using different cooking methods [24]. The cooking method used significantly affects the chemical composition of potatoes, especially their bioactive components. Studies have reported a decrease in bioactive components such as antioxidants and phenolic compounds in potatoes when cooked at high temperatures for extended periods [24–26]. Changes in chemical composition can be attributed to the cooking method itself as well as parameters such as storage conditions and shelf-life duration [26, 27]. Therefore, in addition to fresh consumption, alternative usage methods such as potato flakes and potato flour produced under suitable conditions to preserve nutrients should be preferred. However, due to its high carbohydrate content, the production of potato flour and its use as an ingredient in baked goods are of significant importance. While there are some studies in the literature concerning the production of flour from potatoes [28, 29] limited research has been found on the optimization of purple potato flour production and its utilization [17, 30].

The chemical composition and antioxidant capacity of new-generation vegetables, such as purple potatoes, as well as their alternative applications, have been investigated in previous studies. However, research on the specific properties of these compounds, such as bioaccesbility and anti-diabetic activity, is limited. This study aimed to fill this gap in knowledge by examining how the digestive metabolism affects the biological activity of polyphenol-rich foods such as purple potatoes. Additionally, the study opted for the use of flour form, which allows for the preservation of nutrients and more effective enrichment. When anthocyanin compounds in purple potatoes come into contact with oxygen, they undergo enzymatic oxidation. As a result of this oxidation reaction, the purple color turns brown. To minimize this

oxidation and preserve the purple color, a peroxidase enzyme inactivation process was conducted. What sets this study apart from others is the pre-processing step of peroxidase enzyme inactivation (blanching) conducted to preserve the color of the potatoes. There is no other study available that evaluates the functional properties and in vitro biological activities of purple and yellow potatoes produced in flour form under equal conditions. The purple potato flour produced is expected to be a valuable functional additive, offering an alternative source of dietary fiber, plant-based protein, and essential minerals. With these properties, it holds potential for use in the production of functional products, particularly in gluten-free baked goods, as well as applications in various other industries.

In this study, flour production preserving the nutritional properties and color of purple potatoes grown in Türkiye was carried out. To enable a comparative analysis of the results, yellow potato flour was also produced and analyzed under the same conditions as a control sample. The physicochemical properties, chemical composition (mineral content, organic acid content, and phenolic composition), functional properties (total phenol, antioxidant capacity, total flavonoid, total anthocyanins), and *in-vitro* biological activity analyses were conducted for both the produced purple and yellow potato flours.

Materials and methods

Materials

The purple potatoes (*Solanum tuberosum* L. var. Vitelotte) and yellow potatoes (*Solanum tuberosum* L.) were sourced from Doğa Tohumculuk, a producer located in Nevşehir, in the Central Anatolia Region of Türkiye (38° 12'and 39° 20'N, 34° 11'and 35° 06'E).

Potato flour production

The process flow diagram for the production of potato flour is presented in Fig. 1. The purple and yellow potatoes underwent preliminary preparation steps, including washing and peeling, followed by cutting into uniform dimensions (3 ×3 cm). These potatoes were steam-cooked for 10–12 min for blenching. The cooked potatoes were then spread evenly on drying trays to ensure uniform thickness. Subsequently, they were left to dry in a hot airflow drying unit at 45 °C and 7% relative humidity for 24 h. The dried potatoes were ground using a coffee grinder (Fakir, Türkiye) and passed through a 212 µm sieve. The produced purple potato flour (PPF) and yellow potato flour (YPF) were stored in glass jars under refrigeration conditions (0 °C).

Methods

Nutritional compositions

The moisture content, ash content, protein content, pH, and total acidity of the potato flours were determined according to the AOAC standards methods. The carbohydrate and energy data were calculated using the Atwater general factor system; considering 4.0 kcal/g for protein and carbohydrates, and 9.0 kcal/g for fat [31–33]. The total dietary fiber content was enzymatically determined according to AOAC Method No: 985.29 using α-amylase, amyloglucosidase, and protease.

The color measurement of the samples was determined using the Spectrophotometer (Minolta/CM-139 3600 d, Osaka, Japan) based on the color system of the CIE L^*, a^*, b^*. The results were shown according to parameters specified using the CIE Lab system: L^* ($L^* = 0$ for black, $L^* = 100$ for white), a^*—green color component ($a^* < 0$) or red ($a^* > 0$), b^*—blue component ($b^* < 0$) or yellow ($b^* > 0$). All analyses were performed in triplicate and the mean values were reported.

Determination of organic acid

The organic acid contents of the potato flours were defined using ultra high-performance liquid chromatography (UPLC-Dionex Ultimate 3000 CA, USA) with a specific organic acid column (Acclaim, OA 120 A 250 ×4.0 mm) and detected at 210 nm using the detector (ICS-VWD UV).

Sample preparation 1 g of potato flour and 50 mL of 5 mM H_2SO_4 was added. The solution was shaken at 175 rpm for 3 h using a digital shaker (VWR Advanced 3500, VWR Avantor, USA). Subsequently, the solution was filtered by a 0.45 µm poliyvinylidene fluoride (PVDF) filter before being taken for analysis [34].

Device operating conditions 100 mM Na_2SO_4 (adjusted to pH 2.65 with MSA), flow rate: 0.60 mL/min, temperature: 30 °C, pressure: 93–94 bar, detector: 210 nm, injection volume: 5 µL) of the mobile phase. The citric acid standards were prepared in aqueous solutions using the Supelco (USA) organic acid kit. Linear regression was employed to determine standard curves for quantifying the amount of citric acid. At least five standard solutions within the range of 100–1000 mg/L were prepared for citric acid. The results are presented as mg/100 g DW (dry weight).

Determination of macro and micro minerals

The Na, Cu, Mg, Ca, P, K, Zn, Fe, and Mn content of the potato flours were determined using inductively coupled plasma optical emission spectrophotometer (2100, Perkin Elmer, USA) for all elements, except for 82Se,

