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Yellowness of Selected Legume Flours as Function of Nutritional Composition, Mineral Content and Total Free Phenolic Content

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ABSTRACT

Legume flours from sachu inchi (*Plukenetia volubilis*), pea (*Pisum sativum*), faba bean (*Vicia faba*), chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*) were analysed in a comprehensive study to identify potential sources for the development of yellow colour, which could limit their use in fortified food. The nutritional quality, macroelements and microelements, total free phenolics and fatty acid profile were compared to identify major sources of yellowness. The understanding of the yellowness of legume flours is of particular relevance for the preparation of white creamy products. Faba bean and chickpea had the highest protein content (72.0–73.1 g 100 g⁻¹). The highest fat content was analysed for sachu inchi (11.3 g 100 g⁻¹), and the highest dietary fibre content was analysed in chickpea (12.9 g 100 g⁻¹). Fe content varied from 0.1 (pea) to 5.8 mg 100 g⁻¹ (lentil). Total soluble phenolics content ranged from 82 (chickpea) to 413 mg 100 g⁻¹ (pea). The yellowness index (D1925) ranged from 22.4 to 52.2 and could be correlated in a simplified approach to the content in soluble total phenolics as gallic acid equivalents and the Fe content, which indicates that colour development of legume flours results from a combination of two main factors.

1 | Introduction

Legumes are nutritionally valuable dicotyledonous seeds of the *Fabaceae* family (Cheng et al. 2019; Shavanov 2021). These indispensable oleaginous plants grow in pods and have played an important role as staple foods in most cultures and civilisations (Affrifah et al. 2023; Ambika et al. 2023). Legumes are relatively rich in proteins (20%–45%) with essential amino acids, carbohydrates (60%), dietary fibres (5%–37%), minerals, vitamins and bioactive compounds (Bolarinwa et al. 2019; Kaale et al. 2023). The presence of bioactive compounds with antioxidant properties contributes to the prevention of cancer and cardiovascular

diseases (Singh et al. 2017). Major legumes can be divided into the following subclasses such as nonoilseed legumes (e.g., pulses) and oilseed legumes (e.g., peanuts, soybeans and fresh peas/beans). The term 'pulses' refers to crops that are harvested exclusively as dry grain, which distinguishes them from other vegetable crops that are harvested green (Didinger and Thompson 2021). The classification of major legumes is shown in Figure 1.

Legume consumption has received particular attention in the prevention of some diseases because of their low glycaemic index and slowly digestible starch content, which prevent a

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MAJOR LEGUME CLASSIFICATION

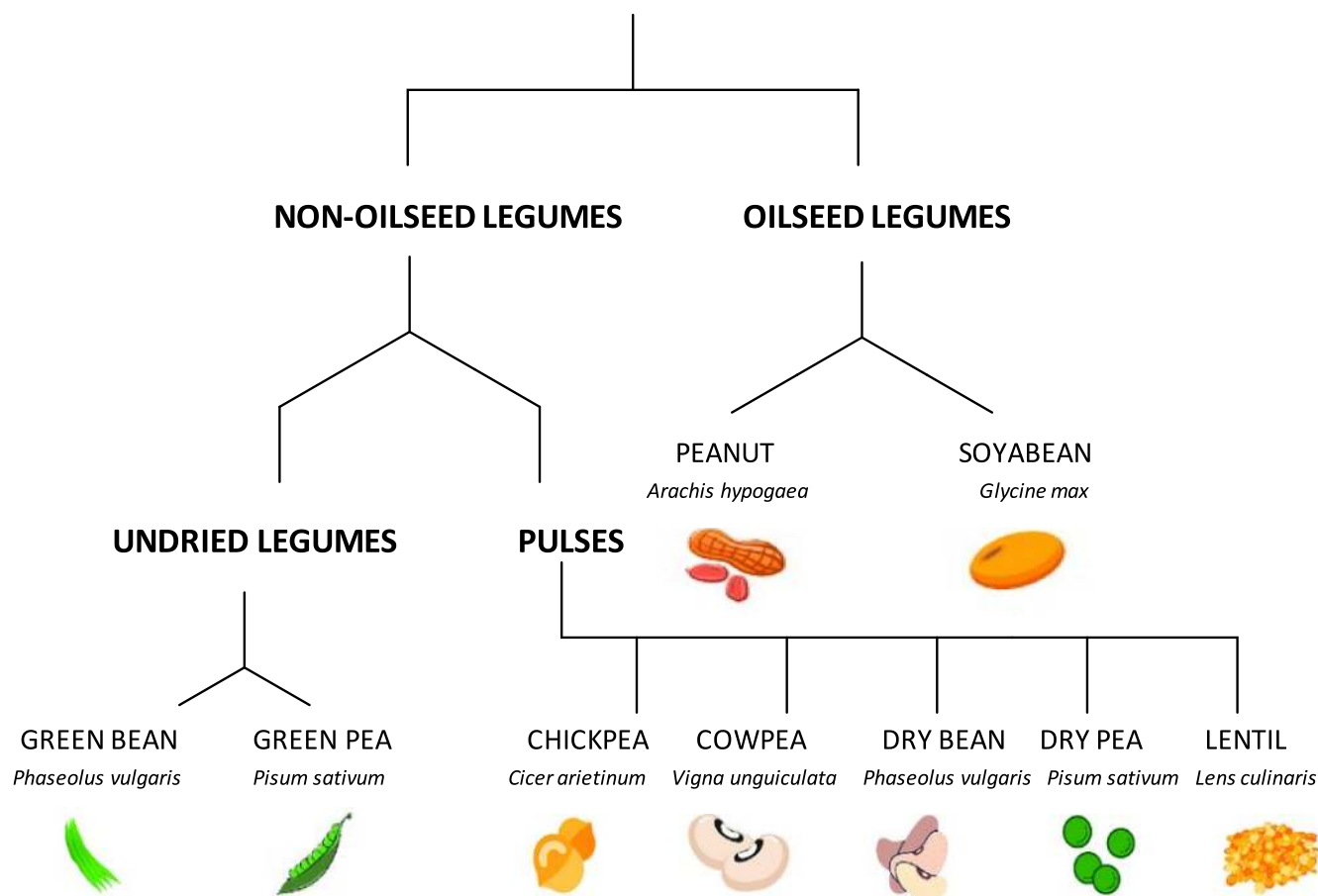


FIGURE 1 | Schematic visualisation of leguminous classification. Reproduced in modified form under CC BY 4.0 license from Diding and Thompson (2021).

sharp rise in blood sugar and promote weight loss (Becerra-Tomás et al. 2018), whereas flavonoids, phenolic acids and tannins have been associated with gastrointestinal functions, antioxidant activity, anti-inflammatory and anticarcinogenic properties (García-Lafuente et al. 2014). Plant-based foods are a growing trend among consumers, which increased the popularity of these flours (Oduro-Yeboah et al. 2023). Legume flours often are perceived as similar products; however, considerable differences in their nutritional composition exist, which depend on the raw materials used and the production process (Summo et al. 2019; Skylas et al. 2023). The high protein content of legume flours makes them a valuable source for fortifying food (Walter et al. 2022). Proximate composition and antinutritional factors have been reported for many legume flours, for example, pea, chickpea and beans (Gultekin Subasi et al. 2024; Millar et al. 2019; Summo et al. 2019). Also, mineral content and trace elements have been analysed in legume flours, for example, for lentil, beans and chickpea with regard to bioavailability as a function of processing (Ramírez-Ojeda et al. 2018), or the content in unwanted heavy metals nickel (Ni), chromium (Cr), lead (Pb) and cadmium (Cd) (Pastor et al. 2024).

Legume flours have high nutritional value; however, their use can be limited by their natural colour. A yellowish tone in legume flours can be attributed to several factors:

- Presence of coloured flavonoid colorants: A high concentration of flavonoids will lead to the development of a more intense yellowish colour. Relevant representatives found in legume flours include quercetin and kaempferol (Amarowicz and Pegg 2008; Panche et al. 2016).
- Processing and storage, for example, dehulling or milling, expose the inner part of the legume, which may intensify the colour. Thermal treatment also leads to changes in the molecular structure of flavonoids, modifying their bioavailability as well as their colour (Rohn et al. 2007; da Costa et al. 2002; Nguyen and Bechtold 2021).
- Presence of intensely coloured metal complexes, for example, Fe complexes with polyphenolic compounds, can lead to the formation of a yellow-brown shade (Fitz-Binder et al. 2025; Melo et al. 2022).
- The oxidation of lipids and lipophilic colorants, for example, chlorophyll and carotenes, can also lead to the

formation of yellowish compounds and volatile degradation products during storage and processing (Ajayi-Banji et al. 2024). Furthermore, the colour of a flour from lupin seeds has been found to change during germination as a consequence of increased content of carotenoids and tocopherol (Estivi et al. 2023).

