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# Nutritional and antinutritional composition of fava bean (*Vicia faba* L., var. minor) cultivars

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ABSTRACT

A dietary shift from resource-demanding animal protein to sustainable food sources, such as protein-rich beans, lowers the climate footprint of food production. In this study, we examined the nutrients and antinutrients in 15 fava bean varieties cultivated in Sweden to select varieties with high nutritional value. On a dry weight basis, the fava beans were analyzed for their content of protein (range 26–33%), amino acids (leucine range: 50.8–72.1 mg/g protein, lysine range: 44.8–74.8 mg/g protein), dietary fiber (soluble fraction range: 0.55–1.06%, insoluble fraction range: 10.7–16.0%), and iron (1.8–21.3 mg/100 g) and zinc contents (0.9–5.2 mg/100 g), as well as for the following antinutrients: lectin (0.8–3.2 HU/mg); trypsin inhibitor (1.2–23.1 TIU/mg) and saponin (18–109 μg/g); phytate (112–1,281 mg/100 g); total phenolic content (1.4–5 mg GAE/g); and vicine(403 μg/g – 7,014 μg/g), convicine (35.5 μg/g – 3,121 μg/g) and the oligosaccharides raffinose (1.1–3.9 g/kg), stachyose (4.4–13.7 g/kg) and verbascose (8–15 g/kg). The results indicate substantial differences between cultivars in relation to their contents of nutrients and antinutrients. Only one of the cultivars studied (Sunrise) have adequate estimated bioavailability of iron, which is of major concern for a diet in which legumes and grains serve as important sources of iron. The nutritional gain from consuming fava beans is significantly affected by the cultivar chosen as the food source.

## 1. Introduction

Grain legumes, such as beans and lentils, have favorable nutritional compositions for human consumption, being low in fat and high in protein, dietary fibers, iron, zinc and vitamins such as folate, riboflavin and thiamine (Tiwari & Singh, 2012). Furthermore, grain legumes contain antioxidants and other bioactive compounds that can contribute to human health (Ganesan & Xu, 2017). Several health benefits have been proposed in relation to the consumption of grain legumes, including reduced risk of colorectal cancer (Aune et al., 2011), improvement of gut health, reduced blood cholesterol levels (Clemente & Olias, 2017), and reduced risk of cardiovascular disease (Sharma, Srivastava, & Prakash, 2011).

However, legumes also contain a number of bioactive compounds that are traditionally classified as antinutrients: phytates, saponins, lectins and protease inhibitors. Even if the levels of several of these compounds can be lowered or eliminated using different processing techniques, they need to be monitored, given that antinutritional compounds can exert negative effects on the human body and reduce the digestibility of nutrients (Gilani, Cockell, & Sepehr, 2005; Khattab & Arntfield, 2009). A plant-based diet that contains grain legumes is generally considered to have a low level of bioavailability of minerals (mainly calcium, iron and zinc) owing to the presence of absorption inhibitors, mainly phytates and polyphenols (Sandberg, 2002; Tako, Beebe, Reed, Hart, & Glahn, 2014). The inhibitory effect of phytates on mineral absorption is linked to the formation of insoluble and indigestible phytate-mineral complexes in the gut (Sandstrom, 1997). Excessive levels of phytates in the diet can lead to deficiencies of zinc and iron as the result of insufficient absorption (Zhou & Erdman, 1995). The main minerals of concern when evaluating the nutritional value of a plant based diet are iron and zinc. Vegetarians have been shown to have lower iron stores, and an increase in iron deficiency anemia, compared with non-vegetarians, mainly explained by the lower bioavailability of iron in plant foods (Haider, Schwingshackl, Hoffmann, & Ekmekcioglu, 2018; Pawlak, Berger, & Hines, 2016). The current prevalence of iron deficiency in women of fertile ages living in Western countries has been estimated to 10–30% (Cooper, Greene-Finestone, Lowell, Levesque, & Robinson, 2012; Hercberg, Preziosi, & Galan, 2001; Lahti-Koski, Valsta,

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Alfthan, Tapanainen, & Aro, 2003; Umbreit, 2005). This proportion is at risk of increasing as a result of the protein shift, especially for individuals in vulnerable groups such as women of fertile ages, children and adolescents. Bioavailability of iron and zinc can be estimated by calculation of the molar ratios of phytate to mineral (Lestienne, Icard-Vernière, Mouquet, Picq, & Trèche, 2005; Lopez, Leenhardt, Coudray, & Remesy, 2002).

The levels of nutrients and antinutritional components can differ significantly between cultivars of the same legume crop. Therefore, it is of importance to screen these compounds to identify cultivars that are most suited to different applications. Nutritional data, as well as data on agricultural properties such as yield and adaptability to different climates and soil types are important for selecting cultivars that are optimal as human or animal feedstuffs (Shang et al., 2016).

The fava bean is a grain legume that, in contrast to the soy bean, can be cultivated in a wide variety of geographic locations, even in regions with a short growing season, such as the Boreal zone (Stoddard & Hämäläinen, 2011). The fava bean has a long history of being used as a food for human consumption, with the oldest seeds of fava bean being traced to the late 10th millennium B.P. in north-west Syria (Tanno & Willcox, 2006). In countries with more advanced economies, legumes are generally underutilized as a food for humans, as the major sources of protein in the diet in these regions are animal products (Tijhuis, Ezendam, Westenbrink, & Rossum, 2012). Among legumes, the fava bean is noteworthy in that it is a crop that can be cultivated to a greater extent in the Nordic region and it plays a role in shifting protein consumption from resource-demanding animal proteins to domestically grown crops, which can also be used as feedstock (Röös et al., 2018). Utilizing locally grown crops is especially important in regions that are currently dependent upon the importation of soy beans for foodstuffs and feedstock.

Apart from the common antinutritional components, fava beans contain vicine and convicine, which in individuals with the x-chromosome-inherited glucose-6-phosphate dehydrogenase (G-6-PD) deficiency can cause acute hemolysis, a disease known as favism (Arese & Flora, 1990). The levels of antinutrients vary according to the specific fava bean cultivar, stage of maturation, climate of cultivation, soil properties etc. (Kumar & Nidhi, 2015). Extensive nutritional data on cultivars of fava beans that are suitable for cultivation in the Nordic climate are, to our knowledge, currently lacking.

The aim of the present investigation was to elucidate the compositions of the nutrients and antinutrients in a number of fava bean varieties cultivated in Sweden, with the goal of suggesting an approach to select varieties that are suitable for creating food products for human consumption.

## 2. Materials and methods

### 2.1. Fava bean cultivars

In total, 15 different cultivars of fava bean (*Vicia faba* L., var. minor) were harvested during the mature stage. Of these, 14 originated from the same field in Grästorp, Sweden, which was planned and cultivated by *Hushållningssällskapet*. These cultivars included the white-flowered cultivars of Banquise, Fernando, Gloria, Sunrise, and Taifun, and the color-flowered cultivars of Fuego, Boxer, Fanfare, Tiffany, Lynx, Birgit, Daisy, GL Emilia, and Stella. In addition, the color-flowered cultivar Alexia was harvested from a different field in the same region (Västra Götaland, Sweden). For all the analyses, dry beans were ground into a fine flour using a rotor mill with a sieve mesh size of 0.5 mm (Retsch GmbH, Haan, Germany).

### 2.2. Analyses of nutrients

#### 2.2.1. Protein Determination

Total nitrogen was determined by complete combustion based on the Dumas principle, using the LECO Trumac nitrogen analyzer (LECO Corporation, St. Joseph, MI, USA) with EDTA as the standard. Combustion of the samples (100 mg) was conducted in a sealed furnace at 1150 °C. The total protein content was calculated using a nitrogen to protein conversion factor of 5.4, which correspond to an average for legume proteins (Mariotti, Tomé, & Mirand, 2008).

