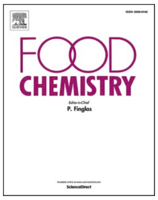
Food Chemistry 409 (2023) 135339

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Effects of fermentation and enzymatic treatment on phenolic compounds and soluble proteins in oil press cakes of canola (*Brassica napus*)

Ye Tian[a], Ying Zhou[a], Marie Kriisa[b], Maret Anderson[b], Oskar Laaksonen[a], Mary-Liis Kütt[b], Maike Föste[c], Małgorzata Korzeniowska[d], Baoru Yang[a,e,*,1]

[a] *Food Sciences, Department of Life Technologies, Faculty of Technology, University of Turku, 20014 Turku, Finland*
[b] *Center of Food and Fermentation Technologies (TFTAK), 12618 Tallinn, Estonia*
[c] *Fraunhofer Institute for Process Engineering and Packaging IVV, 85354 Freising, Germany*
[d] *Department of Functional Food Products Development, Faculty of Biotechnology and Food Science, Wroclaw University of Environmental and Life Sciences, 51-630 Wroclaw, Poland*
[e] *Shanxi Center for Testing of Functional Agro-Products, Shanxi Agricultural University, Taiyuan 030031, China*

ARTICLE INFO

Keywords:
Canola oil press cakes
Fermentation
Enzymatic hydrolysis
Solubilized proteins
Phenolic compounds

ABSTRACT

To develop novel processes for valorizing agro-industry side-streams, canola (*Brassica napus*) oil press cakes (CPC) were treated with lactic acid bacteria, carbohydrase, and protease. Altogether 29 protein-rich liquid fractions were obtained, of which the composition was analyzed using chromatographic and mass spectrometric methods. A clear association was revealed between the treatments and phenolic profile. Applying certain lactic acid bacteria enhanced the release of sinapic acid, sinapine, glycosylated kaempferols, and other phenolic compounds from CPC. Co-treatment using protease and *Lactiplantibacillus plantarum* was effective in degrading these compounds. The fraction obtained after 16 h of hydrolysis (with Protamex® of 2% dosage) and 48 h of fermentation (using *L. plantarum*) contained the lowest phenolic content (0.2 g/100 g DM) and a medium level of soluble proteins (78 g/100 g) among all samples studied. The fractions rich in soluble proteins and low in phenolics are potential food ingredients with improved bioavailability and sensory properties.

1. Introduction

Rapeseed or canola (*Brassica napus*) oil is one of the most produced edible oils in the world nowadays (Chew, 2020). The name of 'Canola' is referred to a specific rapeseed cultivar that contains low contents of glucosinolates and erucic acid. Accumulating evidence suggests that rapeseed oil has a great potential in reducing risk factors of cardiovascular diseases. This is ascribed to high levels of mono- and polyunsaturated fatty acids and an ideal omega-3: omega-6 ratio in this oil (Amiri et al., 2020; Chew, 2020; Ghobadi et al., 2019; Morya et al., 2022; Pourrajab et al., 2022).

Along with a steady growth of world production of rapeseed oil, large amounts of solid wastes (as oil press meals or press cakes) were produced annually. During year 2020–2021, over 29 million tons of rapeseed oil was produced in the world, which led to 41 million tons of oil press meals generated after mechanical oil pressing (Foreign

---

Abbreviations: L. brevis, *Lactobacillus brevis*; *L. plantarum*, *Lactiplantibacillus plantarum*; *L. sanfranciscensis*, *Lactobacillus sanfranciscensis*; *L. paralimentarius*, *Lactobacillus paralimentarius*; *P. pentosaceus*, *Pediococcus pentosaceus*; *B. subtilis*, *Bacillus subtilis*; Pro., Protamex; Vis., Viscozyme; SiM, sinapoyl malate; SiA, sinapic acid; SiHex, sinapoyl hexose; SiGlu, sinapoyl glucose; SiA der, sinapic acid derivative; diSiHexHex, disinapoyl hexose-hexose; diSiHex, disinapoyl hexose; SiN, sinapine; SiN der, sinapine derivative; KaSopGlu, kaempferol 3-*O*-sophroside-7-*O*-glucoside; KaSiSopGlu, kaempferol 3-*O*-(sinapoyl)-sophroside-7-*O*-glucoside; KaSiHexHexHex, kaempferol-sinapoylhexoside-hexoside-hexoside; KaHexPenHex, kaempferol-hexoside-pentoside-hexoside; KaHexHex, kaempferol-hexoside-hexoside; KaSiHexHex, kaempferol-sinapoylhexoside-hexoside; KaHex, kaempferol-hexoside; PrN, progoitrin; GlNFN, gluconapoleiferin; GlNN, gluconapin; GlAN, glucoalyssin; 4-OH-GlBN, 4-hydroxy-glucobrassicin; GlBNN, glucobrassicanapin; Ara, arabinose; Fuc, fucose; Xyl, xylose; Fru, fructose; Gal, galactose; Glu, glucose; Suc, sucrose; GaA, galacturonic acid; Man, mannitol; Ino, myo-inositol; PhA, phosphoric acid; LaA, lactic acid; SuA, succinic acid; MaA, malic acid; CiA, citric acid; Ala, alanine; Gly, glycine; Val, valine; Leu, leucine; Pro, proline; Iso, isoleucine; Ser, serine; Thr, threonine; GlA, glutamic acid; AsA, aspartic acid; Met, methionine; Phe, phenylalanine; Tyr, tyrosine.

[*] Corresponding author at: Food Sciences, Department of Life Technologies, University of Turku, FI, 20014 Turku, Finland.
E-mail address: baoru.yang@utu.fi (B. Yang).

[1] ORCID: 0000-0001-5561-514X.

https://doi.org/10.1016/j.foodchem.2022.135339
Received 28 September 2022; Received in revised form 21 November 2022; Accepted 26 December 2022

Agricultural Service United States Department of Agriculture, 2021). As a major agro-industrial wastes of oil manufacturing, the rapeseed oil press residues still contain 22–52% of crude proteins, 2–12% of crude lipids, and 4–14% of crude fibres (Arrutia et al., 2020). The conventional usage of this material is livestock feeds. Recent research has also discovered the possibility of this material as alternative sources for producing enzymes, antimicrobials, bioactive compounds, platform chemicals, biosurfactants, and biopolymers (Wongsirichot et al., 2022).

For food industry, it is of great interest to convert rapeseed oil press residues into value-added products. Current work in this field focuses on recovering proteins from the material (Ancuţa & Sonia, 2020). Some phytochemicals in rapeseed, such as phytic acids, glucosinolates (primarily as progoitrin, 4-hydroxyglucobrassicin, gluconapin), and phenolic compounds (primarily as tannins, sinapic acid, sinapine, and kaempferol), are considered as major disadvantages of rapeseed-derived proteins for food application. These chemicals are commonly removed during the process of protein purification due to their undesired effect on the nutrition and sensory quality of protein products (Chmielewska et al., 2020; Clarke, 2010). For example, phytic acid and tannins reduce the digestibility and absorption of proteins by forming complex with this nutrient (Ozdal et al., 2013; Wang & Guo, 2021). Glucosinolates, sinapine, and certain kaempferol glucosides confer bitter taste and dark color to protein extracts (Chmielewska et al., 2020; Hald et al., 2019).

Yet, accumulating studies have confirmed that sinapic acid and its derivatives (such as sinapine, 4-vinylsyringol, syringaldehyde, sinapoyl glucose, and sinapoyl malate) exhibit potent anti-oxidative, anti-microbial, anti-inflammatory, and anti-cancer activities (Nićiforović & Abramovič, 2014). High glucosinolates diet interferes the thyroid function of animals, but no clear evidence has been found so far, proving the negative impact of these compounds on human health (Thinh Nguyen et al., 2020). Some studies suggest that glucosinolates are metabolized by human gut microbiota, and the derived metabolites (mainly indoles, nitriles, and isothiocyanates) possess effects against inflammation and different types of cancers (Latté et al., 2011; Sikorska-Zimny & Beneduce, 2021). Therefore, from the perspective of human health and the cost of side-stream valorization, it is advisable to retain some of these chemicals, in particular, phenolic compounds in the protein extracts.

It is challenging to maintain an appropriate level of phenolic compounds in protein products. The content of phenolic compounds should be sufficient to exhibit potential health benefits, while to avoid negative impact on the sensory quality of the products. Current research has suggested that fermentation (using bacteria, yeasts, or fungi) and enzymatic hydrolysis (using carbohydrases, proteinases, or phytase) are effective in altering phenolic content of the by-products from cereals, fruits, and vegetable oil processing (Baker & Charlton, 2020; Tian et al., 2022; Verni et al., 2019). However, in the most of previous research, the phenolic contents in the studied materials were measured only by spectrophotometric methods. Due to lack of accurate determination, little is known about the association between the applied treatments and phenolic composition. The utilization of rapeseed oil press residues in novel food development requires more studies providing systematic characterization of phenolic compounds and in-depth understanding of the impact of varying treatments on phenolic profiles.

Therefore, the present study aimed at revealing the effects of lactic acid bacteria, carbohydrase, and protease on phenolic compounds and soluble proteins. The canola oil press cakes (CPC) were treated by various bacterial fermentation, enzymatic hydrolysis, and co-treatments using both bacteria and enzymes. Detailed changes in composition and contents of phenolic compounds, sugars, acids, and amino acids were monitored by using liquid chromatographic (LC), gas chromatographic (GC), and mass spectrometric (MS) methods. Another goal of the study was to obtain protein-rich fractions with varying content of phenolic compounds suitable for different type of applications. The protein fractions low in phenolic compounds with high bioavailability and optimal sensory properties may be ideal ingredients for new food products or food supplements. Our study provides novel knowledge for valorization of oil manufacturing side-streams, and supports sustainability in food industries.

2. Materials and methods

2.1. Materials and chemicals

Oil press cakes of canola (CPC; 91% of dry matters, mainly contained 27% of crude proteins, 20% of crude fats, and 35% of dietary fibres; Tian et al. 2022) were provided by Myssyfarmi Oy. (Haveri, Finland). Commercial enzymes, Protamex® (serine metalloendoprotease) and Viscozyme® (a mixture of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulose, pectinase, and xylanase) were supplied by Novozymes A/S (Bagsvaerd, Denmark). Reference standards of sugars, inorganic acids, organic acids, amino acids, phenolic acids, flavonols, and glucosinolates were purchased from Sigma-Aldrich (St. Louis, U. S. A.). Reagents of LC and MS grade were purchased from VWR International Oy (Espoo, Finland).

2.2. Bacterial species and preparation of inoculum

Species of *Lactobacillus* spp. (*L. brevis*, *L. sanfranciscensis*, and *L. paralimentarius*), *Lactiplantibacillus plantarum* (previously named as *Lactobacillus plantarum*), *Pediococcus pentosaceus*, *Bacillus subtilis* A, and *Bacillus subtilis* B were obtained from the lab collection of Center of Food and Fermentation Technologies (TFTAK, Tallinn, Estonia).

The bacteria were cultured in MRS (de Man Rogosa Sharpe, Merck, Darmstadt, Germany) and TSB (Tryptic Soy Broth, Merck, Darmstadt, Germany) medium at 30 °C (for lactic acid bacteria) or 37 °C (for the strains of *Bacillaceae* family) until the late exponential phase (ca. 20 h). The bacteria were washed twice in 1 × PBS and maintained as frozen stock in 50% glycerol at − 80 °C. The inocula were prepared from the stocks by diluting in 0.85% of NaCl solution.

