

Enhancing bioactive compounds from coffee cascara via enzymatic treatment and microbial fermentation

Trang Nguyen Phuong Huynh¹ , Thao Thi Thanh Nguyen¹ , Vinh Duong Hoang Nguyen² ,
Hai Hoang Ho¹ , Anh Quynh Nguyen³ , Ly Thi Phi Trinh^{4*} 

¹Khai Minh Viet Enzyme Technology Joint Stock Company, Ho Chi Minh City, Vietnam

²Faculty of Biological Sciences, Nong Lam University, Ho Chi Minh City, Vietnam

³Lab2Life Science and Technology Company Limited, Ho Chi Minh City, Vietnam

⁴Research Institute for Biotechnology and Environment, Nong Lam University, Ho Chi Minh City, Vietnam

*Corresponding author: phily@hcmuaf.edu.vn

Editor: Samuel Pereira de Carvalho

Received in November 9, 2024 and approved in April 9, 2025

ABSTRACT

Coffee cascara is rich in bioactive health-promoting compounds, particularly phenolic compounds with known antimicrobial, antioxidant, and anticancer properties and shows promise for utilization in the food and pharmaceutical industries. Enzymatic treatment and subsequent fermentation have emerged as a viable technique for enhancing the breakdown of complex plant cell structures, releasing phenolic compounds, and generating desirable flavors and active metabolites. This study aims to evaluate the effect of enzymatic treatment and subsequent microbial fermentation on the production of bioactive compounds in coffee cascara. Celluclast was determined to be the most effective for extracting phenolic compounds, yielding a total phenolic content of 25.29 mg gallic acid equivalent/g (mg GAE/g) and demonstrating high antioxidant activity. *Lactobacillus paracasei* C13-treated cascara recorded the highest total phenolic content and antioxidant capacity, with caffeic acid levels 12 – 18 times higher than those observed in other treatments. Combining Celluclast treatment with fermentation by *L. paracasei* C13 significantly increased total phenolic content to 29.56 mg GAE/g and achieved the highest antioxidant activity of 15.40 mg ascorbic acid equivalent/g (mg AAE/g). This process improved the extraction of chlorogenic acid, protocatechuic acid, and caffeic acid to 151.95 mg/100 g, 27.99 mg/100 g, and 22.58 mg/100 g, respectively, while decreasing caffeine levels compared to *L. paracasei* C13 fermentation alone. Enzymatic treatment and subsequent microbial fermentation significantly enhanced bioactive compounds, demonstrating a feasible method for the production of functional food ingredients from coffee cascara.

Key words: Coffee cascara; enzyme-assisted extraction; microbial fermentation; antioxidant capacity; health-promoting compounds.

1 INTRODUCTION

Coffee is one of the most popular beverages with more than 2 billion cups consumed worldwide daily (Roberts, 2021). Global coffee production reached 178 million 60-kilogram bags in 2022 – 2023. Large volumes of residues are discharged during the processing of coffee cherries into coffee beans. Improper disposal of coffee residue wastes seriously impacts landfills and water sources because these wastes contain high levels of biodegradable substances, caffeine, and tannin (Esquivel; Jiménez, 2012). Coffee berries are composed of beans and silver skin, parchment, mucilage, pulp, and outer skin layers (Esquivel; Jiménez, 2012). The remaining part after collecting the coffee beans from within is called coffee cascara, a by-product of wet processing (Esquivel; Jiménez, 2012). Coffee cascara is characterized by secondary metabolites, mainly alkaloids, melanoidins, and phenolic compounds. Coffee cascara contains appreciable amounts of the phenolic compounds chlorogenic acid, protocatechuic acid, and caffeic acid (De Melo Pereira et al., 2020) as a potential source of bioactive compounds with interesting uses in the food and pharmaceutical industries.

