



Nutritional composition and antidiabetic potential of fine and coarse flours from common and tartary buckwheat

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ABSTRACT

This study evaluates the nutritional and bioactive properties and the *in vitro* antidiabetic activity of fine and coarse flours derived from several common and tartary buckwheat (*Fagopyrum esculentum* Moench and *Fagopyrum tataricum* (L.) Gaertn, respectively) varieties. Fine flours were a good source of protein (8.9–13.3 g/100 g), starch (55.9–65.6 g/100 g), riboflavin (117–172 µg/100 g) and had low phytic acid content (0.10–1.04 g/100g), with high differences among varieties. Tartary buckwheat showed the highest riboflavin content and dipeptidyl-peptidase (DPP-IV) inhibitory activity and stood out for its high protein content. Coarse flours, in general, exhibited lower nutritional value than fine flours. Fine flours from all buckwheat varieties had a moderate glycemic index (45.6–60.7 %) and inhibited DPP-IV, α -glucosidase, and α -amylase activities. The findings of this work evidenced the potential of buckwheat to boost the nutritional content of gluten-free diets offering additional health benefits.

1. Introduction

Celiac disease is an immune system-mediated disorder marked by a chronic inflammatory reaction to gluten, which needs strict compliance to a gluten-free diet during whole life (Catassi et al., 2022). While essential for managing the disease, this dietary restriction often results in significant nutritional deficiencies. A number of studies have consistently highlighted deficiencies in micronutrients such as vitamins D, E, B9, B1, B2, and B6 among individuals adhering to gluten-free diets (Ballester-Fernández et al., 2021; González et al., 2018; Hallert et al., 2002). These deficiencies are often attributed to the limited choice of gluten-free grains that are commonly consumed in refined form with lower nutritional value compared to whole grains. Consequently, there is important to investigate and characterize alternative gluten-free grains that offer improved nutritional profiles to boost the quality of gluten-free diets (Cardo et al., 2021).

Buckwheat (*Fagopyrum esculentum* Moench) has emerged as a

noteworthy ingredient for gluten-free diets since it lacks prolamin proteins, which make it safe for people suffering celiac disease and provides excellent nutritional and health benefits, as demonstrated by previous research from our group (Bani et al., 2025). In addition, buckwheat is beneficial for the prevention and management of diabetes due to its moderate glycemic index (Zhu, 2016). It is also rich in ty proteins, essential amino acids, and dietary fiber, and exhibits a unique profile of antioxidants and phenolic compounds, that show anti-inflammatory and antioxidant properties (Kreft & Škrabanja, 2002; Giménez-Bastida et al., 2015). From a nutritional standpoint, buckwheat is noteworthy for its low carbohydrate amount (54.5–78.3 % dry matter), and its high resistant starch content, making it a favorable alternative for diabetic individuals (Yang et al., 2019). Its dietary fiber content, including both soluble and insoluble fractions, is superior to other pseudocereals and is comparable to cereal grains (Alvarez-Jubete et al., 2009). Buckwheat's protein content, ranging from 5.7 % to 16.4 % dry matter, exceeds that of cereals including wheat, rice, and maize, with a well-balanced pattern

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of essential amino acids. While its lipid level is lower than that of major cereals, this pseudocereal has high levels of unsaturated fatty acids (Bonafaccia & Fabjan, 2003). Moreover, this pseudocereal provides an excellent amount of essential bioavailable minerals and vitamins (Rodríguez-Salinas et al., 2020). Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn) is a good source of flavonoids, which has antioxidant and anti-inflammatory properties (Di Lorenzo et al., 2021; Li et al., 2019; Piazza et al., 2024).

Despite the nutritional advantages, the full potential of buckwheat in gluten-free diets remains underexplored, especially regarding the different varieties and processing forms. The type of variety is a key factor influencing the content of nutrients and bioactive compounds. Ad instance, the different levels of starch digestibility in diverse buckwheat varieties can influence their glycemic response. Moreover, the different content of phytic acid in the diverse buckwheat varieties affects the mineral bioavailability. On the other hand, agri-food industry applies milling and sieving methods to market flours with different particle sizes. Particle size has a significant impact on the nutritional composition and functional properties of the resultant flours (Tian et al., 2022). Therefore, a comprehensive investigation into the influence of buckwheat variety and technological factors such as seed matrix structure and particle size on the nutritional and bioactive features of flours is crucial to market high-quality flours. This study aims to fill this gap by evaluating the nutritional composition and bioactive properties of fine and coarse flours (semi-finished products) obtained from different buckwheat varieties. The protein profile, total starch, riboflavin, and phytic acid contents were studied. Additionally, the glycemic index, and the inhibitory activity against dipeptidyl-peptidase (DPP-IV), α -amylase, and α -glucosidase were examined to elucidate the potential antidiabetic effects of these buckwheat varieties. By integrating the nutritional, and bioactive findings, this study seeks to offer insightful information on the health-promoting properties of buckwheat and their practical applications in gluten-free diets.

2. Materials and methods

2.1. Samples

A total of 9 varieties of common buckwheat (*Fagopyrum esculentum* Moench), and 1 variety of Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn) (Table 1) were studied. Common buckwheat samples were supplied in both fine flours (<250 μ m of particle size) and coarse flours (semi-finished products, >250 μ m of particle size), while the Tartary buckwheat was only supplied as fine flour. Samples were stored at 4 °C in darkness.

2.2. Nutritional evaluation

2.2.1. Protein content

The total protein content in buckwheat was analyzed using the Dumas method (AOAC 920.87) with a TruMac elemental analyzer (LECO Corp., St. Joseph, MI) as previously reported (Bautista-Expósito,

Vandenberg, Peñas, Frias, & Martínez-Villaluenga, 2021). A factor of 5.83 was used to obtain total protein concentration. Results were expressed as g/100 g of sample dry weight (d.w.).

2.2.2. Protein profile

To assess the protein profile, 40 mg of buckwheat sample were combined with 2 mL of sample buffer (0.0625 mol/L Tris-HCl pH 6.8, 1.875 % glycerol, 0.5 % SDS, 2.5 % β -mercaptoethanol) mixed with water (1:1 v/v), to reach a concentration of 20 mg/mL. The samples were shaken for 1 h on a plate shaker. A molecular weight marker (broad range, Bio-Rad) was run in parallel to the samples. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a gradient gel (9–19 % acrylamide) according to Ballabio et al. (2011). Proteins were precipitated by immersing the gel in a 20 % trichloroacetic acid (TCA) solution (Sigma-Aldrich, Steinheim, Germany) for 20 min following electrophoresis. Subsequently, Coomassie Brilliant Blue G-250 (Bio-Rad, Hercules, California, USA) was used for gel staining. To remove background staining, the gel was treated with a destaining solution containing 10 % acetic acid and 20 % ethanol (both from VWR International, Fontenay-sous-Bois, France), to which activated charcoal (Merck, Darmstadt, Germany) was added.

