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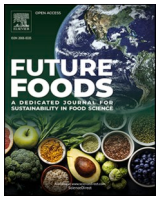

Unlocking the potential of oil by-products: Investigating the influence of co-culture fermentation on phenolic acid degradation in side stream blends from oil and dairy industry

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ABSTRACT

According to the newest trends in food waste management, the priority for valorisation of oil and dairy industry by-products was recognised. New approach of blending side streams of plant and animal origin has been proposed for obtaining future foods with improved nutritional value. Major obstacle in valorising sunflower seed cake (SSC) and whey (W) is their perishability and a relatively high content of phenolics contributing to, among others, protein indigestibility. In the study, a new approach of microwave pre-treatment applied for SSC and W blends was used. Its influence on phenolic acids and antioxidant properties after fermentation with co-culture of lactic acid bacteria and yeast was examined. Microwave drying of blends was longer than that of SSC affecting phenolic acids content. Fermentation by *L. lactis* and *K. lactis* caused pH decrease and increase in total acidity in 48 h effectively limiting the growth of bacterial contaminants. Fermentation decreased the content of phenolic acids, especially in blend composed of SSC and W (8.75:1; *w/w*) with the highest content of these constituents (from 1611.3 down to 838.8 mg/100 g dry matter). The study showed the synergistic effect of pre-treatment method and fermentation in terms of reduction in phenolic acids in new food matrices.

1. Introduction

Dwindling resources as well as intense exploitation and ever-growing generation of food waste and by-products are becoming an urgent, global issue that needs to be addressed. One of the ways of tackling these problems is the development of sustainable food systems that enable providing food in a way that considers economic, environmental, and social aspects of its production. However, sustainable production requires diversified actions and often the creation of new types of foods as well as the integration of various supply chains. One of the promising approaches is the combination of waste and side streams from plant and animal food production in terms of nutrient diversification (Alves and Tavares, 2019). The first attempt has been made for the formulation of blends composed of two important side streams from these sources, i.e., sunflower seed cake and whey in which the structure formation was examined during different thermal treatments (Raak and Corredig, 2023). Such a proposal is a first step in the assessment of the utilization of these by-products with new applications for human consumption. Due to the structural complexity of the sunflower seed cake and the presence of phenolics in a high quantity that may negatively affect the protein digestion and the uptake of nutrients (Ancuța and Sonia, 2020), the strategy for lowering their content is of high importance. Because of that sunflower seed cake being a side stream from oil production is usually used as animal feed. The total phenolics content ranges by 2.9 - 4.2 g/100 g of dry matter in which the dominant constituent is chlorogenic acid and its isomers representing 62 % up to 93 % of the phenolics (Weisz et al., 2009). While chlorogenic acid is a beneficial, health-promoting compound in various plant products including tea,

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coffee, selected fruits, and vegetables (Naveed et al., 2018), it can lead to protein indigestibility and alter organoleptic properties when consumed in relatively high quantities present, such as in sunflower press cake (Ancuța and Sonia, 2020). Microwave technology is considered one of the most promising treatments for this type of raw material in terms of improving the digestibility and bioavailability of proteins, offering a competitive dehydration treatment due to its low energy consumption and homogeneous heat transfer to the product (Hu et al., 2021). In addition, because of the insoluble nature and relatively heterogeneous structure of sunflower seed cake, it is one of the few technologies that allows for minimally destructive yet effective preservation of the material, particularly in terms of microbiological stability. However, the application of this type of treatment may result in the release of components previously bound in the matrix of the material. This includes low-molecular-weight antioxidants (Hu et al., 2021), mainly the above-mentioned chlorogenic acid, the presence of which in such excessive quantities is undesirable. One of the ways of lowering the amount of phenolics in food is the extraction by application of selected chemicals; however, this is often time-consuming and can affect the quality of the final product (Salgado et al., 2012). Another way of dealing with the excessive content of phenolic acids could be fermentation, previously applied for rapeseed press cake (Tian et al., 2023). In this case, the metabolic activities of the microorganisms involved in the process can change the sensorial, physical, and nutritional properties of the food matrix (Shiferaw Terefe and Augustin, 2020). The transformation of substrates may lower the content of the non-nutritive constituents as in the case of certain phenolic acids in sunflower seeds. It was proved that *B. animalis* subsp. *lactis* and *Lb. gasseri* were able to reduce the level of chlorogenic acid after a fermentative step during 48 h (Fritsch et al., 2016). The reduction was attributed, as previously found in other species, to the enzymatic ability to hydrolyse ester bonds in hydroxycinnamates (Couteau et al., 2001). Previously, the fermentation of press cake was made in the presence of whey that is considered as one of the most polluting side streams due to the high organic load with the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of about 27–60 g/L and 50–102 g/L, respectively, and the major one generated by the dairy industry. From the practical point of view, its application in the fermentation process may reduce the BOD and COD demand, diminishing the pollution (Zotta et al., 2020). Furthermore, whey fermentation by lactic acid bacteria (LAB) can improve the organoleptic properties, including the flavour and the texture of foods (Sharma et al., 2017) as well as may simultaneously preserve from bacterial pathogens growth thanks to the acidification of substrate (Leyva Salas et al., 2018). In a previous study on the fermentation of sunflower press cake and whey, a consortium of microorganisms formed by *L. lactis* and *T. delbrueckii* decreased the microbial contamination of 1.15 log CFU/g in 48 h due to the production of lactic acid and ethanol particularly effective against mould (Mangieri et al., 2022). Furthermore, *L. lactis* species is well-recognized as producing bacteriocins, like nisin, lactococcin (Zacharof and Lovitt, 2012) and lacticine. Lacticine 3147 was originally isolated in Irish kefir grains showing a broad antimicrobial spectrum against Gram positive food pathogen bacteria such as *Listeria monocytogenes* and *Bacillus* cereus (Ross et al., 2002).