Interest in transforming the coffee waste into useful products and mitigate the environmental issues has recently increased, with the effective extraction of phenolic compounds and biological active components from coffee cascara attracting much attention. The extraction technique directly impacts the yield and biological activity of phenolic compounds obtained from plant materials. Enzyme-assisted extraction is a potential method because the enzymes disrupt the linkages within the plant cell wall matrix through the hydrolysis process. This method also provides solvent-free biological ingredients for the food and pharmaceutical sectors. Hydrolytic enzymes were appraised for the effective recovery of both bound and free phenolic compounds from various sources such as rose, camellia, and roselle flower (Trinh et al., 2018), and rice bran (Martillanes et al., 2021). Biochemically, the plant cell wall comprises multiple components such as cellulose, hemicellulose, lignin, pectin, and structural proteins. Phenolic hydroxyl groups in phenolic acids or flavonoids link to polysaccharide complexes through hydrogen bonds and hydrophobic interactions or form ether linkages with lignin (Acosta-Estrada et al., 2014). These bound phenolic compounds are not easy to liberate

by conventional solvent extraction techniques. Previous studies have demonstrated that some microorganism-derived enzymes such as cellulase, β -glucosidase, xylanase, and their combinations promote the release of phenolic compounds from plant materials by disrupting the cell wall structure (Hu et al., 2022). In our previous study, enzymatic extraction of phenolic compounds from various flower sources showed higher recovery, lower energy consumption, and reduced solvent consumption compared to conventional methods (Trinh et al., 2018).

Food fermentation has been an integral part of human culture since the Neolithic era. Foods or beverages produced by defined microbial growth and enzymatic transformations of food components are known as fermented foods and maintain or even improve food quality and safety. The lactic acid bacteria (LAB) *Lactobacillus* sp. and *Saccharomyces cerevisiae* are commonly used in food and beverage fermentation. LAB fermentation produces changes in bioactive compounds such as beneficial peptides, short chain fatty acids and polysaccharides while reducing sugars and anti-nutritional compounds, thereby converting phenolic compounds into biologically valuable molecules, enhancing prebiotic and/or probiotic potential, bioaccessibility, and health benefits (Doo et al., 2024). LAB fermentation also results in significant modifications to organoleptic properties such as taste, odor, color or texture, nutrition quality, shelf life, and the economic value of the final products (Szutowaska, 2020). A previous study demonstrated that LAB fermentation of coffee cascara increased antioxidant activity by up to 15% compared to cascara obtained from natural fermentation (Oktaviani et al., 2020). Fermentation by the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, and *Streptococcus* release bioactive phenolic compounds from their precursors and significantly modify the phenolic profile in the food matrix through the enzymatic actions of esterase and glycosidase (Filannino et al., 2018). *Saccharomyces* yeast also plays a significant role in the food industry through the metabolism of carbon sources to major end products (ethanol and carbon dioxide) and many other by-products. Numerous studies have used *S. cerevisiae* to ferment fruits, modifying the composition of phenolic compounds through the hydrolysis of their parent compounds. *Saccharomyces* has special properties, such as producing β -glucosidase activity and other carbohydrase activities that degrade the cell wall matrix to fermentable substances (Amadi et al., 2020). In this study, an effective process combining enzymatic pretreatment and subsequent microbial fermentation of coffee cascara was developed to enhance the extraction yield of bioactive compounds and thus improve product quality, showing potential for manufacturing biofunctional beverages from coffee cascara.

2 MATERIAL AND METHODS

2.1 Materials

2.1.1 Coffee cascara sample

Robusta coffee cascara (*Coffea robusta*) was obtained from coffee plantations in Bao Loc City, Lam Dong Province, Vietnam. Post-harvest, coffee cascara was separated from the coffee beans and dried in an oven at 45°C until the moisture content is below 10%. The dried coffee cascara were ground and sifted through a 0.5 mm sieve to achieve homogenized particle size and then stored in a desiccator to maintain the moisture level below 10%.

2.1.2 Chemicals

Protocatechuic acid, chlorogenic acid, and caffeic acid standards were provided by Sigma-Aldrich (USA) and the acetonitrile, acetic acid, and deionized water solvents were of analytical HPLC grade (Fisher, USA). Folin-Ciocalteu reagent was purchased from Himedia (India) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) was supplied by Tokyo Chemical Industry Co., Ltd, (Japan).

The enzymes were used in accordance with food-grade standards. Celluclast (700 U/g), Pectinex (3300 U/g), and Viscozyme L (300 U/g) were purchased from Novozyme (Denmark).

Plate Count Agar (PCA), *Lactobacillus* Agar according to De Man, Rogosa and Sharpe (MRS), and Yeast Growth Medium (YPD) medium culture were supplied by Himedia company (India).

2.1.3 Microbial strains

Lactobacillus plantarum S5, *Lactobacillus casei* C12, *Lactobacillus paracasei* C13, and *Saccharomyces cerevisiae* SC were provided by Khai Minh Viet Jsc. The *Lactobacillus* spp. were proliferated on MRS medium and incubated anaerobically at 37°C. *S. cerevisiae* was cultured on YPD medium and incubated aerobically at 30°C.