2.3. Fermentation and enzymatic treatments of canola oil press cakes

Raw material of CPC was grinded into fine powders using an ultra-centrifugal mill ZM200 (Retsch GmbH, Haan, Germany) with a 0.5 mm mesh. Approximately 0.25 g of powder samples were taken for treatments and mixed with deionized water at a ratio of 1:8 (*w/v*). The CPC-water suspensions were incubated with the enzymes (Protamex®, enzymatic activity of 1.5 AU/g; or Viscozyme®, 100 FBG/ g; both declared by the manufacturer) or the bacteria (the final concentration was approximately 1×10^7 CFU/mL) in a shaker incubator (KS 3000 i control, IKA, Staufen, Germany) at 150 rpm. The condition of each treatment is given in Supplemental Table 1. The treatments were carried out at native pH of the raw material (pH 6.5) and were performed in duplicates. After treatments, the pH of the samples was adjusted to 6.5 (with 1 M NaOH) to ensure the determination of chemical profile conducted at similar condition. All samples were diluted to final volume of 10 mL with deionized water and centrifuged at 1500×*g* for 10 min. The supernatants were collected and filtered with 0.2 μm of RC filters. All liquid samples were stored at –20 °C for chemical analyses.

2.4. Analysis of dry matter content of treated supernatants

Approximately 0.5 mL of supernatants of treated samples were transferred to the watch-glass and weight accurately. The samples were dried in the thermal oven (Memmert GmbH + Co.KG, Schwabach, Germany) at 105 °C overnight until their weights reached a constant value.

2.5. Analysis of protein content in treated supernatants

The contents of protein in the supernatant samples were quantified by a Pierce™ Modified Lowry Protein Assay Kit (Thermo Scientific™, Rockford, U. S. A.), using bovine serum albumin as calibration standard. The analysis was conducted according to manufacturer's instructions.

2.6. Analysis of phenolic compounds and glucosinolates in treated supernatants

An ultra-high-performance liquid chromatography (UHPLC) equipped with a quadrupole-time-of-flight tandem mass spectrometer (Q-TOF) was applied in the identification of phenolic compounds and glucosinolates in the supernatants (Bruker Corp., Billerica, U. S. A.). The extract of CPC raw material was included in the qualitative analysis as the control group. The CPC powder (0.25 g) was extracted with 10 mL of acetone: methanol: water (7:7:6, *v/v/v*), followed by 20 min of ultrasonication (at room temperature) and 15 min of centrifugation (at 1500 × *g*, room temperature). The collected supernatant from centrifugation was evaporated to dryness with a vacuum rotary evaporator at 50 mbar, 30 °C. The residues were dissolved in 10 mL of Milli Q water and filtered with 0.2 μm of RC filters before identification. The analytical condition of UPLC-Q-TOF were described in our previous study (Tian et al., 2022). Briefly, 35 μL of treated supernatants or CPC extract were injected at a flow rate of 1 mL/min. LC separation was carried out at room temperature, using a Phenomenex Aeris peptide XB-C18 column (150 × 4.60 mm, 3.6 μm, Torrance, U. S. A.). The chromatogram was recorded at wavelength of 320 and 227 nm for phenolics and glucosinolates, respectively. The eluents of 0.35 mL/min were flown into MS system. MS full-scan and MS^2 scan were operated in a range of 20–2000 *m/z* under both negative and positive ionization modes.

The quantification of the phenolic compounds in the treated supernatants was performed on a Shimadzu LC-30AD liquid chromatograph coupled with an SPD-M20A photodiode array detector (Shimadzu Corp., Kyoto, Japan), using a same chromatographic condition described in UPLC-Q-TOF method. The studied compounds were quantified by calibration curves of sinapic acid or kaempferol (Supplemental Table 2).

2.7. Analysis of free sugars, acids, and amino acids in treated supernatants

Free-formed sugars, acids, and amino acids in the treated supernatants were analyzed with the same method described previously (Tian et al., 2022). The studied compounds in the samples were firstly reacted with Tri-Sil reagent (Pierce, Rockford, IL, U. S. A.) to form trimethylsilyl (TMS) esters before chromatographic analysis.

The derivatized compounds were identified by using a Thermo Scientific gas chromatograph-mass spectrometry (GC–MS, Thermo Fisher Scientific, Waltham, U. S. A.), equipped with an SPB-1 column (30 m × 0.25 mm i.d., 0.25 μm, Supelco, Bellefonte, U. S. A.). The characterization of the compounds was suggested by the standard NIST 08 library, and further confirmed by comparing GC retention time with those of external reference standards.

The contents of sugars, acids, and amino acids were determined with an internal standard method by using a Shimadzu GC-2010 coupled with flame ionization detector (Shimadzu corp., Kyoto, Japan). The analytical parameters were the same as applied in the GC–MS identification. Sorbitol (for sugars) and tartaric acid (for acids and amino acids) were used as internal standards. Correction factors of identified sugars, acids, and amino acids were used in quantification of the compounds. The factor of each identified compound was calculated based on concentration and area in the GC chromatogram of both internal and external standards.

2.8. Statistical analyses

The contents of proteins, phenolic compounds, sugars, acids, and amino acids in the treated supernatants were calculated on the basis of dry matter content (DM) of supernatants. The values were expressed as mean ± standard deviation. Statistical differences among data were tested with one way-ANOVA and Tukey's post hoc test ($p < 0.05$) by using IBM SPSS Statistics 26 for Windows (SPSS Inc., New York, U. S. A.). The models of principal component analysis (PCA) with full cross validation were created by Unscrambler 11 (Camo Process AS, Oslo, Norway) to investigate the chemical variation among samples. Hierarchical clustering analysis was performed using open-source platform of MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). Data was normalized with auto-scaling in average values.

3. Results and discussion

3.1. Effects of treatments on phenolic compounds in treated supernatants

3.1.1. Variation of major phenolic groups

The phenolic compounds in the supernatants of treated samples were identified mainly as the derivatives of sinapic acid, sinapine, and kaempferol based on the molecule ions and fragmentation pattern detected in mass spectrometry (Supplemental Table 3). Table 1 shows the contents of the major groups of phenolics in varying treated samples. The concentration of each identified compound in the samples are given in Supplemental Table 4–7.

The applied bacteria exhibited varying effects on the total content of identified phenolics (Table 1). The sample fermented by *L. brevis* showed the highest level of total phenolics (3822 mg/100 g DM), followed by *B. subtilis* A (3070 mg/100 g DM), *B. subtilis* B (2642 mg/100 g DM), and *L. plantarum* (2461 mg/100 g DM). Other bacteria may not contribute to release phenolic compounds from CPC material, showing similar or lower contents of phenolics compared to the control (incubated at same condition for 48 h without bacteria added). For the major groups of phenolics, all studied fermentation increased the total content of free flavonols in supernatants. Higher level of free phenolic acids was detected in the samples treated by *L. brevis*, *L. paralimentarius*, or *B. subtilis*. The use of *L. paralimentarius* or *B. subtilis* A led to a significant degradation of phenolic alkaloids (Table 1). Previous studies have confirmed that the fermentation could affect phenolic contents of CPC differently. Wang et al. (2022) observed an increase in total phenolic content during 72 h of fermentation of rapeseed meals with mixed strains of *Bacillus subtilis* and *Saccharomyces cerevisiae*. Lücke et al. (2019) reported that total content of phenolics in rapeseed press cakes was reduced by 26% after 30–48 h of fermentation using *Rhizopus microsporus* strains. Olukomaiya et al. (2020) found that 7 days of fermentation (using *Aspergillus sojae*, *Aspergillus ficuum*, and their co-cultures) led to a 14–19% decrease in total phenolic content of canola meals, but no significant difference was observed among the treatments using single *Aspergillus* strains or their co-culture. Bigdelian et al. (2021) proposed that the processing condition of fermentation influenced the effect of *Aspergillus niger* strains degrading phenolics in canola meals. The variation in moisture content and temperature may increase the total phenolic content in fermented samples. It was noticed that the data from previous studies may not be comparable to our results since the phenolic contents in the previous studies were measured by Folin-Ciocalteu reagent. The results of Folin-Ciocalteu method were likely interfered by other chemicals containing free hydroxyl groups.

Among enzyme-treated samples, the lowest content of total phenolics was found in the supernatant after 16 h of hydrolysis using 2% of Protamex (Table 1). The degradation of phenolic compounds was remarkedly enhanced by prolonging Protamex treatment. When using same dosage of the protease, extending Protamex hydrolysis from 4 h to 16 h resulted in an approximately 50% decrease in phenolics, including 44% of phenolic acids, 51% of phenolic alkaloids, and 60% of flavonol glycosides. The reduction of phenolic contents was also achieved by increasing the dosage of Protamex. The total content of phenolics was reduced by 21% after the dosage of protease was increased from 0.5% to 2%. Among the supernatants from carbohydrase treatments, the total phenolic content detected in the Viscozyme-hydrolyzed samples (2% of

Table 1

Contents of soluble proteins and major phytochemicals (on the basis of dry matter of the supernatants) in the treated supernatants.