The fatty acid composition of legumes can be divided into the following fractions: 50% polyunsaturated fatty acids (PUFAs), 30% monosaturated fatty acids (MUFAs) and 20% saturated fatty acids (SFAs) (Caprioli et al. 2016). These oleaginous plants are widely cultivated, and the seeds are rich in MUFAs and PUFAs with a high content of essential fatty acids such as omega 6–linoleic acid (C18:2, ω 6) and omega 3 alpha-linolenic acid (C18:3, ω 3) (Saini et al. 2021).

Although a number of coloured ingredients have been identified in legume flours, a general correlation between the yellow colour of flour and the presence of coloured substances has not been established up to now.

In this study, we follow the hypothesis that the yellow colour of protein-enriched legume flour could correlate with the content of selected constituents present in the respective flour. To evaluate this hypothesis, the nutritional quality of nine commercially available protein-enriched flours made from chickpea, faba bean, lentil, pea and sacha inchi was determined, including their fatty acid profile (FAMES) using gas chromatography–mass spectrometry (GC–MS). From the measurement of the diffuse reflectance of the flours their colour was determined in terms of CIELab coordinates and yellowness index (YI), which then were correlated to colour-generating constituents of the flour.

2 | Methods and Materials

2.1 | Materials

The protein-enriched legume flours were provided by the following manufacturers: *Cicer arietinum* CP1 (chickpea₄₄), CP2 (chickpea₆₈) and CP3 (chickpea₉₀) from Döhler GmbH (Darmstadt, Germany); *Vicia faba* FB1 (faba bean₅₀) and *Lens culinaris* LEN (lentil₈₀) from AcerChem B.V. (Rotterdam, Netherlands); *V. faba* FB2 (faba bean₈₈) from Roland Beans GmbH (Bremen, Germany); *Pisum sativum* PEA1 (pea₇₀) from Focus Ingredients GmbH (Trostberg, Germany); *P. sativum* PEA2 (pea₈₀) from Ingredion GmbH (Hamburg, Germany) and *Plukenetia volubilis* SI (sacha inchi) from MRM Nutrition (Oceanside, USA). The derivatising agent for the preparation of fatty acid methyl esters (FAMES) for GC–MS analyses (0.25 M trimethylsulphonium hydroxide in methanol for GC derivatisation) was purchased from Merck (Darmstadt, Germany). The internal and external normalisation of GC–MS were adjusted using the following standards: undecanoic acid methyl ester ($\geq 98\%$) Sigma Aldrich, St. Louis, MO, USA) and FAMES mixture C8–C22 (Supelco, PA, USA). Total phenolic content (TPC) was determined using the Folin–Ciocalteu phenol reagent (Merck, Darmstadt, Germany).

The chemicals and solvents used in the analyses were analytical grade chemicals and were supplied by the following companies: L–aspartic acid ($\geq 99\%$), NaOH ($\geq 98\%$), KOH

($\geq 85\%$), Na₂CO₃ ($\geq 99\%$), CsCl ($\geq 99\%$), La₂O₃ ($\geq 99\%$), KBr ($\geq 99\%$) (Sigma Aldrich, St. Louis, MO, USA); 37% HCl, gallic acid monohydrate ($\geq 99\%$) (Fluka Chemie GmbH, Buchs, Switzerland); 99.9% absolute ethanol (Supelco, Bellefonte, PA, USA); H₂SO₄ (95%–97%); HNO₃ (65%); hexane (C₆H₁₄ [$\geq 98\%$], Merck, Darmstadt, Germany) and chloroform (CHCl₃ [$\geq 99\%$], Sigma Aldrich, St. Louis, Missouri, USA). A solution of phenolphthalein indicator was prepared by dissolving 1 g of phenolphthalein in 100 mL of 95%v/v ethanol.

2.2 | Analytical Methods

2.2.1 | Determination of Moisture Content

For the determination of the moisture content, an exact mass near 2 g of each legume flour was dried at 110°C for 4 h in a hot air oven (Memmert, Schwabach, Germany). After cooling down in a desiccator, the samples were weighed and the moisture content was calculated according to Equation (1). Experiments were performed in two repetitions. The accuracy of the method is estimated with 0.1% moisture content.

$$\text{Moisture [\%]} = \frac{\text{weight of wet sample [g]} - \text{weight of dry sample [g]}}{\text{weight of wet sample [g]}} \times 100 \quad (1)$$

2.2.2 | Determination of Protein Content

Nitrogen analysis was used to determine the protein content of legume flours (Elementar Rapid N III nitrogen analyser, Langensfeld, Germany). The N content was transferred into the protein content with the use of a conversion factor of 5.4 (Mariotti et al. 2008). Experiments were performed in two repetitions. The accuracy of the method is estimated with 0.1% protein content.

2.2.3 | Determination of Fat Content by Soxhlet Method

Approximately 5 g of flour was extracted with 250 mL of hexane for 16 h using a Soxhlet apparatus. The extraction thimbles were plugged with cotton fibres. After extraction had been completed, the hexane was removed by evaporation and the oil-containing flask was weighed. Fat content was calculated according to Equation (2). Experiments were performed in two repetitions. The accuracy of the method is estimated with 0.1% fat content.

$$\text{Fat [\%]} = \frac{\text{weight of flask with fat [g]} - \text{weight of empty flask [g]}}{\text{weight of sample [g]}} \times 100 \quad (2)$$

2.2.4 | Determination of Ash Content

The weight of the empty porcelain crucibles was determined after heating in a muffle furnace (Nabertherm, L9/R, Lilienthal, Germany) for 1 h at 700°C and cooling in a desiccator. Then, an exact mass of 2 g of legume flour was first heated in a fast incinerator until smoke formation had ceased and the residues appeared dark grey. The samples then were transferred into a

muffle furnace and burnt at 700°C to constant weight. After cooling to ambient temperature in a desiccator the light grey or white samples weighed. The ash content was calculated according to Equation (3). Experiments were performed in two repetitions. The accuracy of the method is estimated with 0.01% ash content.

$$\text{Ash [\%]} = \frac{\text{weight of crucible and ash [g]} - \text{weight of empty crucible [g]}}{\text{weight of crucible and sample [g]} - \text{weight of empty crucible [g]}} \times 100 \quad (3)$$

2.2.5 | Determination of Fibre Content

A weighed amount of legume flour was treated with boiling 0.127M H₂SO₄ for a period of 30 min (Merck Thermoreactor TR 105, Darmstadt, Germany). Evaporated water was replenished in regular intervals to maintain the volume at a constant level. The residues were collected by filtration through a cotton cloth and washed with hot water until the filtrate was neutral. The samples then were treated with 0.313M NaOH at a boil for 30 min. The mixtures then were filtered and transferred into a crucible. After drying overnight at 80°C–100°C, the residues were weighed. The samples in the crucibles then were burnt in a muffle furnace (550°C, 3 h). After cooling in a desiccator, the crucible was weighed. The crude fibre weight was calculated according to Equation (4). Experiments were performed in two repetitions. The accuracy of the method is estimated with 0.1% fibre content.

$$\text{Fibre [\%]} = \frac{\text{weight of fibre and crucible [g]} - \text{weight of crucible and ash [g]}}{\text{weight of sample [g]}} \times 100 \quad (4)$$

2.2.6 | Determination of Residuals

The residuals (mainly carbohydrates) can be determined by calculating the percentage that remains after other components have been measured using the Equation (5).

$$\text{Residuals (\%)} = 100 - (\text{moisture} + \text{proteins} + \text{fats} + \text{ash} + \text{fibre}) \quad (5)$$

2.2.7 | Determination of Macroelements and Microelements

Samples were prepared in a muffle furnace at 700°C for 1 h. The ash of each flour (0.10–0.18 g) was dissolved, filtered and made up to 100 mL with 0.1 M HNO₃ for lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe) elements or 0.1 M HCl for calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and with the addition of 0.1% w/w CsCl/La solution as ionisation suppressor. Macroelements and microelements were measured by atomic absorption spectrometry using a ContrAA300 (Analytik Jena GmbH, Jena, Germany) equipped with an autosampler and all determinations were performed in duplicate. CsCl/La solution was added as a releasing agent in the determination of Ca, Mg, Na and K. Standard stock solutions containing 1 g L⁻¹ of each element (Cd, Cu, Zn, Pb, Mn, Fe, Mg, Ca, Na and K) were purchased from Merck (Darmstadt, Germany). Absorbances of macroelements and microelements were recorded at different wavelengths (327-nm Cu, 248-nm Fe, 279-nm Mn, 213-nm Zn, 766-nm K, 588-nm Na, 422-nm Ca and 285-nm Mg). The instrumental conditions and the concentrations of the stock, working and series standard solutions are given in Table S1. The accuracy of the method is estimated

with 0.1 mg 100 g⁻¹ for Mn, Zn and Fe and with 1 mg 100 g⁻¹ for Na, K, Mg and Ca content.