2.2 Methods

2.2.1 Enzymatic treatment of coffee cascara

Coffee cascara was mixed with distilled water at a material-to-water ratio of 1:10 (w/v) and the mixture was pretreated in an autoclave at 121°C for 15 min. The hydrolytic enzymes Celluclast, Pectinase, and Viscozyme were added to the mixture at a loading of 10 mg/g of coffee cascara. The hydrolysis process was conducted in a shaking incubator at 50°C for 0 – 24 hours, with agitation at 200 rpm/min. All the experiments were conducted in triplicate.

2.2.2 Fermentation of coffee cascara

The coffee cascara fermentation was carried out using *L. plantarum* S5, *L. casei* C12, *L. paracasei* C13, and *S. cerevisiae*

SC. For each experiment, cascara was mixed with distilled water at a ratio of 1:10 (w/v) and then autoclaved at 121°C for 15 min. After cooling to room temperature, sugar cane was added at 5% (w/v) and seed cultures (*Lactobacillus* 1.5×10^8 CFU/mL, *Saccharomyces* 1.5×10^6 CFU/mL) were introduced at a seeding ratio of 2% (v/v) of the total medium volume. Cascara fermentation was conducted within 48 hours, at 37°C for bacteria and 30°C for yeast. The fermented cascara broth was sampled at 0, 6, 12, 24, 36, and 48 hours, centrifuged at 10,000 rpm for 15 min, and then taken for further analysis.

2.2.3 Combining enzymatic treatment and microbial fermentation of coffee cascara

Simultaneous enzymatic treatment and fermentation of coffee cascara was conducted using a combination of Celluclast enzyme and *L. paracasei* C13. The aqueous mixture containing 10% (w/v) cascara was sterilized at 121°C and 1.5 atm for 15 min, followed by treatment with Celluclast for 12 hours and fermented by *L. paracasei* C13 using the conditions mentioned previously. Fermented samples were collected at regular intervals of 0, 12, 24, 36, and 48 hours for analysis. The acidity of the fermentation broth throughout the process was measured using a pH meter (Mettler Toledo, USA). The Brix value of the fermentation broth was measured by a Hanna Instruments Optical Refractometer (HI96801, USA).

2.2.4 Determination of total phenolic content

The total phenolic content (TPC) of the samples was measured using the Folin-Ciocalteu method with some modifications (Trinh et al., 2018). Gallic acid was used as the standard, and the results were expressed as mg gallic acid equivalent/g (mg GAE/g). A gallic acid calibration curve was established within a concentration range of 50 – 250 mg/L. For the analysis, 100 µL of the samples or the standards was added to test tubes and mixed with 2 mL of distilled water, followed by the addition of 100 µL of Folin-Ciocalteu reagent. After 5 min, 2 mL of 7% sodium carbonate solution was added to the test tube and allowed to react for 90 min at room temperature in darkness. The absorbance was measured at 760 nm using a UV-Vis Spectrophotometer (UH5300, Hitachi), with results recorded as the mean $\pm$ standard deviation of triplicate measurements for each experiment.

2.2.5 Determination of antioxidant activity

The antioxidant capacity was assessed using a DPPH radical scavenging assay (Kedare; Singh, 2011). Briefly, 0.5 mL of coffee cascara extracts was added to 1 mL of a 0.5 mM DPPH solution in methanol. The mixture was vigorously shaken and left to stand at room temperature in darkness for 15 min. The absorbance was measured at 517 nm using a UV-Vis Spectrophotometer. Ascorbic acid served as the standard

at concentrations ranging from 10 mg/L to 50 mg/L. The antioxidant activity was estimated by measuring the percentage of DPPH radical scavenging. Results were expressed as mg ascorbic acid equivalent/g (mg AAE/g).