#### 2.2.2. Amino acid profiles

Amino acid analysis was carried out using a modified version of the method previously described by (Özcan & Şenyuva, 2006). To 100 mg of fava bean flour, 8 mL of 6 mol/L HCl were added and the mixture was hydrolyzed for 24 h at 110 °C After hydrolysis, the volume was adjusted to 10 mL using Milli-Q water, and an aliquot of 2 μL was injected into the LC-MS system [Agilent 1260 HPLC with a Phenomenex column (C18 (2) 250 μm × 4.6 μm × 3 μm), coupled to the Agilent 6120 Quadrupole in the SIM-positive mode] (Agilent Inc., Santa Clara, CA, USA). The composition of mobile phase A was 3% MeOH, 0.2% formic acid, and 0.01% acetic acid (HAc), and that of mobile phase B was 50% MeOH with 0.2% formic acid and 0.1% HAc. The initial gradient, held for the first 8 min, contained 94% A and 6% B. This gradually changed until it reached 80% A and 20% B after 20 min. This gradient was held for a run time of 27 min, then gradually altered until it reached 94% A and 6% B at a run time of 28 min, and then held again for a total run time of 40 min. Twenty-four amino acids diluted in 0.2 mol/L HAc in the concentration range of 1–20 mg/L were used to derive the standard curve. Due to the use of acidic hydrolysis, tryptophan, cysteine, methionine and tyrosine could not be quantified.

#### 2.2.3. Mineral analyses

A microwave digestion step (Milestone microwave laboratory system; EthosPlus, Sorisole, Italy) was performed as previously described by Fredriksson, Carlsson, Almgren, and Sandberg (2002). Briefly, 0.15 g of sample was mixed with 0.75 mL of concentrated trace metal grade $HNO_3$, 0.15 mL HCl, and 3 mL $H_2O$ in a Teflon vial. The sample was digested to create a transparent solution using a temperature program that reached 180 °C in 15 min and maintained this temperature for an additional 20 min. After cooling, the sample was decanted into a test tube and diluted to a final volume of 10 mL. Acid digestion was performed in duplicate before determining the iron and zinc contents by atomic absorption spectrometry (200 Series AA System; Agilent). Calibration was performed using commercial standards with a concentration range of 0.125–5.0 mg/L. Measurements were carried out using standard flame operating conditions, as recommended by the manufacturer.

#### 2.2.4. Analysis of dietary fiber

Fava bean flours were analyzed by gas chromatography for total dietary fiber according to the Uppsala method (Theander, Aman, Westerlund, Andersson, & Pettersson, 1995) and for soluble and insoluble dietary fiber according to the method of Andersson, Merker, Nilsson, Sørensen, and Åman (1999). Nonresistant starch was removed by α-amylase and amyloglucosidase, and remaining polysaccharides were precipitated by 80% ethanol. Polysaccharides were hydrolyzed by acid and quantified as alditolacetates by gas chromatography. The analyses were performed at the Swedish University of Agricultural Sciences in Uppsala.

#### 2.2.5. Ash and moisture determination

The moisture content of the ground fava beans was determined in duplicate samples of 0.5 g of material that were dried at 105 °C for 20 h, placed in a desiccator for 2 h, weighed, and (0.2 g) then combusted in a furnace at 550 °C for 3 h, cooled to about 300 °C and transferred to a desiccator for 3 h before weighing again.

### 2.3. Analyses of antinutrients

#### 2.3.1. Phytate analysis

Phytate was analyzed as inositol hexaphosphate ($InsP_6$) by high-performance ion chromatography (HPIC) according to Carlsson, Bergman, Skoglund, Hasselblad, and Sandberg (2000). Fava bean flour (0.5 g) was extracted with 10 mL of 0.5 mol/L HCl for 3 h using a laboratory shaker (Heidolph Reax 2; Heidolph Instruments GmbH, Schwabach, Germany). Then, 1 mL was removed, centrifuged, and the supernatant was transferred to an HPLC vial. The chromatography setup consisted of an HPLC pump (model PU-4080i; Jasco Inc., Easton, MD, USA) for the eluent and an RHPLC pump (model PU-4180; Jasco) equipped with a PA-100 guard column and a CarboPac PA-100 column.

$InsP_6$ was eluted with an isocratic eluent of 80% HCl (1 mol/L) and 20% $H_2O$ at 0.8 mL/min, subjected to a post-column reaction with ferrous nitrate, and detected with at 290 nm in a UV–visible HPLC detector (UV-4075; Jasco). Each sample had a run time of 7 min, and the $InsP_6$ concentration was calculated using external standards covering the concentration range of 0.1–0.6 μmol/mL.

#### 2.3.2. Estimations of relative iron and zinc bioavailabilities

The molar ratios Phy:Fe and Phy:Zn were calculated to estimate the relative bioavailabilities of iron and zinc, respectively, in the fava bean cultivars, and to give an indication of the inhibitory effects of phytates on these minerals. A molecular mass for phytate of 660.3 g/mol was used for the calculations.

#### 2.3.3. Vicine and convicine assays

Analyses of flour samples from fava bean cultivars were performed by the Natural Resources Institute of Finland, according to the method of Gutierrez et al. (2006). Briefly, samples (1 g) were extracted with ultrapure water (30 mL) in a hot-water bath (90 °C) for 3.5 h, with shaking every 30 min. The samples were then cooled in a water bath and centrifuged to remove solids. Concentrated HCl (100 μL) was added to the supernatant (10 mL), followed by an additional centrifugation step (10 min, 2500*g*) Samples were filtered through a 0.45-μm Acrodisc GHP membrane filter (Pall Corporation, Port Washington, NY, USA) before analysis by HPLC (Agilent 1100 with a diode array detector, HPLC-DAD; Agilent) on an Atlantis T-3 (2.1 × 150 mm, 3 μm) column (Waters Corp., Milford, MA, USA), followed by elution with a gradient of 50 mmol/L phosphate buffer and methanol at 0.2 mL/min. Detection of vicine (Sigma-Aldrich, St. Louis, MO, USA) and convicine was conducted at 280 nm, and for identification purposes the spectrum from 190 nm to 450 nm was recorded. Quantification of convicine was achieved using the calibration curve for vicine.

#### 2.3.4. Lectin quantification

One gram of bean flour from each cultivar was mixed with 10 mL of PBS in a 50-mL centrifuge tube according to the procedure previously described by Liener and Hill (1953), with some modifications. The mixture was shaken overnight on a microplate shaker (VWR International, Monroeville, PA, USA) at speed setting 350 at 4 °C, and thereafter centrifuged at 14,000*g* for 10 min at 4 °C. The filtrated supernatant was then serially diluted in a 96-well microplate, from no dilution in Well 1 to a 1:128 dilution in Well 8. In the first well, 0.1 mL of extract was placed, and in the other wells was placed 0.05 mL of PBS. The extract was then serially diluted by a factor of 1:2 with volume of 0.05 mL, for each step. From the last well, 0.05 mL of the volume was discarded and finally 0.05 mL of 2% human red blood cells was added to each well. The microplates were then mixed gently on a microplate shaker (VWR International) at room temperature for 2 h.

Hemagglutination was determined visually using a phase microscope (VWR International) and photographed (Moticam, Hong Kong, China), as described previously by Makkar, Becker, Abel, and Pawelzik (1997). From each well, 5 μL were transferred to a glass slide and placed under a cover glass. Dilutions containing blood cells that were forming aggregates with five or more cells were regarded as positive in terms of hemagglutination activity. A blank consisting of 0.05 mL of PBS and 0.05 mL of 2% red blood cells was used.

The hemagglutination activity was calculated according to the equation previously described by (Liener & Hill, 1953). The highest dilution that gave a positive result for hemagglutination was considered to contain one hemagglutinin unit ($HU^{-mg\ flour}$, calculated on a dry-weight basis):

$$HU^{-mg} = \frac{D_a \times D_b \times S}{V} \times \frac{100\%}{100\% - MC}$$

where HU is the hemagglutinin unit per mg of flour, on a dry-weight basis,

$D_a$ is the dilution factor of the extract in Well 1 (equal to 1 unless the original extract was diluted), $D_b$ is the dilution factor in the tube containing 1 HU, S is mL of the original extract/mg flour, V is the volume of extract in Well 1, and MC is the moisture content (%) of the flour.

#### 2.3.5. Determination of saponin

Fava bean flour (1 g) was mixed with 10 mL of 0.9% PBS solution and agitated overnight on a microplate shaker (VWR) at speed setting 350 at 4 °C. The mixture was then centrifuged at 10,000*g* for 15 min at 4 °C. The supernatant was stored at −20 °C until use.