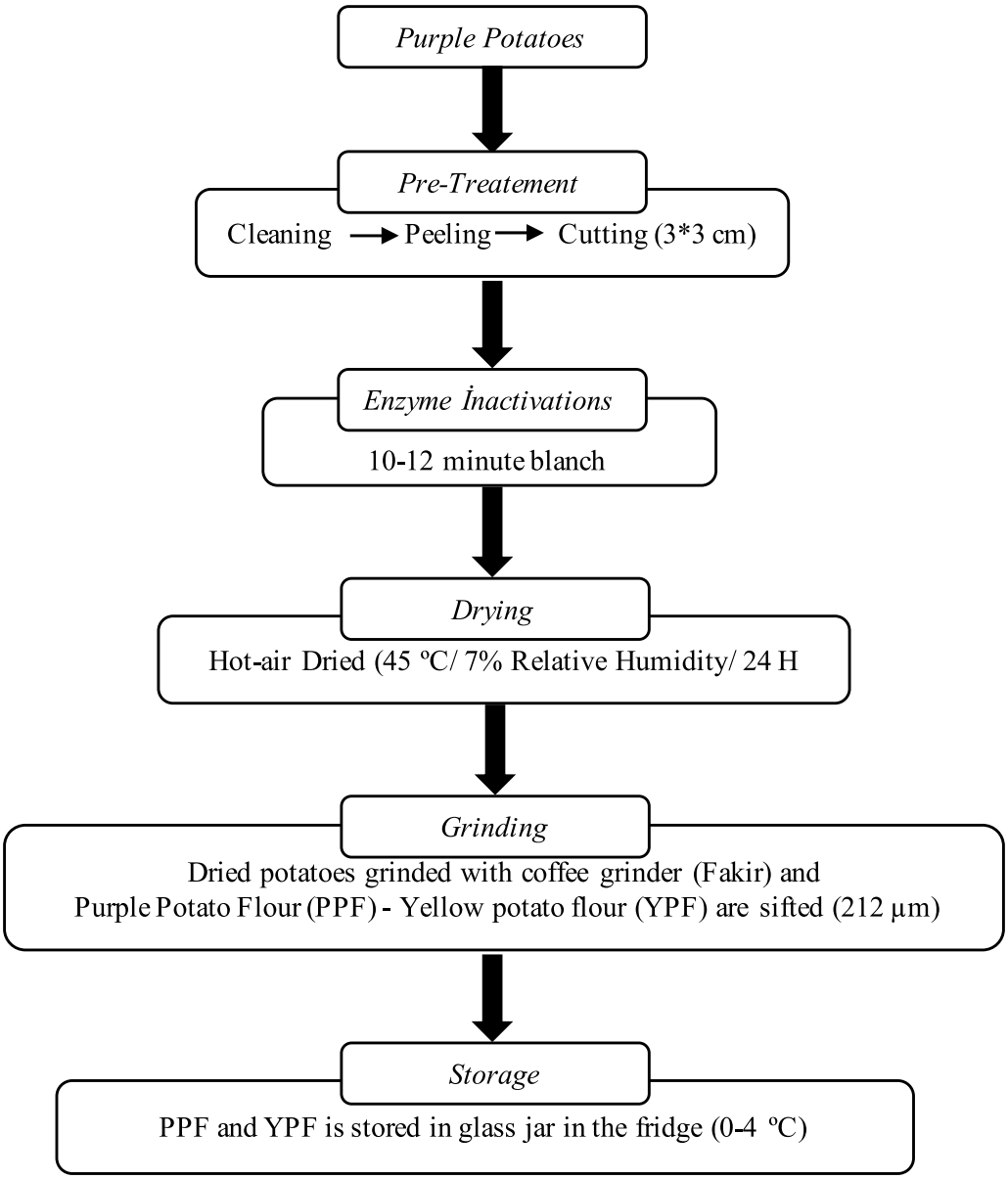


Fig. 1 Potato flour production steps

which was analyzed using inductively coupled plasma-Mass spectrometer ICP-MS (Agilent 7700 × Series Shield Torch System, USA) [32].

Solutions All solutions were prepared using analytical-grade chemicals, and ultra-pure water (18 MΩ cm resistance) obtained through the TKA Ultra Pacific and Genpura water purification system. Nitric acid (67%, Merck, Darmstadt, Germany) was used in the preparation. Argon gas (99.9995% purity, Linde, Türkiye) was used as the carrier gas. The standard solutions (1000 mg/L) for all elements were obtained from Merck (Germany) and used to construct calibration standards. The daily calibration standards were constructed using HNO_3 (0.3%).

Calibration The calibration curves were linearly constructed using standards in the range of 1–20 mg/L for minerals (Ca, P) and 0.1–2 mg/L for Na, Mg, K, Zn, and Fe elements.

Sample preparation For the dissolve process of the samples, a microwave system (Milestone 1200, İtaly) equipped with a rotor containing Teflon-coated polyethylene vessels was employed. Approximately 0.5

g of each sample was taken, and 6 mL of concentrated HNO_3 and 1 mL of H_2O_2 were added. After cooling to an average of 20 °C, the flour samples were placed in 50 mL falcon tubes and diluted with 25 mL of deionized water. The microwave combustion furnace was employed with Step-1 and Step-2 programs. The parameters for each step were set as follows: Ramp (min), Temperature (°C), Holding (min) (Step-1: 10–120-5 and Step-2: 5–200-10) [35].

Extraction of phenolic compounds

Different extraction methods (extractable, hydrolyzable, and bioaccessible) were prepared by modifying the method proposed by Vitali (2009). These extractions were used to analyze antioxidant capacity and phenolic compounds [36].

Extraction of extractable phenols A mixture of 20 mL HCl (37%) and 2.0 g of the flour sample. The solution was then shaken at 250 rpm and 20 °C using a rotary shaker (JB50-D, Shanghai, China) for 2 h. Afterward, it was centrifuged at 3500 rpm for 4 °C/10 min with Sigma 3 K30 (Germany). At the end of the period, the clear upper (supertant) phase in the tube was stored at − 20 °C. The lower phase in the tube was used for the extraction of hydrolyzable phenols. This procedure was repeated two times, and the supernatants were combined.

Extraction of hydrolyzable phenols To the residue remaining from the process mentioned above, a mixture of 20 mL of H_2SO_4-conc/methanol (1:10) was added. The solution was then shaken at 250 rpm and 20 °C using a rotary shaker (JB50-D, Shanghai, China) for 20 h. After the allotted time, the extracts were centrifuged at 3500 rpm for 10 min at 4 °C (Sigma 3 K 30, Germany). The upper phase of the prepared extracts (hydrolyzable) was collected and stored at − 20 °C until using analysis.

In-vitro Bioaccessibility An *in-vitro* enzymatic extraction system mimicking gastrointestinal (GI) conditions was applied with modifications [37].

Oral Phase: Human salivary α-amylase (EC 3.2.1.1) was added to a 2 g sample and stirred, maintaining a pH of 7 for 2 min.

Gastric Phase: Samples from the oral phase were mixed with porcine pepsin (EC 3.4.23.1) in a system adjusted to pH 2. The mixture was then shaken at 250 rpm and 37 °C for 2 h using a rotary shaker (JB50-D, Shanghai, China).

Intestinal Phase: The gastric samples were neutralized to pH 7.2 with 1 M NaOH. Then, pancreatin/bile salt was added based on trypsin activity (100 U mL^{-1} in the final mixture). The mixture was shaken at 250 rpm and 37 °C for 2 h using a rotary shaker. After the allotted time, the extracts were centrifuged at 3500 rpm for 10 min and the upper phase was separated. The analyses were conducted in twice and all obtained extracts were stored at − 20 °C.

Total phenolic content (TPC)

Three extractions (extractable, hydrolyzable, and bioaccessible) of flour samples were determined with the Folin-Ciocalteu colorimetric method [38]. Standards and sample absorbance values readings were taken at a wavelength of 750 nm using a spectrophotometer (Optizen 3220 UV-Mecasys, China). Gallic acid was used as a standard for the determination of TPC content and results calculation has been made using the standart equation $y = 0.2964\times - 0.0012$ ($R^2 = 0.9929$). The samples and standard results were given as mg of gallic acid equivalents per 100 g of fresh weight samples (mg GAE/100 g FW). The total phenolic content was presented as the sum of extractable and hydrolyzable fractions. The analyses were performed three times and given as means ± SD.

Antioxidant capacity (AC)

The potato flour's antioxidant capacities in the hydrolyzable, extractable, and bioaccessible extracts were determined using the Cupric Reducing Antioxidant Capacity (CUPRAC) and Ferric Ion Reducing Antioxidant Power (FRAP) methods [22, 38]. The analytical procedures were applied with modifications to the analysis methods.

CUPRAC method 1.0 $\times 10^{-2}$ M copper(II) chloride solution (1 mL), 7.5 $\times 10^{-3}$ M neocuproine solution (1 mL), 1.0 M ammonium acetate buffer solution (1 mL), and 100 mL of sample extract were taken into a test tube, and the total volume was adjusted to 4 mL with distilled water. After 30 min, the absorbance values were read at 450 nm using a spectrophotometer (Optizen 3220 UV-Mecasys, China) [38]. Results calculation has been made using the standart equation $y = 44{,}593\times + 0.0011$ ($R^2 = 0.9976$).