2.2.6 Determination of phenolic compounds by RP-HPLC

The analysis of protocatechuic acid, chlorogenic acid, and caffeic acid in coffee cascara extracts was conducted using reverse phase high performance liquid chromatography (RP-HPLC, Agilent 1260 Infinity II). The HPLC was equipped with a Diode Array Detector (DAD detector). A Poroshell 120-EC C18 column (100 mm $\times$ 4.6 mm, particle size 2.7 µm) served as the reverse phase column. The mobile phase consisted of a mixture of deionized water with 1% acetic acid (mobile phase A), and acetonitrile with 1% acetic acid (mobile phase B). The program was set as 0 min (5% B); 0 – 2 min (5 – 5% B); 2 – 17 min (5 – 40% B); 17 – 20 min (40 – 40% B); 20 – 21 min (40 – 5% B); 21 – 25 min (5 – 5% B). The sample injection volume was 5 µL, and the elution flow rate was 1 mL/min. Protocatechuic acid (PCA), chlorogenic acid (CGA), and caffeic acid (CA) were used as standards with a 10 - 100 mg/L concentration range. PCA was detected at 270 nm, while CGA and CA were detected at 320 nm (Trinh et al., 2018).

2.2.7 Determination of caffeine

Caffeine was determined in coffee cascara extracts using RP-HPLC analysis equipped with a DAD detector. An analytic Zorbax Eclipse Plus C18 column was used (150 mm $\times$ 4.6 mm, particle size 5 µm), following a reported method with some modifications (Wang et al., 2000). The mobile phase was a mixture of methanol and deionized water at a ratio of 4:6, with a flow rate of 1 mL/min. Caffeine was detected at 254 nm. The caffeine standard was prepared at a concentration range of 1 - 50 mg/L, with results reported as the mean $\pm$ standard deviation of triplicate measurements for each experiment.

2.3 Statistical analysis

All measurements were performed in triplicate, with results expressed as the mean $\pm$ standard deviation. Statistical analysis was conducted using analysis of variance (ANOVA) with the Minitab 16 program. Significant differences were evaluated at $p < 0.05$.

3 RESULTS

3.1 Effect of enzymatic treatment on total phenolic content and antioxidant activity of coffee cascara

The coffee cascara was treated with polysaccharide-degrading enzymes including Celluclast, Pectinex, and

Viscozyme. Figure 1a shows that higher TPC was obtained from enzyme-treated cascara compared to non-treated samples. Enzymatic treatment increased the TPC of cascara in the first 12 hours, reaching 22.53 – 25.29 mg GAE/g and then TPC levels changed insignificantly until 24 hours. Only 14.01 mg GAE/g TPC was released from non-treated samples after 12 hours and TPC levels were insignificantly different for 24 hours processing. Celluclast was the most effective enzyme to extract TPC within 12 hours, followed by Pectinex and Viscozyme. The highest antioxidant activity of cascara extracts (Figure 1b), as measured by DPPH free radical scavenging activity, was 11.22 mg AAE/g using Celluclast treatment, followed by Pectinex and Viscozyme at 8.93 mg AAE/g and 8.96 mg AAE/g, respectively.

3.2 Fermentation of coffee cascara by different microorganisms

The cascara fermentation by different microbes was observed over 48 hours at the optimal temperature for each strain. The acidity of the fermentation broth was assessed using pH measurements, with the results graphically represented in Figure 2a. A sharp drop in pH was observed within 48 h of fermentation, primarily attributed to the activity of LAB. At the end of the fermentation, the pH of LAB sample reached a value of 3.6. The Brix value of the fermentation broth (Figure 2b) decreased significantly during fermentation with *S. cerevisiae* from 9.8 to 4.4 °Bx.

Figure 2c shows the TPC values as the fermentation progressed. In this result studied, *L. paracasei* C13 was the most effective strain for TPC extraction, reaching 24.62 mg GAE/g after 48 hours. *L. plantarum* S5, *L. casei* C12, and *S.*

cerevisiae SC also showed significant TPC levels, slightly lower than those observed in *L. paracasei* C13.

PCA, CGA, CA, and caffeine in the fermented cascara drinks were examined at 36 hours by RP-HPLC methods (Table 1). Cascara fermented by *S. cerevisiae* SC achieved the highest CGA and PCA levels at 151.89 mg/100 g and 15.66 mg/100 g, respectively. The sample fermented with *L. paracasei* C13 exhibited a remarkable increase in CA content (21.29 mg/100 g) and caffeine at the lowest level of 728.63 mg/100 g. Cascara treated with *L. paracasei* C13 demonstrated the highest antioxidant activity of 10.22 mg AAE/g, significantly higher than the control sample at 6.39 mg AAE/g (Figure 2d).