2.3 | Physicochemical Characteristics

2.3.1 | Determination of Acid Value

The acid value (AV) represents the amount of fatty acids that have been liberated by hydrolysis due to the action of moisture, temperature or lipolytic enzymes. It is defined as the consumption KOH (mg) required to neutralise the fatty acids present in 1 g of oil. Experiments were performed in two repetitions. The oil is dissolved in alcohol, and the acid content is determined by titration of the 0.5 M KOH (Equation 6).

$$\text{AV [mg KOH g}^{-1}\text{]} = \frac{56.1 \times V \times M}{W} \quad (6)$$

where *V* is the volume of titrant at the stoichiometric point (mL), *M* is the molarity of KOH and *W* is the sample weight (g).

2.3.2 | Determination of Saponification Value

The saponification value (*SV*) is defined as the quantity of mg of KOH consumed during saponification of 1-g oil by refluxing with an alcoholic KOH solution. The 70 mL of alcoholic KOH (40 g of KOH was dissolved in 1 L of 95% ethanol) solution was added to the flasks containing the extracted oily samples (e.g., extracted from legume flours by Soxhlet extraction in hexane). The samples were heated at 78°C for 2 h. Saponification was complete when no oily matter was present. A phenolphthalein indicator (two to three drops) was added after the samples had cooled down. Afterwards, the samples were titrated with 0.5 M HCL, and the alkali required for saponification was determined by titration of the excess KOH with standard HCL. Experiments were performed in two repetitions. The quantity of alkali required for saponification was determined according the following Equation (7):

$$\text{SV [mg KOH g}^{-1}\text{]} = \frac{56.1 \times M \times (V_0 - V_1)}{W} \quad (7)$$

where *V*₀ is the volume of HCL required for the blank titration (mL), *V*₁ is the volume of HCL used for titration (mL), *M* is the molarity of HCL and *W* is the sample weight sample (g).

2.3.3 | Determination of Density

The dry pycnometer (Blaubrand, Wertheim, Germany) was carefully filled with legume flours to avoid entrapment of air bubbles. Experiments were performed in two repetitions. Equation (8) was used to calculate the density (*D*):

$$\text{Density [g mL}^{-1}\text{]} = \frac{w_2 - w_1}{(w_4 - w_1) - (w_3 - w_2)} \times \rho_{\text{H}_2\text{O}} \quad (8)$$

where *W*₁ is the weight of pycnometer (g), *W*₂ is the weight of pycnometer + sample (g), *W*₃ is the weight of

pycnometer + sample + water (g), W_4 weight of pycnometer + water (g) and ρ_{H_2O} is the density of water was taken with 1 g mL^{-1} .

2.4 | Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM)

Samples were recorded as KBr pellets on an Invenio S spectrometer (Bruker Optik GmbH Ettlingen, Germany) in the spectral range of $4000\text{--}600 \text{ cm}^{-1}$ with a resolution of 2 cm^{-1} and 64 scans per measurement. Scanning electron images of legume flours

(Figure 2) were taken using the benchtop Hitachi TM 3030 (Japan) with a voltage of 15 kV.

2.5 | Determination of Total Phenol Content

The TPC of legume flours was determined using the Folin-Ciocalteu phenol reagent (Tian et al. 2021). In an alkaline medium, the assay relies on the transfer of reducing equivalents from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes, which are presented in the form of blue colour complexes (Ainsworth and Gillespie 2007). The

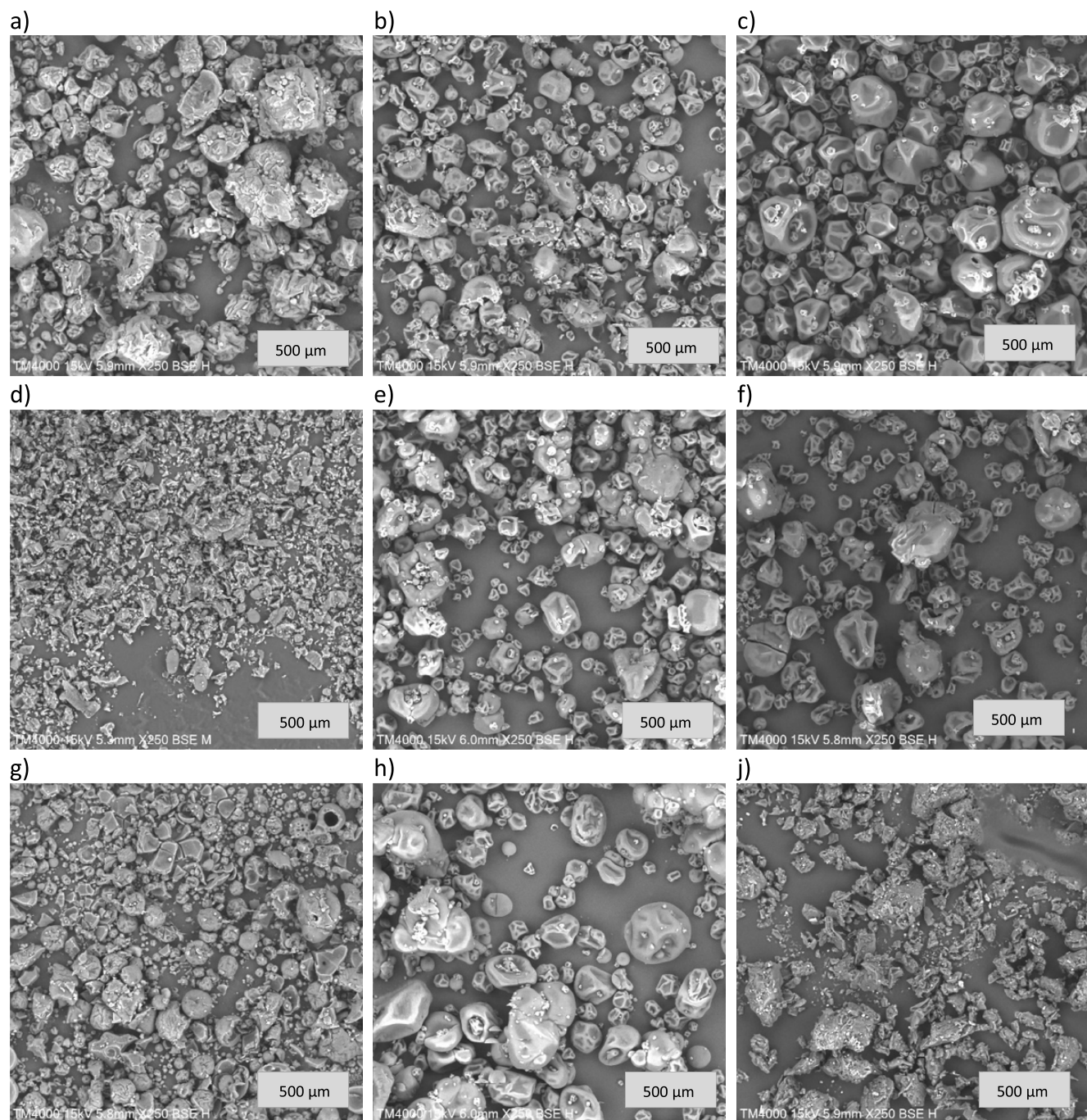


FIGURE 2 | SEM microphotographs of (a) CP1, (b) CP2, (c) CP3, (d) FB1, (e) LEN, (f) FB2, (g) PEA1, (h) PEA2 and (j) SI protein flour (scale bar = $500 \mu\text{m}$).

extraction of TPC from legume flours was carried out using 30% v/v ethanol acidified with 5% v/v HCl as the extraction solvent (Kostic 2014). A mass of 2.5-g sample was treated in a vortex mixer for 20 min with 40-mL solvent (30%v/v ethanol, 5%v/v HCL). After 5-min centrifugation (Heraeus, multifuge 1 L, Thermo Scientific, Waltham, MA, USA), at 4000 rpm, the supernatants were filtered. A volume of 200- μ L filtrate was diluted with 1.4-mL deionised water, and then, 100- μ L Folin–Ciocalteu reagent (Merck, Darmstadt) was added. The reagents were mixed on a vortex mixer (Heidolph Reax Top, Schwabach, Germany), and after 8 min, 300- μ L Na₂CO₃ (200 g L⁻¹) was added. The mixture then was treated for 30 min in a water bath (Julabo GmbH, Seelbach, Germany) at 40°C (Fitz-Binder and Bechtold 2019).