FRAP method FRAP solution was prepared by mixing 250 mL of TPTZ, 250 mL of $FeCl_3$, and 62.5 mL of acetate buffer solutions. The FRAP solution was heated in a water bath at 37 °C. 100 mL of the sample, 3900 mL of distilled water, and 3 mL of the prepared FRAP solution were mixed in a test tube and kept in a water bath at 37 °C for 15 min. After the allotted time, the absorbance values were read at 595 nm using a spectrophotometer (Optizen 3220 UV-Mecasys, China) [22]. Results calculation has been made using the standart equation $y = 14{,}567\times + 0.0084$ ($R^2 = 0{,}9985$). The result values of

the antioxidant capacity were given as micromoles of Trolox equivalents (TE). The analyses were performed three times and given as means ± SD.

Total flavonoid content (TFC)

For the determination of total flavonoid content, the procedure was modified and applied [39]. In this process, 0.3 mL of the extract was mixed with 0.3 mL of 5% $NaNO_2$ and allowed to stand for 6 min. After this period, 0.3 mL of 10% $Al(NO_3)_3$ was added, and the solution was left for another 6 min. Subsequently, 4 mL of 4% NaOH was added, and the solution volume was adjusted to 10 mL with distilled water. The obtained mixture was left in the dark for 12 min after vortexing for 60 s. After the allotted time, the absorbance was measured at 510 nm using a spectrophotometer (Optizen 3220 UV-Mecasys, China). Results calculation has been made using the standart equation y= 0,011x +0,0018 (R^2= 0,9926). The results were expressed as mg of quercetin equivalent per gram (mg QE/100 g FW).

Total anthocyanin content (TAC)

The determination of total anthocyanin content was conducted using the pH-differential method [39]. For this purpose, 4.95 mL of 0.025 M potassium chloride (pH =1) and 0.4 M sodium acetate (pH =4.5) buffers were taken, and 0.5 µL of the sample was added. After 30 min, the absorbances of the samples were measured at 510 nm and 700 nm. The results were calculated using the provided equations (Eqs. 1–2) and calculated as cyanidin-3-glucoside equivalents (mg/100 g).

$$\mathrm{TAC}\left(\mathrm{mg\ ciyanidin-3-glycoside/100g}\right) = \mathrm{A.\ Df.1000.\ M}/\varepsilon.1 \quad (1)$$

$$\mathrm{A} = \left(\mathrm{A}_{510} - \mathrm{A}_{700}\right)_{\mathrm{pH=1}} - \left(\mathrm{A}_{510} - \mathrm{A}_{700}\right)_{\mathrm{pH=4.5}} \quad (2)$$

$$\left(M:\ 449,2\,g/mol;\ \varepsilon:\ 26900;\ Df:\ Dilution\,factor\right)$$

Determination of polyphenol composition

For the extraction of the polyphenolic compounds from the potato flour, a method developed and some modifications [41].

Sample preparation A mixture of hydrochloric acid/methanol/water (1:80:19, v/v) was used for the extraction of the phenolic compounds. Ten milliliters of the extraction mixture was added to 1 g sample and shaken for 2 h at 20 ℃. The supernatant obtained after centrifugation (5 min) was used for analysis. The samples were filtered through a 0.22-µm filter, transferred into a vial, and injected into the LC-QTOF (6550 Agilent, I-Funnel) device.

Analyses The anthocyanins and phenolic acids were identified and quantified using an LC-QTOF/MS–ESI system (6550, Agilent Technologies, USA) equipped with a Poroshell 120 EC- 4.6 ×100 mm, 2.7 µm/C-18 column. The elution of these contents was achieved with a mobile phase comprising two solvents: (A) formic acid (0.1%, v/v) with water and (B) formic acid (0.1%, v/v) with acetonitrile.

For phenolic acids the applied gradient was as follows: 0.6 min, 5% B; 0.5–10 min, 5–95% B; 10–12 min, 95% B; 12–15 min, 95–5% B; 15–20 min, 5% B. The analysis was performed in the negative ionization mode with the mass range set at m/z 50–1000. The optimized instrument conditions were as follows: drying N_2 gas flow rate, 11 L min^{-1}; temperature, 175 °C; nebulizer, 45 psig; capillary voltage, 3500 V; fragmentor, 300.

For anthocyanins The applied gradient was as follows: 0.5 min, 5% B; 0.5–10 min, 5–40% B; 10–22 min, 40–100% B; 22–27 min, 100–5% B; 27–30 min, 5% B. The analysis was performed in the positive ionization mode with the mass range set at m/z 50–1000. The optimized instrument conditions were set as follows: drying N_2 gas flow rate, 13 L min^{-1}; temperature, 275 °C; nebulizer, 35 psig; capillary voltage, 3500 V; fragmentor, 400.

İn-vitro biological activities

In-vitro bioaccessibility To determine the bioaccessibility %, the calculations were performed using the formula in which bioaccessibility is calculated as the percentage ratio of the results obtained from bioaccessible fractions to the sum of results from extractable and hydrolyzable fractions, as shown in Eq. 3 [42].

$$\text{Bioaccessibility \%} = \left(\frac{Biyoaccessible\,fraction}{Extractable\,fractions + Hydrolisable\,fractions}\right) \times 100 \quad (3)$$

In-vitro anti-diabetic activities: α-amylase and α-glucosidase enzyme inhibition methods were used to determine the % anti-diabetic activities. Acarbose was used as a standard for both methods. The results are given as % enzyme inhibition. All the tests were performed in triplicate.

α-amylase inhibitions: 1% starch solution was prepared, kept in 100 °C boiling water for 5 min, and then cooled to 25 °C temperature [43, 44]. The total volume was made up to 100 mL with 0.02 M sodium phosphate buffer. 1 mL sample extract, 500 µL α-amylase enzyme was added and incubated at 37 °C/10 min. After adding 500 µL of starch (1%) solution to the sample and incubating it at 37 °C/10 min. 500 µL 3,5-dinitro salicylic acid (DNSA) was added to terminate the digestion and 100 °C water bath for 5 min. The solution was completed with pure water to a total volume of 4 mL. The absorbance was measured at 540 nm using a spectrophotometer (Optizen 3220 UV-Mecasys). İnhibitions was calculates using the Eq. 4. below.

$$\text{Inhibitions (\%)}: \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}}\right] \times 100 \quad (4)$$

α-glucosidase inhibitions: 200 µL of α-glucosidase solution (100 mg/mL) was added to 200 µL of sample and incubated at 37 °C/10 min [45, 46]. To initiate digestion, 100 µL of 4-Nitrophenyl α-D-glucopyranoside (PNPG) (20 mM) was added to the solution and incubated again at 37 °C/10 min. To terminate the reaction, 1000 µL Na_2CO_3 (0.2 M) was added, and the absorbance using a spectrophotometer (Optizen 3220 UV-Mecasys, China) was measured at 405 nm. İnhibitions was calculates using the Eq. 4.

In-vitro anti-inflamotary activities The reaction mixture is constituted (200 mM, pH = 7, 37 °C) by 100 µL extract and to start the reaction 50 µL hyaluronic acid (5 mg/mL) was incubated for 10 min/37 °C. Then 50 µL hyaluronidase enzyme (7 U/mL units) (Type IV-S: bovine testes) was added mixture and incubated at 10 min/37 °C again. At the end of the period, 100 µL of potassium tetraborate was added and it was incubated in a water bath at ebullition for 5 min. The mixture was cooled at 20 °C and 2 mL of p-dimethylaminobenzaldehyde were added. Afterward, the absorbance was measured at 586 nm [47].