Recently, Mangieri et al. (2022) and Raak et al. (2023) proposed a novel way for the sunflower seed cake valorisation by controlled fermentation in the presence of whey towards the creation of novel sustainable food ingredients being a matter for the development of future foods (Raak et al., 2022). Due to the perishable nature of both ingredients, the pre-treatment method has been proposed to stabilize the components before fermentation. Previously, microwave heating was applied for the extraction of bioactives from sunflower seed cakes (Náthia-Neves and Alonso, 2021) and almond hull (Salgado-Ramos et al., 2022); however, no data has been presented about the effect of microwave drying pre-treatment on sunflower seed cake bioactives in the presence or absence of whey. Therefore, the study investigated the effect of microwave drying on SSC and SSC-whey blends in terms of their influence on the process parameters and physico-chemical properties of products and how such an approach affected phenolic acids before and after fermentation. Taking into account the creation of new plant- and animal-based products by blending sunflower seed cake and whey (Raak et al., 2022), the study specifically aimed at investigating: (1) the influence of microwave drying on the selected properties of sunflower seed cake and its blends in the presence of whey, and (2) the effect of fermentation on the content of phenolic acids in the end products.

2. Materials and methods

2.1. Reagents

All reagents were of analytical grade (Sigma-Aldrich St. Louis, MO, USA).

2.2. Materials

Sunflower seed cake (SSC) was obtained through the commercial cold pressing from the feed consisting of 70 % of whole sunflower seeds and 30 % of dehulled sunflower seeds from a local producer (Złoto Polskie s.c., Kalisz, Poland). Material was milled before further processing on a sieve with aperture size of 1 mm (Retsch SM 2000, Germany). Whey in the powder form was obtained from Bayerische Milchindustrie (Landshut, Germany).

2.2.1. Preparation of the material

The material for the study consisted of grounded SSC and blend of SSC mixed with whey (Fig. 1). Samples were dried using a microwave dryer (Whirlpool JT 359 IX) at 2.5 W/g based on the preliminary tests. The temperature during drying was measured using infrared camera i50 (Flir Systems AB, Stockholm, Sweden). The drying process was carried out until the final moisture content (*Mc*) was below 2%$_{wb}$ (wet basis) in two repetitions ($n = 2$).

Microwaved samples before fermentation were rehydrated at 70 % with sterile, demineralized water (negative control, **Blend1** and **Blend2**), whereas samples that consisted of only SSC were rehydrated at the 70 % with reconstituted bovine sweet whey (6 %; *w/w*) (**SSC1** and **SSC2**). Before fermentation, samples were heated up to 100 °C (microwave oven ZM30TH, Zanussi, Pordenone, Italy) for 1 min. The control sample was rehydrated with water without the addition of whey.

2.2.2. Fermentation and microbiological analysis

The fermentation was carried out by the association of one bacterium, *Lactococcus lactis* B12 strain, and one yeast, *Kluyveromyces lactis* L2 strain, previously isolated and characterised (Mangieri et al., 2022). Fresh cultures for inoculation were prepared following the protocol proposed by Raak et al. (2023). The samples (Fig. 1) (rehydrated at 70 %) were mixed for 5 min with the addition of the microorganisms to obtain a final concentration of around 1×10^5 CFU/g bacteria and 1×10^4 CFU/g of yeast. The samples were transferred into 200 mL sterile plastic containers, incubated at 26 °C for 48 h in static conditions, and closed with a screwed cap. Each experiment was replicated in three independent growth tests. At the beginning (t_{0h}) and the end of fermentation (t_{48h}), the samples were microbiologically analysed by plate count technique in different growth media: Man–Rogosa–Sharpe (MRS) (Difco, Sparks, MD, USA) agar for LAB enumeration with incubation at 30 °C for 48 h, Yeast extract Glucose Chloramphenicol agar (YGC) (Merck, Darmstadt, Germany) for yeasts enumeration with incubation of 26 °C for 72 h, sugar-free agar for mesophilic bacterial contaminants with incubation at 30 °C for 48 h, Tryptone Bile X-GLUC Agar (TBX) (Scharlab, Sentmenat, Spain) for *Escherichia coli*, enumeration with incubation at 37 °C for 48 h and Bacillus cereus Agar (Base) (Sifin, Berlin, Germany) for the detection of *B. cereus* with incubation at 30 °C for 48 h. The samples were chemically analysed at t_{0h} and t_{48h} through pH measurement (Jenway® 3510, Cole-Parmer Ltd, Stone, UK),