3.3 Effective production of phenolic compounds by combining enzymatic treatment and fermentation processes

Cascara pretreated with Celluclast, followed by fermentation with *L. paracasei* C13 achieved the highest TPC value of 29.56 mg GAE/g at 48 hours, whereas fermentation with *L. paracasei* C13 alone resulted in TPC at 24.62 mg GAE/g (Figure 3a). The sample treated with Celluclast also showed an antioxidant capacity of 15.42 mg AAE/g, compared to 10.22 mg AAE/g for *L. paracasei* C13-fermented cascara without enzymatic treatment (Figure 3b). Figure 3c and figure 3d show the production of PCA, CGA, and CA content as the fermentation progressed. The combined treatment at 36 h resulted in CGA, PCA, and CA levels of 151.95 mg/100 g, 27.99 mg/100 g, and 22.58 mg/100 g, respectively. Meanwhile, the cascara fermented by *L. paracasei* C13 alone had CGA, PCA, and CA levels of 124.12 mg/100 g, 15.42 mg/100 g, and 19.10 mg/100 g, respectively

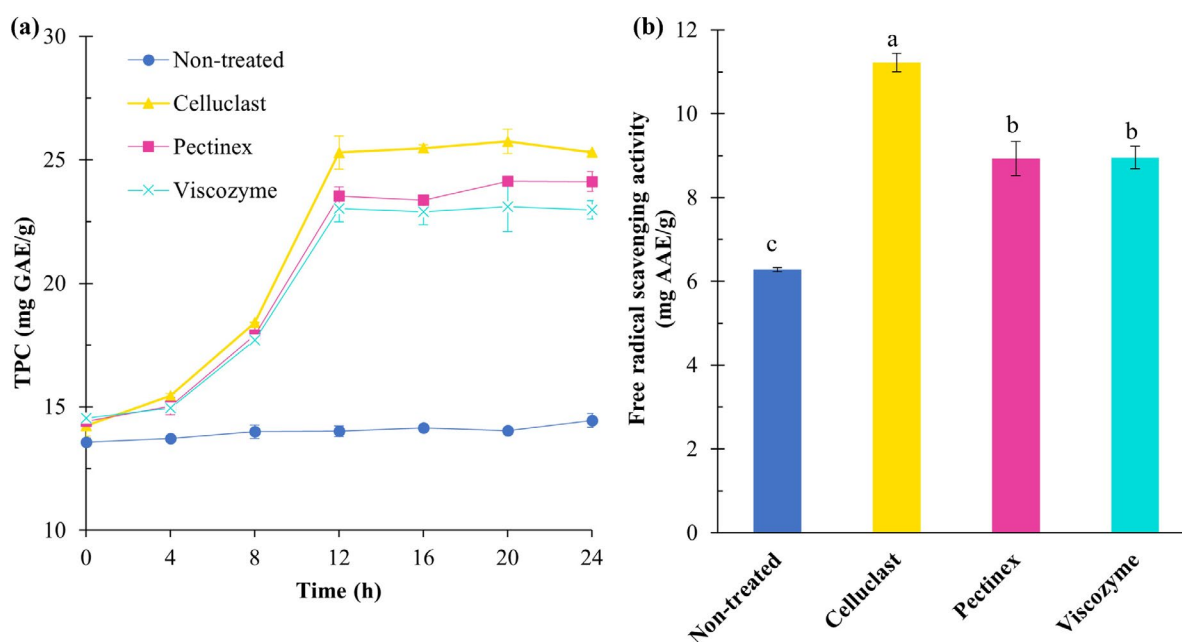


Figure 1: (a) Effect of different enzyme treatments on total phenolic content (TPC), (b) Free radical scavenging activity of the cascara treated with different enzymes at 12 h. Error bars represent standard deviation of three independent experiments.