2.2.3. Total starch content

Quantification of total starch was carried out using a commercial enzymatic kit (K-TSTA-100A, Megazyme, Wicklow, Ireland), following the manufacturer instructions. Briefly, 100 mg of buckwheat samples were weighed in duplicate, with one used as a blank. Samples were combined with 10 mL of 100 mmol/L sodium acetate buffer (pH 5.0) containing 5 mM CaCl_2 and 0.1 mL of thermostable α -amylase, followed by vortexing. The blanks were mixed only with buffer. Both samples and blanks were incubated in a bath (100 °C), and vortexed during 2, 5, and 10 min. After 15 min, they were removed from boiling water, agitated in vortex for 5 s, and incubated in a water bath (50 °C, 5 min). Amyloglucosidase (0.1 mL) was added to the samples, and the incubation continued at 50 °C for 30 min. After cooling, an aliquot of 2.0 mL of samples were centrifuged (15,871 $\times$ g, 5 min), and 1.0 mL of supernatant was mixed with 4 mL of buffer. Sample blanks was processes as the samples except for the addition of enzymes. Samples were extracted in triplicate and extracts were stored at -20 °C. Data were shown as g/100 g of d.w.

2.2.4. Phytic acid content

The sample (1 g) was combined with 20 mL of HCl solution (0.66 mol/L) and stirred vigorously overnight at room temperature. After extraction, 1 mL of the solution was centrifuged (13,000 rpm, 10 min). Then, 0.5 mL of NaOH solution (0.75 M) was added to 0.5 mL of the supernatant, and the content of phytic acid was measured using the Phytic Acid (Phytate)/Total Phosphorus Assay kit (K-PHYT, Megazyme, Wicklow, Ireland). Data were reported in g/100 g d.w.

2.2.5. Riboflavin content

For riboflavin extraction, samples (1 g) were mixed with 0.3 mol/L HCl (7 mL), and subjected (in duplicate) to acid hydrolysis in an autoclave (120 °C, 15 min). After cooling, the 4 mol/L ammonium acetate pH was added to adjust the pH to 5. The samples were then incubated with 1.7 mL of 20 % Taka-Diastase (Sigma-Aldrich, Madrid, Spain) solution for 3 h at 45 °C under agitation. Then, double-distilled was incorporated into the sample water to adjust the volume to 10 mL. Riboflavin quantification was conducted using Reversed-Phase High Performance Liquid Chromatography (e2695, Waters, MA, USA) coupled with a fluorescence detector (2475, Waters, MA, USA). The separation was performed using a Spherisorb ODS2 column (300 $\times$ 3.9 mm, Waters), following the method described by Rico et al. (2020). Briefly, the mobile phase consisted of a mixture of methanol/water/acetic acid (31/68.5/0.5, v/v/v) containing 5 mmol/L sodium hexanesulphonate. The flow rate was 1.5 mL/min and the column temperature was 35 °C. Detection was

Table 1
Buckwheat varieties included in the study and their identification codes.

Variety	Sample	Code
Common buckwheat	Bamby	BA
	Billy	BI
	Darja	DA
	Grigio-Francese	GF
	Kora	KO
	Lileja	LI
	Nojai	NO
	Panda	PA
	Zirka	ZI
Tartary buckwheat	Siberiano	SIB

performed at excitation and emission wavelengths of 445 nm and 520 nm, respectively. Data were expressed as $\mu\text{g}/100\text{ g d.w.}$

2.3. Glycemic index

Although *in vitro* methods are limited in their ability to fully reproduce human physiological responses, they provide a valuable and standardized platform for preliminary screening of carbohydrate digestibility and glycemic potential. *In vitro* glycemic index (GI) estimates have shown moderate correlation with *in vivo* GI values in cereal products (Ferrer-Mairal et al., 2012). Therefore, *in vitro* GI assessment was employed in this study to compare glycemic potential across different buckwheat varieties and flour types, with the understanding that these results are predictive rather than definitive.

GI was determined according to Sanz-Penella et al. (2013), with some modifications. Briefly, 100 mg of sample was weighed in duplicate. For blank samples, 1 mL of 0.2 mol/L HCl-KCl buffer (pH 1.5) was added, while 1 mL of pepsin solution was added to the samples. The mixtures were incubated at 40 °C for 1 h with agitation (2000 rpm) in a Thermomixer™ incubator (Eppendorf Iberica, Madrid, Spain). After incubation, 19 mL of 0.1 mol/L Tris-maleate buffer (pH 6.9) was added to both blanks and samples. Additionally, 10 mL of α -amylase solution (in 0.1 mol/L Tris-maleate buffer, pH 6.9, 3000 U/mL, Megazyme, Wicklow, Ireland) was incorporated into the samples, while the same volume of buffer was added to the blanks. Incubation took place at 37 °C under agitation. Aliquots of 1 mL were collected at intervals (0, 5, 10, 20, 40, 60, 90, 120, and 180 min) to monitor glucose release. Samples were treated at 100 °C for 5 min to inactivate the enzyme, and aliquots were stored at 4 °C. The samples were centrifuged ($9391\times g$, 4 °C, 5 min) and 500 μL of supernatant was mixed with 2 mL of 0.4 mol/L sodium acetate buffer (pH 4.75). Then, 100 μL of amyloglucosidase enzyme (3300 U/mL, Megazyme, Wicklow, Ireland) was added to samples, while the same volume of buffer was incorporated into the blanks. Mixtures were incubated at 60 °C for 45 min with agitation at 1000 rpm followed by heating in a boiling water bath for 5 min. Glucose amount was determined using the D-Glucose Assay Kit (GOPOD, Megazyme, Wicklow, Ireland). The area under the curve (AUC) was calculated by GraphPad Prism 4.00 software, and the hydrolysis index (HI) was obtained by comparing the AUC for the sample to that of the glucose standard. The GI was calculated with the following equation: $\text{GI} = 39.71 + 0.549 \times \text{HI}$ (Jiménez-Pulido et al., 2022).

2.4. Anti-diabetic *in vitro* activity

2.4.1. Sample preparation

Hydro-alcoholic extracts were prepared by adding 10 mL of 60 % ethanol to 0.5 g of samples. Then, samples were incubated with agitation for 2 h at room temperature in darkness (Catena et al., 2019). After centrifugation ($2000\times g$, 15 min, 4 °C), samples were filtered through a Whatman n° 1 filter paper. Extractions were carried out in triplicate. The extracts were purified by passing 4 mL through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA), which was previously activated with methanol (2 mL) and distilled water (3 mL). After purification, samples were subsequently dried (Speed Vac, concentrated SPD 131DDA) and reconstituted with buffer to concentrations of 20 or 50 mg/mL. They were then vortexed and sonicated for 3 min prior to analysis.