Samples	Soluble proteins (mg/g DM)	Phenolics (mg/100 g DM)				Glucosinolates* (mg/100 g DM)
		phenolic acids	phenolic alkaloids	flavonol glycosides	total	
Fermentation						
No bacteria		934.3 ± 16.9^{def}	812.4 ± 8.5^{bc}	384.3 ± 4.8^{e}	2146.5 ± 27.5^{ef}	n.d.
L. brevis	971.7 ± 38.6^{a}	2249.0 ± 105.0^{a}	647.9 ± 64.4^{efg}	793.5 ± 14.2^{a}	3821.6 ± 157.4^{a}	n.d.
L. plantarum	771.6 ± 52.9^{bcdef}	1000.6 ± 27.1^{cde}	899.3 ± 31.3^{a}	560.6 ± 11.8^{c}	2460.5 ± 70.0^{cd}	n.d.
P. pentosaceus	667.4 ± 72.7^{bcdef}	869.1 ± 23.0^{f}	838.3 ± 8.5^{ab}	496.2 ± 8.2^{d}	2268.0 ± 40.6^{de}	n.d.
L. sanfranciscensis	701.0 ± 9.2^{bcdef}	856.2 ± 29.8^{f}	835.5 ± 31.3^{ab}	505.4 ± 22.6^{d}	2197.1 ± 83.6^{e}	n.d.
L. paralimentarius	940.9 ± 72.1^{ab}	1278.3 ± 29.9^{b}	104.5 ± 6.0^{no}	514.4 ± 14.7^{d}	1929.3 ± 47.1^{gh}	n.d.
B. subtilis A	908.1 ± 35.7^{abcd}	2198.5 ± 113.6^{a}	157.1 ± 41.7^{mn}	611.3 ± 48.0^{b}	3069.9 ± 214.4^{b}	n.d.
B. subtilis B	602.0 ± 30.9^{def}	1381.2 ± 24.2^{b}	700.5 ± 9.3^{def}	515.5 ± 7.6^{d}	2642.3 ± 30.1^{c}	n.d.
Viscozyme/Protamex hydrolysis						
0.5% Vis., 16 h	733.9 ± 61.5^{bcdef}	469.6 ± 5.1^{klm}	509.4 ± 7.7^{hi}	265.2 ± 3.6^{hijk}	1261.6 ± 12.5^{mnop}	n.d.
2% Vis., 16 h	742.9 ± 35.1^{bcdef}	542.1 ± 7.2^{ijk}	525.0 ± 14.9^{h}	245.8 ± 5.3^{ijklm}	1329.8 ± 27.8^{lmno}	n.d.
2% Vis., 4 h	728.1 ± 8.5^{bcdef}	920.5 ± 3.7^{ef}	643.0 ± 23.6^{efg}	402.0 ± 7.0^{e}	1985.2 ± 27.7^{fg}	n.d.
0.5% Pro., 16 h	930.1 ± 54.8^{abc}	705.3 ± 2.7^{g}	318.3 ± 13.3^{jk}	326.1 ± 16.1^{f}	1417.8 ± 29.1^{klm}	n.d.
2% Pro., 16 h	811.7 ± 55.9^{bcde}	612.8 ± 15.0^{ghi}	240.0 ± 9.8^{l}	198.4 ± 3.3^{n}	1115.4 ± 26.7^{pqr}	n.d.
2% Pro., 4 h	800.7 ± 5.4^{bcdef}	1092.0 ± 11.7^{b}	490.1 ± 33.6^{hi}	500.2 ± 9.7^{d}	2132.6 ± 32.1^{ef}	n.d.
Viscozyme hydrolysis + Fermentation						
2% Vis., 2 h + No bacteria		1025.5 ± 6.0^{cd}	769.3 ± 18.1^{bcd}	411.3 ± 2.8^{e}	2226.3 ± 12.3^{e}	n.d.
2% Vis., 2 h + *L. brevis*	576.9 ± 27.1^{ef}	650.4 ± 14.5^{gh}	801.4 ± 11.5^{bc}	299.7 ± 7.8^{fgh}	1772.4 ± 33.7^{hi}	n.d.
2% Vis., 2 h + *L. plantarum*	671.3 ± 50.5^{bcdef}	571.5 ± 18.5^{hijk}	708.5 ± 21.5^{de}	269.5 ± 6.7^{hij}	1549.5 ± 45.0^{jk}	n.d.
2% Vis., 2 h + *P. pentosaceus*	481.0 ± 27.0^{f}	545.4 ± 15.0^{ijk}	726.4 ± 16.8^{d}	281.2 ± 8.6^{ghi}	1570.3 ± 40.0^{jk}	n.d.
2% Vis., 2 h + *L. sanfranciscensis*	545.2 ± 29.2^{ef}	412.3 ± 13.7^{m}	610.6 ± 13.2^{g}	225.5 ± 7.4^{klmn}	1248.4 ± 34.2^{mnop}	n.d.
2% Vis., 2 h. + *L. paralimentarius*	549.0 ± 21.0^{ef}	530.9 ± 16.1^{ijkl}	710.2 ± 15.9^{de}	276.0 ± 9.9^{ghi}	1529.7 ± 42.2^{jk}	n.d.
2% Vis., 2 h + *B. subtilis* A	728.0 ± 1.9^{bcdef}	574.7 ± 21.1^{hij}	632.5 ± 24.3^{fg}	284.8 ± 9.7^{ghi}	1499.5 ± 54.8^{jkl}	n.d.
2% Vis., 24 h + *B. subtilis* A	535.6 ± 66.4^{ef}	605.9 ± 11.4^{ghi}	267.0 ± 25.8^{kl}	222.2 ± 5.3^{lmn}	1152.3 ± 37.5^{opq}	n.d.
2% Vis., 2 h + *B. subtilis* B	723.9 ± 27.5^{bcdef}	567.9 ± 13.1^{hijk}	752.1 ± 8.4^{bcd}	286.9 ± 4.1^{fghi}	1619.9 ± 25.1^{ij}	n.d.
2% Vis., 24 h + *B. subtilis* B	491.0 ± 11.9^{f}	554.9 ± 16.6^{hijk}	719.6 ± 12.9^{d}	274.8 ± 7.6^{hij}	1570.1 ± 37.6^{jk}	n.d.
Viscozyme/Protamex hydrolysis + *L. plantarum* fermentation						
0.5% Vis., 16 h + *L. plantarum*	767.5 ± 106.8^{bcdef}	302.9 ± 19.5^{n}	438.1 ± 24.6^{i}	206.0 ± 9.5^{mn}	961.6 ± 53.2^{qr}	n.d.
2% Vis., 16 h + *L. plantarum*	598.0 ± 88.6^{def}	397.8 ± 10.7^{mn}	512.7 ± 10.4^{h}	194.0 ± 4.6^{n}	1117.3 ± 23.1^{pqr}	n.d.
2% Vis., 4 h + *L. plantarum*	584.8 ± 0.7^{ef}	498.1 ± 15.8^{jklm}	614.6 ± 17.2^{g}	249.5 ± 8.2^{ijkl}	1371.9 ± 40.7^{klmn}	n.d.
0.5% Pro., 16 h + *L. plantarum*	851.1 ± 160.9^{bcde}	436.2 ± 24.8^{lm}	226.0 ± 16.9^{lm}	234.5 ± 11.4^{jklmn}	926.7 ± 53.3^{r}	n.d.
2% Pro., 16 h + *L. plantarum*	783.9 ± 33.3^{bcdef}	70.3 ± 25.8^{o}	48.3 ± 1.7^{o}	91.1 ± 10.3^{o}	224.0 ± 39.3^{s}	n.d.
2% Pro., 4 h + *L. plantarum*	617.8 ± 36.3^{cdef}	526.0 ± 19.1^{ijkl}	342.7 ± 22.7^{j}	316.9 ± 12.1^{fg}	1206.3 ± 54.0^{nop}	n.d.

*n.d. means not detected.

enzyme dosage) was significantly decreased from 1985 (after 4 h of incubation) to 1330 mg/100 g DM (after 16 h). Nevertheless, a slight increase in total content of phenolic compounds (primarily as phenolic acids and phenolic alkaloids) were detected when Viscozyme dosage was adjusted from 0.5% to 2% (both incubated for 16 h). Few studies have reported the effect of enzymatic treatment on the phenolic content of CPC. Mahajan and Dua (1998) reported that the incubation with Pepsin, Papain, Trypsin, Ficin, or Hemicellulase (40 °C, for 6 h) significantly reduced polyphenol level in rapeseed de-fatted press cakes. The highest decrease (determined by Folin-Ciocalteu method) was observed when the enzyme concentration was increased to 100 mg/g of protein.

A carbohydrase hydrolysis using 2% of Viscozyme was applied before fermentation to investigate the potential of an enzyme-bacteria co-treatment to degrade phenolic compounds in CPC. The total content of identified phenolics in the co-treated supernatants varied among bacteria (1152–1772 mg/100 g DM, Table 1). After the treatment of Viscozyme-*L. brevis* or Viscozyme-*B. subtilis* A, the total phenolic content in the supernatants was more than 50% lower than the level detected in the samples fermented by *L. brevis* or *B. subtilis* A. The co-treatments using Viscozyme and other studied bacteria caused a 21–43% decrease in total phenolic content compared to the fermentation with the same microorganism. Among the major groups of phenolics, the carbohydrase-bacteria co-treatment effectively reduced the contents of phenolic acids and glycosylated flavonols in the supernatants. In contrast, the co-treatment was less effective than the fermentation in decreasing phenolic alkaloid contents. In some cases, the co-treated samples contained higher level of phenolic alkaloids than the fermented ones. The degradation of phenolic alkaloids was enhanced by optimizing process condition of enzyme-bacteria co-treatment. The Viscozyme-*L. plantarum* co-treated samples showed a decreasing trend in the total content of phenolic alkaloids when Viscozyme hydrolysis was conducted for 2 h (709 mg/100 g DM), 4 h (615 mg/100 g DM), and 16 h (513 mg/100 g DM, Table 1), respectively. Replacing carbohydrase with protease was more effective in reducing phenolic level. After 16 h of hydrolysis with 2% of Protamex followed by 48 h of *L. plantarum* fermentation, the obtained supernatants contained only 224 mg/100 g DM of phenolics, which was 6–10 times lower than the level detected in the samples treated by either *L. plantarum* or the same dosage of Protamex.

3.1.2. Variation of individual phenolic compounds

Statistical models were created to visualize the diversity of phenolic profiles in the treated supernatants. The PCA model in Fig. 1**a** (including 81% of chemical variables in two PCs) suggested that the samples fermented with *L. brevis* or *B. subtilis* A contained high level of sinapoyl malate (SiM), sinapic acid (SiA), kaempferol dihexoside (KaHexHex), and two isomers of kaempferol-sinapoyl glycosides (KaSiHexHex 1 and KaSiHexHexHex 2). The supernatant obtained from *L. paralimentarius* fermentation was rich in kaempferol-hexoside-pentoside-hexoside (KaHexPenHex). Sinapine (SiN) and kaempferol 3-*O*-

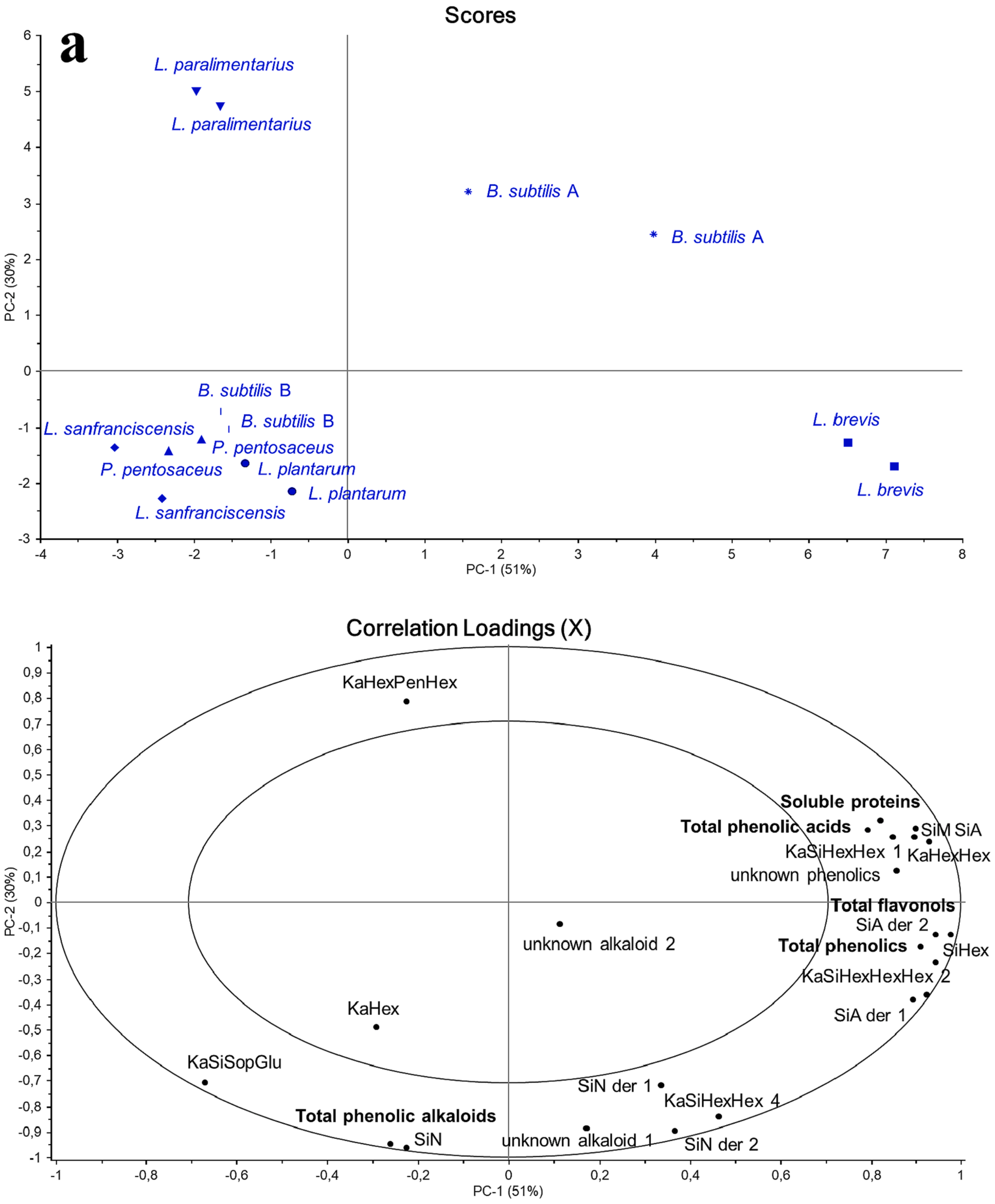


Fig. 1. PCA models for comparison among the supernatants of fermentations. The abbreviation used in the figure are: *L. brevis*, *Lactobacillus brevis*; *L. plantarum*, *Lactiplantibacillus plantarum*; *L. sanfranciscensis*, *Lactobacillus sanfranciscensis*; *L. paralimentarius*, *Lactobacillus paralimentarius*; *P. pentosaceus*, *Pediococcus pentosaceus*; *B. subtilis*, *Bacillus subtilis*; SiM, sinapoyl malate; SiA, sinapic acid; SiHex, sinapoyl hexose; SiA der, sinapic acid derivative; SiN, sinapine; SiN der, sinapine derivative; KaSiSopGlu, kaempferol 3-*O*-(sinapoyl)-sophroside-7-*O*-glucoside; KaSiHexHexHex, kaempferol-sinapoylhexoside-hexoside-hexoside; KaHexPenHex, kaempferol-hexoside-pentoside-hexoside; KaHexHex, kaempferol-hexoside-hexoside; KaSiHexHex, kaempferol-sinapoylhexoside-hexoside; and KaHex, kaempferol-hexoside.

