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Effects of cultivars and dry fraction on nutritional and phytochemical compounds of faba beans (*Vicia faba* L.)

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ABSTRACT

This study aims to examine the variation in bioactive compounds in faba bean seeds among different cultivars with consideration of differences introduced by growing conditions, including minerals, low molecular weight sugars (LMWS), raffinose family oligosaccharides (RFOs), total polyphenols content (TPC), trypsin inhibitor activity (TIA), and vicine and convicine (v-c). Besides, a faba bean protein concentrate from air classification was studied to provide insights into the nutritional modifications resulting from protein refinement. Faba beans had abundant potassium (1391.77 mg/100 g on average) and iron (5.48 mg/100 g). Sucrose was the major LMWS, and verbascose was the primary RFO in faba beans, displaying a higher variability across cultivars compared to growing conditions. The analysis of different faba bean varieties also revealed varying levels of TPC in faba bean seeds (1.29–2.37 mg gallic acid equivalents/g), v-c (1.46–10.5 mg/g; 0.21–2.37 mg/g), and TIA (18.45–39.51 U/mg). Air classification significantly enriched the content of most minerals, as well as verbascose and stachyose, but reduced the content of calcium and raffinose. This indicates that certain components were redistributed during the separation of proteins and starch granules. The study reinforces the role of faba bean varieties in determining essential nutritional attributes while demonstrating limited influence from growing conditions.

1. Introduction

Faba bean (*Vicia faba* L.) is a promising grain legume for developing green and sustainable food systems for the growing world population. As a cool-season legume, faba bean stands out for excellent symbiotic nitrogen fixation, high resilience and yield (Segers et al., 2022). Faba bean seeds, containing abundant bioactive phytochemicals, are a good source of nutrients for human consumption. Faba bean seeds are largely composed of proteins and carbohydrates, which account for up to 30 % (based on protein conversion factor of 5.4) and 64 % of the dry matter, respectively (Millar et al., 2019). Among the total carbohydrates of faba beans, starch and dietary fibres are the major components. Around 10 % of total carbohydrates comprise low molecular weight carbohydrates (LMWC) which can be further divided into low molecular sugars (LMWS) and oligosaccharides. The LMWS contains monosaccharides and disaccharides (also known as simple sugars) such as glucose, fructose and sucrose, whereas oligosaccharides are composed of 3–10 monosaccharide units and play a vital role in plant development under stress (Yan et al., 2022). Stachyose and verbascose are the major oligosaccharides in faba beans (Landry et al., 2016) and, together with raffinose, these are collectively known as the raffinose family oligosaccharides (RFOs). These oligosaccharides have been extensively studied for their impact on human health, particularly due to their association with flatulence and osmotic effects during the digestion of legumes. However, the escape of digestion of RFOs during the gastric phase ensures that they can also exert prebiotic effects in the intestinal phase, promoting the growth of beneficial microorganisms (Martínez-Villaluenga et al., 2008).

Moreover, faba beans are rich in nutrients, like minerals and phytochemicals, that are essential for human health (Rahate et al., 2021). The hidden hunger, referring to micronutrient deficiencies, is an increasing public health concern, with the common deficiency of iron

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affecting billions of people worldwide (Magee and McCann, 2019). Hidden hunger can be addressed by consuming nutrient-dense foods developed by breeding crops with essential trace minerals (Khush, 2001). Among these crops, faba bean is a good candidate, rich in minerals such as phosphorous (P), potassium (K), calcium (Ca), and magnesium (Mg), as well as trace minerals like iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) (Longobardi et al., 2015). Phytochemicals are the secondary metabolites in plants, produced during seed development and maturation. More than a thousand phytochemicals have been identified, with many of these of interest to human dietary well-being, as they exhibit health-associated benefits (Rochfort and Panozzo, 2007). However, some of the seed phytochemicals, largely being plant defense components, are called antinutritional factors (ANFs) due to the negative effects exerted by consuming legume seeds as human food. Among the phytochemicals, polyphenols are a group of compounds showing overall antioxidative, antimicrobial and anti-inflammatory bioactivities (Goswami et al., 2024). Polyphenols are found in plant foods in different forms, from monomeric phenolic acids to polymerized polyphenols. Generally, total polyphenol content (TPC) has been shown to correlate positively with the antioxidant activity of faba beans (Oomah et al., 2011; Chaieb et al., 2011). However, condensed tannins, as the polyphenols primarily concentrated in the seed coat, can bind to proteins and minerals, thereby decreasing the absorption of these nutrients (Zadernowski et al., 2001; Ojo, 2022).

Vicine and convicine (v-c) can cause favism in people lacking glucose-6-phosphate dehydrogenase and are considered the most limiting faba bean ANF (Khazaei et al., 2019). These are pyrimidine glycosides synthesized in the hull and transported to the cotyledons during the development of the pod, leading to fresh faba bean seeds containing the highest v-c concentration compared to other growth stages (Ray and Georges, 2010). To overcome the limitations of v-c, low v-c genotypes were developed since 1989, by Duc et al., through traditional breeding, followed by advanced genome and breeding methods applied to faba beans (Jayakodi et al., 2023). Trypsin inhibitors (TIs) are another phytochemical in faba beans that can inhibit the digestive activity of both trypsin and chymotrypsin by binding to their lysine and arginine residues in the intestinal phase (Asao et al., 1991). There are two types of protease inhibitors in legume seeds: Kunitz-type and Bowman-Birk inhibitors (BBI), both are found in soybean, while in other grain legumes only the BBIs have been characterized (Lajolo and Genovese, 2002). BBIs have been studied as anticarcinogenic agents that may decrease cancer risk due to their efficiency in suppressing the carcinogen-induced transformation (Lajolo and Genovese, 2002).

Along with genomic and breeding advancements, more faba bean varieties have been developed to produce faba beans with distinct nutritional and ANF characteristics (Duc et al., 1989). Fenn et al. (2024) found that growing years and location significantly affected the seed composition of faba beans, including protein content, ash, RFOs, and trypsin inhibitor activity (TIA). The effects of variety and their response to environmental factors, particularly water and soil conditions, on the nutrient composition of faba bean seeds are rarely investigated. Moreover, protein concentrates can increase the utilization of faba beans and the use of dry fractionation/air classification shows compelling advantages in energy consumption and protein integrity (Boye et al., 2010). However, the level of phytochemicals can potentially be increased during the protein enrichment by air classification (Coda et al., 2015). To address these gaps, faba beans from different varieties and growing conditions and a protein fraction from air classification were collected to explore the variation in minerals, LMWS, RFOs, TPC, v-c, and TIA. This study provides insights to support further faba bean breeding and processing strategies for enhancing nutritional quality.