Statistical analysis

The statistical analysis of the data was conducted using the Minitab 22 statistical software. To determine the statistical differences between the values, the LSD (Least Significant Difference) test was applied at a significance level of $P < 0.05$. The evaluation was based on at least three parallel datasets.

Results and discussion

Nutritional composition

The chemical composition results of the potato flour are presented in Table 1. According to the results, it is observed that the moisture content of YPF was significantly higher (10.89%) than that of PPF (6.21%) ($P < 0.05$). When examining the ash content, PPF (4.09%) was found to have significantly higher values than YPF (3.34%) ($P < 0.05$). In a study conducted by Jeriene et al. (2015) with colored potato varieties, they reported that the ash content of the *Vitelotte* variety a purple potato, was 4.95%, and that the dietary fiber content was 1.85%. While the ash content in our study is similar to this previous study, the dietary fiber content was found to be higher in our study compared to theirs. No statistically significant differences were found in the protein values, suggesting that the average values were similar ($P > 0.05$).

YPF (12.47%) was found to have a significantly higher dietary fiber content than PPF (6.98%) (Table 1). While the dietary fiber content of the potato flour is consistent with other vegetable flours in the literature (artichoke flour, turnip flour, Jerusalem artichoke flour, pumpkin flour), it was observed to have a higher fiber content compared to cereal flours (rice, corn, wheat) and pseudo-cereals (quinoa, amaranth, buckwheat, sorghum) [48–53]. Dietary fiber plays a crucial role in preventing various diseases, particularly diabetes and cancer. Identifying sources of dietary fiber and exploring alternative usage possibilities are crucial not only for disease prevention but also due to many positive effects on our

Table 1 Physico-chemical and proximate compositions of potatoes flours*

Physico-chemical Analyses	PPF	YPF
Moisture	6.21 ± 0.23[b]	10.89 ± 0.03[a]
Ash (% DW)	4.09 ± 0.17[a]	3.34 ± 0.09[b]
Fat (% DW)	0.29 ± 0.00[a]	0.14 ± 0.00[b]
Protein (% DW)	6.56 ± 0.52[a]	6.49 ± 0.14[a]
Total dietary fiber (% DW)	6.98 ± 0.43[b]	12.47 ± 0.13[a]
Carbohydrate (% DW)	82.15 ± 0.15[a]	78.48 ± 0.22[b]
Energy (kcal)	329.65 ± 2.12[a]	289.00 ± 0.31[b]
Acidity (%)	0.72 ± 0.00[a]	0.36 ± 0.00[b]
pH	6.28 ± 0.01[b]	6.75 ± 0.01[a]
L^*	42.84 ± 0.01[b]	65.29 ± 0.01[a]
a	8.63 ± 0.01[a]	0.10 ± 0.00[b]
b	−6.76 ± 0.00[b]	13.99 ± 0.01[a]

* Statistically, there is a significant difference between values within different letters on the same row ($P \leq 0.05$). The data are expressed as means ± standard deviations. (*PPF* Purple Potato Flour, *YPF* Yellow Potato Flour, *DW*: Dry Weight)

Table 2 Performance characteristic of mineral determination methods*

ICP-OES	Linear Range	LOD (mg/kg)	LOQ (mg/kg)
Na	0.1–2 mg/L	1.4	13.5
K	0.1–2 mg/L	2.2	7.4
Mg	0.1–2 mg/L	2.1	6.9
Ca	1–20 mg/L	2.7	9.1
P	1–20 mg/L	1.3	4.4
Fe	0.1–2 mg/L	0.3	1.0
Zn	0.1–2 mg/L	0.3	0.8
ICP-MS	**Linear Range**	**LOD (mg/kg)**	**LOQ (mg/kg)**
^{78}Se	1–200 µg/L	0.019	0.063

* LOD Limit of detection, *LOQ* Limit of quantification

health [54]. Our study demonstrated that potato flour was a good source of dietary fiber and could be preferred as an alternative flour source in the production of functional foods. Research on potatoes has identified them as a rich source of dietary fiber [55]. However, studies specifically comparing the dietary fiber content across different potato varieties are limited. The observed differences in fiber content are likely influenced by factors such as starch composition, cultivation practices, soil characteristics, and varietal distinctions.

Color is one of the most important quality criteria in foods, and it is desired that applied processes do not cause color changes [30]. Particularly, thermal processes applied to purple vegetables and fruits with high anthocyanin content are known to cause color losses, leading to an increase in browning as the values of a^* and b^* decrease [56–58]. Additionally, the prolonged drying of purple vegetables at high temperatures can result in undesirable color formation, nutrient loss, and deterioration of sensory properties [23, 59, 60]. Therefore, when producing flour from colored vegetables process criteria should be carefully determined.

According to the color results of the obtained flours, the L^*, a^*, and b^* values for PPF were 42.84, 8.63, and −6.76, while they were 65.29, 0.10, and 13.99 for YPF, respectively. The L^* value is a measure of brightness, and as the product color darkens, the L^* value decreases. As expected, in this study, the dark purple-colored PF gave a lower L^* value than the yellow-colored PF. The b^* value is a measure of yellowness, and accordingly, the b^* value of YPF was higher than that of PPF. The value in the flour samples was 0.10 for YPF and 8.63 for PPF.

Kasnak and Palamutoğlu (2022) reported that the L^* value of yellow potatoes varied between 64.75 and 69.82 in their study with different potato types [61]. Karacabey et al. (2023), investigating the effect of different drying pretreatments on purple potatoes, the L^*, a^*, and b^* values of fresh purple potatoes were found to be 27.07, 10.89, and −6.80, respectively [62]. Comparing these values, it can be observed that the L^* value of the purple potato flour we produced is high and that the a^* value is relatively low. The b value in our study is close to the values reported in that study, indicating that there might not be a browning reaction in the flour. Similar results were obtained in other studies with purple vegetable flours in the literature [63, 64].

Macro and micro minerals

The performance limits of the methods used for mineral determination in PPF and YPF are provided in Table 2 and the mineral quantities are presented in Fig. 2a–b. According to these results, the mineral content for PPF and YPF were determined as K > P > Na > Mg > Ca > Fe > Zn > Mn > Cu > Se. Except for Cu, all mineral quantities in PPF were found to be significantly higher than those in YPF ($P < 0.05$). Potassium was identified as the dominant mineral substance in both potato flours, aligning with the literature [65, 66]. The K values for PPF (1498.69 mg/100 g) and YPF (1470 mg/100 g) were observed to be higher than potassium-rich flours such as banana flour (571.84–978.22 mg/100 g) and purple carrot flour (271.15 mg/100 g) [66, 67]. Vaitkevičienė (2019), in a study involving various types of colored potatoes, reported that *Violetta* purple potatoes showed a predominant values of K (2200 mg/100 g), followed by P (360 mg/100 g) and Mg (114 mg/100 g) [69]. The bioaccessibility of minerals is a critical factor for assessing the functional properties of foods. During the production of flour, low-temperature drying and grinding processes can enhance the breakdown of complex ligands, potentially improving the bioaccessibility of metal ions. However, these processes may also negatively impact bioaccessibility by affecting enzyme inhibitors, leading to the formation of insoluble