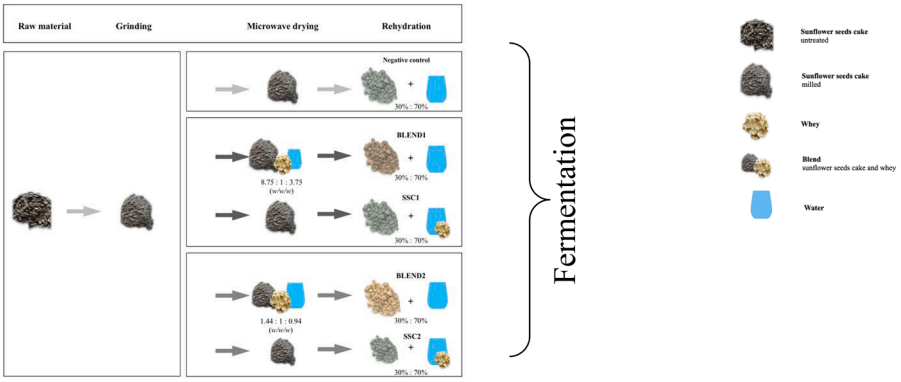

Fig. 1. Scheme of the experimental plan.

and total titratable acidity measured as $°SH_{50}$ (mL of 0.25 M NaOH used for 50 g of sample at pH 8.4). The samples were also examined for the water activity (HygroPalm HP23-AW-A).

2.2.2.1. Isolation and identification. Single bacterial colonies from the plates at higher dilutions of sugar-free agar, TBX agar, and Bacillus cereus agar base were streaked two times in the same medium for the isolation. The bacterial DNA was extracted according to a partially modified method by Green and Sambrook (2012). Single well-isolated colony was picked up and resuspended in 400 μL of TE (Tris–HCl 10 mM, pH 8; EDTA 1 mM) plus 4 μL of lysozyme (50 mg/mL) and incubated at 37 °C. After 30 min, 12.5 μL of sodium dodecyl sulfate (20 %) and 10 μL of proteinase K (20 mg/mL) were added and incubated (30 min). For separation of the nucleic acids, 400 μL of phenol were adjected and centrifuged at 19,830 × *g* for 10 min. The supernatant was recovered followed by the addition of 400 μL of chloroform: isoamyl alcohol (24:1, *v/v*) and centrifuged as before. For precipitating the DNA, the supernatant was supplemented with 800 μL of ethanol (95 %; *v/v*) and 40 μL of sodium acetate (3 M, pH 5.2). After centrifugation at 19,830 × *g* for 30 min, the pellet was washed with 300 μL of ethanol (70 %; *v/v*) and centrifuged at 19,830 × *g* for 15 min. The pellet was dried at 37 °C for 1 h and then resuspended, storing overnight at 4 °C in 50 μL of TE.

The 16S rRNA region was amplified using universal primers BSF8 (5′-AGAGTTTGATCCTGGCTCAG-3′) and BSR1541 (5′-AAGGAGGTGATCCAGCCGCA-3′) (Wilmotte et al., 1993). The thermal conditions of the PCR cycle were 94 °C for 5 min, as initial denaturation, 35 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 5 min. The amplicons were run in a 1 % agarose gel in 0.5 % TBE buffer (Tris-borate-EDTA) stained with ethidium bromide (0.4 μg/mL) at 90 V for 1 h and the images were captured with UV rays transilluminator (Biorad, Hercules, California, USA). The PCR products were purified with the EuroClone® spinNAker purification kit (Pero, Milano, Italia) and then sequenced by an external facility (Eurofins Genomics, Vimodrone, Italy). The obtained sequences were compared by Basic Local Alignment Search Tool (BLAST) (http://www.ebi.ac.uk/blastall/nucleotide.html) and species identification was assigned when homology was more than 98 %.

After fermentation, the samples were freeze-dried (FreeZone freeze dryer; Labconco Corp., MO, USA; 24 h; 65 Pa; −60 °C/+25 °C) and submitted to the analysis of phenolic acids.

2.2.3. Dry matter

The dry matter of the freeze-dried fermented samples was measured ($n = 2$) by the vacuum-oven method (80 °C; 24 h; 300 Pa) according to Figiel (2010) and expressed as a percentage of dry matter (dm).