2.4.2. α -amylase inhibitory activity

The inhibitory activity against α -amylase was quantified as described by Vilcacundo et al. (2017), with some modifications. Initially, 50 μL of sample, positive control (2 mmol/L acarbose), or negative control (distilled water) were mixed with 100 μL of α -amylase solution (2 U/mL, in Tris-HCl 25 mmol/L, pH 7.5, 100 mM KCl buffer). After incubation (20 °C, 5 min in agitation), 100 μL of 1 % soluble starch solution (1 g/100 mL, in 20 mM sodium phosphate buffer, with 6.7 mmol/L sodium chloride, pH 6.9 and boiled for 15 min) was added to samples and

incubated (20 °C, 1000 rpm, 6 min). A volume of 100 μL of color reagent (96 mM solution of 3,5-dinitrosalicylic acid (DNS) was first prepared by dissolving 438 mg of DNS in 20 mL of distilled water heated to 50 °C–70 °C under constant stirring. Separately, 12 mL of distilled water were preheated (50 °C–70 °C), and the entire volume of DNS was slowly added) was added to stop the reaction, and it was then incubated in a water bath (15 min). After cooling, 800 μL of bidistilled water was incorporated into the reaction mixture (850 μL in the standard mixture), and absorbance was measured at 540 nm in a Synergy HT microplate reader (Biotek, Winooski, VT, USA). Maltose concentrations were determined using a standard curve (0–2.23 mg/mL). The percent inhibition of α -amylase activity was determined in relation to the negative control, which was assigned 100 % enzyme activity. All reagents and chemicals used in this assay were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise specified.

2.4.3. α -glucosidase inhibitory activity

The α -glucosidase inhibitory activity was determined according to Satoh et al. (2015) with minor adjustments. A volume of 50 μL of rat intestine α -glucosidase (1 U/mL, in 0.1 mol/L maleate buffer, pH 6.9) was mixed with 100 μL of each sample, positive control (1 mmol/L acarbose), or negative control (distilled water). The mixtures were incubated at 37 °C with shaking at 1000 rpm for 5 min. Following initial incubation, 50 μL of 2 mmol/L maltose was added as the substrate. The reactions were incubated at 1000 rpm and 37 °C for 30 min. Reactions were terminated by placing the tubes in boiling water for 5 min. Glucose concentrations were calculated through a standard curve (0–400 mmol/L). The inhibition percent of α -glucosidase activity was determined in relation to the negative control, which was set to represent 100 % enzyme activity. All reagents and chemicals used in this assay were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Merck Life Science (Darmstadt, Germany), unless otherwise specified.

2.4.4. DPP-IV inhibitory activity

DPP-IV inhibitory activity was evaluated following based on the method reported by Silveira et al. (2013). A volume of 15 μL of recombinant soluble human DPP-IV (0.26 mU per test, in buffer Tris-HCl 50 mmol/L pH 7.5), was mixed with samples bringing the final volume to 50 μL . Samples were incubated (37 °C, 10 min). Diprotin A (Ile-Pro-Ile) served as the positive control. After incubation, 50 μL of H-Gly-Pro-p-nitroaniline (final concentration of 100 $\mu\text{mol/L}$ in buffer Tris-HCl 50 mM pH 7.5) was added to samples. Absorbance was determined at 405 nm every 2 min for 30 min at 37 °C. Subsequently, the results were expressed as the percentage inhibition of enzyme activity by the samples. All reagents and chemicals used in this assay were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Biomol (Enzo Life Sciences, Farmingdale, NY, USA), unless otherwise specified.

2.5. Statistical analysis

Data showed are the mean and standard deviation. The data underwent one-way ANOVA analysis utilizing the least-squared difference test (LSD) with the Statgraphic 5.0 Program (Statistical Graphics Corp., Rockville, MD) for Windows. Principal component analysis (PCA) was performed using R software. Biplots of loadings and scores of the two main Principal Components were realized using ggplot2, ggfortify, and ggrepel R packages 4.1.2. For PCA, data were standardized according to Z-distribution, centering the mean value to 0 and scaling the variance to 1.

3. Results and discussion

3.1. Protein content and profile

Determination of protein content in buckwheat flour is crucial for assessing their nutritional value and potential food applications. The

protein content in both fine and coarse flours from the buckwheat varieties studied is reported in Table 2. Fine flours had a protein content ranging from 8.9 g/100 g d.w. (variety Kora) to 13.3 g/100 g d.w. (variety Zirka), while coarse flour samples showed levels between 2.6 g/100 g d.w. (variety Zirka) and 12.9 g/100 g d.w. (variety Lileja), indicating substantial variation in protein content between buckwheat varieties. The protein content of buckwheat flours is in the range of that previously reported in different buckwheat varieties (10.3–14.13 g/100 g) (Zhu et al., 2016). For all varieties, coarse flours had lower protein content than fine flours, except for Bamby and Grigio-Francese. Particle size has an important impact on flour protein content (Menon et al., 2020). In line with our results, it has been reported that smaller flour particles had higher protein content (Tian et al., 2022). However, other variables such as matrix effects during nitrogen detection, or differences in protein extraction efficiency related to the larger particle size of coarse flours compared to fine flours could also partially responsible for the discrepancy of protein content values observed between both flours.

The protein profile of buckwheat flours was further analyzed using the SDS-PAGE technique. Fig. 1 presents the electrophoretic protein patterns for buckwheat varieties studied, highlighting distinct protein profiles and band intensity, thereby emphasizing varietal differences.

The results revealed a complex protein profile across buckwheat varieties, which appeared highly similar in most cases, although differences in intensity of several protein bands were observed. In agreement with findings in protein content, the fine flour samples generally exhibited a higher protein extraction, as suggested by the increased intensity of the bands, compared to their coarse flour counterparts. Additionally, tartary buckwheat (Siberiano sample) exhibited a different protein profile compared to the common buckwheat samples analyzed. This divergence suggests differences in protein composition between common and tartary buckwheat, which could have implications for both nutritional and functional properties.

3.2. Total starch content

Starch is the primary carbohydrate present in grain-based food products in the human diet. Recent studies have shown that the rapid digestion of starch can trigger a high postprandial glycemic response, increasing the risk of cardiovascular diseases, diabetes, and obesity (Li et al., 2019). In response to these concerns, buckwheat has gained particular interest due to its low to medium glycemic index (GI) and average starch content of approximately 60–70 % in dry weight basis (Zhu, 2016). The total starch content for different buckwheat varieties, in both fine and coarse flours is reported in Table 2. The starch content in flours ranged from 55.9 ± 2.5 % for the Lileja variety to 65.6 ± 0.5 % for

Darja variety. No significant differences ($p > 0.05$) in starch content were observed between tartary buckwheat and the common buckwheat varieties, findings that are consistent with a previous study (Du et al., 2022). In contrast, the coarse flour samples exhibited an overall reduction in starch content compared to their corresponding fine flours, with values ranging from 5.6 ± 0.3 % for the Zirka variety to 16.0 ± 0.4 % for the Lileja variety. This reduction in starch content was particularly pronounced in the coarse flour products from Billy (6.3 ± 0.6 %) and Zirka varieties (5.6 ± 0.3 %). The decrease of starch content in coarse flours (larger particle size) can be attributed to lower surface area with decreases the starch extraction efficiency, as documented previously by different flour components in literature (Luthria et al., 2006; Menon et al., 2020).