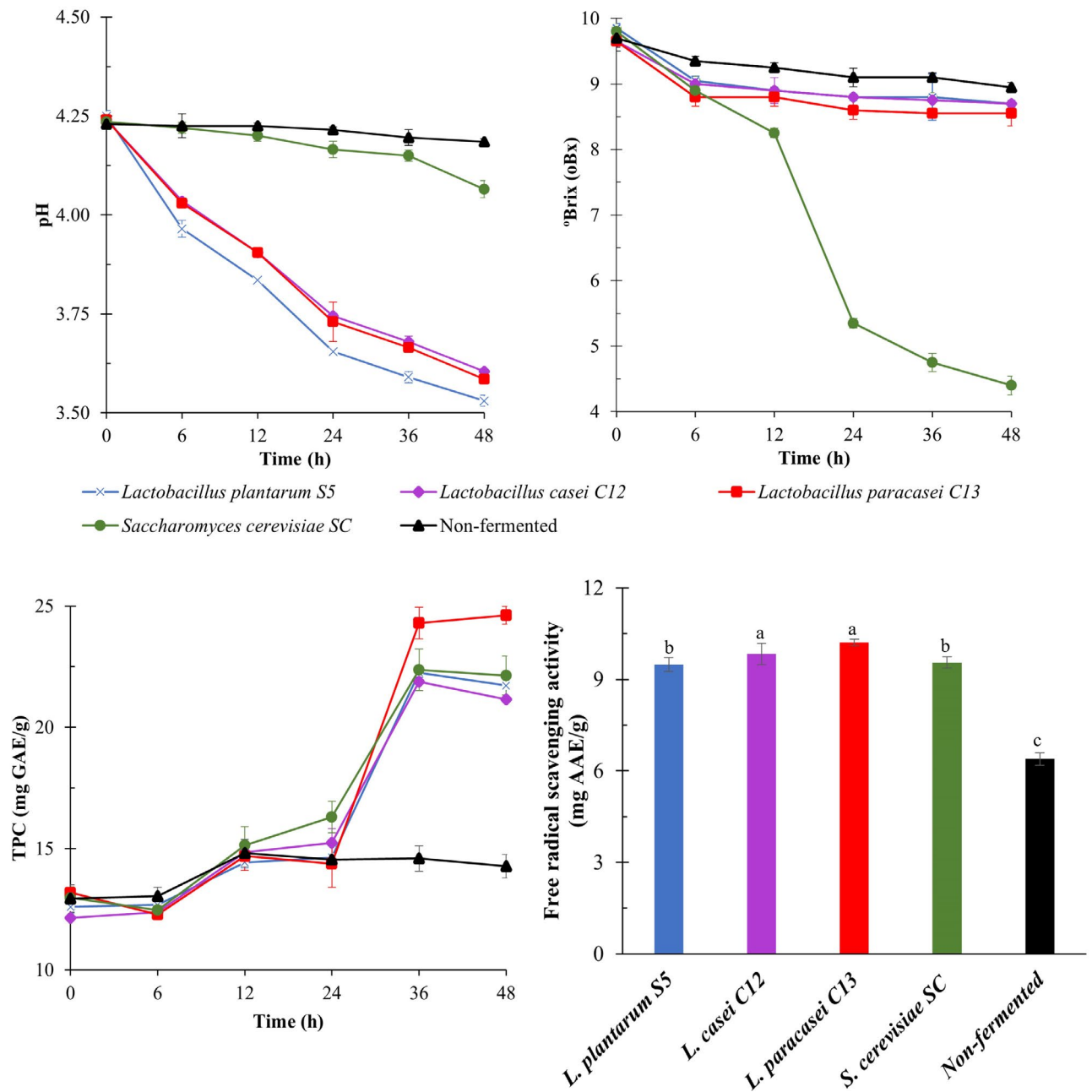


Figure 2: (a) Changes in pH, (b) °Brix, (c) Total phenolic content (TPC) during the fermentation with different microorganisms, and (d) Free radical scavenging activity (mg AAE/g) of fermented cascara drinks at 48 h. Error bars represent standard deviation of three independent experiments.

Table 1: Extraction yield of caffeine and phenolic compounds by different microorganisms at 36 h

	Caffeine (mg/100g)	PCA (mg/100g)	CGA (mg/100g)	CA (mg/100g)
<i>L. plantarum</i> S5	845.45 ± 3.00a	0	129.83 ± 2.59c	1.28 ± 0.06bc
<i>L. casei</i> C12	790.03 ± 1.23c	11.68 ± 0.96b	131.09 ± 0.59bc	1.17 ± 0.16c
<i>L. paracasei</i> C13	728.63 ± 3.33d	12.78 ± 0.22b	136.68 ± 1.44b	21.29 ± 0.13a
<i>S. cerevisiae</i> SC	802.18 ± 2.93b	15.66 ± 0.84a	151.89 ± 1.45a	1.56 ± 0.33bc
Non-fermented	793.63 ± 0.98c	15.42 ± 0.45a	126.98 ± 4.27c	1.68 ± 0.03b

PCA: Protocatechuic acid, CGA: chlorogenic acid, CA: caffeic acid.