sinapoylsophoroside-7-*O*-glucoside (KaSiSopGlu) were concentrated in the samples treated by *L. sanfranciscensis*, *L. plantarum*, *P. pentosaceus*, or *B. subtilis* B. Among these four supernatants (Fig. 1**b** where 79% of chemical variables included in PC-1 and PC-2), the *L. plantarum*-treated sample had higher contents of sinapoyl malate, sinapine, kaempferol-sinapoylhexoside-hexoside, and kaempferol dihexoside than others. Sinapic acid and a kaempferol hexoside (KaHex) were concentrated in the sample fermented by *B. subtilis* B. An unknown alkaloid (unknown alkaloid 2) was detected mainly in the *P. pentosaceus*-treated supernatant.

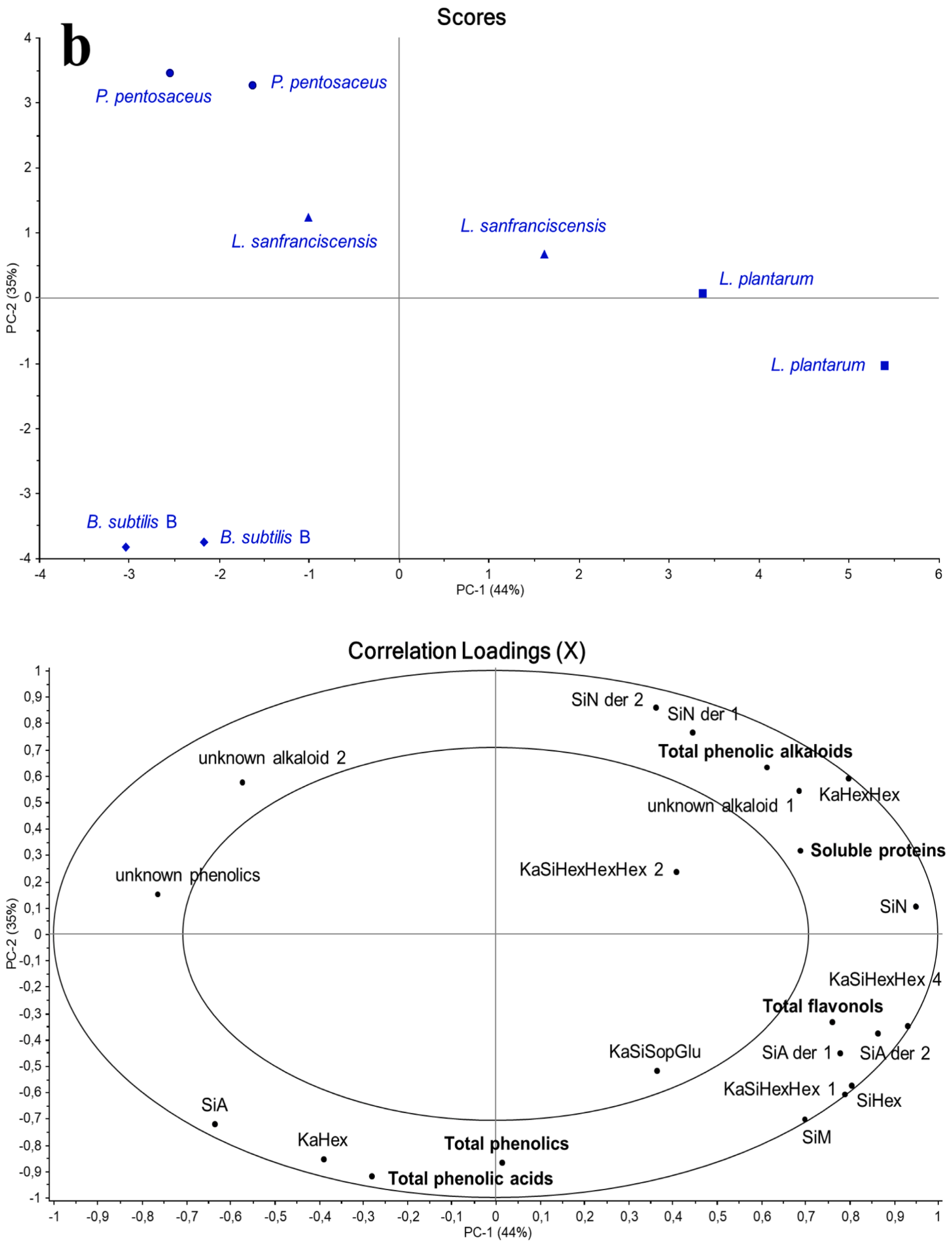


Fig. 1. (*continued*).

Fig. 2 showed the variation in phenolic profile caused by the enzymatic treatments (where 90% of chemical variables included in PC-1 and PC-2). The samples hydrolyzed by either carbohydrase or protease for 4 h had stronger correlations with most of the identified phenolic compounds than those treated for 16 h. This indicated that, compared to the nature of enzyme, the incubation time was the key factor of enzymatic hydrolysis affecting phenolic composition of the supernatants. After 4 h of enzymatic hydrolysis, the Protamex-treated sample was highly associated with kaempferol sinapoyl glycosides, sinapic acid, and its varying derivatives, whereas sinapine correlated mainly to the Viscozyme-treated one. The application of carbohydrase and protease could also lead to the presence of certain phenolics. Kaempferol dihexoside was derived through protease hydrolysis. A kaempferol-sinapoylhexoside-hexoside isomer (KaSiHexHex 2) was detected in the samples after

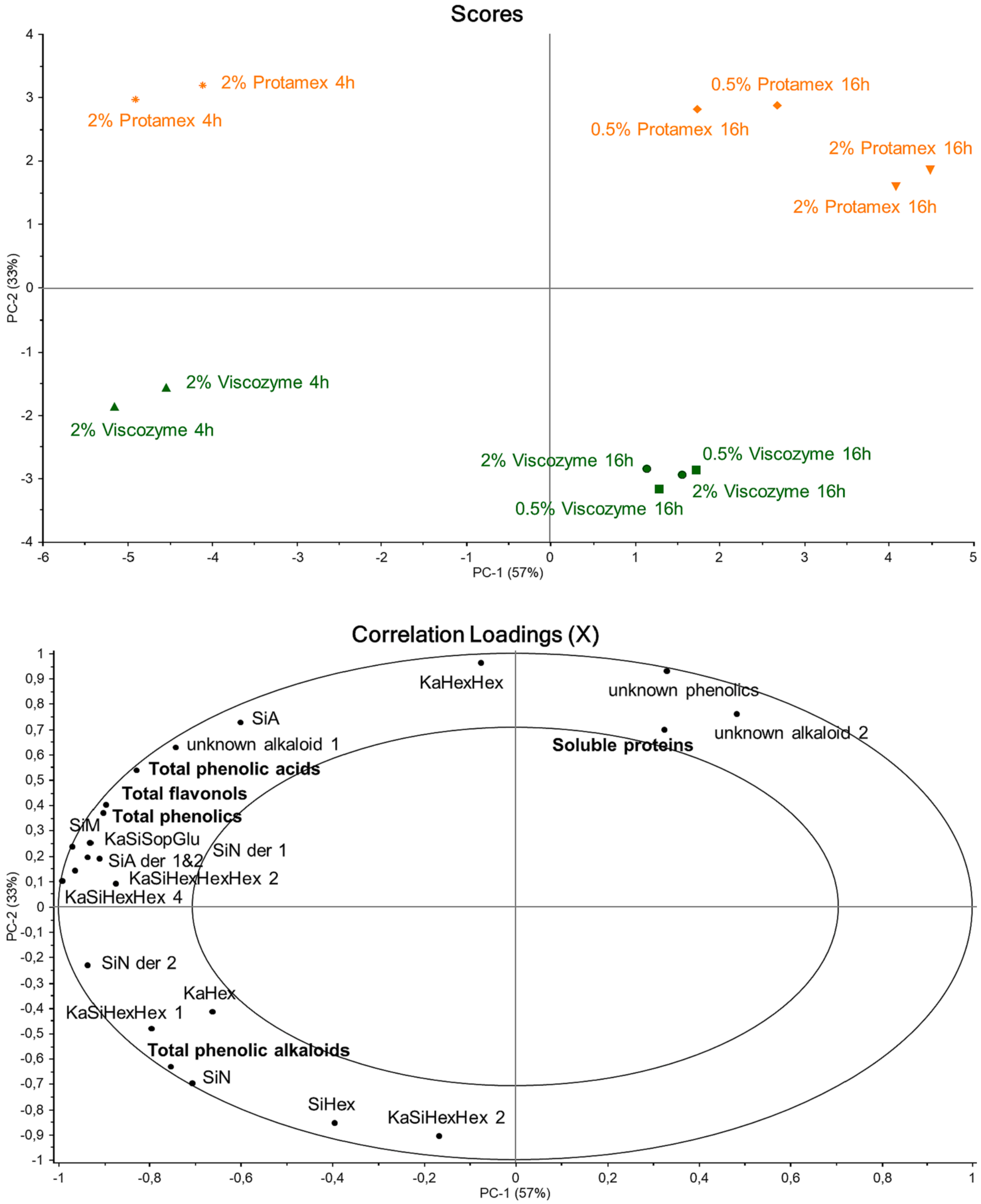


Fig. 2. PCA model for comparison among the supernatants of enzymatic hydrolyses. The abbreviation used in the figure are: SiM, sinapoyl malate; SiA, sinapic acid; SiHex, sinapoyl hexose; SiA der, sinapic acid derivative; SiN, sinapine; SiN der, sinapine derivative; KaSiSopGlu, kaempferol 3-*O*-(sinapoyl)-sophroside-7-*O*-glucoside; KaSiHexHexHex, kaempferol-sinapoylhexoside-hexoside-hexoside; KaHexHex, kaempferol-hexoside-hexoside; KaSiHexHex, kaempferol-sinapoylhexoside-hexoside; and KaHex, kaempferol-hexoside.

carbohydrase treatments. Similar distribution of phenolics in the protease- and carbohydrase-hydrolyzed supernatants was reported in our previous study, where the same CPC raw material was treated by 9% of Viscozyme (at 45 °C for 4 h) and 1% of Protamex (50 °C, 4 h), respectively (Tian et al., 2022).