Saponin was quantified using a competitive ELISA with a monoclonal antibody that recognizes unconjugated soyasaponin I (Frøkiær, Sørensen, Sørensen, & Sørensen, 1995). In brief, diluted samples were incubated with the monoclonal antibody directed against soyasaponin I for 1 h, and then transferred to a microtiter plate (Maxisorb; Nunc, Roskilde, Denmark) that was coated with saponin-BSA conjugate (0.1 μg/mL). A dilution row of pure soyasaponin I was included as the standard. After incubation and washing with PBS-Tween buffer, horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin antibody was added to the wells of the plate. The plates were incubated for 1 h, washed, and developed by the addition of 3,3′,5,5′-tetramethylbenzidine in hydrogen peroxide. The reaction was stopped by the addition of 2 mol/L phosphoric acid and the absorption at 450 nm was measured.

#### 2.3.6. Trypsin inhibitors

Determination of trypsin inhibition was performed according to the method of Kakade (1974). Ground fava beans were extracted as described for saponin analysis. From a stock solution of 1 mg/mL trypsin in 1 mmol/L HCl, a working solution of 200 μg/mL in 0.05 mol/L Tris-HCl (pH 7.5) was made fresh for each analysis. From the trypsin working solution, 20 μL were mixed with 80 μL of extracted sample in a 96-well microplate. The mixture was then serially diluted in 0.05 mol/L Tris HCl buffer (pH 7.5) in 1:1 steps and incubated at 24 °C for 60 min. A working solution of 0.01 mol/L $N_\alpha$-benzoyl-L-arginine 4-nitroanilide hydrochloride (L-BAPA) in Tris-HCl (pH 7.5) was made from a stock solution of 50 mg/mL L-BAPA in DMSO. From the freshly prepared L-BAPA working solution at room temperate, 180 μL were added to each well in the microplate and mixed with the diluted sample extracts. The microplate was then immediately transferred to a kinetic plate reader, set at 410 nm, using a run time of 10 min with10-second interval readings at 37 °C.

#### 2.3.7. Total phenolic content

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method based on the technique of Howard, Clark, and Brownmiller (2003), with some modifications.

Duplicate samples of fava bean flour (0.8 g) were mixed thoroughly with 5 mL of methanol extraction solution (1% trifluoracetic acid in MeOH:$H_2O$, 70:30) and then sonicated for 5 min (Branson Ultrasonics Corporation, Danbury, CT, USA). The mixture was vortexed and sonicated again for 5 min, incubated in a shaking water bath (60 °C, 100 rpm) for 30 min and then cooled on ice for 10 min. The extracts were

vortexed and centrifuged at 5000*g* for 5 min at 4 °C. The supernatant was collected and the pellet was re-dissolved in 5 mL of methanol extraction solution, sonicated as described above, and centrifuged (5000*g* for 5 min at 4 °C). The second supernatant was added to the previously collected supernatant and stored at −20 °C until analysis. Before use, the extracts were centrifuged at 5000*g* for 5 min.

For the TPC analysis, Folin-Ciocalteu reagent was added and the extracts were analyzed spectrophotometrically against a standard curve of gallic acid, measuring the absorbance at 765 nm using the Safire 2 plate reader (Tecan Group Ltd., Männedorf, Switzerland) with the Magellan software. The results for TPC are presented as gallic acid equivalent (GAE) per gram of dry weight (DW).

#### 2.3.8. Determination of oligosaccharides

Quantification of the oligosaccharides raffinose, verbascose and stachyose was performed by HPIC. For this, 100 mg of duplicate samples were mixed with 10 mL of Milli-Q water and set to shake for 1.5 h at ambient temperature using a laboratory shaker (Heidolph Reax 2; Heidolph Instruments GmbH, Schwabach, Germany). The solution was frozen, thawed and vortexed before transfer of 1 mL to an Eppendorf tube (Eppendorf GmbH, Hamburg, Germany), centrifuged at 12,000*g* for 2.5 min, diluted 10-fold and transferred to HPLC vials before injection. The chromatography setup consisted of an HPLC pump (L-6200A intelligent pump; Merck Hitachi, NJ, USA), a microsampler (CMA/200 autosampler), and a CarboPac PA-100 analytical column (Thermo Fisher Scientific, Waltham, MA, USA). The oligosaccharides were eluted with a gradient that consisted of A (Milli-Q $H_2O$), B (NaAc, 1 m/L) and C (NaOH, 1 m/L), and detected in an electrochemical detector (Dionex ED40; Thermo Fisher Scientific). Each sample had a run time of 40 min, the injection volume was 20 μL and the concentration was calculated using an external standard in the concentration range of 5–15 ppm. For the external standard, verbascose (Megazyme, Bray, Co. Wicklow, Ireland), stachyose tetrahydrate (MP Biomedicals, Irvine, CA, USA), and alfa-D-raffinose-pentahydrate (Thermo Fischer Scientific) were used. Data were collected and evaluated using the Borwin Chromatography Software (JMBS Developments, Le Fontanil, France).

#### 2.3.9. Statistical analysis

The results are presented as means and standard deviation, calculated using Microsoft Excel 2002. The contents of iron, phytate, protein and zinc were compared between cultivars using two tailed *t*-test. The cultivar Gloria was used as a reference as this cultivar is currently cultivated as a food crop. Contents were considered higher compared to the reference cultivar if $p < 0.05$. The total phenolic content was compared between colored and white flowered cultivars using a two tailed *t*-test, a difference was found significant if $p < 0.05$.

## 3. Results and discussion

### 3.1. Protein content

The protein contents of the analyzed fava bean cultivars varied from 26.2% in cultivar Alexia to 32.8% in the high-protein cultivar Gloria (Table 1). In a study of German fava bean cultivars (Makkar et al., 1997), the crude protein content was reported to be in the range of 25.7–30.4%. Cultivar Gloria has previously been reported to contain 30.0–33.7% protein (Jezierny, Mosenthin, Sauer, & Eklund, 2009; Makkar et al., 1997), which is comparable with the results of the present study. A protein content range of 22–38% for cultivars of fava bean was reported by Griffiths and Lawes (1978)). These findings illustrate the large variability that exists between fava bean cultivars.

### 3.2. Amino acid compositions

The amino acid compositions of the fifteen fava bean cultivars are presented in Table 2. In this study, the extraction method used for amino acids was acidic hydrolysis, which breaks down tryptophan, cysteine, methionine and tyrosine. However, proteins from grain legumes are considered to be low in the sulfur-containing amino acids (methionine and cysteine), as well as tryptophan, and generally contain high levels of leucine, lysine, aspartic acid, arginine and glutamic acid (Boye, Zare, & Pletch, 2010). In contrast, most cereal grains contain a low level of lysine but very high levels of sulfur-containing amino acids, which is the reason why these two food categories are considered as complementary in a plant-based diet. In the present study, the leucine content varied from 50.8 mg/g protein in cultivar Fanfare to 72.1 mg/g protein in cultivar Boxer. For lysine, the concentrations varied from 44.8 mg/g protein in Fanfare to 74.8 mg/g protein in cultivar Birgit.