2. Materials and methods

2.1. Materials

In this study, a total of 12 commercial faba bean cultivars were collected from individual producers and plant breeders as pulses in multiple regions of Denmark. The cultivars included Apollo, Ba Merkur, Birgit, Capri, Daisy, Fuego, Gl. Arabella, Granit, Lynx, Stella, Taifun and Tiffany, which were harvested between 2020 and 2022 with their morphological characteristics illustrated in Fig. 1. For the cultivar Fuego, faba beans were harvested in three years (Fuego2020, Fuego 2021, Fuego2022), and Apollo harvested in two years (Apollo 2021, Apollo2022) from different farms but the same region, as listed in Table 1. Two samples were also included for the cultivar Lynx but the growing location is only known for one sample. Moreover, a protein fraction sample obtained by air classification from cultivar Fuego2020 was included to explore the impact of dry fractionation on faba bean nutrients. The air classification was conducted using a laboratory classifier, Alpine A 100 MZR model BA 1382/10E. After dehulling, the dehulled faba bean seeds were milled into a fine flour using a two-milling system: a Fitz Knife Mill Comminutor DASO6–16 blades, followed by Alpine 250Z pin mills for fine grinding. For the analysis of faba bean pulses, the faba beans (with seed coat) were milled into a fine flour using an ultra-centrifugal mill (ZM 200, Retch, Germany) with a sieve mesh size of 0.5 mm. All the detected compounds are based on the flour obtained from milling. All chemicals used in this study were purchased from Sigma-Aldrich (MA, USA). Ultrapure water produced by the Milli-Q water system (Millipore Corporation, Merck, Germany) was used to prepare all the solutions.

2.2. Analysis of LMWS and RFOs

The low molecular weight sugars and oligosaccharides were quantified according to Kahala et al. (2023) with small modifications. Briefly, 1 mL of methanol was added to 400 mg of faba bean flour and mixed before adding 30 mL of MilliQ water. The mixture was placed in an ultrasonic water bath for 10 min and subsequently in an 80°C water bath for 1 h with magnetic stirring before centrifugation at 2500 g, room temperature (RT) for 10 min. The supernatant was transferred to a 100 mL volumetric flask. The pellet was extracted again by adding 30 mL of MilliQ water and subsequently incubated for 30 min. This was followed by a second centrifugation step using the same conditions as the first and the supernatant was combined with the first one. The supernatant was then cooled to RT, and 200 μL of Carrez I (15 g potassium hexacyanoferrate (II) trihydrate in 100 mL of water) and Carrez II (21.9 g zinc acetate dihydrate in 100 mL of water) were added. The flask was then filled to the set volume with MilliQ water. An aliquot of the extraction was centrifuged at the same conditions as before, and the supernatant was filtered through a 0.2 μm membrane into a vial for the analysis. All the extractions were conducted in biological triplicate. The extracted sugars were analyzed using a Dionex Integrion HPIC System (Thermo Fisher Scientific, MA, USA) with a pulsed amperometric detector (HPAEC-PAD). A Dionex CarboPac PA210-Fast-4μm (2 mm*150 mm) at 30°C with 12 mM KOH as the eluent. Standards (Sigma-Aldrich, MA, USA) of quantified sugars were prepared in water.

2.3. Analysis of minerals

The mineral composition of the faba beans was analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies, CA, USA) following acid digestion of the samples according to Lokuge et al. (2023), with some modifications. Briefly, 0.5 g of raw faba bean flour was digested in 5 mL of 65 % nitric acid and 2 mL of 30 % hydrogen peroxide mixture using rotor 8SXQ80 in a microwave oven (Multiwave 3000, Anton Paar GmbH, Austria). The following program was applied: 15 min ramp to 1400 W and 15 min hold. After

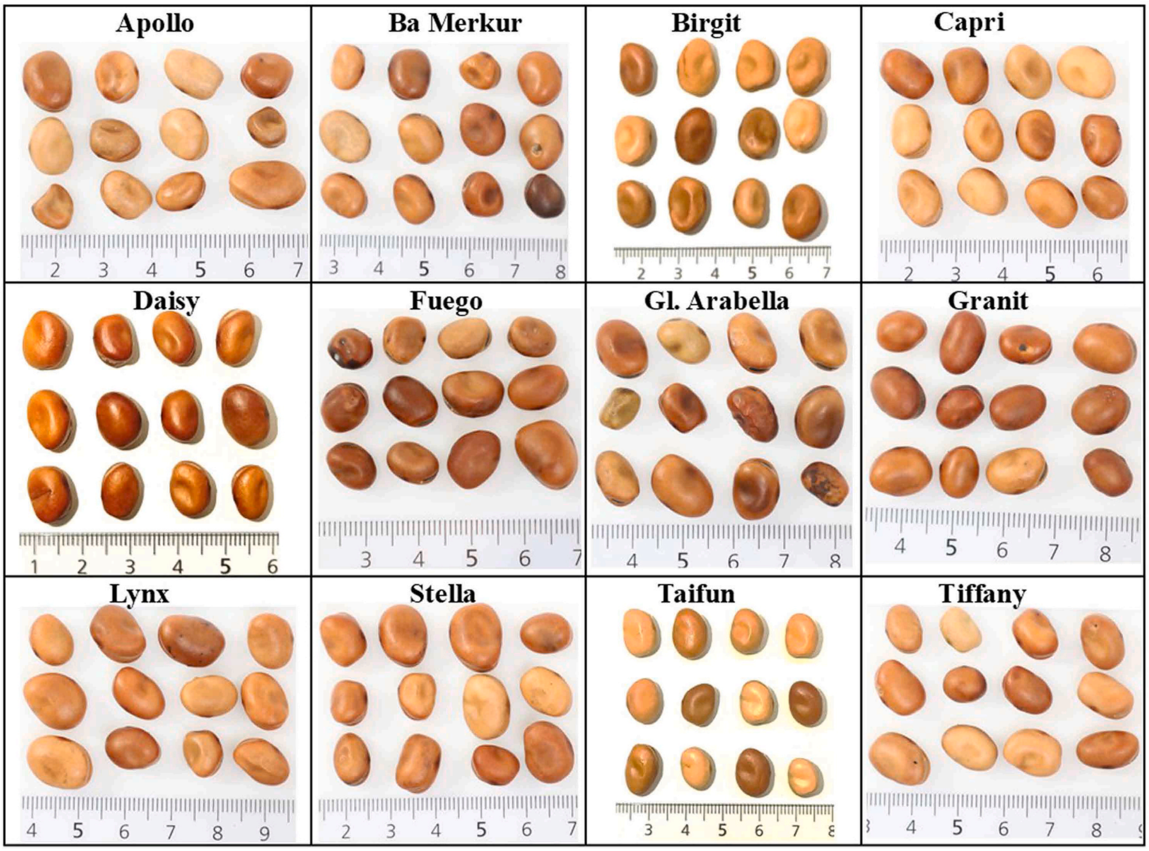

Fig. 1. Seed morphology of 12 different cultivars of faba beans.