In the enzyme-bacteria co-treated samples, the phenolic composition was influenced by the bacterium species and the nature of enzyme. The PCA models in Fig. 3**a** and Fig. 3**b** present the phenolic variation in the supernatants co-treated by carbohydrase (2% of enzyme dosage) and different lactic acid bacteria. As shown in the plots of Fig. 3**a** (containing 77% chemical variables in two PCs), the Viscozyme-*L. sanfranciscensis* treated supernatants showed a negative correlation with most of the detected phenolic compounds. Low level of phenolic compounds was also found in the sample after the co-treatment using Viscozyme (24 h) and *B. subtilis* A, whereas sinapic acid and two unknown compounds (unknown alkaloid 2 and unknown phenolics) were presented in this samples at high contents. Other co-treated samples had similar phenolic profiles. Yet, the supernatant of Viscozyme-*L. brevis* treatment was low in kaempferol 3-*O*-sinapoylsophoroside-7-*O*-glucoside and the Viscozyme (2 h)-*B. subtilis* A treated sample was low in kaempferol-hexoside

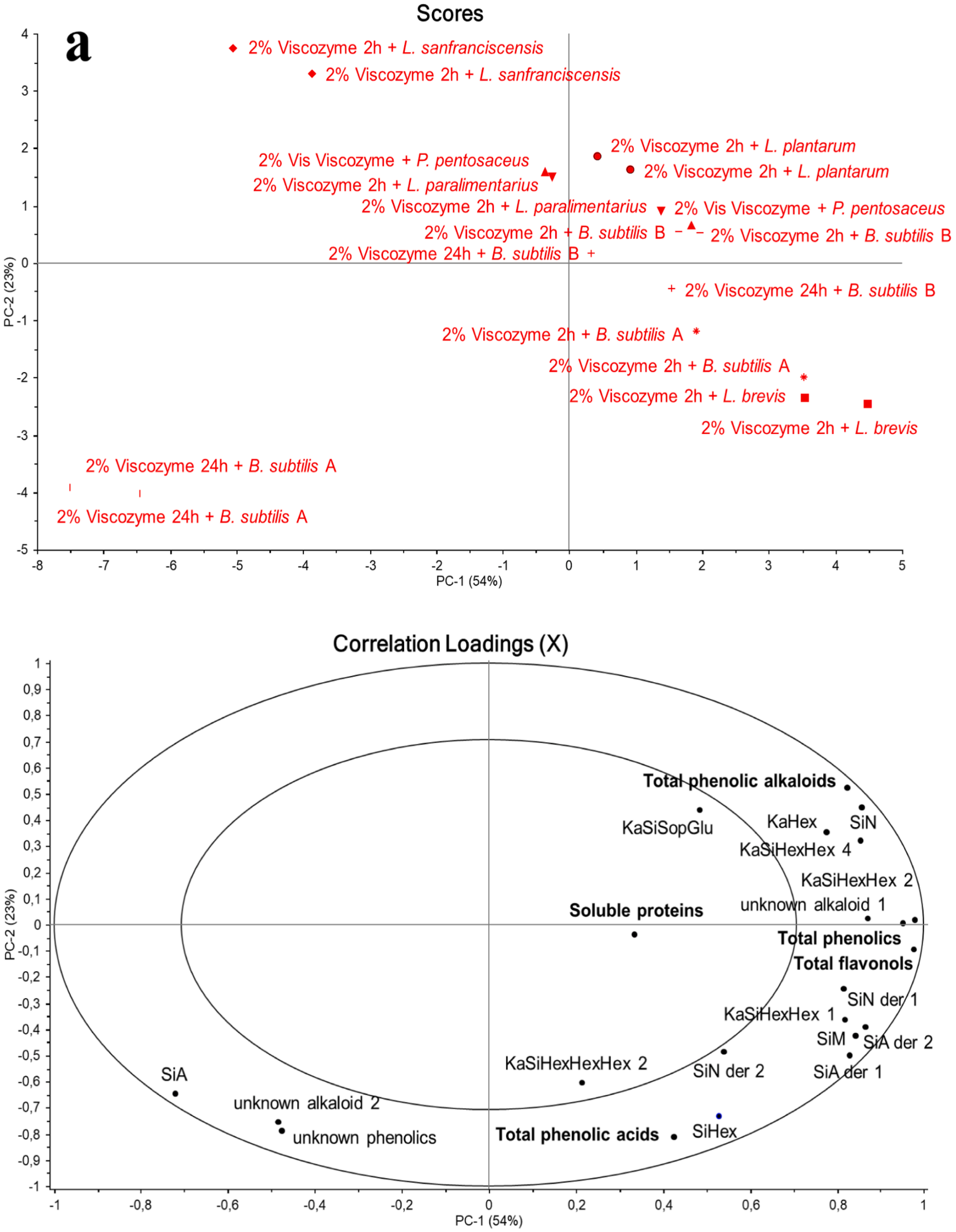


Fig. 3. PCA models for comparison among the supernatants of enzyme-bacteria co-treatments. The abbreviation used in the figure are: *L. brevis*, *Lactobacillus brevis*; *L. plantarum*, *Lactiplantibacillus plantarum*; *L. sanfranciscensis*, *Lactobacillus sanfranciscensis*; *L. paralimentarius*, *Lactobacillus paralimentarius*; *P. pentosaceus*, *Pediococcus pentosaceus*; *B. subtilis*, *Bacillus subtilis*; SiM, sinapoyl malate; SiA, sinapic acid; SiHex, sinapoyl hexose; SiA der, sinapic acid derivative; SiN, sinapine; SiN der, sinapine derivative; KaSiSopGlu, kaempferol 3-*O*-(sinapoyl)-sophroside-7-*O*-glucoside; KaSiHexHexHex, kaempferol-sinapoylhexoside-hexoside-hexoside; KaHexHex, kaempferol-hexoside-hexoside; KaSiHexHex, kaempferol-sinapoylhexoside-hexoside; and KaHex, kaempferol-hexoside.

(Fig. 3**b**, two PCs contained 71% of chemical variables). The impact of applied enzyme is revealed in the model of Fig. 3**c** (including 79% of chemical variables in PC-1 and PC-2). The supernatant of Protamex (for 16 h)-*L. plantarum* co-treatment was separated from other samples due to significantly low contents of phenolics. Other Protamex-*L. plantarum* co-treated samples were associated strongly with sinapic acid. The samples obtained after Viscozyme-*L. plantarum* treatment had a positive correlation with phenolic alkaloids, mainly as sinapine. Two isomers of kaempferol sinapoyl di-hexoside (KaSiHexHex 1 and 2) were also presented in these samples at high levels.

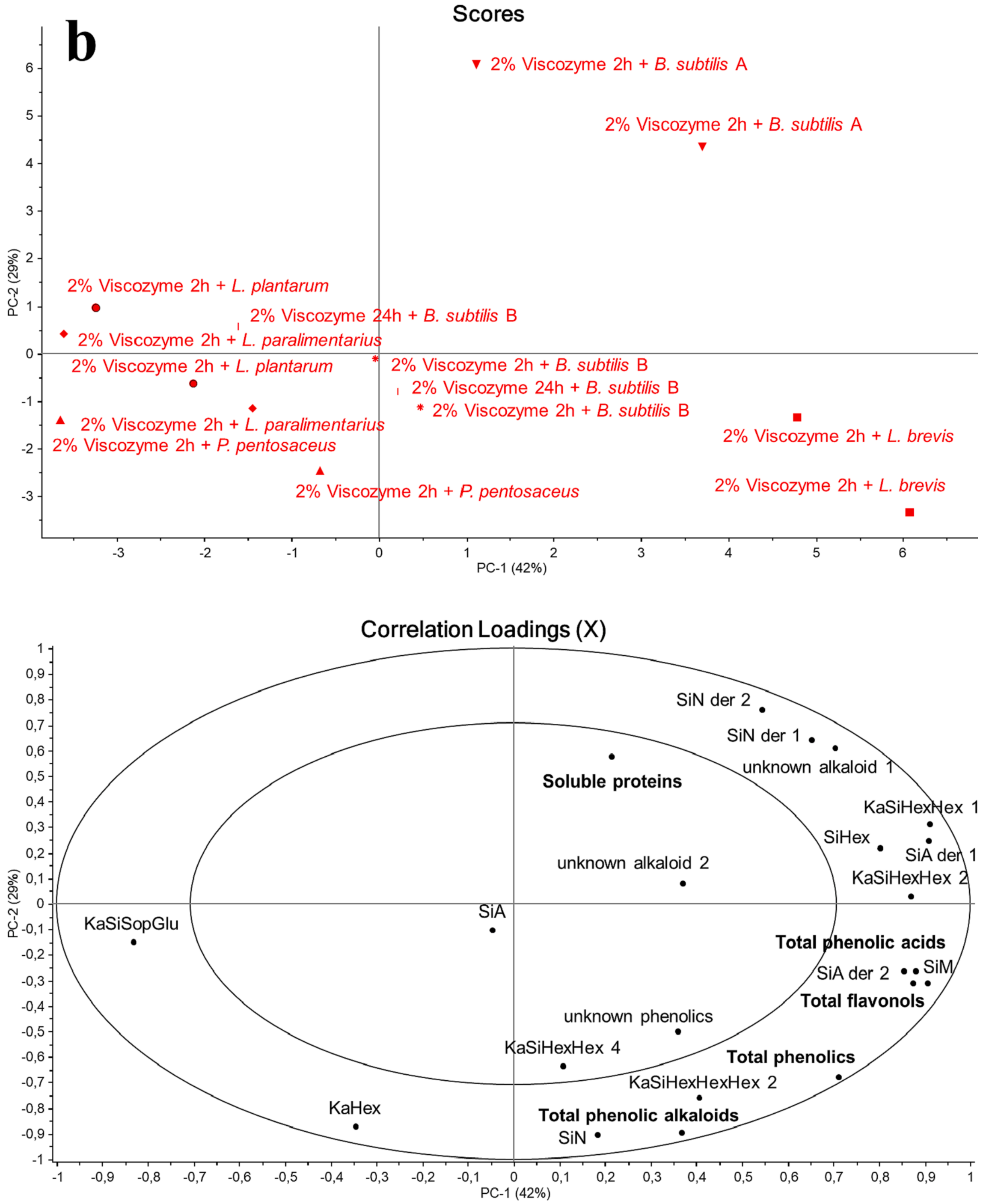


Fig. 3. (*continued*).

The impacts of fermentation and enzymatic hydrolysis on phenolics has been seldom investigated on molecule level. The available studies focused on only few yeasts or yeast-derived enzymes, and limited numbers of phenolics. Laguna et al. (2019) monitored the contents of certain phenolics in enzyme-treated rapeseed meals with HPLC. During 4 h of hydrolysis using *Aspergillus niger*-derived carboxylic esters hydrolase, a steady increase in sinapic acid contents was observed, whereas the level of sinapine was reduced significantly. Bigdelian et al. (2021) extracted phenolic compounds from *Aspergillus niger*-fermented canola meals and analyzed them with HPLC. The chromatographic results showed that syringic acid, sinapic acid, and ferulic acid were present in the fermented samples at remarkably high contents. High level of kaempferol aglycone was also detected after fermentation. This might be the result of degradation of kaempferol glycosides, triggered by some *Aspergillus niger*-produced glycosidases.