**Table 1**
Protein, phytate, ash and mineral content of the 15 fava bean cultivars.

| Flower type | Cultivar | Protein | Phytate | Fe | Zn | Ash |
|---|---|---|---|---|---|---|
| Colored | Alexia | $22.7^{\pm 0.10}$ * | $1281^{\pm 15}$ * | $3.8^{\pm 0.02}$ * | $3.8^{\pm 0.06}$ * | 3.41 |
| Colored | Birgit | $24.0^{\pm 0.07}$ * | $773^{\pm 13}$ | $3.2^{\pm 0.07}$ * | $1.3^{\pm 0.05}$ | 3.23 |
| Colored | Boxer | $25.8^{\pm 0.03}$ * | $880^{\pm 18}$ | $5.5^{\pm 0.00}$ * | $1.6^{\pm 0.01}$ | 2.96 |
| Colored | Daisy | $24.8^{\pm 0.04}$ * | $720^{\pm 13}$ * | $4.7^{\pm 0.04}$ * | $1.2^{\pm 0.03}$ * | 2.83 |
| Colored | Emilia | $25.5^{\pm 0.04}$ * | $742^{\pm 5}$ * | $4.8^{\pm 0.04}$ * | $1.5^{\pm 0.05}$ | 2.72 |
| Colored | FanFare | $24.9^{\pm 0.06}$ * | $612^{\pm 10}$ * | $4.0^{\pm 0.13}$ | $1.22^{\pm 0.05}$ | 3.10 |
| Colored | Fuego | $24.5^{\pm 0.03}$ * | $724^{\pm 1.9}$ * | $4.3^{\pm 0.15}$ | $1.4^{\pm 0.01}$ | 3.17 |
| Colored | Lynx | $23.4^{\pm 0.01}$ * | $313^{\pm 6}$ * | $7.0^{\pm 0.18}$ * | $1.4^{\pm 0.02}$ | 3.15 |
| Colored | Stella | $22.8^{\pm 0.24}$ * | $735^{\pm 29}$ | $3.9^{\pm 0.05}$ * | $1.2^{\pm 0.01}$ * | 3.19 |
| Colored | Tiffany | $23.3^{\pm 0.24}$ * | $748^{\pm 18}$ | $3.9^{\pm 0.02}$ * | $0.9^{\pm 0.00}$ * | 3.19 |
| White | Banquise | $24.3^{\pm 0.04}$ * | $946^{\pm 1}$ * | $1.8^{\pm 0.02}$ * | $3.6^{\pm 0.10}$ * | 3.13 |
| White | Fernando | $27.1^{\pm 0.03}$ * | $810^{\pm 26}$ | $4.4^{\pm 0.27}$ | $1.3^{\pm 0.01}$ | 3.02 |
| White | Gloria | $28.3^{\pm 0.03}$ * | $820^{\pm 6}$ | $5.1^{\pm 0.02}$ | $1.5^{\pm 0.02}$ | 3.24 |
| White | Sunrise | $24.6^{\pm 0.03}$ * | $112^{\pm 4.2}$ * | $21.3^{\pm 0.29}$ * | $5.2^{\pm 0.06}$ * | 3.36 |
| White | Taifun | $25.0^{\pm 0.03}$ * | $823^{\pm 21}$ | $3.9^{\pm 0.22}$ | $1.3^{\pm 0.02}$ * | 3.27 |

Data presented as means of duplicates ± standard deviation.
Phytate and mineral content are presented as mg/100 g DW.
Ash and protein are presented as percentage on a DW basis.
*Indicate a significant difference compared to the reference cultivar Gloria.

### 3.3. Dietary fiber

The contents of dietary fiber in the fifteen cultivars of fava bean are presented in Table 3.

The endogenous enzymes of the human digestive tract are unable to metabolize dietary fiber, which instead serves as the main energy source for bacteria in the colon (Hamaker & Tuncil, 2014). Soluble dietary fiber reduces the postprandial blood sugar and insulin responses (Sierra et al., 2002) and may have beneficial effects in protecting against several forms of cancer, reducing blood pressure, and exerting an anti-inflammatory effect in the digestive tract (Chawla & Patil, 2010; Scheppach et al., 2004). Insoluble dietary fiber reduces the gastrointestinal transit time and increase the fecal bulk (Weickert et al., 2006). In this study, the soluble fraction of dietary fiber ranged from 0.55% of the total DW in cultivar Tiffany to 1.06% in cultivar Fanfare, and the insoluble fiber fraction ranged from 10.70% in cultivar Daisy to 15.96% in Stella. As expected, insoluble DF was the most abundant form, with glucomannan and uronic acids appearing as the largest fractions in all

**Table 2**
Amino acid compositions of the 15 cultivars of fava bean, expressed as mg of amino acid per g of protein. Calculations on daily requirements and recommendations are based on data from WHO/FAO/UNU (FAO/WHO/UNU, 2007).