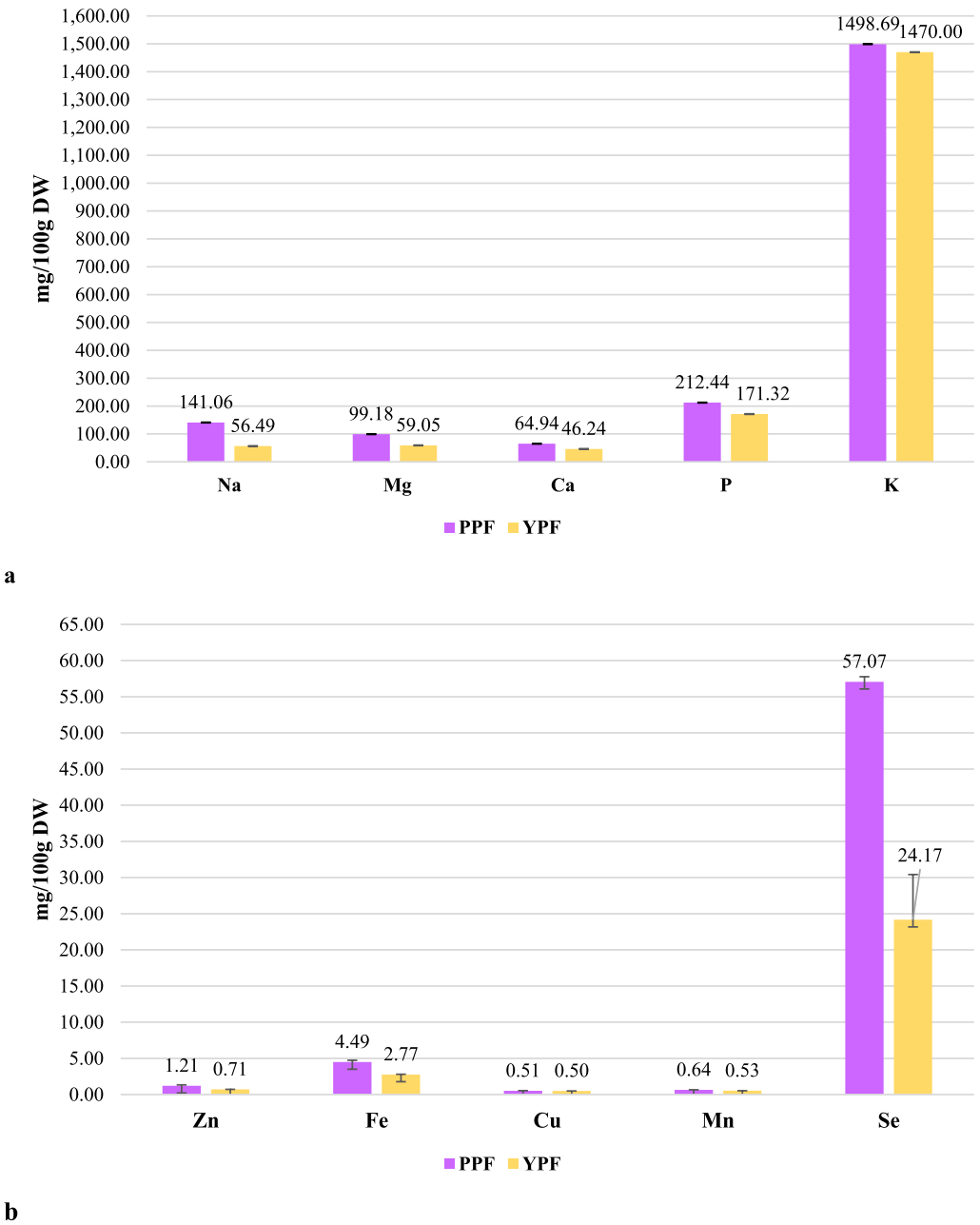


Fig. 2 Potato flours **a** macro and **b** micro mineral content. (PPF: purple potato flour; YPF: yellow potato flour; DW: dry weight

metal compounds (precipitates) [70, 71]. A comprehensive evaluation of these effects requires considering the bioaccessibility of minerals and their dynamic interactions within the body in a complex and holistic manner. The mineral substance results and their ranking in our study align with this research. The favorable mineral content of the functionally produced potato flours indicates their high nutritional value. Considering the daily recommended intake of macro and micro minerals, it was determined that potato flour could serve as a good source of K, P, Na, Mg, Ca, Fe, and Zn in our diet.

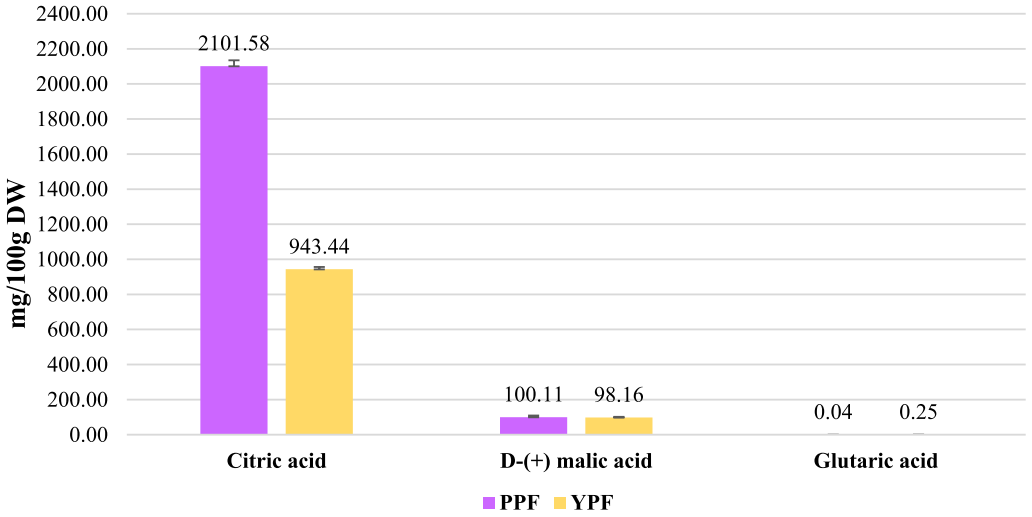


Fig. 3 Organic acid compositions of potatoes flours. (PPF: purple potato flour; YPF: yellow potato flour; DW: dry weight)

Organic acids

Organic acids can be defined as food components with high antioxidant properties found in vegetables and fruits [72]. The results of Citric acid content for PPF and YPF are provided in Fig. 3**.** Upon examination, the citric acid content of PPF (2101.58 mg/100 g) was found to be significantly higher than that of YPF (943.44 mg/100 g) ($P < 0.05$). In YPF, the D-(+) malic acid content was determined to be 98.16 ± 3.63 mg/100 g DW. Similarly, D-(+) malic acid was identified in PPF, with both potato types showing relatively high malic acid levels. Different studies in the literature indicated that the dominant organic acid in potatoes was citric acid [73, 74] reporting that the amount of citric acid varied between 4.21 and 11.3 g/kg in different potato genotypes [75].