Table 1
Overview of Fuego and Apollo faba beans collected from different harvest years.

Cultivar	Sample Name	Harvest Year	Region	Farm
Fuego	Fue0	2020	South	1
	Fue1	2021	South	2
	Fue2	2022	South	1
Apollo	Apo1	2021	South	2
	Apo2	2022	South	2

digestion, the samples were diluted in MilliQ water, with a 5-fold dilution for microminerals and a 60-fold dilution for macrominerals. A series of blank and Certified Reference Material (CRM) (NIST 3234, Soy Flour) samples were prepared under the same conditions to ensure the accuracy and precision of the analysis. All diluted samples were introduced to ICP-MS using He mode with a cell gas flow rate of 4.3 mL/min for analyzing the selected isotopes of Na, Ca, P, K, Mg, Mn, Cu, Fe, and Zn. Yttrium was used as the internal standard, and the external standards were used to quantify the minerals in the sample. The final concentration of the above minerals in the sample was expressed in mg/100 g raw faba bean flour. All samples were detected in biological duplicates.

2.4. Determination of total polyphenol content (TPC)

Total polyphenolic content was determined using the Folin-Ciocalteu (F-C) assay according to Sánchez-Rangel et al. (2013) with slight modifications. Briefly, 200 mg of faba bean flour was mixed with 1.5 mL of 70 % (v/v) ethanol. The mixture was vortexed for 1 min and shaken at 2000 rpm for 60 min, followed by centrifugation at 20,817 g, RT, for 10 min. The supernatant was collected and the pellet was extracted with 250 µL 70 % (v/v) ethanol for another 30 min. The mixture was centrifuged to collect the supernatant. This extraction was repeated and all the supernatant was combined and stored at −20ºC until analysis. All extractions were conducted in biological triplicate. Before analysis the extracts were centrifuged at 5000 g for 5 min. (Labba et al., 2021). Gallic acid was used as the standard. The absorbance at 765 nm was measured using the plate reader with the Gen5 1.11 software (Agilent Technologies, CA, USA). The TPC was expressed as mg gallic acid equivalents (GAE)/g.

2.5. Quantification of vicine and convicine (v-c)

The extraction of v-c was according to Pulkkinen et al. (2015) with some modifications. Briefly, 50 mg of raw faba bean flour was mixed with 6 mL MilliQ water. The mixture was shaken at 1000 rpm for 20 min before centrifugation at 14,000 g for 15 min at RT. Then the supernatant was collected and filtered through Whatman Mini-UniprepTM 0.2 µm membrane filters (Cytiva, Buckinghamshire, UK) before analysis. All extractions were conducted in biological triplicate. A commercial vicine standard was obtained and 0.4 mg/mL of uridine was used as the internal standard. The quantification of v-c by LC-ESI/MS was developed and optimized according to previous methods (Pulkkinen et al., 2015), with some modifications as outlined below. A silica-based C18 column with a particle size of 3 µm (4.6x150mm) from Atlantis (Waters, USA) was used with 0.1 % Formic acid (FA) in water solution as solvent A and 0.1 % FA in acetonitrile as solvent B. The method started with 98 % of solvent A over 4 min, and decreased to 50 % of solvent A over the next 12 min. Thereafter it decreased to 5 % of solvent A over 1 min and remained at 5 % for 5 min. The flow rate was 0.5 mL/min, the injection volume was 1 µL, and the column was kept at 30°C. The mass spectrometry analysis was conducted on a Single Q (Agilent Technologies, Palo Alto, CA, USA) and the MS fragmentor was set to 70 eV.

2.6. Determination of trypsin inhibitor activity (TIA)

The TIs were extracted under acid conditions according to Valdebouze et al. (1980). Briefly, 3 g of raw faba bean flour was mixed with 30 mL of MilliQ water (pH adjusted to 2.9 with 1 M HCl). The mixture was stirred at 250 rpm at RT for 30 min before centrifugation at 20, 817 g, RT, for 15 min. The supernatant was collected and stored at −20°C until analysis. The detection of TIA was according to Kakade et al. (1974), with some modifications. Before the analysis, 200 µg/mL trypsin in 0.05 M Tris-HCl (pH 7.5) and 0.01 M Nα-Benzoyl-DL-arginine p-nitroanilide hydrochloride (BAPNA) in 0.05 M Tris-HCl (pH 7.5) were prepared. The TI extractions were diluted with MilliQ water (pH adjusted to 2.9 with 1 M HCl) to reach a 30–70 % inhibition. Subsequently, 80 µL samples were added to a 96-well plate, followed by adding 20 µL trypsin solution. The trypsin solution was excluded in blank samples, and MilliQ water (pH adjusted to 2.9 with 1 M HCl) was used as the control. The plates were incubated in an air-heated incubator at 37°C for 10 min, followed by the addition of 180 µL BAPNA in 0.05 M Tris-HCl (pH 7.5). Then, the absorbance (abs) at 405 nm was measured by a kinetic plate reader (BioTek Instruments, USA) with settings of 90 min with 5 min interval reading at 37°C installed by the software program Gen5TM (version 1.11). All the analyses were conducted in biological triplicate. The TIA was expressed as trypsin units inhibited (TIU), where one trypsin unit was defined as an increase of 0.01 abs. units at 405 nm. The TIA was calculated based on the following equation:

$$TIA(TIU) = \frac{(AC - ABC) - (AS - ABS)}{0.01} \times DF$$

where AC is the abs. of the control, AS is the abs of the sample, ABC is the abs of the blank control, ABS is the abs of the blank sample, and DF is the dilution factor. All the above-mentioned abs are at a specific reaction time (min) at 405 nm. The TIA was further calculated in TIU/mg faba bean flour.

2.7. Statistical analysis

One-way ANOVA with Tukey's range test was used to measure the significance among cultivars. A two tailed *t*-test was used to compare the variation of phytochemical contents from faba beans (variety Fuego, Apollo and Lynx, respectively) harvested in different growing years or from different regions. P value < 0.05 was considered as significant. The variance of minerals and simple sugars was represented as the coefficient of variation, calculated as the standard deviation divided by the mean concentration of each compound. The PCA and correlation analysis were conducted by SIMCA version 18.0.1.