| | Amino acid content (mg/g protein) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino acids | Alexia | Banquise | Birgit | Boxer | Daisy | Emilia | FanFare | Fernando | Fuego | Gloria | Lynx | Stella | Sunrise | Taifun | Tiffany | mg/kg BW per day[A] | mg/g protein[B] |
| **Essential** | | | | | | | | | | | | | | | | | |
| Histidine | $28.2^{\pm0.5}$ | $30.8^{\pm0.5}$ | $35.9^{\pm1.2}$ | $33.6^{\pm0.2}$ | $31.6^{\pm1.8}$ | $27.5^{\pm2.8}$ | $27.3^{\pm0.5}$ | $30.8^{\pm1.5}$ | $29.1^{\pm0.1}$ | $31.0^{\pm0.9}$ | $13.3^{\pm1.0}$ | $29.3^{\pm1.5}$ | $28.5^{\pm0.0}$ | $30.3^{\pm0.3}$ | $31.3^{\pm0.0}$ | 10 | 15 |
| Isoleucine | $23.4^{\pm1.0}$ | $25.9^{\pm0.6}$ | $20.7^{\pm0.4}$ | $33.1^{\pm2.3}$ | $26.7^{\pm1.8}$ | $24.4^{\pm3.5}$ | $20.9^{\pm0.7}$ | $24.6^{\pm1.1}$ | $21.4^{\pm0.0}$ | $25.8^{\pm0.3}$ | $23.7^{\pm0.5}$ | $27.1^{\pm1.5}$ | $22.3^{\pm0.7}$ | $24.0^{\pm0.9}$ | $23.9^{\pm1.0}$ | 20 | 30 |
| Leucine | $55.0^{\pm0.4}$ | $59.8^{\pm0.8}$ | $52.8^{\pm0.4}$ | $72.1^{\pm1.3}$ | $61.6^{\pm4.7}$ | $56.9^{\pm5.4}$ | $50.8^{\pm1.4}$ | $58.9^{\pm2.7}$ | $51.5^{\pm0.1}$ | $58.0^{\pm4.9}$ | $55.7^{\pm1.6}$ | $63.3^{\pm2.8}$ | $54.8^{\pm1.0}$ | $57.4^{\pm0.9}$ | $58.4^{\pm0.8}$ | 39 | 59 |
| Lysine | $49.7^{\pm0.4}$ | $51.6^{\pm0.3}$ | $74.8^{\pm0.3}$ | $65.4^{\pm5.2}$ | $51.4^{\pm3.3}$ | $47.2^{\pm4.7}$ | $44.8^{\pm0.7}$ | $48.9^{\pm2.5}$ | $46.3^{\pm0.1}$ | $52.4^{\pm1.1}$ | $48.9^{\pm1.3}$ | $54.4^{\pm3.0}$ | $47.7^{\pm0.7}$ | $51.7^{\pm0.9}$ | $51.6^{\pm0.7}$ | 30 | 45 |
| Phenylalanine | $30.5^{\pm0.9}$ | $31.3^{\pm0.8}$ | $32.1^{\pm0.4}$ | $36.6^{\pm1.2}$ | $32.4^{\pm2.7}$ | $29.9^{\pm3.3}$ | $27.4^{\pm0.4}$ | $31.1^{\pm1.6}$ | $27.5^{\pm0.0}$ | $33.3^{\pm0.2}$ | $23.0^{\pm0.1}$ | $33.6^{\pm1.6}$ | $29.0^{\pm0.5}$ | $30.6^{\pm0.1}$ | $31.7^{\pm0.4}$ | 25[C] | 38[C] |
| Threonine | $28.6^{\pm0.1}$ | $32.1^{\pm0.1}$ | $38.0^{\pm0.3}$ | $37.7^{\pm1.0}$ | $33.8^{\pm3.3}$ | $29.3^{\pm2.8}$ | $26.6^{\pm0.2}$ | $31.0^{\pm1.4}$ | $27.4^{\pm0.5}$ | $32.1^{\pm0.4}$ | $29.6^{\pm1.2}$ | $32.9^{\pm1.3}$ | $29.0^{\pm0.3}$ | $30.8^{\pm0.4}$ | $31.2^{\pm0.2}$ | 15 | 23 |
| Valine | $36.0^{\pm0.7}$ | $38.7^{\pm0.5}$ | $33.2^{\pm0.5}$ | $42.3^{\pm0.5}$ | $39.8^{\pm2.4}$ | $37.2^{\pm2.5}$ | $32.9^{\pm0.7}$ | $37.4^{\pm2.2}$ | $35.7^{\pm0.6}$ | $38.4^{\pm0.8}$ | $36.6^{\pm1.3}$ | $39.5^{\pm1.5}$ | $34.6^{\pm0.0}$ | $37.5^{\pm1.0}$ | $37.2^{\pm0.7}$ | 26 | 10 |
| **Non essential** | | | | | | | | | | | | | | | | | |
| Alanine | $49.1^{\pm0.1}$ | $57.9^{\pm0.1}$ | $52.8^{\pm0.1}$ | $53.8^{\pm1.2}$ | $57.3^{\pm3.6}$ | $45.6^{\pm3.1}$ | $48.4^{\pm0.3}$ | $54.4^{\pm4.5}$ | $47.6^{\pm0.2}$ | $55.7^{\pm0.2}$ | $53.7^{\pm1.9}$ | $48.9^{\pm2.6}$ | $52.4^{\pm0.4}$ | $54.9^{\pm0.6}$ | $56.5^{\pm1.5}$ | – | – |
| Arginine | $75.1^{\pm0.6}$ | $98.7^{\pm0.8}$ | $121.0^{\pm2.5}$ | $107.3^{\pm2.4}$ | $94.4^{\pm6.1}$ | $85.1^{\pm6.0}$ | $85.3^{\pm0.2}$ | $99.5^{\pm5.9}$ | $88.5^{\pm0.3}$ | $114.7^{\pm1.2}$ | $52.1^{\pm2.4}$ | $86.4^{\pm6.0}$ | $90.0^{\pm1.7}$ | $95.6^{\pm1.9}$ | $103.9^{\pm1.7}$ | – | – |
| Asparginine | $89.3^{\pm0.9}$ | $101.7^{\pm0.6}$ | $117.8^{\pm2.1}$ | $111.4^{\pm4.6}$ | $99.4^{\pm9.0}$ | $111.5^{\pm6.2}$ | $84.1^{\pm3.1}$ | $99.5^{\pm7.7}$ | $86.5^{\pm0.9}$ | $120.5^{\pm6.8}$ | $91.5^{\pm0.2}$ | $97.0^{\pm15.5}$ | $94.5^{\pm2.6}$ | $100.8^{\pm12.5}$ | $104.9^{\pm4.9}$ | – | – |
| Aspartic acid | $95.0^{\pm0.0}$ | $107.1^{\pm1.1}$ | $106.7^{\pm0.2}$ | $107.1^{\pm0.1}$ | $102.5^{\pm7.1}$ | $89.1^{\pm7.1}$ | $88.0^{\pm0.4}$ | $101.0^{\pm7.1}$ | $86.4^{\pm1.0}$ | $109.5^{\pm0.5}$ | $96.8^{\pm1.3}$ | $96.7^{\pm6.1}$ | $94.8^{\pm0.8}$ | $101.4^{\pm0.3}$ | $102.0^{\pm1.3}$ | – | – |
| Cysteine | $10.0^{\pm0.0}$ | $10.2^{\pm0.4}$ | $11.3^{\pm0.1}$ | $9.9^{\pm0.6}$ | $10.7^{\pm0.6}$ | $8.5^{\pm1.0}$ | $9.4^{\pm0.4}$ | $10.2^{\pm0.4}$ | $10.3^{\pm0.0}$ | $10.0^{\pm0.4}$ | $9.9^{\pm0.5}$ | $10.0^{\pm0.4}$ | $9.8^{\pm0.3}$ | $10.5^{\pm0.2}$ | $10.4^{\pm0.1}$ | – | – |
| Glutamic acid | $133.2^{\pm2.8}$ | $151.5^{\pm2.6}$ | $187.9^{\pm0.1}$ | $154.5^{\pm0.7}$ | $150.2^{\pm8.7}$ | $130.1^{\pm9.4}$ | $125.6^{\pm0.9}$ | $148.1^{\pm8.7}$ | $125.0^{\pm1.6}$ | $154.2^{\pm2.4}$ | $136.6^{\pm1.3}$ | $141.0^{\pm8.2}$ | $137.2^{\pm2.4}$ | $145.3^{\pm0.6}$ | $149.8^{\pm0.2}$ | – | – |
| Glycine | $32.7^{\pm0.2}$ | $37.8^{\pm0.6}$ | $39.5^{\pm0.8}$ | $30.2^{\pm1.9}$ | $36.0^{\pm2.0}$ | $27.2^{\pm1.5}$ | $31.9^{\pm0.0}$ | $34.6^{\pm2.4}$ | $32.1^{\pm0.0}$ | $35.8^{\pm0.2}$ | $35.0^{\pm1.1}$ | $28.8^{\pm2.2}$ | $35.8^{\pm1.2}$ | $35.8^{\pm1.0}$ | $37.5^{\pm1.0}$ | – | – |
| Proline | $32.9^{\pm0.2}$ | $37.8^{\pm0.1}$ | $45.6^{\pm0.2}$ | $48.2^{\pm2.3}$ | $38.1^{\pm2.1}$ | $35.0^{\pm3.4}$ | $32.9^{\pm0.2}$ | $36.9^{\pm2.7}$ | $33.5^{\pm0.3}$ | $37.8^{\pm0.1}$ | $36.0^{\pm1.2}$ | $41.1^{\pm2.9}$ | $35.7^{\pm0.1}$ | $36.8^{\pm0.4}$ | $37.6^{\pm0.1}$ | – | – |
| Serine | $41.3^{\pm1.1}$ | $45.6^{\pm0.2}$ | $52.1^{\pm0.0}$ | $48.9^{\pm3.0}$ | $46.0^{\pm2.4}$ | $40.2^{\pm3.5}$ | $39.5^{\pm0.3}$ | $44.7^{\pm2.6}$ | $40.7^{\pm0.3}$ | $45.5^{\pm1.4}$ | $43.1^{\pm1.2}$ | $44.2^{\pm2.9}$ | $42.6^{\pm0.5}$ | $44.4^{\pm1.1}$ | $46.1^{\pm0.4}$ | – | – |
| Taurine | $30.3^{\pm0.7}$ | $32.0^{\pm2.1}$ | $41.5^{\pm0.6}$ | $34.1^{\pm1.2}$ | $30.1^{\pm2.1}$ | $18.6^{\pm0.2}$ | $26.4^{\pm0.7}$ | $28.5^{\pm2.7}$ | $24.9^{\pm1.0}$ | $33.8^{\pm1.9}$ | $30.6^{\pm2.8}$ | $22.5^{\pm0.2}$ | $30.8^{\pm2.2}$ | $26.8^{\pm0.6}$ | $32.2^{\pm0.9}$ | – | – |

Results are expressed on a dry weight basis. Values are means (n = 2) ± standard deviation.
A Amino acid recommendations calculated as mg/kg body weight per day for adults, based on a total protein requirement of 0.66 g/kg BW per day according to WHO/FAO/UNU 2007[34].
B Amino acid recommentations expressed as mg/g protein consumed for adults according to WHO/FAO/UNU 2007[34].
C Phenylalanine and Tyrosine combined.

**Table 3**
Dietary fiber contents of the different fava bean cultivars, presented as percentages on a dry-weight (DW) basis.

| | Soluble | Insoluble | Total |
|---|---|---|---|
| Alexia | 0.60 | 15.29 | 15.89 |
| Birgit | 0.56 | 13.62 | 14.19 |
| Boxer | 0.66 | 12.13 | 12.79 |
| Daisy | 0.67 | 10.70 | 11.37 |
| Emilia | 0.69 | 13.71 | 14.40 |
| FanFare | 1.06 | 13.05 | 14.11 |
| Fuego | 0.79 | 14.84 | 15.64 |
| Lynx | 0.66 | 14.84 | 15.50 |
| Stella | 0.62 | 15.96 | 16.59 |
| Tiffany | 0.55 | 13.23 | 13.78 |
| Banquise | 0.55 | 11.64 | 12.19 |
| Fernando | 0.80 | 14.26 | 15.06 |
| Gloria | 0.71 | 12.95 | 13.66 |
| Sunrise | 0.76 | 14.29 | 15.05 |
| Taifun | 0.65 | 12.18 | 12.82 |

the cultivars (contents in the ranges of 4.93–8.45% and 1.82–2.94%, respectively). In the soluble fraction, arabinose, rhamnose and soluble uronic acids showed the highest contents (ranges of 0.06–0.14%, 0.15–0.22% and 0.24–0.41% respectively). In Singh, Bhardwaj, and Singh (2014) a total dietary fiber content of fava bean was reported in the range of 12.25–13.49%, which is in line the range found in the present study.