The absorbance of the solutions was determined at 765 nm (HITACHI U-2000 double beam spectrophotometer, Tokyo, Japan). Total phenolics content is given as gallic acid equivalent (GAE) (Equation 9):

$$TPC [mg\ g^{-1}] = \frac{\text{volume of extract [mL]} \times \text{concentration [mg mL}^{-1}]}{\text{weight of extract [g]}} \quad (9)$$

Figure S11 shows the standard calibration curve using the GAE equation of $y = 0.0112x + 0.0057$, whereby x is the concentration of extracts in mg mL⁻¹ and y is the absorbance at 765 nm. A correlation coefficient value (R^2) of 0.9976 was obtained from the linear calibration curve of gallic acid.

2.6 | Determination of Fatty Acid Profile

An exact mass near 5 g of each sample was weighed and extracted in a Soxhlet apparatus with 250 mL of hexane for 16 h. At the end of the extraction process, the liquid fraction of the flask was filtered through a Whatman No. 1 filter paper and the solvent was removed under reduced pressure using a rotary evaporator (IKA RV 10 digital with VACSTAR vacuum pump, Staufen, Germany). The oily residue was collected, evaporated under argon, weighed and stored in glass vials at -20°C until it was analysed. The sample for GC–MS measurement was prepared as follows: The oily residue was added to 400 μ L of CHCl₃, followed by the addition of 100 μ L of 0.25 M trimethylsulphonium hydroxide in methanol (Merck, Darmstadt, Germany) to achieve derivatisation to volatile FAMES (Ačanski et al. 2015; Pastor et al. 2020).

Fatty acids were analysed by GC–MS QP2010 (Shimadzu, Kyoto, Japan) in EI mode. Hydrogen was used as a carrier gas at a flow rate (total flow, 41.7 mL min⁻¹), whereas separation was performed on the Zebron ZB-5MSi GC column ($l = 30$ m; $df = 0.25$ mm; $t = 0.25$ μ m). The injector temperature was 280°C, whereas the oven temperature was held at 130°C for 5 min and then increased to a final temperature of 240°C. A split ratio of 1:50 was used for the injection of 1- μ L sample solution. Chromatograms of FAMES were obtained in TIC mode (total ion chromatogram) and mass spectra were recorded in the range 40–400 m z⁻¹, allowing a comprehensive analysis of the methyl esters. The interface and ion source temperatures were 352°C

and 220°C, respectively, whereas the solvent separation time was set to 2.0 min.

For quantitative determination of FAME content, both internal and external standard methods were used. Undecanoic acid methyl ester (Sigma Aldrich, St. Louis, MO, USA) was used as the internal standard for internal normalisation, whereas FAME mixture C8–C22 (Supelco, PA, USA) was used as the standard for external normalisation. Fatty acid composition was related to the total amount of fatty acids in the sample (mol %). Experimental data were processed using LabSolution software.

2.7 | Colour Measurement

The CIELab colours of the flours were determined by measurement of the diffuse reflectance of legume flour. The data then were used for calculation of the YI, which is a valuable tool to estimate the impact of a chosen product on the final colour of a product. The K/S values of the legume flours were determined by colour measurement using a wavelength range of 400–700 nm and excluding the specular component. The measurement was performed with a Konica Minolta CM 3610d spectrophotometer (Chiyoda City, Tokyo, Japan) in d/8 configuration, equipped with pulsed xenon lamps as light source. The diameter of the measurement area was 8 mm, and the reflectance data were transformed into the L*, a* and b* coordinates of the CIE colour space (D65 illuminant, 10° observer) using the onboard software. Colour depth (K/S) was estimated using the Kubelka–Munk Equation (10), where R is the fractional reflectance at the minimum peak (600 nm).

$$K/S = \frac{(1-R)^2}{2R} \quad (10)$$

The YI quantifies the colour of a specimen in the range from clear or white to yellow and is calculated from spectrophotometric data. The YI, which is recommended by ASTM E313–20 and DIN6167, is calculated using the following Equation (11) (Anon 2022):

$$YI = \frac{100x(C_x X - C_y Z)}{Y} \quad (11)$$

where X , Y and Z are CIE tristimulus values and $C_x = 1.28$ and $C_z = 1.062$.

2.8 | Statistical Analysis

Data in Tables 1 and 2 are given as mean and standard deviation from the results of two repetitions. On the basis of a mean value for the standard deviation of each variable, the statistical test for comparison of independent groups was used to calculate a threshold value indicating the minimum difference between two mean values to become statistically significant at a confidence level of 95% (*) or 99% (**). The thresholds allow statistical assessment of the difference between two results obtained for different flour (Tables 1, 2 and 4).

TABLE 1 | Proximate composition and physicochemical properties of legume flours (mean $\pm$ std. dev.) and threshold (TH) value for statistically significant difference between two results at a confidence level of * = 95% and ** = 99%.

	CP1	CP2	CP3	FB1	FB2	LEN	PEA1	PEA2	SI	TH
Moisture (%)	2.2 $\pm$ 0.1	3.3 $\pm$ 0.1	3.7 $\pm$ 0.5	6.8 $\pm$ 0.1	6.3 $\pm$ 0.1	3.5 $\pm$ 0.2	4.1 $\pm$ 0.3	6.1 $\pm$ 0.1	4.1 $\pm$ 0.5	*1.9
Protein (%)	29.2 $\pm$ 0.1	59.0 $\pm$ 0.1	73.1 $\pm$ 0.1	52.9 $\pm$ 0.1	72.0 $\pm$ 0.4	70.5 $\pm$ 0.1	67.6 $\pm$ 0.4	65.3 $\pm$ 0.1	52.2 $\pm$ 0.2	**3.6
Fat (%)	8.4 $\pm$ 0.3	4.8 $\pm$ 0.3	0.5 $\pm$ 0.1	3.5 $\pm$ 0.2	4.7 $\pm$ 0.3	4.3 $\pm$ 0.1	0.2 $\pm$ 0.2	1.3 $\pm$ 0.1	11.3 $\pm$ 0.1	**4.6
Ash (%)	3.64 $\pm$ 0.01	6.05 $\pm$ 0.55	7.39 $\pm$ 0.12	5.66 $\pm$ 0.02	9.52 $\pm$ 0.73	10.45 $\pm$ 0.37	5.08 $\pm$ 0.01	7.93 $\pm$ 0.80	8.96 $\pm$ 0.27	*3.2
Fibre (%)	12.9 $\pm$ 0.3	5.2 $\pm$ 0.1	1.1 $\pm$ 0.1	7.3 $\pm$ 0.2	4.3 $\pm$ 0.1	0.5 $\pm$ 0.4	3.0 $\pm$ 0.1	2.2 $\pm$ 0.1	3.8 $\pm$ 0.1	**3.3
Carbs (%)	43.7	21.7	14.2	23.8	3.2	10.8	20.0	17.2	19.7	**12.6
SV (mgg ⁻¹)	161.1 $\pm$ 0.9	165.1 $\pm$ 0.9	168.7 $\pm$ 0.2	181.1 $\pm$ 0.7	187.1 $\pm$ 0.9	110.2 $\pm$ 0.7	124.1 $\pm$ 0.6	129.8 $\pm$ 0.1	145.7 $\pm$ 0.7	**12.6
AV (mgg ⁻¹)	3.22 $\pm$ 0.1	2.02 $\pm$ 0.01	5.52 $\pm$ 0.1	3.35 $\pm$ 0.01	2.98 $\pm$ 0.1	4.56 $\pm$ 0.1	2.62 $\pm$ 0.01	2.98 $\pm$ 0.01	2.23 $\pm$ 0.1	**1.2
D (gmL ⁻¹)	0.51 $\pm$ 0.02	0.30 $\pm$ 0.04	0.40 $\pm$ 0.02	0.38 $\pm$ 0.02	0.50 $\pm$ 0.04	0.77 $\pm$ 0.01	0.63 $\pm$ 0.03	0.94 $\pm$ 0.04	0.95 $\pm$ 0.03	*0.25