Aside from the changes among different supernatants, a variation in phenolic composition was also found between CPC raw material and treated samples (Supplemental Fig. 1). Certain phenolics originally presented in the CPC raw material, such as kaempferol 3-*O*-sophoroside-7-*O*-glucoside (compound 4), sinapoyl glucose (compound 5), kaempferol-sinapoylhexoside-hexoside-hexoside isomers (compounds 12 and 16), a sinapine derivative (compound 14), disinapoyl hexose-hexose (compound 17), and disinapoyl hexose isomers (compounds 18 and 19), were not detected in the treated samples. Nine phenolic

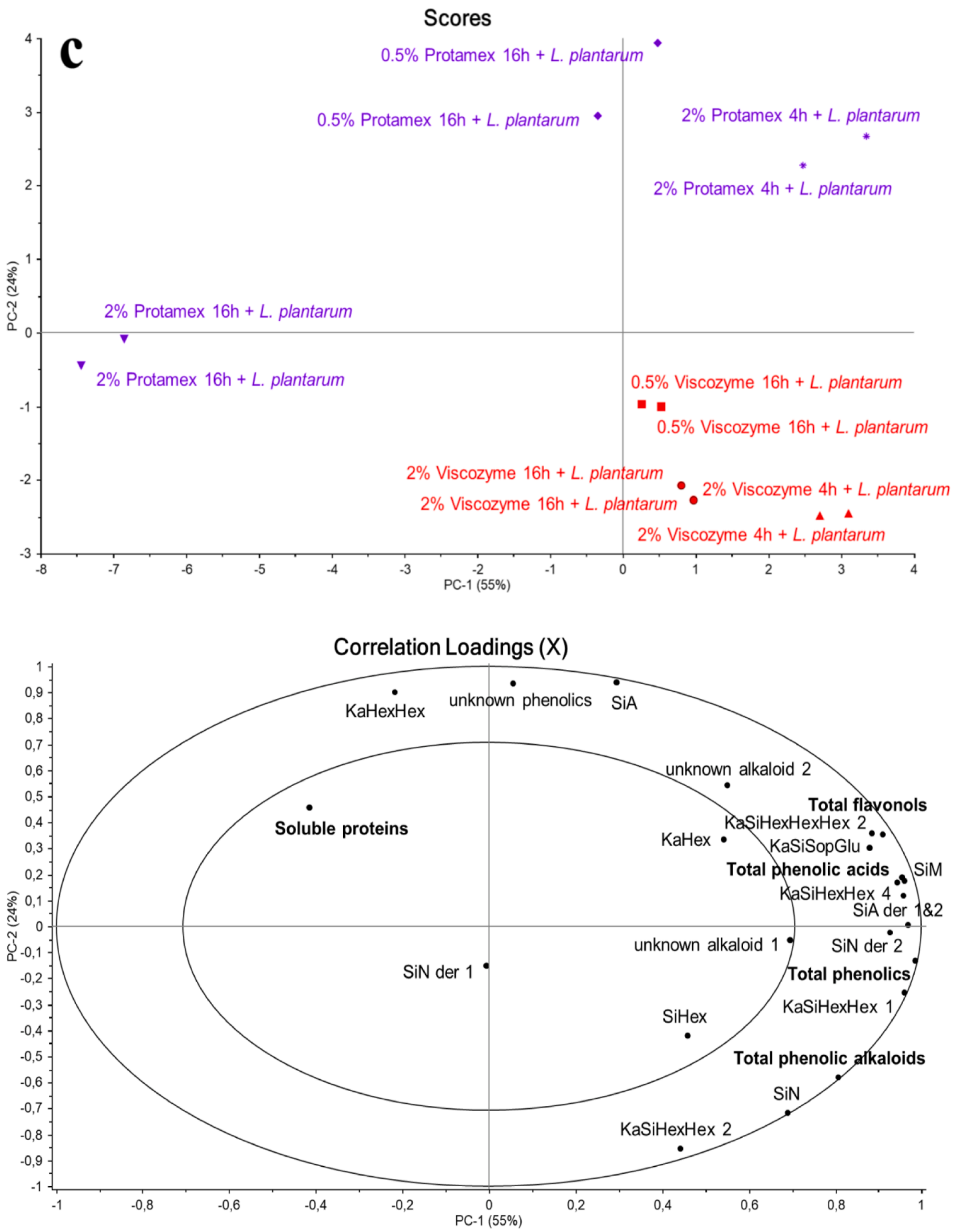


Fig. 3. (*continued*).

compounds (compounds P1–P9) were generated during treatment, mostly as kaempferol glycosides. However, the variation in phenolic profile might have not been due to the applied lactic acid bacteria and enzymes. Similar changes were also found in the samples incubated at same condition without any bacteria and enzymes added.

3.2. Effects of treatments on protein content in treated supernatants

Table 1 shows protein content on the basis of dry matter of different supernatants. Among all the fermented samples, the one treated with *L. brevis* had the highest level of soluble proteins (972 mg/g DM). Higher content of soluble proteins was also found in the samples treated by *L. paralimentarius* (941 mg/g DM) and *B. subtilis* A (908 mg/g DM). In comparison with these three species, other lactic acid bacteria were less effective in solubilizing proteins from CPC material since they led to lower soluble protein levels. The fermentation using certain *Lactobacillus* species or *Bacillus subtilis* was a feasible approach of enriching proteins in canola oil press residues as discussed previously. The enrichment of proteins was ascribed to a wide range of enzymes produced by the applied bacteria, such as phytase, carbohydrase, protease, and lipase, which help to release the protein from cellular structures (Olukomaiya et al., 2019). Other bacteria and yeasts were also used in CPC fermentation, including *Enterococcus faecium*, *Saccharomyces cerevisiae*, *Aspergillus* spp., *Candida utilis*, *Rhizopus microspores*, and *Mortierella alpine* strains. Employing these bacteria and yeasts contributed to an increase in the crude protein content. This positive effect was highly dependent on the species of the microorganism applied (Ferreira et al., 2020; Lücke et al., 2019; Olukomaiya et al., 2019, 2020). However, few studies have compared the ability of microorganisms enriching proteins under the same culture conditions. The results from previous studies were not comparable since the fermentations in these studies were performed in different conditions.

For enzymatic hydrolysis, the soluble protein content was influenced by the nature of enzyme (Table 1). The Protamex-treated supernatants were rich in soluble proteins (801–930 *vs* 728–743 mg/g DM) compared to Viscozyme-hydrolyzed ones. Previous research confirmed that carbohydrase showed lower effectivity than protease in increasing total content of proteins (Niu et al., 2012; Rommi, 2016; Tian et al., 2022). Viscozyme, as a mixture of carbohydrases, may have no direct effect in extracting proteins. Sari et al. (2015) suggested that carbohydrase degraded biomass cell wall and released protein, but did not appear to increase yields of protein extraction. In our previous research, high ratio of solubilized proteins was found in the Viscozyme-treated supernatant; however, compared to other enzyme-treated supernatants, the total protein content in this sample was lower (Tian et al., 2022).

In comparison with fermentation and enzymatic hydrolysis, most of the studied enzyme-bacteria co-treatments showed a negative impact on solubilizing proteins (Table 1). Introducing a 2 h of carbohydrase treatment (2% of Viscozyme added) before bacterial fermentation resulted in a 13–51% of reduction in soluble protein content compared to the fermentation using same bacterium species. Among carbohydrase-bacteria co-treated samples, higher level of soluble proteins was found in the supernatants treated by both Viscozyme and *B. subtilis*. For enzyme-bacteria co-treatment, replacing carbohydrase with protease might enhance the extractability of proteins, since Protamex-*L. plantarum* co-treated supernatants contained more solubilized proteins than Viscozyme-*L. plantarum* treated ones (618–851 *vs* 585–768 mg/g DM). Moreover, the changes in process condition of enzyme-bacteria co-treatment also led to the variation in soluble protein contents. The use of 0.5% of enzymes (for either protease or carbohydrase) and 16 h of enzymatic incubation might contribute to the accumulation of solubilized proteins in supernatants.

An overall comparison of the treated supernatants is given by the clustering heatmap in Fig. 4. Twenty-six samples were classified into two clusters based on the contents of soluble proteins and phenolic compounds. The red zones in the map represented high amounts of the compound. The first cluster consisted of the samples after fermentation or after 4 h of enzymatic hydrolysis. These supernatants contained high contents of phenolic compounds, mainly as sinapic acid and its derivatives, as well as most of identified kaempferol glycosides. The samples fermented by *L. brevis*, *L. paralimentarius*, or *B. subtilis* A contained higher levels of solubilized proteins than other samples in this cluster. For the co-treatments using Viscozyme and lactic acid bacteria, sinapine and an isomer of kaempferol sinapoyl di-hexoside (KaSiHexHex 2) were rich in the obtained supernatant, while other phenolic compounds were mostly presented at low levels. High amount of soluble proteins was found in some Viscozyme-*L. plantarum* treated samples. The co-treatment combining 16 h of Protamex hydrolysis and 48 h of *L. plantarum* fermentation was effective in degrading phenolic compounds. As the main factor responsible for bitter taste and dark color of protein extracts, sinapine was not detected in the supernatant after the co-treatment. Other identified phenolics were present at considerably lower contents. This co-treated supernatant also contained a medium level of soluble proteins in comparison with other samples.