A detailed description of the dietary fiber contents of the analyzed fava beans can be found in Supplementary Table 1.

### 3.4. Mineral content

The zinc and iron contents of the analyzed fava bean cultivars are presented in Table 1. The iron content ranged from 1.8 mg/100 g DW in cultivar Banquise to 21.3 mg/100 g DW in cultivar Sunrise. The zinc content ranged from 0.9 mg/100 g DW in cultivar Tiffany to 5.2 mg/100 g in cultivar Sunrise. This indicates a variation of almost 12-fold for the iron content and almost 6-fold for the zinc content among the analyzed fava bean cultivars. The results in this study are comparable to previously published data on zinc and iron concentrations in fava beans, with the exception of the iron content of cultivar Sunrise in the present study, which can be considered higher than previously described. In a study of forty different fava bean cultivars grown in Saudi Arabia, the iron content was in the range of 14.6–15.8 mg/100 g and the zinc content was in the range of 5.8–7.2 mg/100 g (Khan et al., 2015). These results are comparable with the values deduced in the present study. In Cabrera, Lloris, Giménez, Olalla, and López (2003) lower ranges of iron and zinc contents for fava beans were reported (7.8–8.2 mg/100 g and 4.3–5.0 mg/100 g, respectively).

### 3.5. Phytate content and estimated relative bioavailabilities of zinc and iron

The phytate contents of the analyzed fava bean cultivars are presented in Table 1. The levels ranged from 112 mg/100 g in cultivar Sunrise to 1281 mg/100 g in cultivar Alexia, indicating a 10-fold variation among the analyzed cultivars. Of the fifteen cultivars studied, two had a phytate content $>$ 400 mg/100 g, which is considerably lower than the previously reported levels of phytate in fava beans that were analyzed using comparable methods. For Chinese fava bean, (Luo, Xie, & Luo, 2012) reported a phytate content of 823 mg/100 g. A similar phytate content, 746 mg/100 g, was reported by Honke, Sandberg, and Kozłowska (1999) for Polish fava beans. A content similar to those of the cultivars with the highest phytate content analyzed in this study was reported for Bolivian fava beans (1170 mg/100 g) by Lazarte, Carlsson, Almgren, Sandberg, and Granfeldt (2015). It has previously been suggested that apart from the cultivar, also climatic conditions, location, irrigation conditions, soil factors and crop year contribute to the variability in phytate contents (Kumar et al., 2005; Oomah et al., 2011; Urbano et al., 2000).

A common indicator of the bioavailabilities of zinc and iron in a food product is the molar ratio of phytates to these minerals. The inhibition of zinc absorption by phytate is dose-dependent (Fredlund, Isaksson, Rossander-Hulthén, Almgren, & Sandberg, 2006). The Phy:Fe molar ratios of the analyzed fava bean cultivars are presented in Fig. 1, the Phy:Zn molar ratios in Fig. 2. One of the analyzed cultivars (Sunrise) had a Phy:Zn molar ratio within the range of what is considered by the European Food Safety Authority (EFSA) to represent high bioavailability ($\leq$5) (EFSA, 2017). The other fourteen cultivars analyzed had molar ratios of Phy:Zn $>$ 15, which is considered to reflect low bioavailability (EFSA, 2017).

Similar to the Zn absorption, the inhibition of iron absorption by phytates is dose-dependent, with a noticeable inhibitory effect even at low levels (Brune, Rossander-Hultén, Hallberg, Gleerup, & Sandberg, 1992; Hallberg, Brune, & Rossander, 1989; Hurrell et al., 1992). Cultivars Sunrise and Lynx showed the lowest molar ratios of Phy:Fe (0.4 and 3.8, respectively). Only cultivar Sunrise had a Phy:Fe molar ratio that corresponds to a high bioavailability of iron and zinc. Each of the other cultivars were above the threshold that estimates a good availability. Therefore, the fava bean cultivars estimated in this paper except for cultivar Sunrise has a low estimated bioavailability of iron and zinc.

### 3.6. Vicine and convicine concentrations

The vicine and convicine contents of the fava bean cultivars are presented in Table 4. Cultivar Emilia showed the lowest contents of vicine and convicine (403.2 μg/g and 35.5 μg/g, respectively). The convicine content of cultivar Emilia was below the limit of detection (100 μg/g). In the other fourteen cultivars of fava bean, the vicine contents ranged from 1,185 μg/g in cultivar Tiffany to 7,014 μg/g in cultivar Gloria and the convicine contents ranged from 378 μg/g in cultivar Tiffany to 3121 μg/g in cultivar Banquise. Khazaei et al. (2019) reported that the low-vicine cultivar Mélodie contained 290 μg/g vicine and 14 μg/g convicine. Other cultivars have been reported to contain 4640–5640 μg/g convicine and 940–3,090 μg/g vicine, demonstrating substantial variability among the fava bean cultivars.

### 3.7. Lectin quantification

In the analyzed fava bean cultivars, the contents of lectin ranged from 0.8 HU/mg in cultivar Daisy to 3.2 HU/mg in cultivar Taifun. Shi, Arntfield, and Nickerson (2018) reported that Canadian fava beans and Spanish fava beans contained lectin at 5.52 HU/mg and Alonso, Aguirre, and Marzo (2000) reported a lectin content of 49.3 HU/mg, with both studies measuring the hemagglutination of rabbit erythrocytes. The hemagglutinin assay that is commonly used to estimate the lectin content of fava beans is a semi-quantitative method based on the ability of phytohemagglutinin (PHA) to agglutinate erythrocytes (Etzler, 1985). The hemagglutinin assay does not have high precision, as it is affected by the properties of the erythrocyte surface, blood type, species, metabolic state of the cells, and conditions of the assay, as well as by variable molecular properties of the lectins (Lis & Sharon, 1986). Due to the nature of the hemagglutinin assay, significant variations of the contents of lectin in fava beans have been reported. Furthermore, the lectin contents of plants are influenced by environmental factors and growth conditions (Jiang, Ma, & Ramachandran, 2010).

### 3.8. Saponin content

The saponin contents of the analyzed fava bean cultivars ranged from 18.3 μg/g for cultivar Banquise to 109 μg/g DW for cultivar Sunrise (Table 4). This indicates substantial variability among the cultivars. It is difficult to compare the levels of saponin between studies reported in the

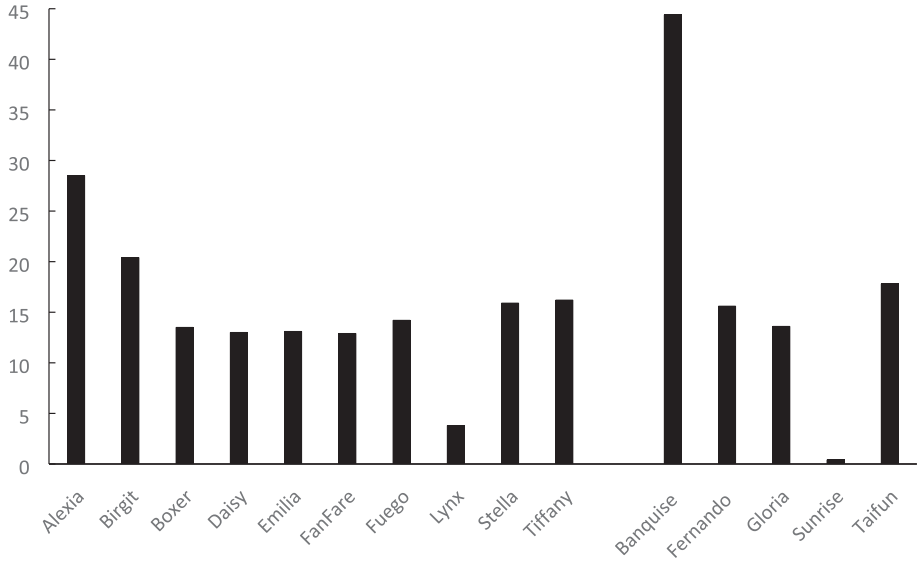


**Fig. 1.** The molar ratio phytate to iron in fifteen fava bean cultivars. A molar ratio less than 0.4 indicates a high iron bioavailability (Hurrell & Egli, 2010).