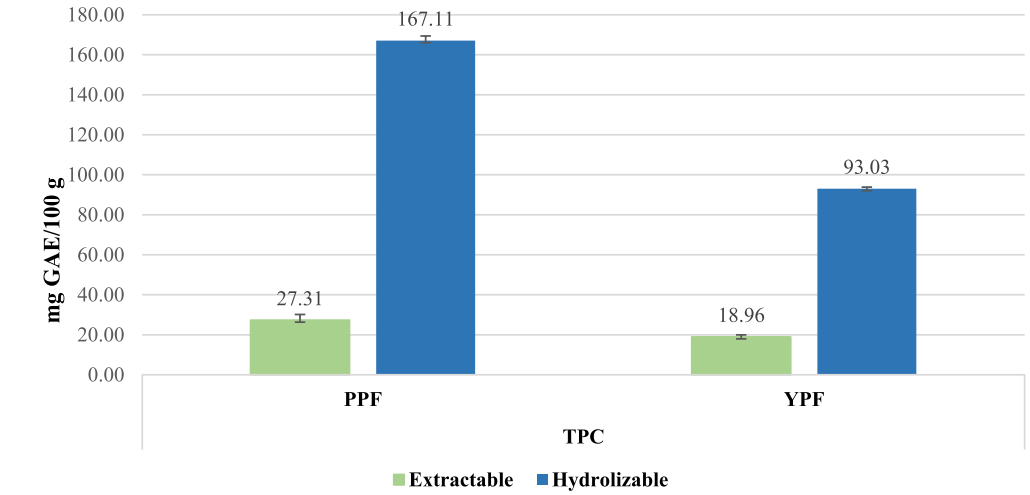
Total phenolic content (TPC)

PPF and YPF's TPC results are presented in Fig. 4a and Fig. 5a. The TPC amounts were found to be 194.41 mg GAE/100 g DW for PPF and 111.99 mg GAE/100 g DW for YPF. It was determined that the TPC content of PPF was significantly higher than that of YPF ($P < 0.05$). In different studies in the literature, the TPC value of potatoes was shown to vary between 54 and 359 mg GAE/100 DW among different genotypes. Moreover, colored potato genotypes, especially Heijigang (299.13 mg GAE/100 g DW), Purple Majesty (63.54 mg GAE/100 g FW), and Mountain Rose (61.41 mg GAE/100 g FW), were reported to have at least twice total phenolic content compared to classic yellow types [16, 17, 76]. This difference is attributed to genetic makeup and pigmentation levels. Additionally, some studies indicated that the TPC value in vegetables was influenced by climatic conditions, agrotechnical processes, phenolic stability, and post-harvest processes [10].

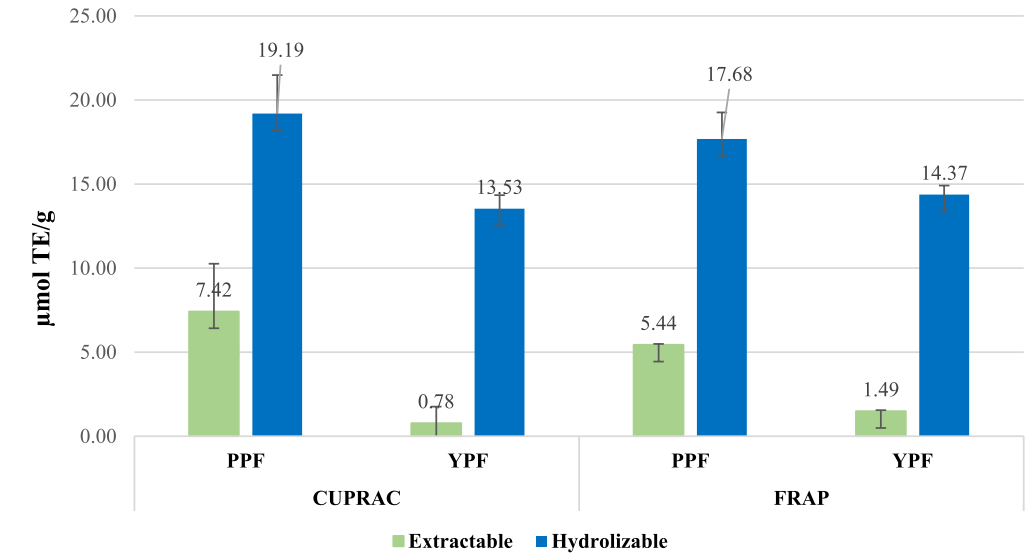
Antioxidant capacity

PPF and YPF's extractable and hydrolyzable fractions of PPF and YPF sample results are presented in Fig. 4b. The results of the total antioxidant capacity of potato flours according to the CUPRAC and FRAP methods are presented in Fig. 5b as the sum of extractable and hydrolyzable fractions. In the CUPRAC method, PPF showed 26.61 µmol TE/g, and YPF showed 14.31 µmol TE/g, while in the FRAP method, PPF showed 23.12 µmol TE/g, and YPF showed 15.86 µmol TE/g. It was observed that the antioxidant capacity of PPF was significantly higher than that of YPF in both methods ($P < 0.05$). Across all flour samples, it was noticed that the hydrolyzable fractions consistently exhibited higher values than the extractable fractions for both antioxidant methods (Fig. 5b). In a study on dried purple potatoes, Lee et al. (2016) reported antioxidant amounts of 89.95 mg/100 g for purple potatoes and 1.56/100 g for yellow potatoes [64]. Similarly, Soare et al. (2020) found that colored potato varieties (Blue Congo, Purple Majesty, Mountain Rose, Cranberry Red) exhibited significantly higher antioxidant capacities compared to classic types [16]. These results align with our study, showing that, according to the CUPRAC method, the antioxidant capacity of purple potato flour is significantly higher than that of yellow potato flour ($P < 0.05$).

The obtained results, when compared with studies on colored vegetable flours in the literature, indicate that the antioxidant capacities of the vegetables were higher than those of purple carrot powder (62.18 µmol TE/100



a



b

Fig. 4 **a–b** Extractable and hydrolyzable fractions of PPF and YPF samples. **a** (TPC, Total phenol content was calculated as the sum of extractable and hydrolysable fraction. The results were expressed as gallic acid equivalents (GAE) per kilogram of dry weight (mg GAE/100 g); **b** Antioxidant capacity methods: CUPRAC, cupric ion reducing power assay; FRAP, ferric reducing antioxidant power; The results are expressed in terms of micromoles Trolox equivalents per kilogram of dry weight (µmol TE/g). TPC: mg GAE/100 g; CUPRAC: µmol TE/g; FRAP: µmol TE/g; PPF: purple potato flour; YPF: yellow potato flour)

g DW) [77], turnip flour (99.55 µmol TE/100 g DW) [78] and pumpkin flour (0.64 µmol TE/100 g DW) [53]. It was suggested that purple potato flour was suitable for use as a functional ingredient in developing products as an antioxidant source.