3.3. Effects of treatments on other components in treated supernatants

As shown in Supplemental Table 8, the profiles of other nutrients in

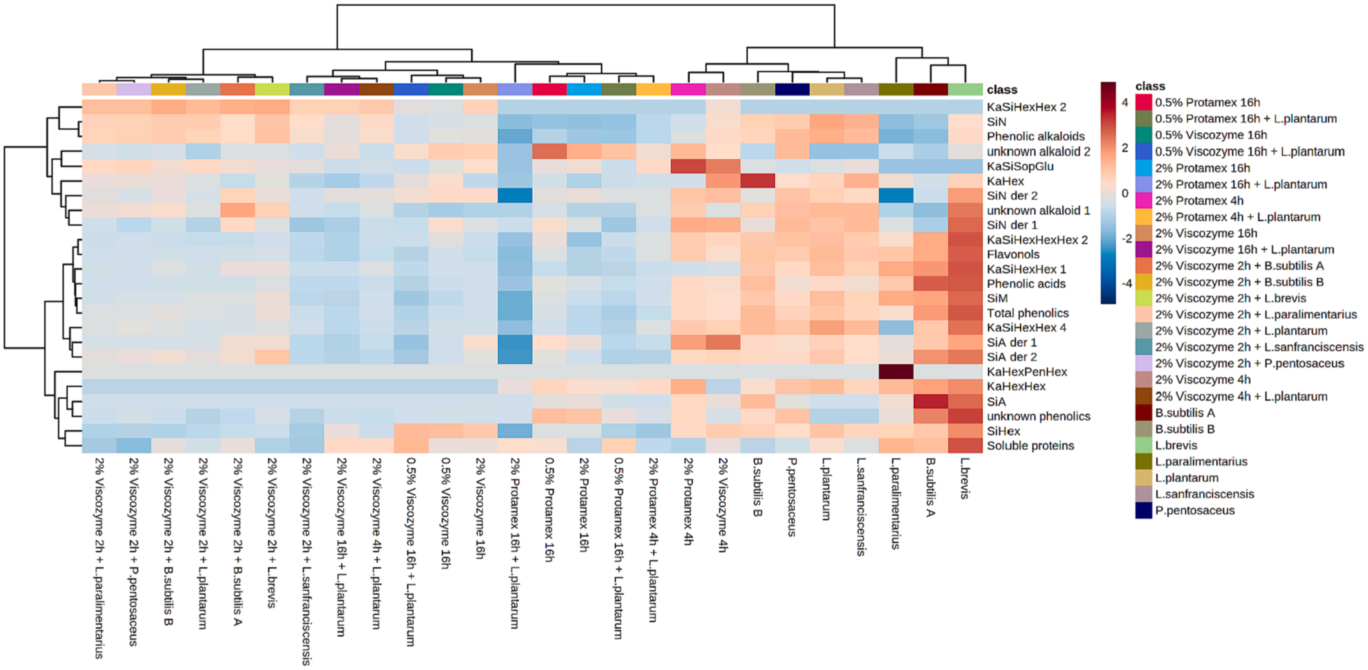


Fig. 4. Hierarchical clustering analysis of soluble proteins and phenolic compounds in the treated supernatants. The abbreviation used in the figure are: *L. brevis*, *Lactobacillus brevis*; *L. plantarum*, *Lactiplantibacillus plantarum*; *L. sanfranciscensis*, *Lactobacillus sanfranciscensis*; *L. paralimentarius*, *Lactobacillus paralimentarius*; *P. pentosaceus*, *Pediococcus pentosaceus*; *B. subtilis*, *Bacillus subtilis*; SiM, sinapoyl malate; SiA, sinapic acid; SiHex, sinapoyl hexose; SiA der, sinapic acid derivative; SiN, sinapine; SiN der, sinapine derivative; KaSiSopGlu, kaempferol 3-*O*-(sinapoyl)-sophroside-7-*O*-glucoside; KaSiHexHexHex, kaempferol-sinapoylhexoside-hexoside-hexoside; KaHexPenHex, kaempferol-hexoside-pentoside-hexoside; KaHexHex, kaempferol-hexoside-hexoside; KaSiHexHex, kaempferol-sinapoylhexoside-hexoside; and KaHex, kaempferol-hexoside.

the treated CPC samples were also affected by applied treatments. The statistical model in Fig. 5 presents the distribution of sugars, acids, and amino acids in the supernatants of enzymatic hydrolyses and enzyme-*L. plantarum* co-treatments (where two PCs contained 88% of chemical variables). The usage of protease enhanced the release of free amino acids into the supernatants. The sample co-treated by Protamex and *L. plantarum* correlated strongly with proline (Pro), glycine (Gly), and aspartic acid (AsA). The Protamex-hydrolyzed supernatant was highly related to serine (Ser) and tyrosine (Tyr). Free sugars were highly associated with the addition of carbohydrase. The supernatant of Viscozyme hydrolysis contained high levels of fructose (Fru) and glucose (Glu), whereas arabinose (Ara), xylose (Xyl), and galacturonic acid

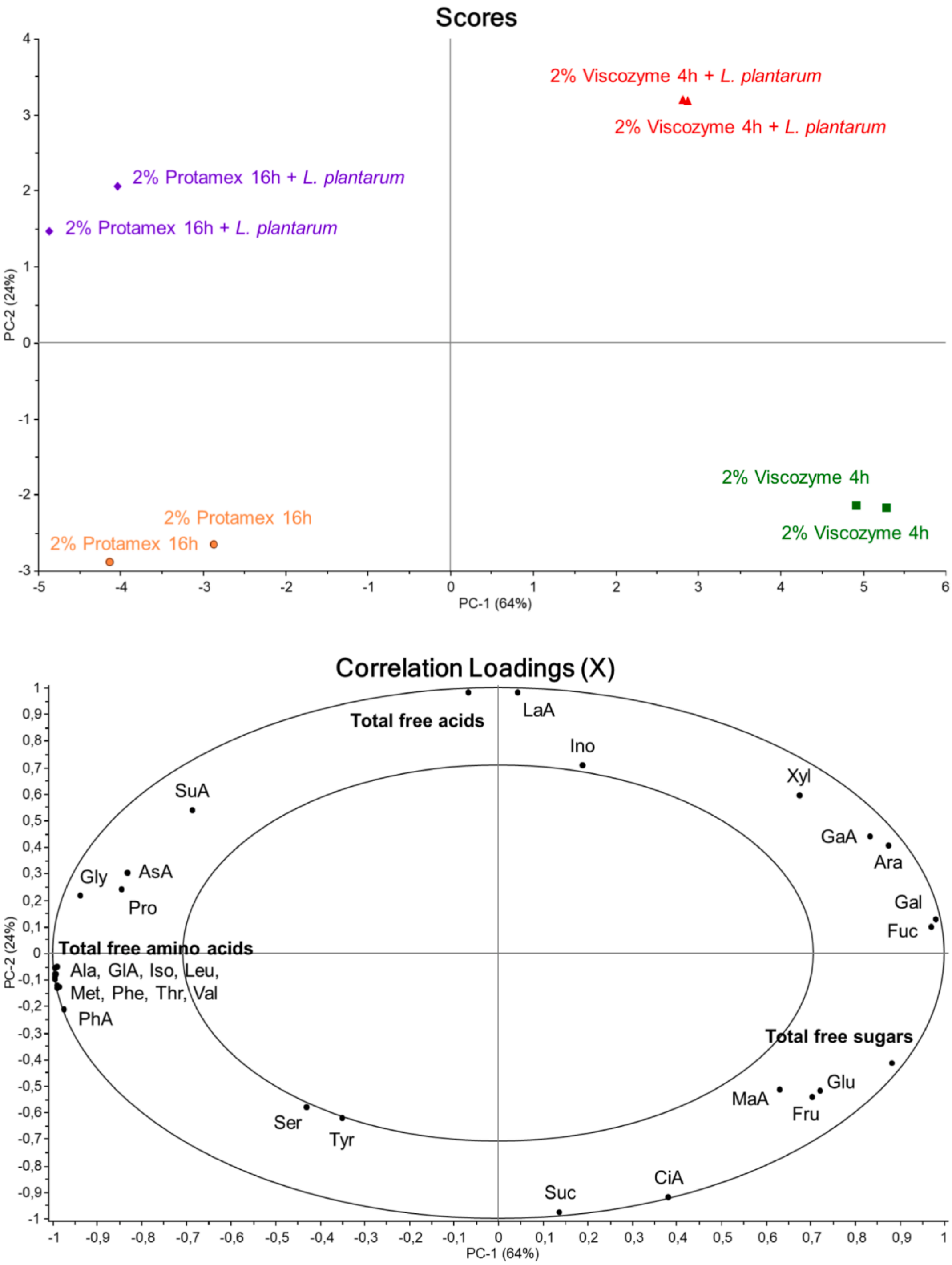


Fig. 5. PCA model for variation in free sugars, acids, and amino acids among the supernatants of enzyme hydrolyses and enzyme-*L. plantarum* co-treatments. The abbreviation used in the figure are: *L. plantarum*, *Lactiplantibacillus plantarum*; Ara, arabinose; Fuc, fucose; Xyl, xylose; Fru, fructose; Gal, galactose; Glu, glucose; Suc, sucrose; GaA, galacturonic acid; Man, mannitol; Ino, myo-inositol; PhA, phosphoric acid; LaA, lactic acid; SuA, succinic acid; MaA, malic acid; CiA, citric acid; Ala, alanine; Gly, glycine; Val, valine; Leu, leucine; Pro, proline; Iso, isoleucine; Ser, serine; Thr, threonine; GlA, glutamic acid; AsA, aspartic acid; Met, methionine; Phe, phenylalanine; and Tyr, tyrosine.

(GaA) concentrated in Viscozyme-*L. plantarum* co-treated one. Among identified acids, lactic acid (LaA) was rich in the sample after the enzyme-*L. plantarum* co-treatment. The addition of Protamex was responsible for the increase in phosphoric acid (PhA) content. The Protamex-*L. plantarum* treated sample had a strong correlation with succinic acid (SuA). Malic acid (MaA) and citric acid (CiA) were presented mostly in the Viscozyme-hydrolyzed supernatant.

Glucosinolates, as the major group of phytochemicals of CPC (Tian et al., 2022), were not detected in all treated CPC samples (Table 1, Supplemental Fig. 1). Previous research suggested that both fermentation and enzymatic hydrolysis were effective in degrading glucosinolates. The content of glucosinolates in rapeseed/canola oil press residues was decreased by 16–95% after the fermentation using *Lactobacillus delbrueckii*, *Bacillus subtilis*, *Actinomucor elegans*, *Rhizopus microspores*, or *Aspergillus* spp. strains (Cheng et al., 2022). Mahajan and Dua (1998) found that hemicellulase effectively reduced glucosinolate content in rapeseed de-fatted press cakes. Nevertheless, in our study, the applied lactic acid bacteria, carbohydrase, and protease were not responsible for the absence of glucosinolates, since glucosinolates was not found in the control sample either (Table 1). The degradation of glucosinolates might have been due to myrosinase remained in treated CPC samples. This endogenous enzyme was not inactivated before the treatments were performed, since the common deactivation approach (heating at 70 °C or in boiling water bath) might have negative influence on certain nutrients and bioactive compounds of CPC (Dijkstra et al., 2003). Moreover, isothiocyanates, the breakdown products of glucosinolates formed by myrosinase, were not detected in the MS spectra of the samples. This might be due to the fact that the applied LC method was not suitable for analyzing isothiocyanates. For isothiocyanate characterization, a pre-column derivatization was commonly required (Andini et al., 2020; Pilipczuk et al., 2017). Andini et al. (2020) identified 15 isothiocyanates from methanol extracts of *Sinapis alba*, *Brassica napus*, and *Brassica juncea*. These compounds were first reacted with *N*-acetyl-L-cysteine reagent, of which the derivatives were identified using reversed-phase LC-MS.

4. Conclusion

Twenty-nine treatments were applied in our study, including fermentation with diverse lactic acid bacteria, enzymatic hydrolysis with carbohydrase and protease, and enzyme-bacteria co-treatments. In the obtained liquid fractions, phenolic compounds, glucosinolates, sugars, acids, and amino acids were thoroughly investigated at molecular level. A clear association between the chemical composition of CPC and the applied treatments was revealed. The co-treatment using protease and *Lactiplantibacillus plantarum* showed excellent capacity in degrading phenolic compounds and solubilizing proteins. Based on our best knowledge, this is the first research that includes such a large number of treatments and systematically investigates the impact of the treatments on nutrients and phytochemicals in CPC. Our results provide a novel option of converting oil press residues into value-added food products. Retaining certain amounts of phenolic compounds would provide the CPC protein products with health-beneficial functions. By optimizing the process parameters of bioprocessing using enzymes and fermentation, the contents of phenolics present in the fraction can be maintained at appropriate level that may not cause unpleasant taste and flavor. The definition of this level requires a thorough sensorial evaluation with trained panelists. Applying such fractions in foods will fortify the nutrient level and increase the health-promoting effects of food. Future studies should combine bioprocessing with sensory evaluation in order to optimize the processing methods.

CRediT authorship contribution statement

Ye Tian: Methodology, Investigation, Funding acquisition, Writing – original draft, Writing – review & editing. **Ying Zhou:** Formal analysis, Investigation, Writing – original draft. **Marie Kriisa:** Methodology, Investigation, Writing – review & editing. **Maret Anderson:** Methodology, Investigation. **Oskar Laaksonen:** Conceptualization, Investigation, Writing – review & editing, Project administration. **Mary-Liis Kütt:** Conceptualization, Writing – review & editing. **Maike Föste:** Conceptualization, Writing – review & editing. **Małgorzata Korzeniowska:** Conceptualization, Writing – review & editing. **Baoru Yang:** Conceptualization, Investigation, Funding acquisition, Writing – review & editing, Supervision, Project administration.

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.