3 | Results and Discussion

3.1 | Proximate and Physicochemical Properties of Legume Flours

The proximate composition of legume flours includes moisture, ash, fat, protein and carbohydrate contents, which are presented in Table 1. The AOAC (Official Methods of Analysis of AOAC International, 18th Edition, Chapters 3 and 4) methods were used to estimate the proximate composition of protein flours (anon. 2005). The obtained results showed that the protein content as one of the essential components of selected legume flours ranged between 29.2% and 73.1%. The highest crude protein contents were found in CP3 (73.1%) and FB2 (72.0%) flours. A statistically significant difference in the protein content of CP3 and FB2 was observed at a confidence level of 99% in samples with protein content below 60%.

The large variability in the fat content may be due to differences in plant origin, cultivar, subspecies, harvest time, harvest season, growing conditions, processing conditions (roasting before extraction) and method of defatting during protein enrichment (Madurapperumage et al. 2021). The estimated fat content in SI flour of 11.3 $\pm$ 0.1% is significantly higher (confidence level 99%) than in the results for legume flours with a fat content below 5%. With the increase in protein content, the fat content of chickpea flour decreases from 8.4% in CP1 to 0.5% in CP3. In addition, the data show that the moisture content was found to be in the range (2.2%–6.8%), whereas the fibre content was estimated to be between 0.5% and 12.9%. Digestible carbohydrates (3.2%–43.7%) are the main source of dietary energy needed to maintain muscles, skin, bones and so forth. According to experimental results, the residues (mainly, carbohydrate content) vary between 0.5% and 12.9%.

Major physicochemical properties of legume flours such as density, acidic and saponification values are summarised in Table 1. The procedures for estimation of saponification and acidic numbers have been adapted from the following source: Food Safety and Standards Authority of India (2012).

3.2 | Minerals Content

As illustrated in Table 2, the content of macrominerals and microminerals (in mg per 100 g⁻¹ of sample) in legumes exhibited a slight variation among samples of the same type. The primary factors that contribute to the observed variability in mineral composition are likely to be related to the soil composition, climatic conditions, seasonal fluctuations, physiological status and the maturity of the legume (Ramírez-Ojeda et al. 2018).

The chickpea flours (CP1, CP2 and CP3) were found to have a similar chemical composition for Zn and Mg. With higher protein content, the chickpea protein flours showed a lower content of Fe, Ca, Mg and K, whereas the Na content increased. The PEA1 had a lower K content compared with other legume flours. The statistical analysis of the K content of PEA1 to the nearest result (PEA2) with the use of the *t*-test for independent samples indicated significance at a confidence level above 99.9%. In view of the results obtained, it was possible to verify that chickpea

flours and sachu inchi had a relatively higher content of minerals among the legumes analysed. These results corroborate the work developed by Wang et al. (2010), which indicated that chickpea is an excellent source of minerals. Nevertheless, it was observed that the FB1 had a lower Na content compared with the other samples.

Fe content in the flours varied from 0.1 mg 100 g⁻¹ in PEA1 to 5.8 mg 100 g⁻¹ in LEN, which exhibited the highest Fe content. The statistical analysis of the Fe content of PEA1 indicated a significant difference in the value of CP1 (4.7 ± 0.1 mg 100 g⁻¹ Fe) and higher values at a 99% confidence level. The contents of Pb, Cd and Cu were below the detection limit of the applied AAS method.

3.3 | FT-IR

The legume flours were characterised by FT-IR spectra. As can be seen in Figure 3 for CP1, a broad shoulder at 3343 cm⁻¹ was due to OH stretching, whereas shoulders at 2926 and 2855 cm⁻¹ were assigned to -CH₂ vibration. The bands at 1659 cm⁻¹ are assigned to the amide I C=O stretching (Carbonaro et al. 2012) and 1547 cm⁻¹ to the amide II region C-N stretching and NH bending. A strong band at 1746 cm⁻¹ was attributed to C=O

carbonyl of fatty components. Characteristic vibrations of carbohydrates were observed at 1408 cm⁻¹ owing to -CH bending and 1020/1080 cm⁻¹ for C-O vibration. The spectra of other legume flours can be found in Figures S3–S10.

3.4 | Determination of Fatty Acid Composition Using GC-MS Spectrometry

Fatty acids are carboxylic acids comprising long, saturated and unsaturated aliphatic chains. The analysis of fatty acids in legume flours reveals that the content of these compounds can be classified into two main categories: SFAs and unsaturated fatty acids (Purvis et al. 2024). The determination of the fatty acid profile involved three steps such as extraction of fatty acids from the sample matrix, derivatisation and GC-MS analysis. Undecanoic acid methyl ester was added as an internal standard, and in addition, FAME mixture was used for external normalisation.

As is shown in Figure 4, four major peaks in CP1 legume flour were identified with their corresponding retention times: C16:0 with a retention time of 14.043 min, C18:0 with a retention time of 16.261 min, C18:1 with a retention time of 16.007 min and C18:2 with a retention time of 15.946 min. The composition was calculated as a percentage of methyl ester area. The fatty acid

TABLE 2 | Macroelements and microelements in plant flours (mean ± std. dev.) detected with atomic absorption spectrophotometry and threshold (TH) value for statistically significant difference between two results at a confidence level of * = 95% and * * = 99%.

(mg 100 g ⁻¹)	CP1	CP2	CP3	FB1	FB2	LEN	PEA1	PEA2	SI	TH
Mn	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	* > 1
Zn	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.5 ± 0.1	0.2 ± 0.3	0.3 ± 0.1	0.4 ± 0.1	* > 1
Fe	4.7 ± 0.1	3.6 ± 0.2	2.9 ± 0.3	0.4 ± 0.2	0.3 ± 0.2	5.8 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	4.8 ± 0.1	* 1.5
Ca	46 ± 1	17 ± 1	13 ± 1	20 ± 1	20 ± 1	9 ± 1	8 ± 1	15 ± 1	124 ± 1	* 9
Mg	182 ± 1	27 ± 1	12 ± 1	65 ± 1	18 ± 1	11 ± 1	29 ± 1	25 ± 1	512 ± 1	* 9
Na	106 ± 1	125 ± 1	163 ± 1	19 ± 1	60 ± 1	49 ± 1	44 ± 1	50 ± 1	52 ± 1	* 9
K	1150 ± 1	1550 ± 1	335 ± 1	665 ± 1	248 ± 1	235 ± 1	57 ± 1	75 ± 1	833 ± 1	* 9

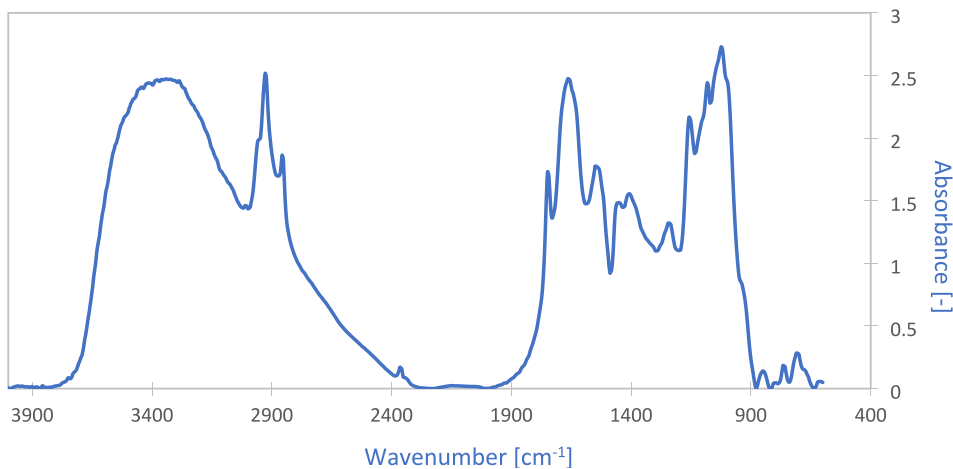


FIGURE 3 | FT-IR spectra of CP1 legume flour.

composition with corresponding peak area ratio of analysed legume flours is given in Table 3. The respective chromatographs can be found in Figures S12–S19. The major compounds were unsaturated fatty acids such as oleic (C18:1 ω 9) and linoleic (C18:2 ω 6) acids followed by SFAs such as palmitic (C16:0) and stearic (C18:0) acids. The content in linolenic acid (C18:3) was below the detection limit of the GC–MS method used.