2.2.4. Quantification of phenolic acids by UPLC

The extraction of samples was performed as described by Wojdyło et al. (2013) with 30 % methanol (methanol/water/acetic acid/ascorbic acid: 30/68/1/1; *v/v/v/w*). The qualitative and quantitative determination of phenolic acids was performed in duplicate ($n = 2$) using the Acquity UPLC system (Waters Corp., Milford, MA, USA) equipped with a BEH C18 analytical column (2.1 mm × 100 mm; 1.7 μm), maintained at 30 °C and set at a flow rate of 0.42 mL/min. The samples were kept at 4 °C. Acetonitrile (100 %) was employed as a strong washing solvent, while a mixture of acetonitrile and water (10 %) served as a milder one. The mobile phase consisted of 4.5 % formic acid (solvent A) and acetonitrile (solvent B). The elution proceeded as follows: from 0 to 10 min, a linear gradient from 1 % to 25 % B; from 10 to 11.5 min, a linear gradient from 25 % to 100 %; from 11.5 to 12.5 min, subsequent column washing, and finally reconditioning for 2.5 min. The determination of phenolic acids was carried out at $\lambda = 320$ nm. Retention times (R_t) and spectra were cross-referenced with those obtained from pure standards for which the calibration curves were constructed at concentrations ranging from 0.05 to 5 mg/mL ($r^2 \leq 0.9998$). Results were interpreted using Empower 3 software and expressed as mg/100 g dm.

2.2.5. Antioxidant capacity in vitro

The antioxidant capacity *in vitro* of non-fermented and fermented samples in 80 % methanolic extracts (*v/v*) was measured by TEAC ABTS (Re et al., 1999) and FRAP (Benzie and Strain, 1996) methods. Results gained were expressed in mmol Trolox equivalent per 100 g dm ($n = 2$). The Synergy H1 spectrophotometer (BioTek Instruments Inc., USA) was used for the measurements.

2.2.6. Statistical analysis

RStudio (version 2023.03.0, Build 386, Posit Software) was applied to perform the statistical analysis for the samples in the study with the use of ANOVA (one-way analysis of variance) and HSD Tukey's tests using the 'agricolae' package ($p < 0.05$).

3. Results and discussion

3.1. Phenolic acids content before fermentation

The composition of the SSC-derived products affected the pretreatment parameters that were linked to the presence of whey in the blends. Microwave drying of SSC without whey lasted for 12 min and the temperature of the sample reached 160 °C (Fig. 2). The addition of reconstituted whey to SSC (**Blend1** and **Blend2**) prolonged the drying process to 28 min due to the higher initial moisture content of samples ($Mc = 32.44 \pm 0.69$ for **Blend1** and **Blend2**, and $Mc = 7.69 \pm 0.38$ for SSC sample); however, the quantity of whey in blends did not affect this factor.

In the samples analysed, four phenolic acids were quantified, namely, chlorogenic, neochlorogenic, benzoic acid derivative and protocatechuic acid. Such composition was similar to this indicated by de Oliveira Filho and Egea (2021) in sunflower seeds by-products. The sum

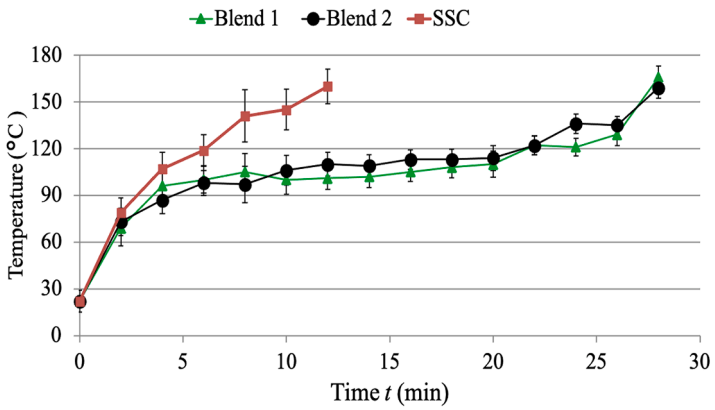

Fig. 2. The temperature profile of sunflower seed cake-derived products during microwave drying. SSC – sunflower seed cake; Blend – sunflower seed cake and whey.

of identified compounds in non-fermented samples ranged from 725 up to 1611 mg/100 g dm (Table 1). The dominant phenolic acid was chlorogenic acid which consisted of, on average, 74 % of all identified phenolic acids in the non-fermented products.

The temperature profile (Fig. 2) indicated that the maximum temperature reached for all the samples was around 160 °C. In the case of microwave drying of **Blend1** for almost the whole process duration the temperature was maintained at around 100 °C (from the 6th to 20th min), which is typical for this method. During microwave drying, the intense evaporation of water provides a cooling effect that prevents the heating up of the material. On the other hand, at the final stages of drying, when there is little moisture left and internal diffusion becomes the main driving force of the process, the sample temperature increases (Li et al., 2010). This phenomenon could contribute to the release of phenolic acids, mainly chlorogenic acid from the material matrix (Özcan et al., 2020). For the microwave-dried SSC samples, the maximum temperature was at the similar level reaching 160 °C, but the longer duration of thermal treatment had an impact on the bioactives as the content of the sum of identified phenolic acids in SSC consisted of more than 50 % of these constituents present in **Blend1**. On the other hand, a higher rate of reconstituted whey in **Blend2**, did not differentiate the temperature profile; however, the content of chlorogenic acid in the sample was 2-fold lower when compared to **Blend1**. It was indicated that addition of whey resulted in a decrease of phenolic acids content before drying as SSC.