Different letters in the same column represent significant differences ($p < 0.05$)

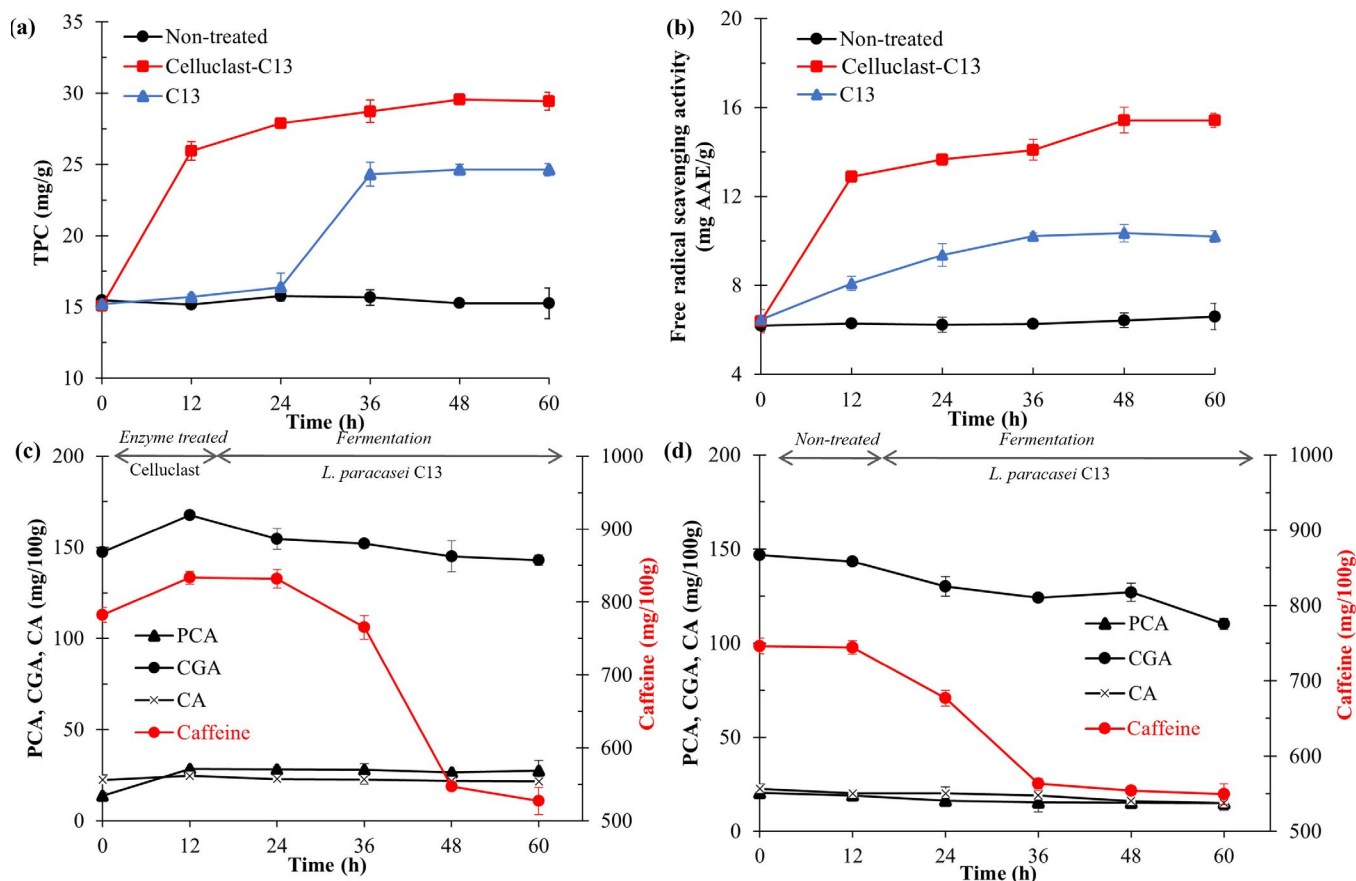


Figure 3: (a) Total phenolic content (TPC) and (b) Free radical scavenging activity of of cascara drinks fermented by *L. paracasei* C13 with and without Celluclast treatment; (c) Production of caffeine and phenolic compounds by Celluclast - *L. paracasei* C13 and (d) fermented by *L. paracasei* C13 alone. PCA: Protocatechuic acid, CGA: chlorogenic acid, CA: caffeic acid. Error bars represent standard deviation of three independent experiments.