Because of the low fat content in CP3, a separation between C18:1 and C18:2 was not possible by GC; thus, the value given in Table 3 represents the sum of both. High levels of palmitic acid were found in CP3 (39.3%) and FB2 (27.5%). The percentages of stearic acid (octadecanoic acid or C18:0) were more homogeneous among all samples. The levels of MUFA analysed (oleic acid or 9-(Z)-octadecenoic acid or C18:1, ω 9) ranged from

7.01% to 68.9%. Oleic acid was particularly high in SI (68.9%) and CP1 (58.8%) but very low in PEA1 (7.01%). On the contrary, PUFAs were particularly high in PEA1 (80.4). Linoleic acid (9,12-(Z,Z)-octadecadienoic acid or C18:2, ω 6) was identified as a highly abundant PUFA in the majority of analysed legumes (Fanali et al. 2011; Follegatti-Romero et al. 2009; Maurer et al. 2012).

As illustrated in Figure 5, the distinctive ions were successfully identified in the mass spectra of legume flour (CP1). The mass spectra exhibited a fragmentation pattern, with the most abundant ion (100% abundance) being the base ion (Chiu and Kuo 2020). The mass spectrum may be employed as a basis for estimating the compound at a specific retention time when compared with the mass spectrum of the MS library data

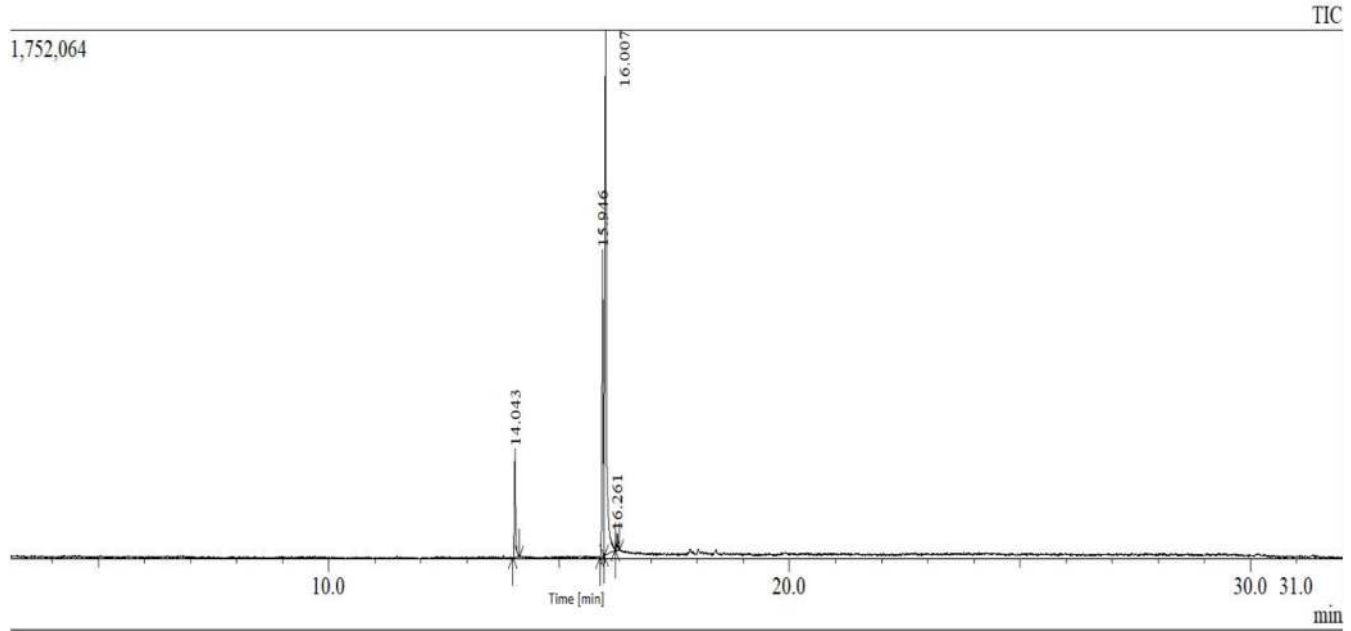


FIGURE 4 | GC–MS chromatogram of CP1 legume flour: palmitic acid (14.043 min), stearic acid (16.261 min), oleic acid (16.007 min) and linoleic acid (15.946 min).

TABLE 3 | FAMES identified in legume flours by GC–MS analysis with their peak area ratio percentage.

	Fat	C10:1	C16:0	C18:2	C18:1	C18:0
	%	%	%	%	%	%
CP1	8.42 ± 0.32	—	10.2	29.2	58.8	1.8
CP2	4.79 ± 0.33	2.7	16.9	29.1	49.7	1.6
CP3	0.59 ± 0.03	—	39.3	^a	53.6 ^b	7.1
FB1	3.54 ± 0.19	—	13.4	44.9	39.6	2.1
LEN	4.72 ± 0.25	—	20.7	29.8	49.5	—
FB2	4.29 ± 0.04	—	27.5	28.9	34.3	9.3
PEA1	0.22 ± 0.02	—	4.1	80.4	7.0	8.5
PEA2	1.32 ± 0.02	—	12.5	35.3	47.2	5
SI	11.3 ± 0.09	—	1.7	26.7	68.9	2.7

^a4-Decenoic acid (C10:1); palmitic acid (C16:0); linoleic acid (C18:2); oleic acid (C18:1); stearic acid (C18:0).

^bSum of C18:2 and C18:3.

(Nicolescu 2017). The initial peak observed in the GC–MS chromatogram (Figure 4), with a retention time of 14.043 min, was identified as palmitic acid methyl ester ($C_{17}H_{34}O_2$). The mass spectrum of this peak is presented in Figure 5a, which displays a molecular ion (M^+) peak at a molecular weight of $m/z=270$, followed by fragmentation peaks at $m/z=227$, 171, 143, 129, 111 and 74.

The second peak in the chromatogram (Figure 4) with a retention time of 15.946 min corresponded to linoleic acid methyl ester ($C_{19}H_{34}O_2$). The mass spectrum is provided in Figure 5b and displays molecular ion peaks at $m/z=294$, 263, 220, 178, 150, 135, 109, 67 and 55. Furthermore, the third and fourth peaks, with retention times of 16.007 and 16.261 min (Figure 4), respectively, were identified as oleic acid methyl ester ($C_{19}H_{36}O_2$) and stearic

acid methyl ester ($C_{19}H_{38}O_2$). The mass spectra are presented in Figure 5c, displaying molecular ion peaks at $m/z=296$, 264, 222, 180, 137 and 55 for oleic acid methyl ester and at $m/z=298$, 255, 199, 143, 111 and 74 for stearic acid methyl ester in Figure 5d.