3.2.1. Phytic acid content

Phytic acid is a compound derived from the progressive phosphorylation of myo-inositol. It forms complexes with essential cations including calcium (Ca^{2+}), iron (Fe^{2+}), and zinc (Zn^{2+}), reducing their absorption, acting as an anti-nutrient in the digestive tract (Lönnerdal, 2002). Therefore, reducing phytate content in plants is desirable to enhance the nutritional value of foods intended for human consumption. Assessing the phytic acid content in buckwheat samples is crucial for this purpose. The phytic acid content in both fine and coarse flours for different buckwheat varieties, is reported in Table 3. Phytic acid content in fine flours varied significantly ($p < 0.05$), ranging from 0.1 g/100 g–1.0 g/100 g. Regarding fine flours, Lileja and Nojai varieties showed the lowest phytic acid content, while Zirka variety had the highest levels ($p < 0.05$), followed by Bamby and Siberiano varieties.

No significant differences ($p > 0.05$) in phytic acid content were found between fine and coarse flours for Bamby, Darja, Grigio-Francese and Panda varieties. However, coarse flours derived from Billy, Kora, Lileja, and Nojai varieties showed higher phytic acid content than their respective fine flours ($p < 0.05$). Zirka was the only variety where the coarse flour showed a lower content of this antinutrient than its fine flour counterpart. Phytic acid is an important antinutrient to be considered in gluten-free products since celiac individuals often suffer from micronutrient deficiencies (Carrizo et al., 2020). The phytic acid levels found in the buckwheat fine flours analyzed in this work were lower than those previously reported for buckwheat and quinoa grains (Altıkardes & Güzel, 2024), and rice bran (Lehrfeld & Morris, 1992), suggesting their nutritional suitability for individuals with celiac disease.

3.3. Riboflavin content

Various studies have reported deficiencies in several vitamins, particularly vitamin D, E, B9, B1, B2, and B6, in individuals adhering to a gluten-free diet (Ballester-Fernández et al., 2021; González et al., 2018; Hallert et al., 2002). In general, B vitamin deficiencies are often ascribed to insufficient dietary intake rather than malabsorption, which underscores the importance of adequate dietary planning for people suffering celiac disease (Hallert et al., 2002). As such, it is crucial not only to monitor vitamin levels through biochemical analysis but also to ensure a nutrient-rich diet to prevent and treat deficiencies (Cardo et al., 2021). The riboflavin content in both fine and coarse flours for different buckwheat varieties, is collected in Table 3. Buckwheat samples showed significant variability in riboflavin content across varieties and product forms (fine and coarse flours). Specifically, the riboflavin concentration in the fine flours varied from 117 $\mu\text{g}/100$ g in Nojai variety to 172 $\mu\text{g}/100$ g in Billy variety. The Siberiano variety had a riboflavin content of 156 $\mu\text{g}/100$ g, ranking among the highest, suggesting its potential as a good source of this vitamin. These values are consistent with previous findings by Rybicka and Gliszczynska-Swiglo (2017), who reported riboflavin levels in buckwheat flour ranging from 60 μg to 220 $\mu\text{g}/100$ g. Furthermore, buckwheat fine flours showed a content of this vitamin similar to that reported in other traditional (chickpea, maize, millet,

Table 2

Protein and starch contents (g/100 g d.w.) in fine and coarse flours obtained from different buckwheat varieties.

Sample	Protein		Starch	
	Fine flour	Coarse flour	Fine flour	Coarse flour
BA	9.7 ± 0.3 ^{b,A}	9.9 ± 0.4 ^{e,A}	61.6 ± 5.8 ^{bc,A}	11.8 ± 1.0 ^{d,B}
BI	10.8 ± 0.1 ^{e,A}	4.5 ± 0.5 ^{b,B}	61.6 ± 1.6 ^{bc,A}	6.3 ± 0.6 ^{a,B}
DA	10.6 ± 0.2 ^{de,A}	11.9 ± 0.1 ^{8,B}	65.6 ± 0.5 ^{c,A}	10.3 ± 0.3 ^{c,B}
GF	10.5 ± 0.2 ^{d,A}	10.8 ± 0.5 ^{f,A}	63.2 ± 2.6 ^{bc,A}	10.2 ± 0.2 ^{c,B}
KO	8.9 ± 0.1 ^{a,A}	5.7 ± 0.2 ^{c,B}	63.3 ± 1.3 ^{bc,A}	11.2 ± 0.6 ^{d,B}
LI	12.2 ± 0.2 ^{g,A}	12.9 ± 0.1 ^{h,B}	55.9 ± 2.5 ^{a,A}	16.0 ± 0.4 ^{e,B}
NO	11.4 ± 0.8 ^{f,A}	6.9 ± 0.2 ^{d,B}	63.4 ± 4.2 ^{bc,A}	9.7 ± 0.3 ^{bc,B}
PA	10.1 ± 0.1 ^{c,A}	7.0 ± 0.5 ^{d,B}	65.2 ± 1.6 ^{c,A}	9.1 ± 0.4 ^{b,B}
ZI	13.3 ± 0.1 ^{h,A}	2.6 ± 0.03 ^{a,B}	60.2 ± 4.0 ^{ab,A}	5.6 ± 0.3 ^{a,B}
SIB	11.1 ± 0.3 ^f	–	60.1 ± 3.8 ^{ab}	–

Data are expressed as mean $\pm$ standard deviation of three replicates. Different lower-case letters within a column indicate statistical differences among varieties for the same product form ($p < 0.05$). Different upper-case letters within a row indicate statistical differences among products for the same variety ($p < 0.05$).

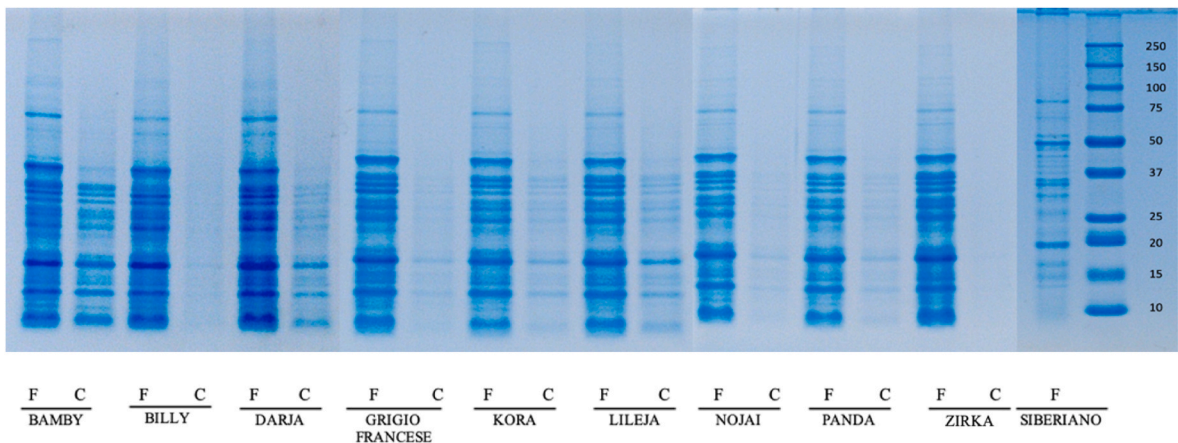


Fig. 1. Protein profile of buckwheat fine (F) and coarse (C) flour samples.