3. Results and discussion

3.1. Low molecular weight sugars and oligosaccharides (LMWC)

As shown in Table 2, sucrose was the most abundant LMWS in faba beans with an average of 25.95 mg/g of flour, with Gl. Arabella (31.12 mg/g) being the variety with the highest abundance, which is in line with previous research, showing sucrose to account for 1.32–3.8 % dry weight (DW) of faba bean flours (Landry et al., 2016). The contents of glucose and fructose were present at low levels, in line with Goyoaga et al. (2011), whereas the detected galactose ranged from 5.51 to 12.12 mg/g flour but was negligible in this study. The LMWS content and composition varied considerably across cultivars based on previous findings on carbohydrate analysis of faba beans (Jeganathan et al., 2022). Sucrose is the primary energy source during growth by providing carbon for the seed and remains unaffected by water stress during seed filling (Egli and Bruening, 2004). Thus, the variance observed in sucrose content among the three Fuego samples may result from differences in environmental conditions during faba bean growth. Myo-inositol, varying from 8.40 (Apollo 2021) to 11.92 mg/g flour (Tiffany), was the second most abundant LMWS in faba beans. The level of myo-inositol in the air classification sample did not differ significantly from the content of the faba bean pulses. Myo-inositol shares the same molecular weight as glucose but differs in chemical structure, and is widely present in plant foods with multiple functions, such as improving insulin sensitivity in both clinical and animal studies (Croze and Soulage, 2013). The monosaccharides detected in faba beans, including glucose, fructose, galactose and rhamnose, were present in trace amounts, indicating that faba bean was a good candidate for a low glycemic index (GI) food. The faba bean concentrate sample from air classification showed no significant difference in the levels of monosaccharides compared to the faba bean pulses. The variety of Taifun showed the highest glucose content (0.68 mg/g flour) but no detectable contents of fructose and rhamnose. Apollo 2021 displayed the highest content of fructose (0.25 mg/g) and galactose (0.31 mg/g), whereas Lynx and Capri showed the highest concentration of rhamnose (0.21 mg/g). Rhamnose is found in plant cell walls and is critical in

Table 2
Concentration of low molecular weight sugars (mg/g flour) in faba beans.

Sample	Glucose	Fructose	Galactose	Rhamnose	Sucrose	myo-Inositol
Apo1	na	na	0.31 ± 0.02^{a}	0.08 ± 0.03^{ab}	23.20 ± 2.29^{de}	na
Apo2	0.24 ± 0.04^{b}	0.08 ± 0.03^{abc}	0.11 ± 0.01^{cd}	0.17 ± 0.11^{ab}	24.00 ± 3.01^{de}	9.42 ± 0.36^{bc}
Ba Merkur	0.19 ± 0.01^{b}	0.12 ± 0.07^{abc}	0.10 ± 0.01^{cd}	0.08 ± 0.05^{ab}	29.01 ± 1.23^{abc}	10.92 ± 0.06^{abc}
Birgit	0.21 ± 0.03^{b}	0	0.12 ± 0.01^{cd}	0.11 ± 0.06^{ab}	23.17 ± 1.35^{de}	9.77 ± 0.40^{bc}
Capri	0.17 ± 0.04^{b}	0	0.08 ± 0.07^{d}	0.20 ± 0.03^{a}	22.32 ± 0.22^{e}	11.21 ± 0.27^{ab}
Daisy	0.23 ± 0.02^{b}	0	0.12 ± 0.02^{cd}	0.03 ± 0.02^{ab}	24.97 ± 1.00^{cde}	10.05 ± 0.25^{abc}
Fue0	0.15 ± 0.05^{b}	0	0.13 ± 0.02^{cd}	0.12 ± 0.01^{ab}	27.34 ± 0.77^{abcd}	9.55 ± 0.57^{bc}
Fue1	0.26 ± 0.02^{b}	0.17 ± 0.08^{ab}	0.21 ± 0.01^{bc}	0.02 ± 0.01^{ab}	20.81 ± 1.29^{e}	10.66 ± 1.39^{abc}
Fue2	0.26 ± 0.06^{b}	na	0.27 ± 0.08^{ab}	0.08 ± 0.03^{ab}	29.78 ± 0.55^{ab}	9.25 ± 0.86^{bc}
Gl. Arabella	0.21 ± 0.02^{b}	0.19 ± 0.08^{ab}	0.16 ± 0.02^{bcd}	0.08 ± 0.01^{ab}	31.12 ± 1.13^{a}	10.27 ± 0.96^{abc}
Granit	0.22 ± 0.02^{b}	0.23 ± 0.03^{a}	0.16 ± 0.00^{cd}	0.07 ± 0.00^{ab}	26.62 ± 1.09^{bcd}	10.13 ± 0.29^{abc}
Lynx	0.21 ± 0.01^{b}	0.10 ± 0.02^{abc}	0.20 ± 0.05^{bc}	0.21 ± 0.06^{a}	29.12 ± 0.89^{abc}	10.38 ± 0.53^{abc}
Stella	0.16 ± 0.04^{b}	0.07 ± 0.01^{bc}	0.21 ± 0.03^{bc}	na	24.54 ± 2.24^{de}	8.94 ± 0.92^{c}
Taifun	0.51 ± 0.33^{a}	0	0.18 ± 0.04^{bcd}	0	27.11 ± 0.15^{abcd}	10.75 ± 0.06^{abc}
Tiffany	0.23 ± 0.04^{b}	0.16 ± 0.13^{ab}	0.18 ± 0.00^{bcd}	0	24.02 ± 0.20^{de}	11.92 ± 0.92^{a}
Ave.[1]	0.23	0.09	0.17	0.10	25.95	10.28
AC	0.19 ± 0.07	0	0.15 ± 0.03	0.21 ± 0.13	19.90 ± 0.78*	11.41 ± 0.69
P value[2]	0.538	–	0.337	0.489	0.009	0.001
CV-V[3]	0.58	0.95	0.28	0.78	0.10	0.08
CV-G[4]	0.27	1.41	0.35	0.64	0.18	0.08

[na] Not available due to high SD among biological triplicates; [1] Average value of different faba bean samples; * p values < 0.05, indicating significance; [2] p value by Student T test between Fuego 2020 and Air classification sample, [3] coefficient of variation across varieties; [4] coefficient of variation growing years;

forming pectic polymers and cell wall glycoproteins (Jiang et al., 2021). Fructose and rhamnose were detected at the lowest levels in the faba bean samples but varied considerably between both growing years and varieties. This indicates that these LMWS are more susceptible to, e.g. annual variations in growth, with temperature and sunlight playing important roles in regulating their growth.