Similarly, a lower content of phenolic acids in the case of **SSC1** and **SSC2** in comparison to negative control can be linked to the initial products composition, as SSC was subjected to drying prior to fermentation while whey was added without any pre-treatment as in the case of **Blend1** and **Blend2**.

3.2. Fermentation

The sunflower seed cake samples subjected to different pre-treatments were fermented using the association of *L. lactis* B12 and *K. lactis* L2 strains. Then, the samples were analysed for the capability to grow in the substrate by the inoculated bacteria and yeasts, and also for the proliferation of microbial contaminants (Table 2). The inoculated microorganisms were able to colonise these materials despite the presence of non-nutritive constituents, i.e., phenolic compounds. On average, the lactococci well increased cell number of +4.07 Log_{10}CFU/g after 48 h of incubation, meanwhile, the yeasts of +2.36 Log_{10}CFU/g. No significant differences were found by ANOVA in the plate counts of lactococci at each sampling time, whereas there were for yeast counts but only at 48 h (p-value = 0.024). Nevertheless, by subtracting the logarithmic value of the final count from the logarithmic value of the initial count, for each sample, it was possible to compare the cell increase in the blends: statistical analysis revealed that no significant differences were found for lactococci growth (p-value = 0.635) neither for yeasts growth (p-value = 0.146). Unexpectedly the microbial consortium was able to ferment the negative control, that had no whey, with a final pH of 4.66 ± 0.09 (Table 2), final LAB concentration of 9.20 ± 0.51 Log_{10}CFU/g and final yeasts concentration of 5.29 ± 0.37 Log_{10}CFU/g, with no growth over the limit of detection for any contaminants. The **Blend1** showed an increase of 4.05 and 2.52 Log_{10}CFU/g for LAB and yeast, respectively. Among the contaminants, presumptive colonies of *B. cereus* (2.69 ± 1.29 Log_{10}CFU/g) in **Blend1** at 48 h were observed. Likewise, in the **Blend2** the number of presumptive *B. cereus* were over the limit of detection with 2.85 ± 1.48 (t_{0h}) Log_{10}CFU/g (Table 2). The samples **SSC1** and **SSC2** showed an increase in cell number for the inoculated microorganisms, as shown in Table 2 with a

Table 1
The content of phenolic acids in sunflower seed cake-derived samples before and after fermentation (mg/100 g dm).

	Before fermentation					After fermentation				
SSC (%)	100	90		60		100	90		60	
Sample name	Negative control	SSC1	Blend1	SSC2	Blend2	Negative control	SSC1	Blend1	SSC2	Blend2
chlorogenic acid	822.33 ± 2.00c*	764.65 ± 4.11c	1147.36 ± 24.15d	553.51 ± 0.82b	551.60 ± 16.00b	599.25 ± 4.35b	517.81 ± 0.68ab	546.10 ± 5.13b	370.66 ± 1.99a	486.40 ± 11.35ab
neochlorogenic acid	110.17 ± 3.19ab	120.01 ± 3.63bc	167.71 ± 3.76e	101.46 ± 6.04ab	112.68 ± 8.00ab	148.70 ± 11.71de	135.38 ± 4.74cd	157.79 ± 2.89e	94.43 ± 0.90a	116.35 ± 5.36bc
benzoic acid derivative	80.37 ± 2.00d	71.39 ± 4.11cd	184.53 ± 24.15f	50.06 ± 0.82ab	100.24 ± 16.00e	58.81 ± 4.35bc	52.90 ± 0.68ab	84.05 ± 5.13de	36.70 ± 1.99a	90.04 ± 11.35de
protocatechuic acid	31.54 ± 2.01c	29.13 ± 2.99bc	111.68 ± 4.24f	19.57 ± 0.82ab	71.21 ± 8.95e	21.02 ± 1.03a-c	20.20 ± 0.68ab	50.84 ± 1.85d	13.46 ± 0.67a	62.65 ± 5.97de

SSC - sunflower seed cake; Blend - SSC and whey; *Values followed by the same lowercase letters within the same row were not significantly different ($p < 0.05$) (Tukey's HSD test).

Table 2
Results of microbiological analysis expressed in $Log_{10}CFU/g \pm$ the relative standard deviation ($n = 3$). Mean values followed by the same lowercase letters within the same column were not significantly different ($p < 0.05$) (Tukey's HSD test).