Total flavonoid content (TFC)

The TFC results for the potato flours were determined as 58.08 mg QE/100 g FW for PPF and 25.74 mg QE/100 g FW for YPF (Fig. 5c). It was observed that the total flavonoid content of PPF was significantly higher than that of YPF ($P < 0.05$). Soare et al. (2020) reported that TFC results for different types of colored potatoes ranged from 11.02 to 40.96 mg QE/100 g [16]. In a study

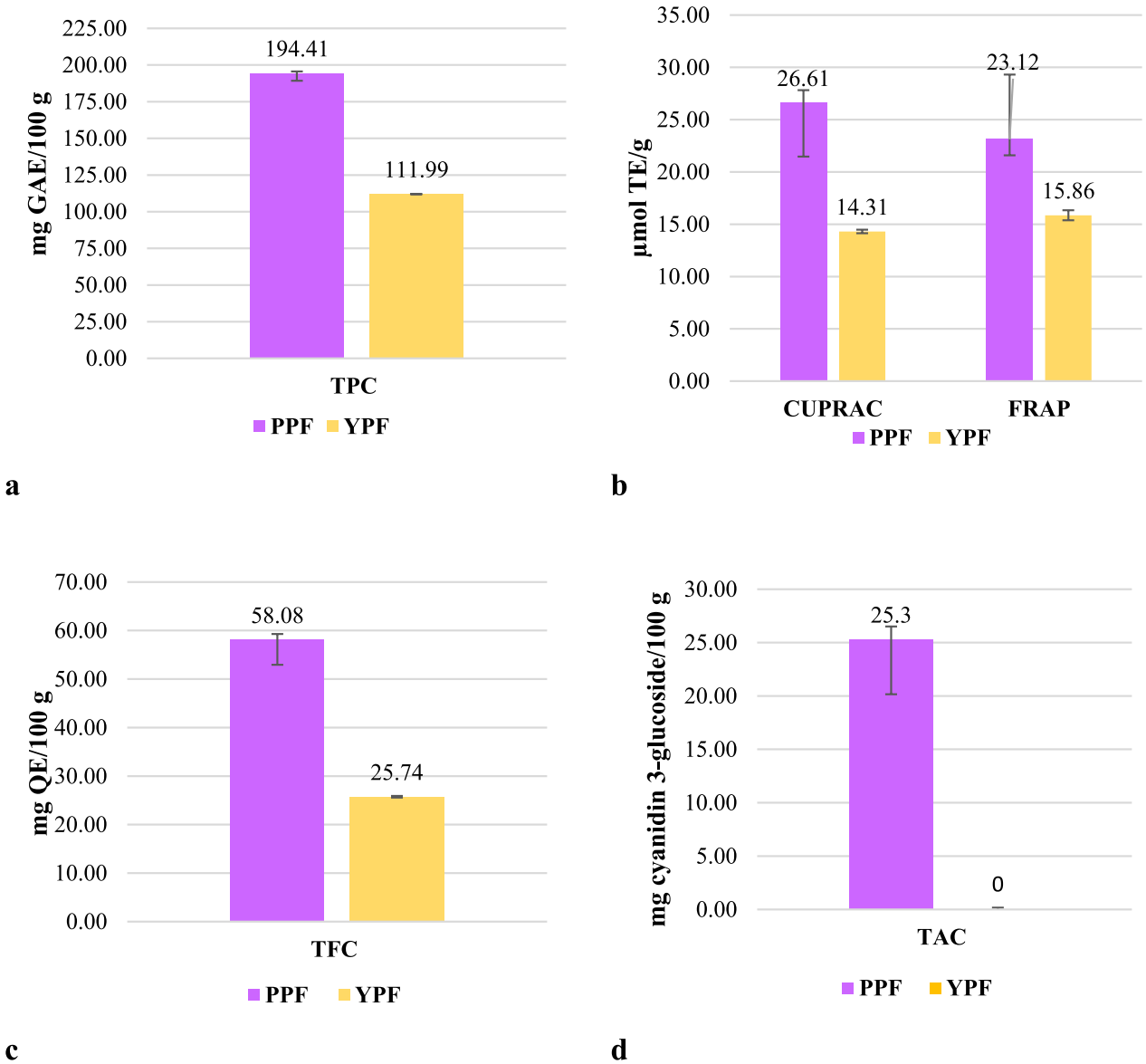


Fig. 5 **a–d** Functional properties of potatoes flours. *TPC* total phenol content, *CUPRAC* cupric ion reducing power assay, *FRAP* ferric reducing antioxidant power, *TFC* total flavonoid content, *TAC* total anthocyanin content, *PPF* purple potato flour, *YPF* yellow potato flour. The results are expressed in terms of TPC **a**: mg GAE/100 g; CUPRAC **b**: µmol TE/g; FRAP **b**: µmol TE/g; TFC **c**: mg QE/100 g; TAC **d**: mg cyanidin 3-glucoside/100 g)

conducted on flours obtained through freeze-drying, they mentioned that purple potato flours (76.69 mg QE/100 g DW) had a higher flavonoid content than yellow potato flour (55.12 mg QE/100 g DW) [17]. These results are consistent with our current study.

Total anthocyanin content

Anthocyanins are a group of polyphenols found in red, purple and blue fruits and vegetables [79]. The total anthocyanin content of PPF was determined to be 25.30 ± 1.89 mg cyanidin-3-glucoside/100 g (Fig. 5d), while as expected anthocyanins were not detected in YPF. Similar studies in the literature reported that blue and purple potato types were rich in anthocyanins [15, 63]. In a study, it was indicated that the high amount of anthocyanins found in *Vitoletta* purple potato exhibited anticancer properties [80]. There are also studies suggesting that the skin powder of colored potatoes contains more anthocyanins than the powder of the vegetable itself [81]. The amount of anthocyanins can vary depending on the degree of pigmentation of the vegetable, the parts in which it is found (skin, root, etc.), and the choice of

Table 3 Phenolic compounds in potato flours*

Phenolic classes	Phenolic compounds (mg/100 g DW)	PPF	YPF
Phenolic acids	Chlorogenic acid	143.79 ± 1.81[a]	27.43 ± 0.26[b]
	D-(+) malic acid	100.11 ± 9.16[a]	98.16 ± 3.63[b]
	Suscinic acid (Butanedionic acid)	7.57 ± 0.02[b]	18.37 ± 1.02[a]
	Ferrulic acid	10.12 ± 0.1[a]	0.87 ± 0.00[b]
	p_hydroxy benzoic acid (4 hydroxy benzoic acid)	2.31 ± 0.01[a]	2.29 ± 0.01[b]
	Caffeic acid	1.86 ± 0.01[a]	0.42 ± 0.00[b]
	(Y) p-cumaric acid	0.29 ± 0.02	N.D
	3-Hydroxybenzoic acid	N.D	0.23 ± 0.00
	Glutaric acid	0.04 ± 0.00[b]	0.25 ± 0.00[a]
	3,4 Dihydroxybenzoic acid (Protocatechuic acid)	N.D	0.32 ± 0.00
Flavan-3-ol	Catechin	0.21 ± 0.00[b]	0.22 ± 0.00[a]
Flavonols	Rutin hydrate (Quercetin-3-O-rutinoside hydrate)	0.20 ± 0.00[b]	0.29 ± 0.00[a]
Flavonoid	Quercetin (Quercetin −3-O-rhamnoside)	0.26 ± 0.02[a]	0.04 ± 0.01[b]
Flavanol (kateşinler)	Epicatechin gallate	N.D	0.03 ± 0.00
Anthocyanidins	Cyanin chloride Cyanidin chloride Malvidine chloride	0.45 ± 0.00 0.85 ± 0.00 31.41 ± 1.61	N.D N.D N.D

* Mean values represented by the same line within the same column are not significantly different at $P \leq 0.05$. The data are expressed as means ± standard deviations. (*PPF* Purple Potato Flour, *YPF* Yellow Potato Flour, *N.D* Not determined, *DW* dry weight)