The content of the RFOs, including raffinose, stachyose and verbascose across the faba bean samples, is shown in Fig. 2. The total amount of oligosaccharides detected ranged from 28.44 (Granit) to 40.76 mg/g (Fuego 2021) across different faba beans. Verbascose (20.14–27.06 mg/g) was the major component of total detected oligosaccharides, which aligned with the findings of Landry et al. (2016), where 2.4 % DW of seeds was quantified as verbascose. Stachyose ranged from 6.39 to 8.38 mg/g, and raffinose was the least abundant component with an average concentration of 1.95 mg/g in faba bean seeds. Fuego 2021 displays a significantly higher concentration of raffinose (7.51 mg/g) compared to the average level of 1.95 mg/g. The content of raffinose was eliminated by air classification, whereas stachyose and verbascose increased significantly to 9.92 and 46.60 mg/g, respectively. Air classification utilizes milling and controlled airflow to separate protein particles from large starch granules. The enrichment of proteins, together with RFOs, in the fine fraction of air classification was also observed previously, with a decrease in the LMWS (do Carmo et al., 2022). These results illustrate the redistribution of the LMWC between the coarse and fine fractions after air classification, with LMWS tending to accumulate in the coarse fraction while RFOs are enriched in the fine fraction. These oligosaccharides are α-galactosides, which can cause flatulence in individuals lacking the hydrolytic enzyme α-galactosidase in the intestinal phase. The undigested oligosaccharides are metabolized by the gut microbiota, leading to excessive gas production in the lower intestine (Onyenekwe et al., 2000). On the other hand, the RFOs serve as prebiotics by promoting the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* species (Kanwal et al., 2023). Among different faba bean varieties, a significant difference was observed in the contents of oligosaccharides. No significant difference was observed in faba beans from different harvest years. These results corresponded with the findings of Fenn et al. (2024), which showed that variety accounted for the major variations of oligosaccharides.

3.2. Minerals

Mineral composition covering K, P, Ca, Mg, Fe, Zn, Cu, and Mn in different faba bean samples is shown in Table 3. The detected minerals accounted for 2–2.8 % of faba bean flour, with K (1391.77 mg/100 g on average) being the most abundant element, aligning with the previous study (Meng et al., 2021). The highest level of K was detected in Tiffany (1731.43 mg/100 g), and the lowest in Capri (1046.93 mg/100 g). The concentration of P in faba beans ranged from 440.49 (Capri) to 765.45 mg/100 g (Gl. Arabella). The highest concentration of Ca was detected in Fuego 2020 (161.89 mg/100 g), which was significantly higher than levels found in Fuego 2021 and 2022 (105.29 and 99.93 mg/100 g, respectively). The Gl. Arabella variety showed the highest levels of Mg (167.91 mg/100 g), which was significantly higher than Daisy (121.24 mg/100 g) and Ba Merkur (119.67 mg/100 g). The concentration of micronutrients, including Fe, Zn, Cu and Mn ranged from 4.19 to 6.68, 3.50–6.79, 0.85–2.27 and 1.12–2.16 mg/100 g, respectively. However, the bioavailability of Fe and Zn is generally low in legume seeds due to their binding to phytates (Grases et al., 2001; Sandberg, 2002). Phytates are well-known chelating agents that can bind to divalent cations forming complexes that are insoluble in the intestine and, as a result, inhibit the absorption of these minerals (Wainaina et al., 2022). In the air classification sample, the minerals were significantly enriched, except for Ca, indicating a negative combination of proteins and Ca. The accumulation of minerals in legume seeds encompasses several processes, such as uptake and translocation from the soil into the roots, mobilization between different tissues and final redistribution into the seeds. Generally, the accumulation of K can be 38 % higher in the pod compared to the seeds, while P can accumulate 40 % more in the seeds than in the pods (Etemadi et al., 2018). In the seeds, 50–80 % P is stored as phytate that can further bind to proteins and small amounts of myo-inositol (Croze and Soulage, 2013). It was reported that the phytates were concentrated with proteins in the

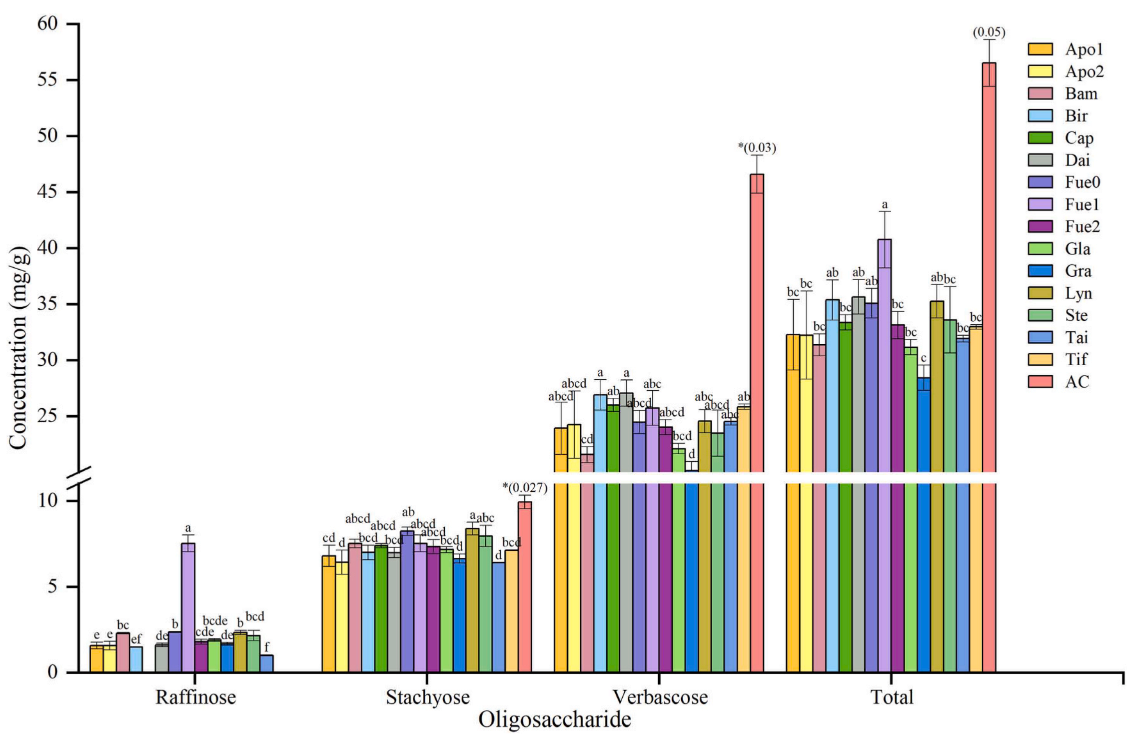

Fig. 2. Concentration of raffinose family ologosaccharides (RFOs) in different faba bean samples. Different letters between samples indicate significant differences (p value < 0.05) for each RFOs in and total RFOs in content.