Table 3
Phytic acid (g/100 g d.w.) and riboflavin (μg/100 g d.w.) contents in fine and coarse flours obtained from different buckwheat varieties.

Sample	Phytic acid		Riboflavin	
	Fine flour	Coarse flour	Fine flour	Coarse flour
BA	0.85 ± 0.10 ^{e,A}	0.97 ± 0.05 ^{f,A}	131 ± 13 ^{ab,A}	82.6 ± 4.9 ^{a,B}
BI	0.22 ± 0.00 ^{b,A}	0.77 ± 0.07 ^{e,B}	172 ± 12 ^{e,A}	89.2 ± 6.2 ^{a,B}
DA	0.69 ± 0.03 ^{d,A}	0.63 ± 0.03 ^{c,A}	152 ± 8 ^{cd,A}	102 ± 6 ^{b,B}
GF	0.61 ± 0.04 ^{cd,A}	0.54 ± 0.03 ^{c,A}	155 ± 14 ^{cd,A}	133.5 ± 3.8 ^{e,B}
KO	0.55 ± 0.01 ^{c,A}	0.88 ± 0.03 ^{f,B}	132 ± 12 ^{ab,A}	139.6 ± 7.4 ^{e,A}
LI	0.10 ± 0.01 ^{a,A}	0.59 ± 0.05 ^{c,B}	141 ± 8 ^{bc,A}	156 ± 8 ^{f,B}
NO	0.12 ± 0.00 ^{a,A}	0.17 ± 0.00 ^{b,B}	117 ± 4 ^{a,A}	114.5 ± 5.2 ^{cd,A}
PA	0.57 ± 0.04 ^{c,A}	0.75 ± 0.06 ^{e,A}	127 ± 7 ^{ab,A}	105 ± 9 ^{bc,B}
ZI	1.04 ± 0.06 ^{f,A}	0.04 ± 0.00 ^{a,B}	149 ± 12 ^{cd,A}	115 ± 9 ^{d,B}
SIB	0.84 ± 0.01 ^e	–	156 ± 9 ^d	–

Data are expressed as mean ± standard deviation of three replicates. Different lower-case letters within a column indicate statistical differences among varieties for the same product form (p < 0.05). Different upper-case letters within a row indicate statistical differences among products for the same variety (p < 0.05).

rice) and alternative (acorn, amaranth, chestnut, oat, teff) gluten-free flours (Rybicka & Gliszczynska-Swiglo, 2017).

The riboflavin content in the coarse flours ranged from 82.6 μg/100 g in the Bamby variety to 156 μg/100 g in the Lileja variety. Although no differences in riboflavin content were observed between fine and coarse flours in Kora and Nojai varieties, most of the varieties showed higher riboflavin content in fine flours than in the coarse flours. The only exception was Lileja variety, where coarse flours had an 11 % higher riboflavin content than the corresponding fine flour.

3.4. Glycemic index

Scientific evidence demonstrated that gluten-free diet is often low in dietary fiber due to the need to avoid naturally fiber-rich foods, such as wheat, and to the high consumption of gluten-free products typically elaborated with refined flours that exhibit low fiber content. Furthermore, an increase in the GI and glycemic load of gluten-free meals has been observed (Vici et al., 2016). Therefore, it is essential to assess the GI of gluten-free grains, as well as their impact on enzymes involved in carbohydrate metabolism. GI is a measure that evaluates the effect of consuming carbohydrate-rich foods on blood glucose levels. The GI ranks carbohydrates on a scale from 0 to 100 based on their postprandial impact (after a meal) on blood sugar levels. This index is defined as the incremental area under the blood glucose response curve associated with a 50 g portion of carbohydrates from the food being tested, expressed as a percentage of the response to an equivalent amount of

carbohydrates from a standard reference food, consumed by the same individual (Liu et al., 2009). The GI values were estimated *in vitro* for common and tartary buckwheat fine flours to evaluate their potential for glycemic control. *In vitro* models for GI estimation offer a standardized, reproducible, and ethically viable approach for preliminary screening of carbohydrate digestibility and glycemic potential. However, they do not recapitulate the complex physiological and metabolic processes involved in human digestion, including interindividual variability in gastric emptying rates, enzymatic activity, hormonal regulation, gut microbiota interactions, and glucose homeostasis. Consequently, *in vitro* GI values should be interpreted as indicative approximations, not as substitutes for clinical (*in vivo*) measurements. The GI values for buckwheat fine flours are shown in Table 4. Only fine flours were analyzed since the coarse flours had low total starch content (Table 2). The GI values for buckwheat fine flours ranged from 45.6 in Darja variety to 60.7 in the Siberiano variety. Common varieties such as Billy, Kora and Lileja exhibited values around 50. According to standard GI classification (Guo, 2009), all analyzed flours fall within the low or moderate GI categories, indicating a potentially beneficial glycemic response. Although the Siberiano variety presented the highest GI among all the varieties tested, numerous investigations have demonstrated that tartary buckwheat has significant potential to improve the symptoms of type 2 diabetes mellitus (T2DM). For instance, Wu et al. (2022) reported that tartary buckwheat noodles administered to type 2 diabetic rats alleviated the complications associated to T2DM, largely attributed to the high content of resistant starch and dietary fiber. Additionally, clinical studies have also confirmed the ability of Tartary buckwheat consumption to reduce insulin resistance, mitigate renal damage in diabetic individuals and improve blood lipid metabolism (Qiu, Liu, et al., 2016).

Table 4
Glycemic index of fine flours obtained from different buckwheat varieties.

Sample	GI
BA	46.4 ± 0.1 ^a
BI	50.8 ± 0.3 ^c
DA	45.6 ± 0.8 ^a
GF	53.4 ± 0.4 ^d
KO	50.8 ± 1.0 ^{bc}
LI	50.0 ± 0.0 ^b
NO	52.6 ± 0.2 ^d
PA	52.8 ± 1.6 ^d
ZI	52.7 ± 0.1 ^d
SIB	60.7 ± 2.1 ^e

Data are expressed as mean ± standard deviation of three replicates. Different lower-case letters within a column indicate statistical differences among varieties.

Resistant starch (RS) is known to resist hydrolysis by pancreatic amylase, reducing the rate and extent of glucose absorption in the small intestine. Moreover, RS has been shown to directly suppress α -amylase and α -glucosidase activities *in vitro*, which is consistent with the inhibitory patterns observed in our samples (Li et al., 2023). Additionally, fermentation of RS in the colon generates short-chain fatty acids that improve insulin sensitivity and glucose metabolism.

Other studies suggest that bioactive compounds in buckwheat, such as rutin and other polyphenols, interact with starch to inhibit its digestion by enzymes like α -amylase. This inhibition slows the glucose release into the bloodstream, contributing to the lower glycemic index of buckwheat-based foods. Studies on breads made with buckwheat flour highlight that their lower glycemic impact is partially due to the action of these compounds binding to starch, reducing enzymatic digestion (Takahama & Hirota, 2010). However, these observations are primarily derived from *in vitro* studies or limited clinical trials, and more comprehensive research is needed to confirm these mechanisms *in vivo* and across diverse populations. Overall, these results suggest that the observed GI values may be influenced not only by the fiber and resistant starch content of the flour, but also by the presence and activity of polyphenols and other bioactive compounds. The combined effect of these components provides a plausible mechanistic basis for the observed glycemic modulation, reinforcing the potential role of buckwheat as a functional ingredient in the management of T2DM.