3.5 | Nutritional Composition, Mineral Content, TPC and YI

The highest values of YI (DI1925) were observed with CP1, CP2 and PEA2 with a protein content of 49.4 ± 0.7 , 52.2 ± 0.7 and $44.9 \pm 0.2\%$, respectively. The lowest YI was observed with FB1, FB2 and LEN with 22.4 ± 0.1 , 31.2 ± 0.1 and $31.2 \pm 0.6\%$, respectively. On the basis of the highest standard deviation of

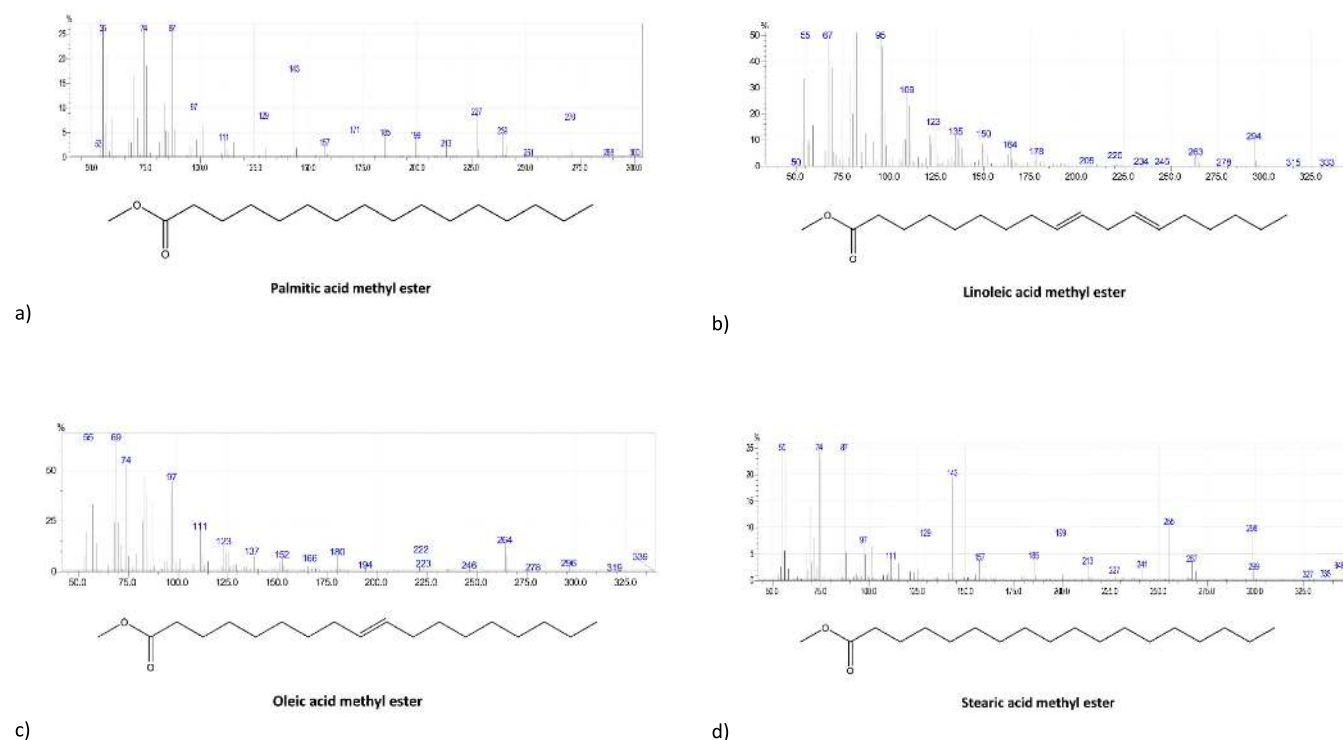


FIGURE 5 | Mass spectra of the fatty acid profile of CP1 legume flour: (a) palmitic acid methyl ester, (b) linoleic acid methyl ester, (c) oleic acid methyl ester and (d) stearic acid methyl ester.

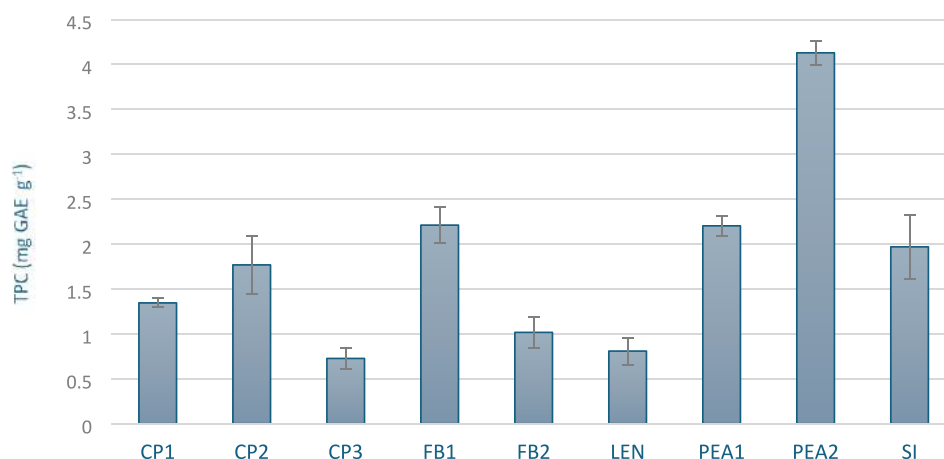


FIGURE 6 | Comparison of total phenolic content (TPC) in different flours derived from legumes.

± 0.7 YI (Table S4), statistical analysis indicates that a difference between the yellow indices above 8 is statistically significant at the 99% confidence level.

Differences in yellowness of legume flour could also be related to various factors of the nutritional composition, for example, protein content, fat content and fibre content. Besides tyrosine and tryptophane, the other amino acids in proteins do not contribute to the yellow colour. In case the yellow colour would originate from the protein content, flours with the lowest protein content should exhibit lower YI, which is not observed. Similarly fat content and fibre contents of the flours did not correlate to the observed results in YI.

Among the macroelements and microelements, only Fe is present in sufficient concentration to contribute to the YI through

the formation of intensively coloured Fe complexes with polyphenolic compounds, for example, flavonoids. Alkali and earth alkali elements Na, K, Mg and Ca are not able to form intensively coloured complexes with other compounds in the flour. Concentrations of Cu and Mn are too low to make a significant contribution to the YI. Therefore, the only possible explanation for the variation in YI is the presence of coloured polyphenols and Fe.

TPC shows large variability in different protein flours and also depends on the source and the region of cultivation (Amarowicz and Pegg 2008). Among the various known phenolic compounds, legumes contain mainly phenolic acids, flavonoids and condensed tannins (Amarowicz and Pegg 2008). These compounds are distributed differently in the seed coat (mainly flavonoids) and in the cotyledon (mainly nonflavonoids such