Table 3
Concentration of major elements (mg/100 g flour) in faba beans.

Sample	K	P	Mg	Ca	Fe	Zn	Cu	Mn
Apo1	1179.46 ± 340.54^{bc}	527.50 ± 115.75^{bcd}	129.97 ± 1.95^{abc}	110.70 ± 12.11^{de}	5.87 ± 0.03^{abcd}	4.13 ± 0.05^{b}	1.36 ± 0.02^{abc}	1.12 ± 0.02
Apo2	1607.15 ± 143.07^{ab}	717.03 ± 34.09^{ab}	147.98 ± 14.40^{abc}	122.20 ± 2.67^{cde}	6.15 ± 0.67^{abc}	5.15 ± 3.15^{ab}	2.27 ± 0.93^{a}	1.99 ± 1.12
Ba Merkur	1265.26 ± 12.56^{abc}	490.86 ± 3.08 ^{cd}	119.67 ± 2.46^{c}	106.12 ± 2.43^{def}	5.44 ± 0.18^{bcd}	4.43 ± 0.17^{b}	1.35 ± 0.06^{abc}	1.30 ± 0.04
Birgit	1550.57 ± 5.79^{abc}	667.31 ± 0.95^{abc}	162.09 ± 1.37^{ab}	138.91 ± 6.31^{abc}	6.47 ± 0.30^{ab}	8.83 ± 0.15^{a}	1.94 ± 0.07^{ab}	2.17 ± 0.16
Capri	1046.93 ± 1.82^{c}	440.49 ± 0.16^{d}	153.40 ± 2.17^{abc}	151.59 ± 1.14^{ab}	6.18 ± 0.28^{abc}	4.91 ± 0.13^{b}	1.72 ± 0.06^{abc}	2.05 ± 0.09
Daisy	1296.57 ± 161.53^{abc}	557.97 ± 72.13^{bcd}	121.24 ± 7.61^{bc}	127.65 ± 5.06 ^{cd}	4.85 ± 0.07^{de}	4.95 ± 0.19^{b}	1.72 ± 0.01^{abc}	1.29 ± 0.02
Fue0	1408.03 ± 16.34^{abc}	619.45 ± 34.21^{abcd}	150.80 ± 2.12^{abc}	161.89 ± 0.21^{a}	5.59 ± 0.04^{abcd}	5.44 ± 0.07^{ab}	1.82 ± 0.02^{abc}	2.16 ± 0.05
Fue1	1377.90 ± 23.55^{abc}	585.22 ± 11.22^{abcd}	125.58 ± 5.58^{bc}	105.29 ± 6.72^{def}	3.85 ± 0.58^{e}	3.86 ± 0.58^{b}	1.09 ± 0.24^{bc}	1.32 ± 0.27
Fue2	1212.85 ± 299.26^{abc}	479.45 ± 123.06 ^{cd}	131.26 ± 13.76^{abc}	99.93 ± 4.73^{ef}	4.74 ± 0.26^{de}	5.24 ± 0.04^{ab}	1.25 ± 0.10^{bc}	1.77 ± 0.01
Gl. Arabella	1512.39 ± 10.56^{abc}	765.45 ± 3.90^{a}	167.91 ± 1.49^{a}	154.65 ± 6.21^{ab}	6.68 ± 0.07^{a}	4.83 ± 0.04^{b}	1.83 ± 0.03^{abc}	2.15 ± 0.01
Granit	1337.06 ± 38.32^{abc}	639.98 ± 15.07^{abcd}	129.64 ± 16.65^{abc}	85.43 ± 3.53 ^{f}	5.02 ± 0.51^{cde}	3.86 ± 0.01^{b}	0.85 ± 0.00^{c}	1.30 ± 0.01
Lynx	1516.22 ± 5.51^{abc}	719.01 ± 2.73^{ab}	138.96 ± 0.99^{abc}	141.10 ± 3.00^{abc}	6.41 ± 0.04^{ab}	6.79 ± 1.81^{ab}	1.68 ± 0.01^{abc}	1.50 ± 0.01
Stella	1373.26 ± 159.40^{abc}	582.80 ± 57.69^{abcd}	146.59 ± 28.95^{abc}	134.12 ± 12.24^{bc}	4.99 ± 0.13^{cde}	3.50 ± 0.16^{b}	1.35 ± 0.05^{abc}	1.39 ± 0.05
Taifun	1461.45 ± 23.42^{abc}	682.05 ± 5.18^{abc}	137.97 ± 2.33^{abc}	119.57 ± 2.72^{cde}	4.85 ± 0.16^{de}	6.16 ± 0.06^{ab}	1.84 ± 0.05^{abc}	1.57 ± 0.09
Tiffany	1731.43 ± 1.21^{a}	584.03 ± 3.84^{abcd}	158.03 ± 1.91^{abc}	141.84 ± 0.96^{abc}	5.10 ± 0.00 ^{cd}	4.31 ± 0.04^{b}	1.51 ± 0.03^{abc}	1.54 ± 0.01
Ave.[1]	1391.77	603.91	141.41	126.73	5.48	5.09	1.57	1.64
AC	2394.95 ± 163.99	1285.83 ± 85.35	217.01 ± 12.87	69.10 ± 3.55*	6.19 ± 0.21	8.93 ± 0.29*	2.87 ± 0.08*	2.30 ± 0.05
P value[2]	0.075	0.062	0.088	0.017	0.154	0.038	0.033	0.217
CV-V[3]	0.12	0.15	0.11	0.15	0.13	0.28	0.19	0.20
CV-G[4]	0.08	0.13	0.10	0.28	0.18	0.18	0.28	0.24

[1] Average value of different faba bean samples; [2] p value by Student T test between Fuego 2020 and Air classification sample; * p values < 0.05, indicating significance; [3] coefficient of variation across varieties; [4] coefficient of variation across growing years; different letters in each column mean significant difference.

fine fraction (do Carmo et al., 2022). The variance of different elements regarding variety and harvest years reflected by the coefficient of variance for the different elements showed that especially Zn, but also K and P varied more among varieties than other elements, whereas Fe, Cu, Mn, and Ca varied the most across growing years.