	LAB		Yeasts		Other bacteria		*E. coli*		*B. cereus*	
Samples	t_{0h}	t_{48h}	t_{0h}	t_{48h}	t_{0h}	t_{48h}	t_{0h}	t_{48h}	t_{0h}	t_{48h}
Negative control	5.06 ± 0.51a	9.20 ± 0.51a	3.92 ± 0.26a	5.29 ± 0.37a	< 2	< 2	< 2	< 2	< 2	< 2
SSC1	5.14 ± 1.27a	9.16 ± 0.06a	4.16 ± 0.12a	6.92 ± 0.09b	2.30 ± 0.30a	2.33 ± 0.44a	< 2	< 2	< 2	< 2
Blend1	4.96 ± 0.95a	9.05 ± 0.46a	4.00 ± 0.55a	6.52 ± 0.58b	2.13 ± 0.23a	3.16 ± 1.44a	< 2	< 2	< 2	2.69 ± 1.20
SSC2	5.16 ± 0.56a	9.49 ± 0.52a	4.38 ± 1.08a	6.73 ± 0.49b	2.77 ± 1.08a	2.76 ± 1.32a	< 2	< 2	< 2	< 2
Blend2	4.71 ± 0.60a	8.47 ± 0.20a	3.94 ± 0.07a	6.76 ± 0.09b	2.33 ± 0.44a	< 2	< 2	< 2	2.85 ± 1.48	–

SSC - sunflower seed cake; Blend - SSC and whey.

limited number of contaminant bacteria. In addition, the presence of *E. coli* was not detected in any of the samples. Similarly, moulds were not observed in any sample, therefore it was possible to estimate their counts remained lower than 3 $Log_{10}CFU/g$ in the tested experimental conditions and, for this reason, they were not reported in Table 2. The isolates found at concentrations higher than 10^4 CFU/g were identified through 16S rRNA gene partial sequencing. From the plates of sugar-free agar of **Blend1** at t_{48h} *Enterobacter hormaechei* was identified, while from the PEMBA agar plates of **Blend2** at t_0, the presence of *B. cereus* was confirmed. Also, in the same sample *Enterococcus gallinarum* was identified.

As regards the variation of pH, acidity and water activity (a_w), data are shown in Table 3. The fermentation process resulted in a decrease in pH on average from 5.84 ± 0.22 to 4.76 ± 0.21 and an increase in total acidity from 11.30 ± 2.86 to 35.14 ± 6.86. The water activity of the samples ranged from 0.941 to 0.949. The ANOVA revealed that significant differences were noticed among the different blends both in the pH values at time 0 and at 48 h, and the same occurred for the values of total titratable acidity at the two sampling times. Significantly **Blend2**, which contained the higher amount of whey, started (t_{0h}) with a lower pH value and higher acidity and ended up (t_{48h}) being less acidic than the other samples. This phenomenon may be due to the lower availability of lysine in this sample with respect to the others, essential for the growth of lactococci, because of the greater content of lactose that react with the amino acid *via* the Maillard reaction in thermally treated materials (Naranjo et al., 1998).

To compare the samples with each other, the variations undergone during fermentation were assessed by calculating the difference between the initial and the final pH for each sample, and in the same way the titratable acidity values. The average pH variation highlighted in **Blend2** was significantly lower from that of the other samples (p-value < 0.001), as well as the developed acidity is the lowest of the investigated blends (p-value < 0.001). Fig. 3 showed the box plots of the variations in pH and total titratable acidity detected in the different blends.

Table 3
Results of pH, total titratable acidity and activity water (a_w) ± the relative standard deviation. Mean values followed by the same lowercase letters within the same column were not significantly different ($p < 0.05$) (Tukey's HSD test).

	pH		Total titratable acidity		a_w
Sample	t_{0h}	t_{48h}	t_{0h}	t_{48h}	
Negative control	6.03 ± 0.09c	4.66 ± 0.09a	9.17 ± 1.04a	36.17 ± 3.33bc	0.941± 0.001a
SSC1	5.95 ± 0.03bc	4.57 ± 0.04a	9.50 ± 0.00a	40.67 ± 1.89c	0.948 ± 0.021a
Blend1	5.80 ± 0.08b	4.77 ± 0.14a	11.50 ± 2.18a	41.00 ± 1.32c	0.941 ± 0.001a
SSC2	5.95 ± 0.04bc	4.66 ± 0.03a	10.17 ± 0.58a	33.67 ± 1.04b	0.946 ± 0.002a
Blend2	5.48 ± 0.04a	5.12 ± 0.03b	16.17 ± 1.04b	24.17 ± 0.58a	0.949 ± 0.001a

SSC - sunflower seed cake; Blend - SSC and whey.

3.3. Phenolic acids content after fermentation

In the study, a selected microbial consortium composed of *Lactococcus lactis* B12 and *Kluyveromyces lactis* L2 strains was tested for fermentation of microwave-dried SSC products. The process caused a decrease in the sum of phenolic acids content that was 27 % lower in the case of fermented negative control when compared to a non-fermented counterpart (Table 1). This could be linked to the enzymatic ability to break down ester bonds in hydroxycinnamates as it was noted for other species (Couteau et al., 2001). The initial composition of blends (SSC and whey) had a significant impact on the presence of phenolic acids after fermentation, with a reduction in their content of almost 50 % in the case of **Blend1** and 10 % in the case of **Blend2** (Table 1).