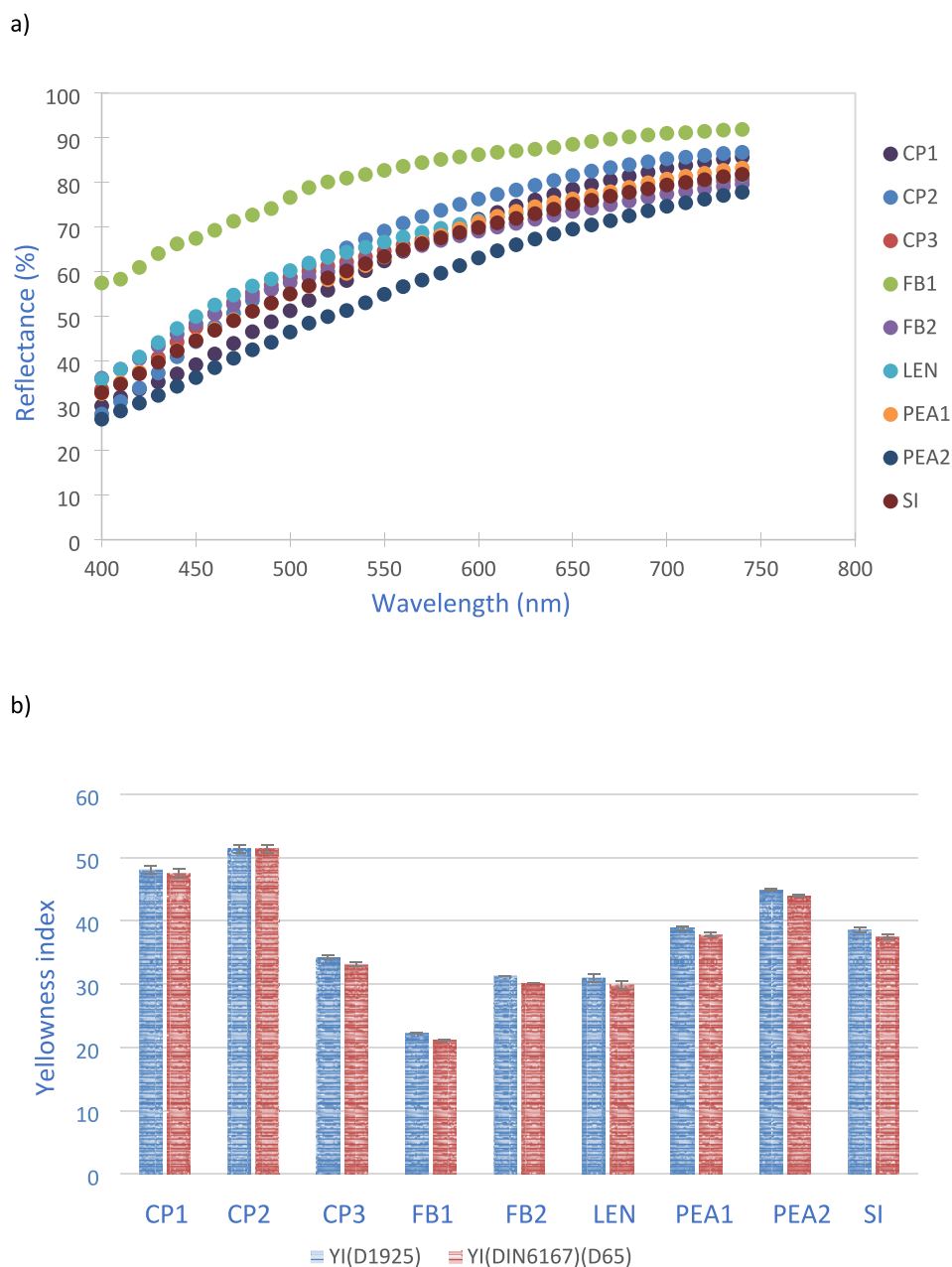


FIGURE 7 | (a) Diffuse reflectance of different legume flours derived from legumes; (b) yellowness index Y(D1925) and Y(DIN6167)(65) for legume flours.

TABLE 4 | CIELab values for legume flours (CIELab coordinates for D65 illumination) and threshold (TH) value for statistically significant difference between two results at a confidence level of * = 95% and ** = 99%.

	CP1	CP2	CP3	FB1	FB2	LEN	PEA1	PEA2	SI	TH
L*	88.7 ± 0.3	74.2 ± 0.2	84.4 ± 0.2	92.3 ± 0.2	83.6 ± 0.04	85.1 ± 0.3	83.5 ± 0.2	79 ± 0.08	82.9 ± 0.3	**15.5
a*	4.28 ± 0.03	1.36 ± 0.02	0.78 ± 0.06	0.02 ± 0.01	1.49 ± 0.01	0.45 ± 0.09	3.1 ± 0.02	3.99 ± 0.02	2.17 ± 0.02	**0.35
b*	23.1 ± 0.32	20.3 ± 0.12	16.7 ± 0.18	11.3 ± 0.05	14.6 ± 0.01	15.2 ± 0.24	18.3 ± 0.13	20.3 ± 0.08	18.3 ± 0.16	**1.7

as hydroxycinnamic acid and hydroxybenzoic acids) (Shahidi and Ambigaipalan 2015). The TPC reported in different protein flours is presented in Figure 6. The TPC in LEN in the present study was 0.81 mg GAE g⁻¹, which was in the range reported by Levent et al. (2023). Table S2 shows that the TPC of different CP1, CP2 and CP3 flours and PEA1 and PEA2 cultivars ranged from 0.72 to 1.76 mg GAE g⁻¹ and 2.20–4.13 mg GAE g⁻¹, respectively. Changes in phenolic compounds of FB1 and FB2 cultivars varied between 1.01 and 2.21 mg GAE g⁻¹. Differences in TPH between PEA2 and both LEN and CP3 were statistically significant at a confidence level of 99%. In the case of chickpea, pea and faba bean flours, it is very difficult to compare the obtained results with those of other authors because of the different cultivar sources, methods of extraction, determination and calculation of plant results used by them. The low content of TPC in SI flour of 1.96 mg GAE g⁻¹ is in the range with the obtained results by Singanusong and Jiamyangyuen (2020).

Significant results of the colour analysis by measuring the diffuse reflectance show that PEA2 was the darkest, whereas FB1 was the lightest of all the legumes (Figure 7a). These values are shown in Table S3. The distributions of the yellowness index Y(D1925) and Y(DIN6167)(65) are shown in Figure 7b. The CIELab parameters are given in Table 4.

Several factors contribute to the development of colour in legume flours. A considerable part of the soluble phenolic compounds, for example, flavonoids, exhibits yellow colour; thus, the content in total soluble phenolics should correlate with the observed yellowness. Analysis of the results indicated that differences in TPC content between the legume flours were not sufficient to explain the observed YI results (Figure S20).

A similar result was obtained by analysing the Fe content in the flours (Figure S21). However, by combining TPC and Fe content as possible sources of yellow colour in the form of a combined colour factor, a correlation between the colour factor and the YI could be observed (Figure 8). The colour factor was calculated by a weighted addition of the TPC (mg 100 g⁻¹) and Fe content (mg 100 g⁻¹) according to Equation (12):

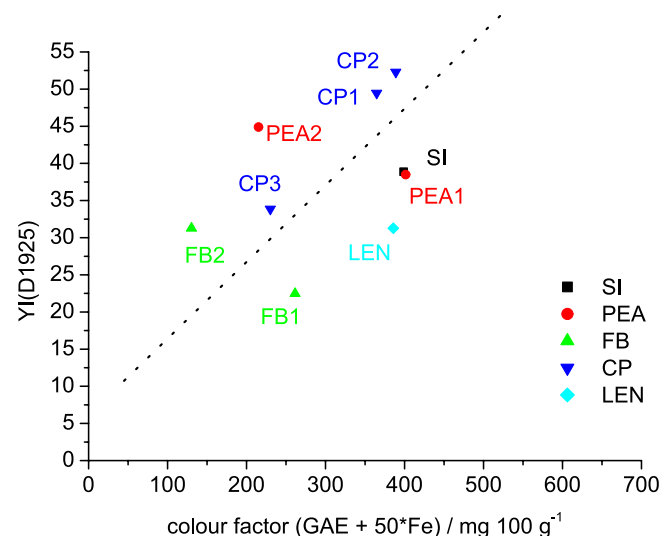


FIGURE 8 | Correlation between yellowness of different legume flours and the colour function.

$$\text{Colour factor} = \text{TPC (mg 100 g}^{-1}\text{)} + 50 \times \text{Fe content (mg g}^{-1}\text{)} \quad (12)$$

By a weighted combination of TPC and Fe content as main sources for a yellowish colour, a general relationship was obtained for the explanation of the yellowness of different legume flours.

4 | Conclusions

For the enrichment of food products, the proximate composition and mineral content of legume flours are fundamental properties for assessing their nutritional value. The yellow colour of certain legume flours may present limitations in their utilisation, particularly in the production of white, creamy products. In this study, protein-enriched legume flours from sacha inchi, pea, faba bean, chickpea and lentil were analysed for their proximate composition. The lowest yellowness index Y(D1925) was observed for faba bean (FB1), whereas the highest YI was observed for chickpea flours (CP1 and CP2). Protein, fat and dietary fibre content of the different flours did not correlate to the observed variations in yellowness. A correlation between the YI and the content of total soluble phenolics and Fe content could be established. This demonstrated that soluble yellow-coloured flavonoids and the presence of Fe-hydroxides represent two main factors, both contributing to the development of a yellow colour.

The colour of legume flours also depends on specific conditions during processing, thermal treatment and storage, which is a limitation of the present study. Also, plant variety and conditions of growth, for example, quality of soil and climate, and time of harvesting will influence colour development. Comparison of different varieties and growth conditions as well as the influence of processing steps on the development of yellow colour thus should be addressed in future studies.

A further improvement in understanding yellowness can be expected by including insoluble polyphenolic compounds, which may also contribute yellow-brown colour formation.

Author Contributions

Jelica Kovačević: conceptualization, methodology, investigation, formal analysis, visualization, writing – original draft. **Tung Pham:** conceptualization, supervision, funding acquisition, resources. **Thomas Bechtold:** conceptualization, methodology, visualization, writing – review and editing.

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Disclosure

Authors used [deepl.com/write](https://www.deepl.com/write) in order to correct English spelling and grammar errors. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available upon request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.