In addition, recent evidence highlights the presence of bioactive peptides in buckwheat capable of inhibiting dipeptidyl peptidase-IV (DPP-IV), thereby prolonging incretin hormone action and enhancing glucose-dependent insulin secretion (Kehinde et al., 2022). This mechanism complements the enzyme inhibition results reported in section 3.9 and adds a protein-mediated layer of glycemic regulation.

Together, these components interact in a coordinated fashion to modulate postprandial glycemia, supporting the potential use of buckwheat flours in dietary interventions for type 2 diabetes management. These findings underscore the importance of considering both compositional and functional properties when evaluating glycemic response and antidiabetic efficacy of gluten-free flours.

3.5. *In vitro* anti-diabetic activity

T2DM is a major and rapidly growing global health concern marked by high blood glucose levels and impaired insulin function (Hossain, Mamun, & Islam, 2024). T2DM leads to severe complications including cardiovascular diseases, neurological disorders, vision impairment, renal dysfunction, and even limb loss. Although current management strategies, such as lifestyle changes, pharmacotherapy, and insulin, are available, they often face limitations due to side effects and short-term efficacy (Patil, Mandal, Kumar Tomar, & Anand, 2015). Considering these challenges, there is a heightened interest in exploring novel therapeutic options. This shift in focus has led to a growing emphasis on natural sources with potential anti-diabetic properties. Researchers are increasingly investigating the bioactive compounds in various foods, aiming to uncover new dietary interventions that could offer more effective and sustainable management of T2DM (Vilcacundo et al., 2017).

Dipeptidyl-peptidase IV (DPP-IV) has a key role in diabetes and autoimmune diseases. Inhibiting this enzyme allows glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide, involved in insulin production, to extend their beneficial action in the body (Chang et al., 2001). Similarly, α -amylase cleaves α -1,4-glycosidic bonds in amylose, producing maltose and glucose. Inhibiting α -amylase slows carbohydrate absorption, thereby affecting blood glucose and insulin levels after meals, making it an effective strategy for managing T2DM (Martinez-Gonzalez et al., 2019). Additionally, α -glucosidase hydrolyzes non-reducing α -glucose residues, releasing glucose molecules. Inhibition of α -glucosidase slows carbohydrate digestion and absorption, helping maintain lower postprandial blood glucose levels and

reducing insulin demand (A. Hossain, Das, Ghosh & Sil, 2020).

Thus, the inhibition of DPP-IV, α -amylase, and α -glucosidase is of significant interest in developing therapeutic strategies for managing T2DM, closely linked to celiac disease. Table 5 presents the inhibitory activity of buckwheat fine and coarse flours against DPP-IV, α -amylase, and α -glucosidase. All samples were assayed at a concentration of 10 mg/mL, apart from DPP-IV, that was tested at a final concentration of 5 mg/mL. Although variability exists among the samples, Lileja variety exhibited the strongest DPP-IV activity inhibition with the fine and coarse flours showing 98.7 % and 97.4 %, respectively. Fine flours from Darja, Grigio-Francese, and Siberiano varieties also presented high DPP-IV inhibition percentages, while Billy, Kora, Nojai, Panda, and Zirka had the lowest inhibitory activity. Research confirms that buckwheat is a potent source of DPP-IV inhibitory peptides. Wang et al. (2015) demonstrated that buckwheat hydrolysates exhibited significant DPP-IV inhibitory activity, with IC₅₀ values ranging between 0.13 mg/mL and 8.15 mg/mL depending on the type of enzymatic digestion and peptides released. Peptides derived from other grains such as amaranth, quinoa, chia and wheat have also been shown to inhibit DPP-IV activity, with some studies reporting values similar to those observed in buckwheat (Nongonierma et al., 2017; Valenzuela-Zamudio, Hidalgo-Figueroa, Ortíz-Andrade, Hernández-Álvarez, 2022). In most cases, coarse flours showed greater DPP-IV inhibitory activity than the corresponding fine flours, except for Grigio-Francese, and Lileja varieties, where no significant differences ($p < 0.05$) between product forms were noticed. Considering the high inhibitory potential of the Lileja sample, both in fine and coarse flour forms, against the DPP-IV enzyme (Table 5), this sample was further tested at a lower concentration (1 mg/mL) and exhibited significant inhibition (9.2 ± 0.4 for fine flour and 69.5 ± 3.9 for coarse flour), confirming its notable inhibitory activity even at lower concentrations.

Regarding α -amylase inhibition, Lileja fine flour exhibited the highest inhibitory activity 37.30 %, while Zirka showed the lowest one. Coarse flours showed higher inhibitory activity than the corresponding fine flours in Bamby, Billy, Grigio-Francese and Panda varieties. Conversely, Darja, Kora, Lileja, Nojai, and Zirka showed higher

Table 5

DPP-IV, α -amylase and α -glucosidase inhibition (% vs. control) in fine and coarse flours obtained from different buckwheat varieties.

Sample	DPP-IV		α -amylase		α -glucosidase	
	Fine flour	Coarse flour	Fine flour	Coarse flour	Fine flour	Coarse flour
BA	9.9 $\pm$ 0.8 ^{a,A}	27.7 $\pm$ 3.6 ^{ab,B}	17.7 $\pm$ 1.2 ^{ab,A}	26.4 $\pm$ 2.1 ^{a,B}	80.9 $\pm$ 3.0 ^f	ND
BI	13.7 $\pm$ 1.8 ^{a,A}	34.9 $\pm$ 1.3 ^{cd,B}	20.3 $\pm$ 2.2 ^{abc,A}	39.2 $\pm$ 2.0 ^{a,B}	34.6 $\pm$ 3.6 ^{b,A}	24.1 $\pm$ 2.9 ^{b,B}
DA	26.0 $\pm$ 4.1 ^{b,A}	38.9 $\pm$ 5.3 ^{d,B}	34.0 $\pm$ 2.5 ^{c,A}	22.2 $\pm$ 2.3 ^{c,B}	55.4 $\pm$ 4.2 ^{d,A}	62.6 $\pm$ 5.1 ^{c,A}
GF	25.0 $\pm$ 3.7 ^{b,A}	24.7 $\pm$ 0.8 ^{a,A}	ND	8.6 $\pm$ 0.3 ^{ab}	10.0 $\pm$ 1.3 ^a	ND
KO	12.6 $\pm$ 0.9 ^{a,A}	31.1 $\pm$ 1.2 ^{bc,B}	33.2 $\pm$ 2.7 ^{bc,A}	21.1 $\pm$ 1.1 ^{a,B}	31.6 $\pm$ 3.6 ^b	ND
LI	98.7 $\pm$ 6.1 ^{c,A}	97.4 $\pm$ 4.4 ^{e,A}	37.3 $\pm$ 0.8 ^{d,A}	11.5 $\pm$ 1.5 ^{a,B}	40.4 $\pm$ 2.5 ^{c,A}	16.2 $\pm$ 2.4 ^{ab,B}
NO	13.0 $\pm$ 1.6 ^{a,A}	38.7 $\pm$ 3.1 ^{d,B}	18.6 $\pm$ 1.1 ^{abc,A}	13.6 $\pm$ 0.0 ^{b,B}	70.1 $\pm$ 2.9 ^e	ND
PA	13.8 $\pm$ 1.5 ^{a,A}	29.4 $\pm$ 4.4 ^{abc,B}	ND	25.2 $\pm$ 2.7 ^a	31.6 $\pm$ 1.9 ^{b,A}	13.5 $\pm$ 1.5 ^{a,B}
ZI	11.9 $\pm$ 1.8 ^{a,A}	25.3 $\pm$ 2.7 ^{a,B}	3.2 $\pm$ 0.2 ^a	ND	58.7 $\pm$ 4.9 ^d	ND
SIB	25.2 $\pm$ 1.8 ^b	–	17.6 $\pm$ 0.6 ^{ab}	–	13.1 $\pm$ 1.8 ^a	–