Data availability

Data will be made available on request.

Acknowledgements

MSc. Juuso Korpela, MSc. Mikael Fabritius, and PhD Annelie Damerau (University of Turku) are appreciated for assistance in analyses of sugars, acids, amino acids, glucosinolates, and phenolic compounds. We thank Anastassia Taivosalo (TFTAK) for the help with the CPC treatment experiments and analyses.

Funding

This research belongs to the project “PROWASTE: Protein-fibre biorefinery for scattered material streams”. The project was initiated by the ERA-NET Cofund FACCE SURPLUS (in the frame of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI). FACCE SURPLUS has received funding from the European Union’s Horizon 2020 research and innovation programme (under grant agreement No. 652615, Finnish Ministry of Agriculture and Forestry participated as a member of FACCE Governing Board). The study of phenolics and glucosinolates has been also funded by Turku University Foundation, Finland (identification No. 080888).

Appendix. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.135339.

References

Amiri, M., Raeisi-Dehkordi, H., Sarrafzadegan, N., Forbes, S. C., & Salehi-Abargouei, A. (2020). The effects of canola oil on cardiovascular risk factors: A systematic review and meta-analysis with dose-response analysis of controlled clinical trials. *Nutrition, Metabolism and Cardiovascular Diseases, 30*(12), 2133–2145. https://doi.org/10.1016/j.numecd.2020.06.007

Ancuţa, P., & Sonia, A. (2020). Oil press-cakes and meals valorization through circular economy approaches: A review. *Applied Sciences, 10*(21), 7432. https://doi.org/10.3390/app10217432

Andini, S., Araya-Cloutier, C., Sanders, M., & Vincken, J.-P. (2020). Simultaneous analysis of glucosinolates and isothiocyanates by reversed-phase ultra-high-performance liquid chromatography− electron spray ionization−tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 68*, 3121–3131. https://doi.org/10.1021/acs.jafc.9b07920

Arrutia, F., Binner, E., Williams, P., & Waldron, K. W. (2020). Oilseeds beyond oil: Press cakes and meals supplying global protein requirements. *Trends in Food Science and Technology, 100*, 88–102. https://doi.org/10.1016/j.tifs.2020.03.044

Baker, P. W., & Charlton, A. (2020). A comparison in protein extraction from four major crop residues in Europe using chemical and enzymatic processes-a review. *Innovative Food Science and Emerging Technologies, 59*, Article 102239. https://doi.org/10.1016/j.ifset.2019.102239

Bigdelian, E., Labbafi, M., Safari, M., Rezaei, K., & Razavi, S. H. (2021). Investigating qualitative parameters and antioxidant activity of phenolic compounds extracted from canola meal using solid-state fermentation by *Aspergillus niger*. *Journal of Food* *and Bioprocess Engineering, 4*(1), 1–10. https://doi.org/10.22059/JFABE.2020.306126.1056

Cheng, H., Liu, X., Xiao, Q., Zhang, F., Liu, N., Tang, L., … Jiang, X. (2022). Rapeseed meal and its application in pig diet: A Review. *Agriculture, 12*(6), 849. https://doi.org/10.3390/agriculture12060849

Chew, S. C. (2020). Cold-pressed rapeseed (*Brassica napus*) oil: Chemistry and functionality. *Food Research International, 131*, Article 108997. https://doi.org/10.1016/j.foodres.2020.108997

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., & Rosicka-Kaczmarek, J. (2020). Canola/rapeseed protein–nutritional value, functionality and food application: A review. *Critical Reviews in Food Science and Nutrition, 1–21*. https://doi.org/10.1080/10408398.2020.1809342

Clarke, D. B. (2010). Glucosinolates, structures and analysis in food. *Analytical Methods, 2*(4), 310–325. https://doi.org/10.1039/b9ay00280d

Dijkstra, D. S., Linnemann, A. R., & Van Boekel, T. A. J. S. (2003). Towards sustainable production of protein-rich foods: Appraisal of eight crops for western Europe. PART II: Analysis of the technological aspects of the production chain. *Critical Reviews in Food Science and Nutrition, 43*(5), 481–506. https://doi.org/10.1080/10408690390246332

Ferreira, M., Fernandes, H., Peres, H., Oliva-Teles, A., Belo, I., & Salgado, J. M. (2020). Bio-enrichment of oilseed cakes by *Mortierella alpina* under solid-state fermentation. *LWT, 134*, Article 109981. https://doi.org/10.1016/j.lwt.2020.109981

Foreign Agricultural Service United States Department of Agriculture. (2021). Oilseeds: World markets and trade. *Foreign Agricultural Service/USDA*. https://www.fas.usda.gov/data/oilseeds-world-markets-and-trade.

Ghobadi, S., Hassanzadeh-Rostami, Z., Mohammadian, F., Zare, M., & Faghih, S. (2019). Effects of canola oil consumption on lipid profile: A systematic review and meta-analysis of randomized controlled clinical trials. *Journal of the American College of Nutrition, 38*(2), 185–196. https://doi.org/10.1080/07315724.2018.1475270

Hald, C., Dawid, C., Tressel, R., & Hofmann, T. (2019). Kaempferol 3-*O*-(2-*O*-sinapoyl-*β*-sophoroside) causes the undesired bitter taste of canola/rapeseed protein isolates. *Journal of Agricultural and Food Chemistry, 67*(1), 372–378. https://doi.org/10.1021/acs.jafc.8b06260

Laguna, O., Odinot, E., Bisotto, A., Baréa, B., Villeneuve, P., Sigoillot, J. C., … Lecomte, J. (2019). Release of phenolic acids from sunflower and rapeseed meals using different carboxylic esters hydrolases from *Aspergillus niger*. *Industrial Crops and Products, 139*, Article 111579. https://doi.org/10.1016/j.indcrop.2019.111579

Latté, K. P., Appel, K. E., & Lampen, A. (2011). Health benefits and possible risks of broccoli - An overview. *Food and Chemical Toxicology, 49*(12), 3287–3309. https://doi.org/10.1016/j.fct.2011.08.019

Lücke, F. K., Fritz, V., Tannhäuser, K., & Arya, A. (2019). Controlled fermentation of rapeseed presscake by *Rhizopus*, and its effect on some components with relevance to human nutrition. *Food Research International, 120*, 726–732. https://doi.org/10.1016/j.foodres.2018.11.031

Mahajan, A., & Dua, S. (1998). Improvement of functional properties of rapeseed (*Brassica campestris* var toria) meal by reducing antinutritional factors employing enzymatic modification. *Food Hydrocolloids, 12*(3), 349–355. https://doi.org/10.1016/S0268-005X(98)00030-7

Morya, S., Menaa, F., Jiménez-López, C., Lourenço-Lopes, C., BinMowyna, M. N., & Alqahtani, A. (2022). Nutraceutical and pharmaceutical behavior of bioactive compounds of miracle oilseeds: An overview. *Foods, 11*(13), 1824. https://doi.org/10.3390/foods11131824

Nićiforović, N., & Abramovič, H. (2014). Sinapic acid and its derivatives: Natural sources and bioactivity. *Comprehensive Reviews in Food Science and Food Safety, 13*(1), 34–51. https://doi.org/10.1111/1541-4337.12041

Niu, Y. X., Li, W., Zhu, J., Huang, Q., Jiang, M., & Huang, F. (2012). Aqueous enzymatic extraction of rapeseed oil and protein from dehulled cold-pressed double-low rapeseed cake. *International Journal of Food Engineering, 8*(3). https://doi.org/10.1515/1556-3758.2530

Olukomaiya, O., Fernando, C., Mereddy, R., Li, X., & Sultanbawa, Y. (2019). Solid-state fermented plant protein sources in the diets of broiler chickens: A review. *Animal Nutrition, 5*(4), 319–330. https://doi.org/10.1016/j.aninu.2019.05.005

Olukomaiya, O., Fernando, W. C., Mereddy, R., Li, X., & Sultanbawa, Y. (2020). Solid-state fermentation of canola meal with *Aspergillus sojae*, *Aspergillus ficuum* and their co-cultures: Effects on physicochemical, microbiological and functional properties. *LWT, 127*, Article 109362. https://doi.org/10.1016/j.lwt.2020.109362

Ozdal, T., Capanoglu, E., & Altay, F. (2013). A review on protein-phenolic interactions and associated changes. *Food Research International, 51*(2), 954–970. https://doi.org/10.1016/j.foodres.2013.02.009

Pilipczuk, T., Kusznierewicz, B., Chmiel, T., Przychodzeń, W., & Bartoszek, A. (2017). Simultaneous determination of individual isothiocyanates in plant samples by HPLC-DAD-MS following SPE and derivatization with *N*-acetyl-*L*-cysteine. *Food Chemistry, 214*, 587–596. https://doi.org/10.1016/J.FOODCHEM.2016.07.125

Pourrajab, B., Sharifi-Zahabi, E., Soltani, S., Shahinfar, H., Shidfar, F., & Sharifi-Zahabi, E. (2022). Comparison of canola oil and olive oil consumption on the serum lipid profile in adults: A systematic review and meta-analysis of randomized controlled trials. *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408398.2022.2100314

Rommi, K. (2016). *Enzyme-aided recovery of protein and protein hydrolyzates from rapeseed cold-press cake*. University of Helsinki.

Sari, Y. W., Mulder, W. J., Sanders, J. P. M., & Bruins, M. E. (2015). Towards plant protein refinery: Review on protein extraction using alkali and potential enzymatic assistance. *Biotechnology Journal, 10*(8), 1138–1157. https://doi.org/10.1002/biot.201400569

Sikorska-Zimny, K., & Beneduce, L. (2021). The metabolism of glucosinolates by gut microbiota. *Nutrients, 13*(8), 2750. https://doi.org/10.3390/nu13082750

Thinh Nguyen, V. P., Stewart, J., Lopez, M., Ioannou, I., & Allais, F. (2020). Glucosinolates: Natural occurrence, biosynthesis, accessibility, isolation, structures, and biological activities. *Molecules, 25*(19). https://doi.org/10.3390/molecules25194537

Tian, Y., Kriisa, M., Föste, M., Kütt, M.-L., Zhou, Y., Laaksonen, O., & Yang, B. (2022). Impact of enzymatic pre-treatment on composition of nutrients and phytochemicals of canola (*Brassica napus*) oil press residues. *Food Chemistry, 387*, Article 132911. https://doi.org/10.1016/j.foodchem.2022.132911

Verni, M., Rizzello, C. G., & Coda, R. (2019). Fermentation biotechnology applied to cereal industry by-products: Nutritional and functional insights. *Frontiers in Nutrition, 6*, 42. https://doi.org/10.3389/fnut.2019.00042

Wang, R., & Guo, S. (2021). Phytic acid and its interactions: Contributions to protein functionality, food processing, and safety. *Comprehensive Reviews in Food Science and Food Safety, 20*(2), 2081–2105. https://doi.org/10.1111/1541-4337.12714

Wang, Y., Sun, H., Han, B., Li, H. Y., & Liu, X. L. (2022). Improvement of nutritional value, molecular weight patterns (soluble peptides), free amino acid patterns, total phenolics and antioxidant activity of fermented extrusion pretreatment rapeseed meal with *Bacillus subtilis* YY-1 and *Saccharomyces cerevisiae* Y. *LWT, 160*, Article 113280. https://doi.org/10.1016/j.lwt.2022.113280

Wongsirichot, P., Gonzalez-Miquel, M., & Winterburn, J. (2022). Recent advances in rapeseed meal as alternative feedstock for industrial biotechnology. *Biochemical Engineering Journal, 180*, Article 108373. https://doi.org/10.1016/j.bej.2022.108373