The content of chlorogenic acid being the dominant component of phenolic acids in SSC-derived products, lowered after fermentation. Previously, it was proved that lactic acid bacteria and bifidobacteria may diminish the content of chlorogenic acid in sunflower protein concentrate and flour (Fritsch et al., 2016; Pöri et al., 2023). To the best of authors' knowledge, no evidence was found in the literature that *Lactococcus lactis* or *Kluyveromyces lactis* can hydrolyse ester bonds in hydroxycinnamates. Fritsch et al. (2016) showed that chlorogenic acid degradation caused by the fermentation process was stronger in the case of sunflower protein concentrate in which higher content of protein and lower content of chlorogenic acid was noticed. In the case of analysed blends (**Blend1** and **Blend2**), higher whey content and lower chlorogenic acid amount in **Blend2** (Fig. 1), did not accelerate the reduction of chlorogenic acid after fermentation. This phenomenon might be attributed to the: (1) lower proportion of SSC (the source of this compound) in the composition compared to **Blend1**, and (2) formation of chlorogenic acid-whey protein complexes (Zhang et al., 2023) during pre-treatment (i.e., microwave drying), making it less accessible to the fermentation process. A decrease of approx. 32 % in chlorogenic acid content after fermentation was noted for **SSC1** and **SSC2** samples. As previously reported, the degradation of the chlorogenic aqueous solution is affected by time and pH (Narita and Inouye, 2013), which may have indirectly altered the extractability of the phenolics from the matrices throughout the fermentation process.

Neochlorogenic acid, which consisted of approx. 12 % of phenolic acids present in non-fermented sunflower seed cake-derived products, turned out to be the most stable among identified phenolics, indicating that the fermentation process had no substantive effect on changes in its concentration. The only statistically significant differences were found in the case of the **SSC1** sample, which, unlike the other phenolic acids, had a higher content after fermentation. Ambiguous behaviour of selected phenolic acids was observed after fermentation that was explained by probable higher activity of chlorogenic acid esterase that led to the degradation of chlorogenic acid and an increase in other phenolic acids content (Pöri et al., 2023). Previously, Jiang et al. (2022) found out that the fermentation of red raspberry juice with yeast resulted in a simultaneous decrease in chlorogenic acid and an increase in neochlorogenic acid content. A similar observation was made with naturally fermented coffee beans after 24 h, and the authors attributed this phenomenon to the isomerization of chlorogenic acid to the

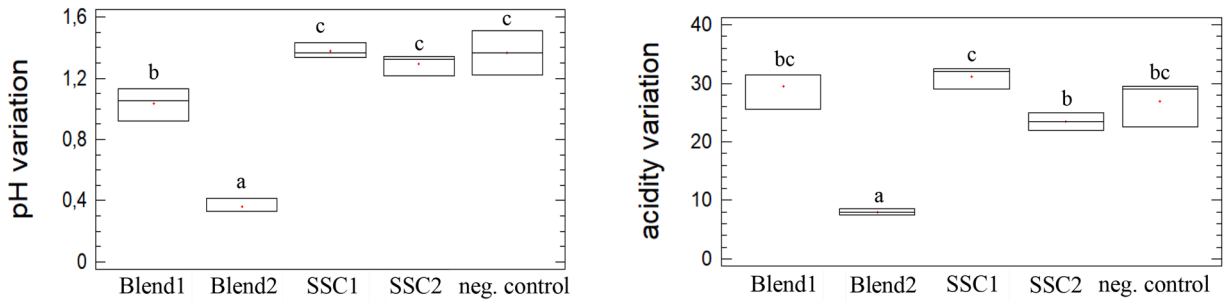

Fig. 3. The variation in pH and total titratable acidity observed after 48 h of fermentation in the different samples. Values with different superscripts are significantly different. SSC – sunflower seed cake; Blend – sunflower seed cake and whey, neg. control – negative control.

neochlorogenic form (Oktavianawati et al., 2020). From this, it can be assumed that the same transformations occurred in the case study, resulting in an opposite trend in the neochlorogenic acid content compared to the rest of the compounds identified within this group.

The dihydroxybenzoic acid derivative and protocatechuic acid accounted for, respectively, approx. 9 % and 5 % of all phenolic acids, and followed the same pattern when the influence of the fermentation process depending on the matrix composition was considered (Table 1). Namely, in the case of all SSC-derived products, a decreasing tendency was observed, however, in particular cases, the differences were not statistically significant (Table 1). Similarly to chlorogenic acid, the greatest reduction in their content was reported for the **Blend1**, followed by negative control. Interestingly, the only divergent behaviour from the chlorogenic acid trend was exhibited by the **Blend2** sample, which for dihydroxybenzoic acid derivative and protocatechuic acid showed a higher content of these compounds than **SSC1** and **SSC2**. This might be linked to the influence of the surrounding matrix, which affects the reactivity of individual components and therefore their presence in the final product (Capuano et al., 2018).