3.3. Phytochemical analysis

Fig. 3a displays the variation in TPC, TIA, and v-c across varieties. Air classification significantly increased TIA and v-c concentrations. The TPC content of the air classification sample did not vary significantly from the raw faba bean flour. Before air classification the seeds are normally dehulled, thereby also removing condensed tannins significantly (Ojo, 2022). Among the faba bean pulses, the concentration of TPC ranged from 1.29 (Stella) to 2.37 mg GAE/g (Daisy), and the cultivars could be divided into two groups according to the TPC. The high TPC group (1.86–2.37 mg GAE/g) included Apollo, Birgit, Ba Merkur, Daisy, Fuego and Lynx, while the low TPC group (1.29–1.67 mg GAE/g) included Capri, Gl. Arabella, Granit, Stella, Taifun and Tiffany. TPC content has earlier been reported to vary considerably from 1.4 to 5.7 mg GAE/g DW in different faba bean varieties grown in Europe and Australia (Labba et al., 2021; Valente et al., 2019; Johnson et al., 2021). The TPC of faba beans is largely affected by genetic factors (Baginsky et al., 2013) and can be deduced by the color of the flowers according to Labba et al. (2021), where faba beans with coloured flowers showed higher TPC compared to those with white flowers. The TIA across all the faba bean samples varied from 18.45 (Taifun) to 39.51 U/mg (Gl. Arabella) which divided the faba beans into three groups accordingly. Variety Gl. Arabella, Tiffany and Capri were the high TIA group (34.43–39.51 U/mg), followed by the mid TIA group (27.05–31.31 U/mg), including Apollo, Birgit, Ba Merkur, Fuego and Lynx, and then the low TIA group (18.45–22.50 U/mg) covering Daisy, Granit, Stella and Taifun, implying the effects of variety on TIA of faba beans. These TIA levels found in the current study were higher compared to those reported earlier in faba beans (Valdebouze et al., 1980; Labba et al., 2021). This may be due to different extraction and detection methods applied and even ways of calculating activities depending on the definition of TIA, which largely complicates the comparison of TIA across

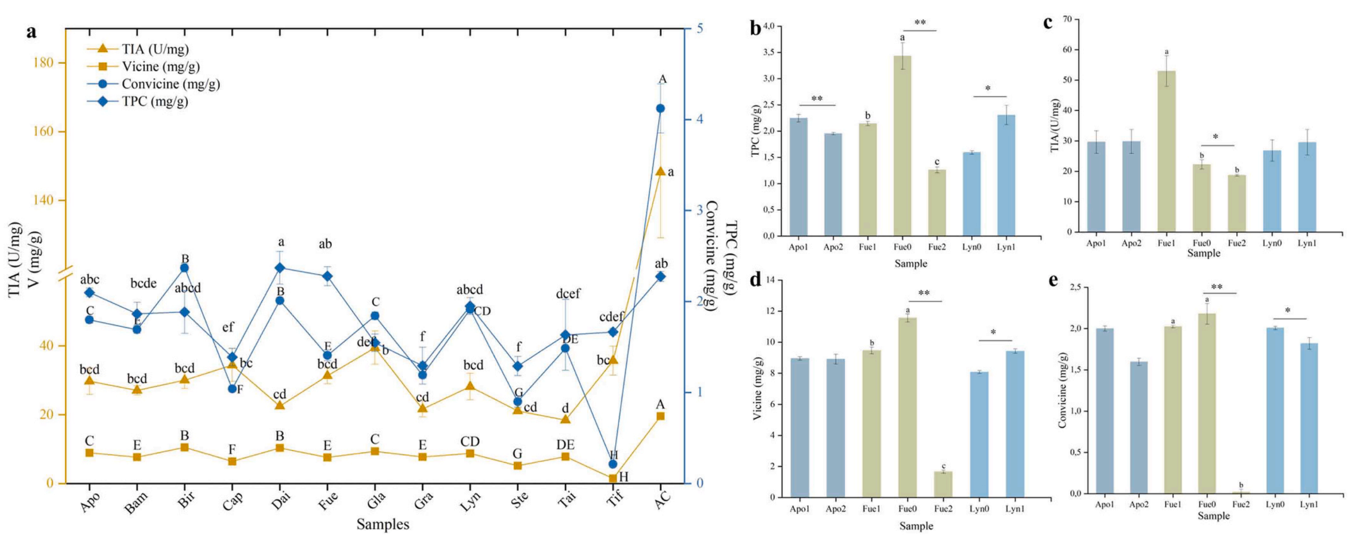

Fig. 3. **a.** Overview of trypsin inhibitor activity (TIA), vicine, convicine, and total polyphenol contents (TPC) in different varieties of faba beans and air classification sample; concentration of **b.** TPC; **c.** TIA; **d.** vicine; **e.** convicine in faba beans from different growing years and regions. For Fig. 3a, different letters indicate significant differences among samples (p value < 0.05). For Figs. 3b-3d; * (p value < 0.05), ** (p value < 0.01).

studies. Nevertheless, levels and trends obtained within a study can be deduced and faba beans have been shown to exhibit lower TIA compared to soybeans and peas (Valdebouze et al., 1980; Guillamón et al., 2008). In this study, the highest level of v-c was detected in Birgit (10.5 and 2.37 mg/g, respectively) with the lowest in Tiffany (1.46 and 0.21 mg/g, respectively). These results are in line with a previous study showing that Tiffany is a low v-c cultivar (Labba et al., 2021). As breeding develops, commercial low v-c cultivars will be more broadly available (Khamassi et al., 2013). However, apart from Tiffany, the v-c concentration of Fuego, Granit, and Taifun was considerably lower in v-c in this study, as also documented previously (Labba et al., 2021), indicating that the growing conditions also affect the accumulation of v-c in faba bean seeds.

Besides compositional differences among varieties, the variation of these compounds across different harvest years and regions was also further studied (Fig. 3b-d). The highest TPC (3.5 mg GAE/g) was detected in Fuego harvested in 2020, which was significantly higher than those from the same cultivar but under different growing conditions. The same pattern was also observed in Apollo from different years and in Lynx from different regions. Genotype was the first contributor affecting TPC level among different faba bean cultivars, by influencing phenolic biosynthetic pathways, which further spread to the color of faba bean flowers (Zanotto et al., 2020). For TIA, Fuego from different growing years showed significant differences. However, the TIA was not significantly different among Apollo and Lynx samples, indicating that TIA was affected more by genotype than by growing conditions. This contrasts the results of a previous study where the effect of growing location was more pronounced than differences between varieties or growing years (Fenn et al., 2024). For v-c, there was no significant difference in Apollo from different harvest years, whereas the concentration of v-c changed significantly in Fuego and Lynx from different growing conditions.

3.4. PCA analysis

The PCA analysis was applied to visualise the variation in the dataset when combining results for all measured compounds of faba beans across different varieties and growing years (Fig. 4). The first and second principal components together explained 38 % of the total variance. Especially samples from the variety Fuego from three different growing years displayed clear separations from each other. PC1 separated Fuego 2020 from Fuego 2021 and Fuego 2022, respectively. Fuego 2020 clustered closely together with Birgit, which seems to be driven by higher contents of minerals and v-c in these samples, in contrast to Granit, Stella and especially Fuego 2022, which were characterized by higher contents of LMWS. Variation along PC2 between Fuego 2021 and the remaining samples was mainly driven by raffinose, and among the other faba bean samples, variation along PC2 is driven by TIA, followed by stachyose and glucose.