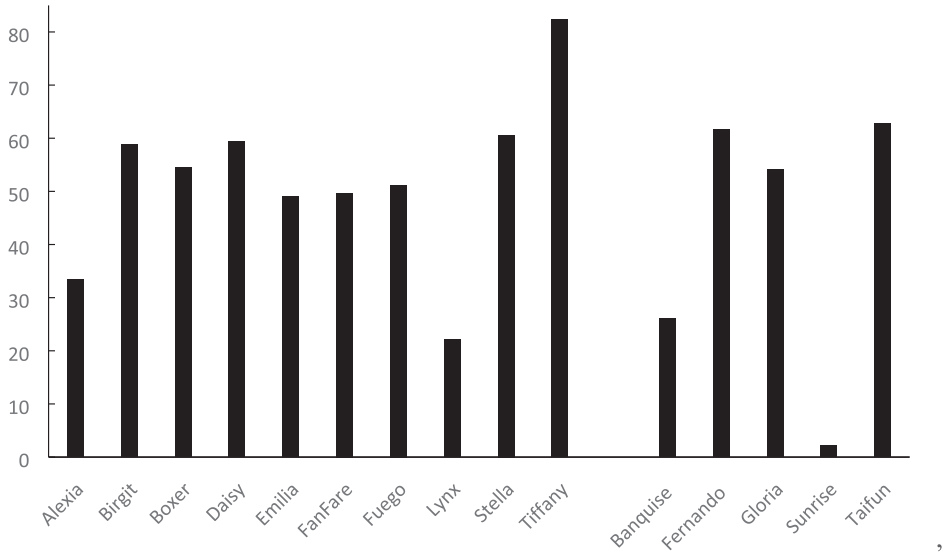


**Fig. 2.** The molar ratio phytate to zinc in fifteen fava bean cultivars. A molar ratio less than 5 indicates a high Zn bioavailability (EFSA, 2019).

literature owing to the large discrepancies in the data, which are attributed to the various analytic methods used, including different extraction procedures and reference compounds.

Saponins have been suggested to have plasma cholesterol-lowering effects in humans (Singh, Singh, Singh, & Kaur, 2017), and epidemiologic studies have suggested that saponins play a role in protection from cancer, although given their bitter taste some saponins might not be desirable as components food products (Shi et al., 2004).

### 3.9. Trypsin inhibitors

Trypsin inhibitors in the diet inhibit the activities of the pancreatic enzymes trypsin and chymotrypsin, thereby reducing the levels of digestion and absorption of dietary proteins, as well as causing pancreatic hyperplasia. Thus, it is necessary to process crops that contain trypsin inhibitors, such as grain legumes (Savelkoul, Van Der Poel, & Tamminga, 1992). The trypsin inhibition activities, expressed in trypsin inhibition units (TIU) per mg of DW, of the tested fava bean cultivars ranged from 1.2 TIU/mg DW for cultivar Lynx to 23.1 TIU/mg DW for cultivar Banquise (Table 4). Of the fifteen cultivars analyzed, six had trypsin activities $>$ 10 TIU/mg. These six cultivars showed higher levels of activity than the values previously reported for fava beans by different groups, i.e., 5.45 TIU/mg DW by Millar, Gallagher, Burke, McCarthy, and Barry-Ryan (2019), 5.96 TIU/mg DW by Shi, Mu, Arntfield, and Nickerson (2017), and 7.2 TIU/mg DW by Hernández-Infante, Sousa, Montalvo, and Tena (1998). However, the trypsin inhibition activity seen in the present study is lower than that of soya bean, which has been reported to have an activity of 45.89 TIU/mg DW (Shi et al., 2017).

### 3.10. Total phenolics content

The TPC values for the fifteen different cultivars of fava bean ranged from 1.4 mg GAE/g in cultivar Banquise to 5.0 mg GAE/g in cultivar Fuego (Table 4), indicating an approximately four-fold variation in TPC

**Table 4**
Antinutrient composition of the 15 fava bean cultivars.

| Cultivar | Vicine | Convicine | Lectin | Saponins | TPC | TIU |
|---|---|---|---|---|---|---|
| Alexia | $5647^{\pm 208}$ | $2032^{\pm 56}$ | n/a | $87.1^{\pm 0.7}$ | $2.8^{\pm 0.06}$ | $3.6^{\pm 0.10}$ |
| Birgit | $5859^{\pm 102}$ | $2109^{\pm 52}$ | 1.6 | $32.1^{\pm 0.7}$ | $3.4^{\pm 0.07}$ | $11.6^{\pm 0.28}$ |
| Boxer | $6818^{\pm 111}$ | $2707^{\pm 37}$ | n/a | $24.5^{\pm 1.3}$ | $3.7^{\pm 0.27}$ | $5.5^{\pm 0.22}$ |
| Daisy | $4285^{\pm 79}$ | $1784^{\pm 34}$ | 0.8 | $32.3^{\pm 2.8}$ | $3.6^{\pm 0.08}$ | $6.1^{\pm 0.07}$ |
| Emilia | $403^{\pm 5.9}$ | $35.8^{\pm 10}$ | 0.8 | $24.9^{\pm 0.4}$ | $3.3^{\pm 0.06}$ | $3.9^{\pm 0.9}$ |
| FanFare | $6185^{\pm 106}$ | $2637^{\pm 46}$ | 0.8 | $59.7^{\pm 1.0}$ | $4.7^{\pm 0.03}$ | $14.3^{\pm 0.06}$ |
| Fuego | $6102^{\pm 72}$ | $2366^{\pm 35}$ | 1.6 | $45.5^{\pm 1.6}$ | $5.0^{\pm 0.06}$ | $13.7^{\pm 0.06}$ |
| Lynx | $5251^{\pm 10}$ | $2319^{\pm 5}$ | 3.2 | $42.3^{\pm 0.6}$ | $4.7^{\pm 0.02}$ | $1.2^{\pm 0.06}$ |
| Stella | $3848^{\pm 263}$ | $1360^{\pm 6}$ | 0.8 | $37.4^{\pm 2.4}$ | $4.0^{\pm 0.26}$ | $3.8^{\pm 0.45}$ |
| Tiffany | $1185^{\pm 48}$ | $378^{\pm 6}$ | 0.8 | $30.5^{\pm 9.5}$ | $4.0^{\pm 0.03}$ | $10.8^{\pm 0.85}$ |
| Banquise | $4995^{\pm 114}$ | $3121^{\pm 47}$ | 3.2 | $18.3^{\pm 1.5}$ | $1.4^{\pm 0.01}$ | $23.1^{\pm 1.11}$ |
| Fernando | $6415^{\pm 96}$ | $2188^{\pm 24}$ | 0.8 | $86.8^{\pm 4.3}$ | $2.3^{\pm 0.04}$ | $4.5^{\pm 0.85}$ |
| Gloria | $7014^{\pm 67}$ | $1906^{\pm 16}$ | 0.8 | $59.5^{\pm 3.5}$ | $1.8^{\pm 0.02}$ | $12.0^{\pm 0.60}$ |
| Sunrise | $5499^{\pm 92}$ | $2039^{\pm 12}$ | 0.8 | $109.0^{\pm 1.9}$ | $1.7^{\pm 0.01}$ | $1.9^{\pm 0.08}$ |
| Taifun | $6389^{\pm 110}$ | $2766^{\pm 37}$ | 3.2 | $71.0^{\pm 1.6}$ | $1.6^{\pm 0.03}$ | $4.2^{\pm 0.28}$ |

Average of triplicates ± SD. Vicine, convicine and saponins are presented as ug/g, lectin as hemagglutinin units (HU)/mg, total phenolic content (TPC) as mg gaelic acid equivalents (GAE)/g and trypsin inhibitor as trypsin inhibiting units (TIU)/mg. Data is presented on a dry weight basis.

content among the studied genotypes. As expected, the TPC was significantly higher in the color-flowered cultivars (range, 2.8–5.0 mg GAE/g) than in the white-flowered cultivars (range, 1.4–3.2 mg GAE/g). The results obtained in this study are within the range of TPC values previously reported for fava beans by Saleh, Hassan, Mansour, Fahmy, and El-Bedawey (2019), but lower than the TPC values reported by Chaieb, González, López-Mesas, Bouslama, and Valiente (2011). Iron-binding polyphenols are known inhibitors of iron absorption (Brune, Hallberg, & Skånberg, 1991; Brune, Rossander, & Hallberg, 1989). However, these compounds can have positive health effects, as polyphenols have antioxidant properties through which they counteract the initiation and propagation of oxidative processes (Amarowicz & Pegg, 2008).