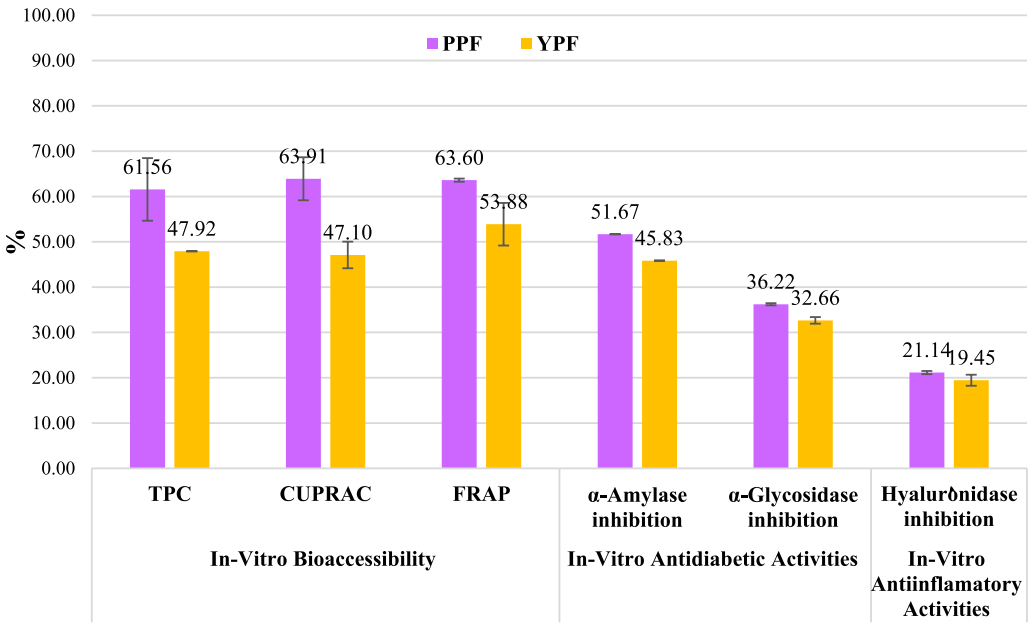


Fig. 6 *İn-Vitro* biological activities of potatoes flours. *TPC* Total phenol content, *CUPRAC*, cupric ion reducing power assay, *FRAP* ferric reducing antioxidant power, *PPF* purple potato flour, *YPF* yellow potato flour

solvents (methanol, ethanol, and distilled water) used in the analysis [82]. Similarly, many studies showed that the anthocyanin content of purple grains, such as purple corn flour [83, 84], purple wheat flour [85] and purple rice flour [86] was higher compared to traditional grains.

Determination of polyphenol composition

The phenolic components of the potato flours included various groups, such as phenolic acids, flavan-3-ols, flavonoids, flavonols, and anthocyanin compounds as identified (Table 3). The predominant phenolic component identified in PPF was chlorogenic acid at 143.79 ± 1.81 mg/100 g DW. The other polyphenols found in PPF included ferulic acid, catechin, quercetin, cyanidin chloride, and malvidin chloride. The compounds belonging to the anthocyanidin group, such as cyanin chloride, cyanidin chloride, and malvidin chloride, were detected in PPF but not in YPF. Purple potatoes primarily contain phenolic compounds such as pelargonidin, malvidin, petunidin, and delphinidin [87]. It was observed that these anthocyanin compounds, along with rutin and quercetin, exhibited high antioxidant activity and also possessed anti-inflammatory properties [88].

In-vitro biological activities

In-vitro bioaccessibility The bioaccessibility of total phenolic content in PPF was determined to be approximately 61% for both the FRAP and CUPRAC methods, while it was around 63% (Fig. 6). In YPF, the bioaccessibility of total phenolic content was found to be 47%, with values of 53% and 47% for the FRAP and CUPRAC methods, respectively. Upon evaluation of the results, it was observed that the bioaccessibility of PPF was higher than that of YPF.

When examining the *in-vitro* % bioaccessibility results of different flour samples in the literature, it was observed that sweet potato flour [28] ranged around 22%, that soybean flour [28] varied between 22 and 45% and that sorghum flour [89] showed a range of 5.26–26.31%. Aydın and Göçmen (2015) reported that pumpkin flour had a *in-vitro* bioaccessibility of around 30.78% and that hydrolyzable phenols gave higher results than extractable phenols [90].

In-vitro anti-diabetic activities α-glycosidase is an enzyme found in the small intestine epithelium. It plays a role in breaking down carbohydrates consumed in the diet into glucose. For this purpose, it is utilized in anti-diabetic activity studies to understand the effect of a food matrix on our body's glucose levels and its relationship with diabetes in an in vitro environment.

PPF and YPF α-amylase enzyme inhibitions were determined to be respectively 51.67% and 45.83%. PPF and YPF α-glycosidase enzyme inhibitions were determined to be respectively 36.27% and 23.66% (Fig. 6). According to these results, it was seen that PPF had better enzyme inhibition activities than YPF ($P < 0.05$). In both methods, it was determined that purple potato flour gave the closest value to acarbose standards. Similarly, the latest studies have reported higher anti-diabetic activity of gluten-free flours as fruit, vegetable, and grain flours [91–93]. In studies conducted with plant-based foods, the evaluation of antidiabetic activity is typically interpreted alongside the food's antioxidant activity and phenolic compounds [94]. These results indicate that the α-glycosidase enzyme activity of potatoes is not entirely dependent on the amount of phenols present, but that the hydroxyl group in the structure of phenolic compounds, especially flavonoids, is higher. Additionally, the fact that the flours are rich in non-phenolic compounds such as organic acids, dietary fiber, and minerals has also contributed to their strong antidiabetic effects [95–97]. Therefore, consumption of purple and yellow potato flour may provide anti-diabetic benefits.

In-vitro anti-inflammatory activities PPF and YPF hyaluronidase enzyme inhibitions was determined to be respectively 21.14% and 19.45% (Fig. 6). The value of the quercetin used as the standard % is 44.25. The ability of plants to inhibit enzymes varies depending on the region where the plant grows, the growing conditions, and its phenolic composition. This makes comparison of our results with the literature difficult. Therewithal purple potato flour is a good source of flavonoid and phenolic compounds. Studies have shown that phenolic compounds such as rutin, quercetin, catechins, and chlorogenic acid can inhibit amylase and glycosidase enzymes and act as an anti-diabetic and anti-inflammatory agent by delaying carbohydrate absorption [97–102].

The data obtained in our study are in line with these literature findings. These results indicate that the use of purple potato flour in our daily diet could be highly beneficial for health. According to the study results, purple potato flour appeared to be a highly valuable source of anthocyanins, phenolic compounds, and functional properties. Additionally, it was found that purple potato flour had higher amounts of mineral content, antioxidant capacity, phenolic compounds, and *in-vitro* biological activities compared to other gluten-free flour.

Conclusion

As interest in healthy eating increases, people's eating habits and interest in trendy foods are growing day by day. Along with this, foods are increasingly being consumed based on their functional properties. Gluten-free products like purple potato flour, which have a long shelf life, are suitable for daily nutrition and are economically viable, can be used as alternative flour sources. Additionally, purple potato flour is odorless and easily integrates into almost all baked goods, providing

convenience in use. It also offers potential as a natural colorant in dairy desserts and children's snacks. Besides its physical advantages, purple potato flour can also be used as a functional food due to its high nutritional properties. Purple potato flour is, therefore, considered a good gluten-free alternative, especially for individuals with celiac disease or those who prefer gluten-free or functional nutrition and can be used in our daily diet. Given the limited research on purple potato flour, many areas related to the functionalization of products such as cakes, biscuits, and bread can be explored in future studies.

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Author contributions
Conceptualization, D.D.A., Y.S.; data curation and methodology, M.S and D.D.A.; formal analysis, M.S and D.D.A.; investigation, D.D.A. and M.S.; writing—original draft, Y.S, D.D.A, M.S.; writing—review and editing, Y.S., D.D.A., M.S. All authors have read and agreed to the published version of the manuscript.

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