3.4. Antioxidant capacity

In general, among the non-fermented samples, the ability to scavenge the $ABTS^{\bullet+}$ radical cations and to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions was the highest for **Blend1** which was approx. 2-times higher when compared to negative control and **SSC1** values (Fig. 4a and 4b) which was linked to the content of phenolic acids.

Fermentation reduced the antioxidant capacity of analysed samples and a strong positive correlation between the sum of identified phenolic acids and the TEAC as well as FRAP was indicated ($R = 0.91$ and 0.96, respectively) confirming the contribution of phenolic acid, namely chlorogenic acid in antioxidant potential of sunflower seed cake-derived products (Amakura et al., 2013). However, only in **Blend2** the differences between non-fermented and fermented samples were not statistically significant. This may be due to the higher content of whey in the mixed sample that had weaker influence on the ability to scavenge $ABTS^{\bullet+}$ radical cations and to reduce ferric ions than phenolic acids.

4. Conclusions

In the study, a pre-treatment protocol for the oil and dairy industry side streams before turning in novel fermented products was applied. To this aim, the sunflower seed cake-derived products mixed with whey were microwave-treated before submitting to the fermentation process. The pre-treatment method influenced the composition of the sunflower seed cake-whey blends in contrast to the single component samples (sunflower seed cake). It was indicated that blends composition influenced the microwave drying as addition of whey prolonged the duration of the process. This had an impact on the initial content of phenolic acids and antioxidant capacity of the samples. A significantly higher content of the dominant constituent, chlorogenic acid was observed. The acidification of the blends, resulting from the fermentation process, effectively limited the growth of bacterial contaminants. What is more, it was indicated that microbial metabolic activities diminished the content of phenolic acids present in samples analysed, thus this strategy may be successfully applied for the reduction of these constituents in sunflower seed cake-derived products. Fermentation caused a reduction of phenolic acids in the samples (down to even 50 %) that were dependent on the microwave treated compositions. It can be suggested that this approach can be effectively employed to decrease these components in new products derived from sunflower seed cake.

Future considerations

Blending side streams from different food industry origins have received special attention due to waste management and the potential modification and diversification of nutritional compositions present in new formulations (liquid, semi-liquid and solid). However, due to the

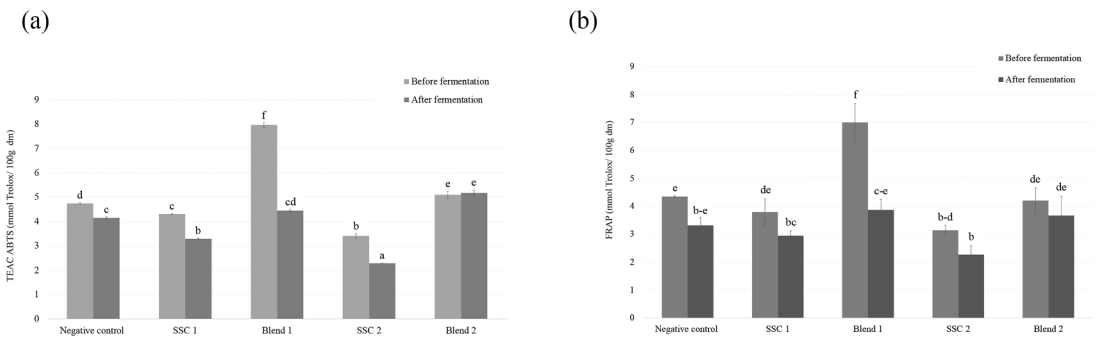

Fig. 4. Antioxidant capacity measured by TEAC ABTS (a) and FRAP (b) assays in sunflower seed cake-derived products (mmol Trolox/100 g dm). Values followed by the same lowercase letters were not significantly different ($p < 0.05$) (Tukey's HSD test). SSC – sunflower seed cake; Blend – sunflower seed cake and whey, neg. control – negative control.

consumer perception of these by-products, the co-creation of new blended products requires the application of novel processing techniques towards acceptable sensory characteristics and improved nutritional properties. These approaches are considered in the FERBLEND project (https://ferblend.webspace.tu-dresden.de/; Raak et al., 2022), including the new issue of pre-treatment by microwave drying as it can stabilise the ingredients before fermentation. Future research will focus on the application of different drying techniques for fermented blend products to obtain easy-to-handle powders that can be distributed in different food formulations to improve nutritional properties and co-create new platform products (e.g. spreads) with novel approaches to their characterisation in terms of physical, techno-functional and sensory properties.

CRediT authorship contribution statement

Anna Michalska-Ciechanowska: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Klaudia Masztalerz:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Nicola Mangieri:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Roberto Foschino:** Writing – original draft, Methodology, Funding acquisition, Data curation, Conceptualization. **Krzysztof Lech:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Aneta Wojdyło:** Writing – original draft, Supervision, Methodology, Investigation, Data curation. **Paulina Nowicka:** Methodology, Investigation. **Jessica Brzezowska:** Writing – original draft, Investigation.

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.

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