### 3.11. Oligosaccharide analysis

The fava bean contents of raffinose, stachyose and verbascose, belonging to the raffinose family of oligosaccharides (RFOs), are presented in Table 5. The concentration ranges were 1.1–3.9 g/kg DW for raffinose, 4.4–13.7 for stachyose, and 8–15 g/kg DW for verbascose. Landry, Fuchs, and Hu (2016) reported a positive relationship between seed size in different fava bean cultivars and RFO content in 40 different fava beans, with a raffinose content in the range of 2.7–13.0 g/kg, a stachyose content of 9.0–25.0 g/kg DW, and verbascose content of 6.7–50.3 g/kg DW. The variability in concentration of RFOs is considerably high among fava bean cultivars. The contents of these non-digestible oligosaccharides may be of concern for the human diet, since RFOs are known as compounds that can cause various degrees of flatulence and discomfort in healthy individuals, and can trigger symptoms of inflammatory bowel syndrome (IBS) as a result of their fermentation by anaerobic microorganisms in the digestive tract (Shepherd, Parker, Muir, & Gibson, 2008). On the other hand, RFOs are prebiotics that can provide positive health effects for the consumer, such as increasing the bifidobacterial population in the gut, promoting mineral absorption, improving the immune system response, and decreasing risk factors associated with obesity, metabolic syndrome and colon cancer (Johnson, Thavarajah, Combs, & Thavarajah, 2013; Mussatto & Mancilha, 2007; Rastall, 2013). Even a low level of RFOs can help to improve the acceptability of grain legumes as food products.

**Table 5**
The levels of oligosaccharides raffinose, stachyose and verbascose in the 15 fava bean cultivars, presented as g/kg on a dry-weight (DW) basis.

| Oligosaccharide content (g/kg DW) | | | | | | |
|---|---|---|---|---|---|---|
| Cultivar | Raffinose | | Stachyose | | Verbascose | |
| Alexia | 1.4 | ±0.12 | 6.7 | ±0.00 | 8.0 | ±0.02 |
| Birgit | 2.0 | ±0.03 | 4.4 | ±0.18 | 11.3 | ±0.36 |
| Boxer | 1.7 | ±0.37 | 7.0 | ±0.41 | 12.5 | ±0.20 |
| Daisy | 2.3 | ±0.13 | 4.8 | ±0.49 | 13.5 | ±0.18 |
| Emilia | 1.3 | ±0.04 | 6.1 | ±0.07 | 11.2 | ±0.12 |
| FanFare | 1.1 | ±0.04 | 5.6 | ±0.09 | 12.6 | ±0.17 |
| Fuego | 3.9 | ±1.69 | 8.4 | ±1.54 | 11.3 | ±0.07 |
| Lynx | 2.1 | ±0.00 | 7.3 | ±0.59 | 14.9 | ±0.20 |
| Stella | 1.3 | ±0.10 | 8.9 | ±0.06 | 10.7 | ±0.53 |
| Tiffany | 1.2 | ±0.11 | 7.4 | ±0.26 | 10.7 | ±0.52 |
| Banquise | 1.5 | ±0.08 | 11.0 | ±0.09 | 11.1 | ±0.16 |
| Fernando | 1.5 | ±0.08 | 9.3 | ±0.05 | 15.0 | ±0.14 |
| Gloria | 2.7 | ±0.06 | 9.7 | ±1.79 | 11.3 | ±0.16 |
| Sunrise | 2.4 | ±0.06 | 7.1 | ±0.20 | 13.6 | ±0.72 |
| Taifun | 2.8 | ±0.11 | 13.7 | ±0.30 | 14.6 | ±0.47 |

Data presented as means of duplicates ± standard deviation.

### 3.12. Selection of fava bean varieties

The results presented in this study demonstrate the substantial compositional variations that can exist within a single plant species, with both nutritional and antinutritional compounds showing a broad spectrum of contents. Large variations in nutritional components between cultivars also reveal the difficulties associated with choosing the appropriate crop for a specific geographic area or for specific soil properties. Also, weather conditions can affect the nutritional quality of a crop. For example, during the growing season of the fava beans for this study, it was unusually dry and warm which might have affected the level of several compounds. Rather than simply considering protein yields, from the nutritional viewpoint, it is better to adopt a broader perspective that includes both nutritional and antinutritional factors. Identifying the most important factors depends on the end-product and whether one is considering a whole bean or a highly processed end-product, such as extruded protein, where the levels of several antinutritional factors and nutrients are affected. In the cases of vicine and convicine, which exert severe hemotoxic effects on individuals with favism, the importance of in-depth knowledge of the nutritional and food technologies within the food industry is exemplified. The prevalence of favism varies with geographic area, ranging from 16% in tropical Africa to 1% in Europe (WHO, 1989). Thus, depending on the consumer, it will be more or less important to limit the levels of these compounds through the use of appropriate cultivars and post-harvest processing.

In the context of a dietary protein shift from resource-demanding animal products to a diet that is higher in plant-based products, consuming a variety of plant proteins, including grain legumes that have a high content of lysine, is essential to ensure adequate overall amino acid intake (Mariotti, 2017). Another aspect of protein quality is digestibility, which is negatively affected by the antinutrients present in

the food matrix. Therefore, plant protein is generally considered to have lower digestibility, the extent of which is affected by the processing method and, as demonstrated in this study, the cultivar used, due to the large differences in antinutritional components between varieties. Other factors that influence protein quality are specific demands related to the individual consuming the protein, such as energy balance, physiologic status, health status, and age (Millward, Layman, Tomé, & Schaafsma, 2008).

Grain legumes are often highlighted as a replacement for meat products. In this case, the content of iron and zinc and their relative bioavailabilities are factors that should play significant roles, given that legumes are important sources of iron and zinc in a plant-based diet. Since vegetarians have a higher risk of developing low iron stores, as compared to non-vegetarians (Pawlak, Berger, & Hines, 2018), partly due to the lower bioavailability of non-heme iron caused by antinutritional components (phytate, polyphenols), iron should receive greater attention when it comes to sustainable dietary patterns. Cultivars with a high Phy:Fe molar ratio can be expected to contribute only marginally to the absorbed iron. With this in mind, cultivars such as Sunrise that show low Phy:Fe and Phy:Zn molar ratios are most suitable for human consumption. However, the end application and agricultural aspects need to be taken into account when selecting the plant variety.

## 4. Conclusions

This study provides an analysis of the nutritional and antinutritional composition of fifteen different varieties of fava bean cultivated in the same region and during the same growth season. The results indicate substantial differences between cultivars in relation to their contents of nutrients and antinutrients, leading to variations in bioavailability of nutrients such as iron and zinc between the cultivars. The nutritional gain from consuming fava beans is affected by the cultivar chosen as the food source. Of the analyzed cultivars, only one (Sunrise) showed a high bioavailability of iron and zinc. This is a major dietary concern when facing a large shift in dietary preferences into a more plant based food pattern, especially for groups at risk of developing iron and zinc deficiency. However, the Sunrise cultivar did not have the highest protein content, illustrating the need to look beyond protein when considering a dietary shift. From a large-scale societal perspective, it is important to make well-informed decisions based on nutritional knowledge when contemplating a dietary shift, in order to avoid potential harms, such as an increased prevalence of iron deficiency. These decisions include choosing the right cultivar, as well as using processing methods aimed at lowering the level of phytate.

Author's contributions

Inger-Cecilia M. Labba (ICML) and Ann-Sofie Sandberg (ASS) planned and conceived the study. ICML performed the chemical analyses and wrote the first draft of the manuscript. Hanne Frøkiær and ASS were responsible for the methodology. ASS had the final responsibility for the submission of the manuscript. All authors contributed to the interpretation of the results and provided critical feedback, from draft to final version of the article.

## 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 material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.110038.

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