Data are expressed as mean $\pm$ standard deviation of three replicates. Different lower-case letters within a column indicate statistical differences among varieties for the same product form ($p < 0.05$). Different upper-case letters within a row indicate statistical differences among products for the same variety ($p < 0.05$). ND: not detected.

inhibitory activity in their fine flour forms that in their coarse flour counterparts. *In vitro* studies have demonstrated that buckwheat contains albumin, a protein that competitively inhibits α -amylase, thereby reducing starch digestion. This contributes to lower postprandial blood glucose spikes, making buckwheat beneficial for people with T2DM (Ninomiya et al., 2018).

In terms of α -glucosidase inhibition, the Bamby fine flour showed the highest value (80.9 %), followed by Nojai (70.1 %) and Zirka (58.7 %), and Grigio-Francese and Siberiano fine flours had the lowest inhibitory activity against this enzyme. In consistency with our results, the research by Ikeda et al. 2017 revealed that different buckwheat varieties have varying levels of α -glucosidase inhibitory activity, with tartary buckwheat showing particularly high inhibition compared to common buckwheat (Ikeda et al., 2017). Coarse flour products exhibited lower α -glucosidase inhibitory activity than the fine flours for all the buckwheat varieties analyzed, except for Darja, where no significant differences were observed between both product forms ($p < 0.05$). Our results suggest that the type of processing influence significantly the antidiabetic potential of buckwheat, making fine flours more suitable processed ingredients in terms of T2DM management. Begum et al. (2024) showed that buckwheat-based bread significantly reduced postprandial glucose levels in subjects with T2DM compared to regular wheat bread, largely due to the high polyphenol content, especially phenolic acids. The finer flour in the buckwheat bread contributed to improved glycemic control, as finer milling increases the surface area available for enzymatic digestion and polyphenol absorption (Begum et al., 2024; Sofi et al., 2023). However, these inhibitory percentages should be interpreted with caution, as they are preliminary results obtained at a single extract concentration. While the observed values suggest a potentially strong inhibitory effect on DPP-IV, α -amylase and α -glucosidase activity, the use of only one concentration limits the ability to draw definitive conclusions about the potency and efficacy of the different samples. In order to strengthen these findings, it will be necessary to perform additional

assays using a range of extract concentrations. This will allow the generation of dose-response curves and enable the calculation of key pharmacological parameters, such as the half maximal inhibitory concentration (IC_{50}), which provides a more accurate and quantitative measure of the inhibitory potential.

3.6. Principal Component Analysis

To better interpret the complex relationships among chemical and biochemical parameters across all buckwheat varieties, a Principal Component Analysis (PCA) was carried out separately for fine and coarse flours.

For fine flours (Fig. 2), two primary components were found, which together accounted for roughly 57 % of the total variance. The first component (PC1) accounted for 29.97 % of the variance and was primarily positively influenced by DPP-IV inhibitory activity and protein content (Pro), and negatively by starch content (Sta). The second component explained 27.05 % of the variance and showed a negative correlation with glycemic index (GI) and several chemical parameters (phytic acid [Phy], riboflavin [Rib], protein [Pro]), while being positively influenced by antidiabetic indicators such as α -amylase (α -amy), α -glucosidase (α -glu), and DPP-IV inhibitory activities.

The PCA not only highlighted the most influential variables but also allowed us to distinguish specific trends and behaviors among the buckwheat varieties. Based on the PCA score plot, the varieties (BA, BI, DA, GF, KO, LI, NO, PA, SIB, ZI) were grouped into four main clusters: group 1 comprised Zi and Sib varieties, which were characterized by high GI and low α -amylase inhibitory activity, suggesting a less favorable antidiabetic profile. Group 2 included BI and GF, which exhibited intermediate characteristics. Group 3, containing most of the samples (BA, DA, KO, NO, PA), was distinguished by high starch and α -glucosidase inhibitory activity but lower GI, Riboflavin, and Protein contents, indicating a trade-off between energy content and nutritional quality.

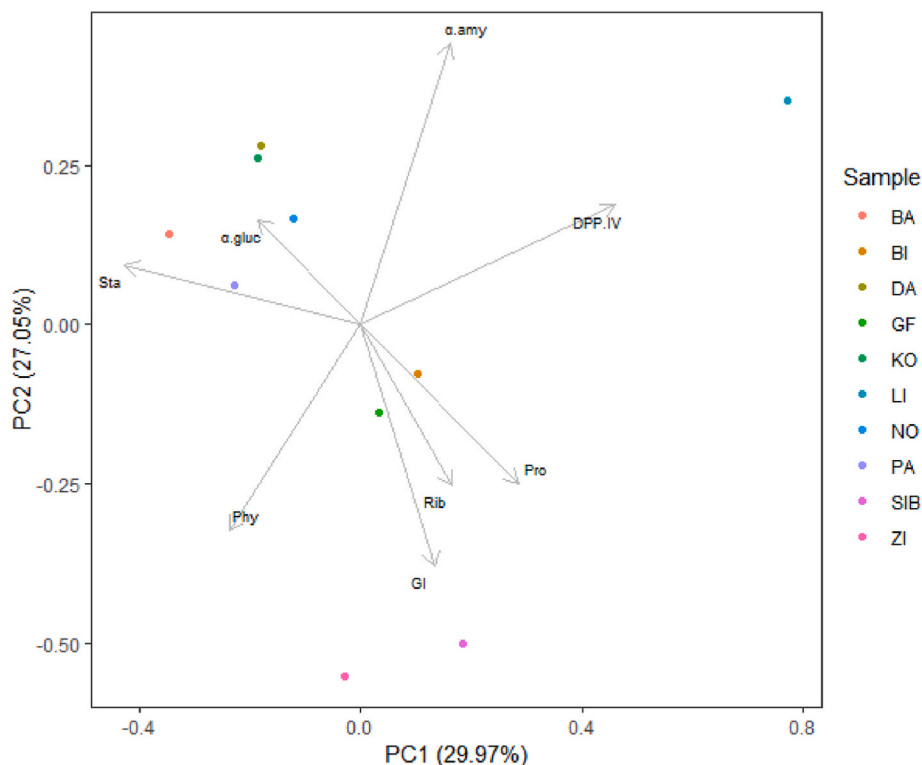


Fig. 2. Biplot of main Principal Components (PCs) identified in the PC Analysis of fine flours, considering chemical variables (protein, Pro; starch, Sta; riboflavin, Rib; and phytic acid, Phy; and glycemic index, GI) and anti-diabetic variables (α -amylase, α -amy; α -glucosidase, α -glu, and dipeptidyl-peptidase, DPP-IV inhibitory activities). PC loadings are reported as arrows and PC scores are shown as dots.