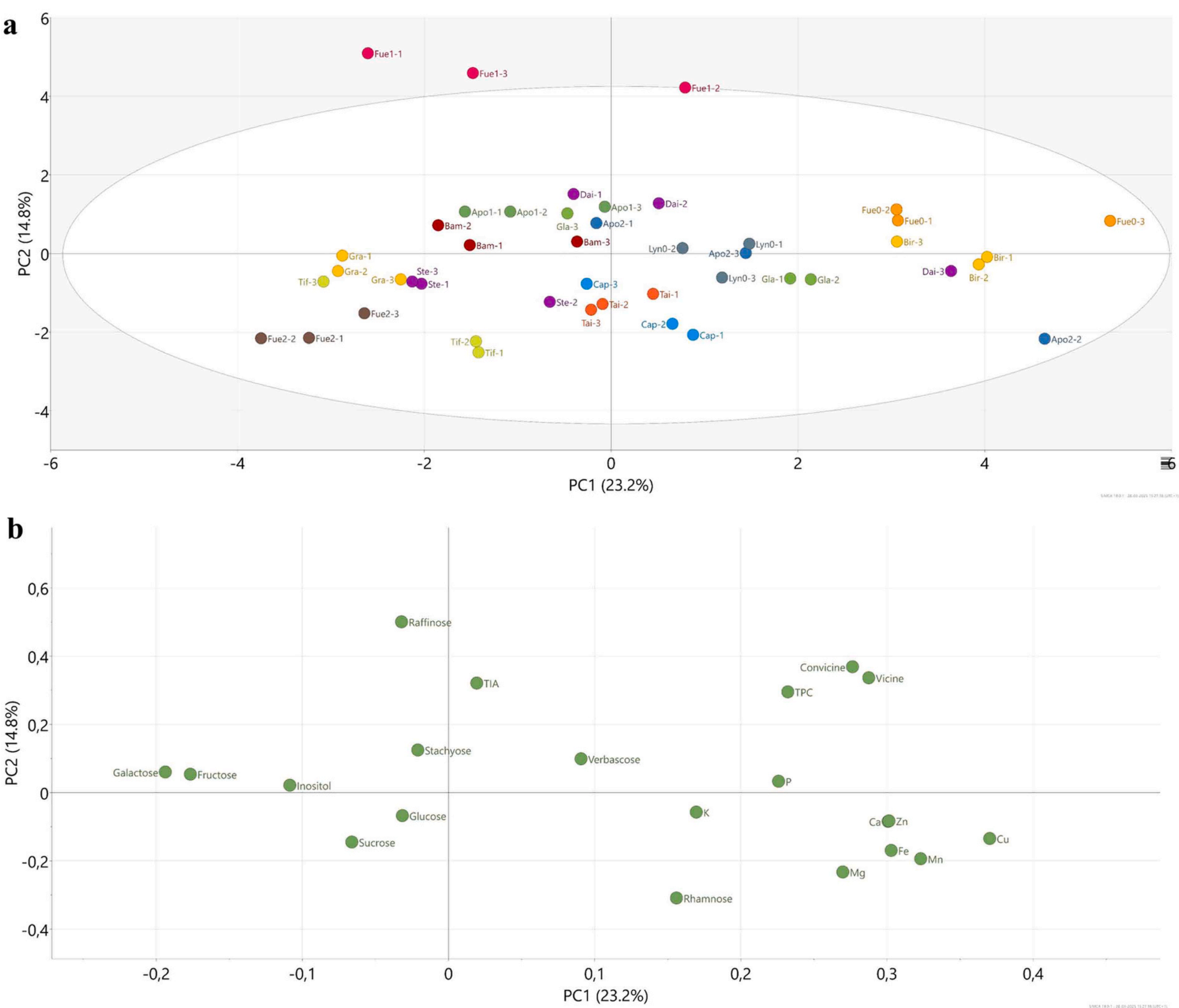

Fig. 4. PCA **a.** score plot; and **b.** loading plots of dttected compounds from different faba bean cultivars.

4. Conclusion

This study investigated nutritional composition, including minerals and LMWS, and phytochemicals (oligosaccharides, polyphenols, trypsin inhibitor and v-c) among 12 different faba bean varieties. Each variety showed individually distinct levels of these components across samples. The variation in these components at different growing conditions and dry fraction processing was further investigated. Sucrose was the most abundant LMWS and was more affected by growing years than by varieties. The RFOs accounted for 3.35 % of faba bean flour, with verbascose (24.31 mg/g) being the most abundant component, which was further enhanced by air classification. Potassium at an average level of 1391.71 mg/g was the most abundant micronutrient measured in faba beans. The variation in minerals was less susceptible to both varieties and growing years. Air classification significantly increased TIA and minerals, except Ca. Tiffany was a low v-c cultivar detected (1.46, 0.21 mg/g). The growing year showed less contribution to the variation in the contents of the detected compounds, though this may reflect constraints in the limited data collected from this study. These results highlight the potential of faba beans as a nutritional food ingredient, contributing to mineral supplements and low GI food while at the same time emphasising the importance of variety selection and processing methods in optimising their nutritional benefits. Future work can focus on how specific growing conditions can be leveraged to fortify the nutritional profiles of faba beans. Although air classification facilitates advantages in protein fractionation, further optimization is still required to enhance the nutrients and reduce ANFs. This knowledge can guide future breeding, processing, and utilisation strategies to maximise the nutritional potential of faba beans in food applications.

Author statement

The authors have made revisions according to reviewers and editor' s comments.

CRediT authorship contribution statement

Marie Louise Pontoppidan Witt: Methodology, Data curation. **Kübra Yilmaz Gøkhan:** Writing – review & editing, Methodology, Data curation. **Tianzhen Xiao:** Writing – original draft, Visualization, Validation, Project administration, Methodology, Funding acquisition, Data curation. **Lotte Bach Larsen:** Writing – review & editing, Validation, Supervision. **Nina Aagaard Poulsen:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition. **Minna Kahala:** Writing – review & editing, Validation, Methodology. **Santeri Kankaanpää:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Markus Nurmi:** Writing – review & editing, Methodology. **Gayani Madushani Sirinayake Lokuge:** Writing – review & editing, Methodology, Data curation. **Karin Loft Eybye:** Writing – review & editing, Resources, Methodology.

Declaration of Competing Interest

The authors declare no competing interest.

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Data Availability

Data will be made available on request.

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