(Bamby, BA; Billy, BY; Darja, DA; Grigio-Francese, GF; Kora, KO; Lileja, LI; Nojai, NO; Panda, PA; Siberiano, SIB; Zirka, ZI).

Group 4, consisting solely of the LI variety, stood out due to its high DPP-IV inhibitory activity, suggesting a potentially stronger *in vitro* antidiabetic effect.

Two main components were identified for coarse flour extracts (Fig. 3), representing together about 70 % of the variance. The first component (PC1) explained 46.31 % of the variance and was influenced in a positive manner mainly by α -amylase inhibitory activity and negatively by protein, riboflavin and starch content and DPP-IV inhibitory activity. The second component (PC2), explaining 23.12 % of the variance, was positively influenced by riboflavin content and negatively correlated with phytic acid, α -amylase inhibitory activity, starch, and protein levels. The PCA allowed us to discern key patterns among the buckwheat varieties in terms of their biochemical and nutritional profiles. For instance, samples BA, BI, and LI were clearly separated along the principal components, each showing distinct biochemical characteristics: BA was associated with high phytic acid content, BI showed elevated α -amylase inhibitory activity, and LI stood out due to its high DPP-IV inhibitory potential. In contrast, ZI and NO samples clustered separately and were characterized by lower overall chemical and bioactive compound content, suggesting a comparatively limited nutritional profile. These findings contribute to a more detailed understanding of how specific varieties may be preferentially used in functional food applications.

4. Conclusions

The results of this work show the suitability of buckwheat as a gluten-free ingredient alternative to wheat for individuals with celiac disease. This study integrated chemical and biochemical analyses to comprehensively assess nutritional and bioactive properties of fine and coarse flour products from different buckwheat varieties. Fine flours proved to be excellent sources of protein, starch, and riboflavin, and showed low phytic acid content. Coarse flours, in general, had lower

nutritional attributes. Fine flours from all buckwheat varieties had moderate glycemic indices (45.6–60.7) and demonstrated inhibitory activity against dipeptidyl-peptidase (DPP-IV), α -amylase and α -glucosidase enzymes. Inhibiting these enzymes can help reduce postprandial blood sugar spikes by slowing down the absorption of carbohydrates. In conclusion, this study confirms buckwheat as a valuable pseudocereal for both celiac disease management and glycemic control, making it beneficial not only for individuals with gluten sensitivity but also for those suffering T2DM. The particle size was a key factor influencing nutritional and bioactive properties of buckwheat flours. The insightful information provided by this study regarding the nutritional and potential health-promoting properties of buckwheat flours as influenced by both crop variety and particle size will be valuable for the food industry to tailor the nutritional and bioactive features of the produced flours. Further research is needed to explore the influence of different milling and fractionation techniques in the nutritional and functional properties of flours. Moreover, the study of the influence of particle size on nutritional and bioactive composition of flours obtained from other pseudocereals and cereals requires further attention.

CRediT authorship contribution statement

Corinne Bani: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Cristina Martínez-Villaluenga:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Chiara Di Lorenzo:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Patrizia Restani:** Writing – review & editing, Visualization, Validation, Supervision, Data curation, Conceptualization. **Sara Bautista-Expósito:** Writing – review & editing,

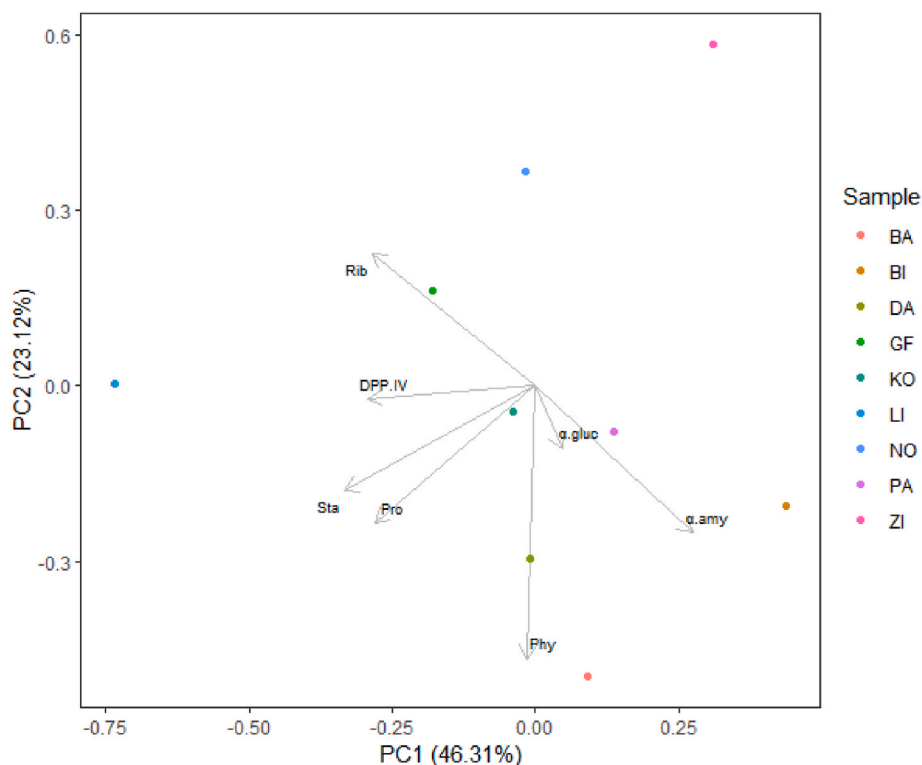


Fig. 3. Biplot of main Principal Components (PCs) identified in the PC Analysis of coarse flours, considering chemical variables (protein, Pro; starch, Sta; riboflavin, Rib; and phytic acid, Phyt) and anti-diabetic variables (α -amylase, $\alpha.amy$; α -glucosidase, $\alpha.gluc$, and dipeptidyl-peptidase, DPP-IV inhibitory activities). PC loadings are reported as arrows and PC scores are shown as dots.

(Bamby, BA; Billy, BY; Darja, DA; Grigio-Francese, GF; Kora, KO; Lileja, LI; Nojai, NO; Panda, PA; Zirka, ZI).

Supervision, Methodology, Investigation, Formal analysis, Data curation. **Carola Pozzoli:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation. **Francesca Mercogliano:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Elena Peñas:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2025.118404>.

Data availability

Data will be made available on request.

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