4 DISCUSSION

4.1 Effect of enzymatic treatment on total phenolic content and antioxidant activity of coffee cascara

Phenolic compounds, commonly known as polyphenols, represent a diverse class of secondary plant metabolites. These compounds demonstrate multiple health benefits such as antioxidant, antimicrobial, anticancer, anti-inflammatory, and antitumor properties with antiviral, anti-allergic, antithrombotic, and cardioprotective effects (Gligor et al., 2019). Thus, these bioactive components have attracted increasing attention for application in the production of high-quality foods and beverages.

Bioactive compounds commonly occur in plant materials in free and bound forms. Enzymatic treatment separates the bound bioactive components by breaking the linkages within the cell wall matrix, leading to enhanced release of phenolic compounds (Acosta-Estrada et al., 2014). The enzymatic process addresses the limitations of solvent extraction methods and is also a green technique that provides natural bioactive ingredients for the food and pharmaceutical industries (Gligor

et al., 2019). Celluclast is a mixture of endoglucanases, cellobiohydrolases, and β -glucosidases that is widely used to hydrolyze various lignocellulosic feedstocks. Celluclast treatment facilitates the release of bound phenolic compounds from coffee cascara by breaking glycosidic linkages within the plant cell wall matrix. In this study, Celluclast yielded the highest TPC, followed by Pectinex and Viscozyme. All enzymes used improved TPC by 1.6 – 1.8 times compared to the non-treated sample. The data obtained were consistent with previously published results. The phenolic content of oat bran significantly increased when treated with Celluclast (338.7 μ g/g), Viscozyme (326.8 μ g/g), and amyloglucosidase (61.4 μ g/g), compared to untreated bran (121.7 μ g/g) (Alrahmany, et al., 2013). Celluclast was found to be effective in extracting pectin from several agro-industrial residues (Babbar et al., 2016). Celluclast has reported to improve the extraction yield, total flavonoids, total polyphenols, and DPPH free-radical scavenging activity of spent coffee grounds (Scully; Jaiswal; Abu-Ghannam, 2016). Pectinex was suitable for the treatment of pectin-rich materials such as pomelo peel (Huang et al., 2014) and beetroot residue while Viscozyme has cellulolytic

and pectolytic activity that are effective for the extraction of phenolics, flavonoids, and antioxidants from banana peel (Gao et al., 2022). Enzyme-assisted extraction resulted in higher yields of phenolic compounds and also significantly increased free radical-scavenging activity (Gómez-García; Martínez-Ávila; Aguilar, 2012). Numerous studies demonstrated a correlation between TPC and DPPH scavenging activity.

4.2 Fermentation of coffee cascara by different microorganisms

In this study, a notable decline in pH occurred within the initial 48 h of fermentation, mainly attributed to the activity of lactic acid bacteria. LAB produced organic acids, primarily lactic acid, leading to a decrease in pH during fermentation. This pH reduction serves as a crucial signal for the acidification process, playing a vital role in preserving and enhancing the flavor of the fermented product (Degrain et al., 2020). Our findings aligned with previously reported results for other fermented beverages. For example, tomato juices processed with *L. sanfranciscensis* showed a decrease in pH during incubation (Zhu et al., 2020), with decrease in pH from 5.0 to 3.9 within 72 hours of coffee pulp juice fermentation using *L. casei* (Myo; Nantarat; Khat-Udomkiri, 2021). The pH values of cascara fermented by *S. cerevisiae* SC (4.10 ± 0.02) were higher than those obtained from LAB fermentation (Figure 2a), indicating that the acidity levels of cascara beverages were impacted by the metabolism of the specific microorganisms employed in the fermentation process. The sensory properties of drinks were also notably influenced by the pH levels of the fermentation broth (Poláková et al., 2023).

The Brix value of the fermentation broth was used to assess the soluble solid contents, typically dissolved sugar levels. This decline in the Brix value was attributed to sugar transformation into alcohol, a natural product of yeast fermentation by *S. cerevisiae* (Filannino et al., 2018). However, there was a negligible decrease in the °Bx of the cascara drinks during lactic acid fermentation (Figure 2b) because *Lactobacillus* primarily metabolized simple sugars into organic acids, which had an insignificant effect on °Bx (Xu et al., 2020).

Cascara fermentation triggers structural breakdowns of the plant cell wall matrix, leading to the liberation of bound phenolic compounds (Filannino et al., 2018). Consequently, the assessment of phenolic compounds serves to study the efficiency of the fermentation process. Some phenolics are covalently attached to plant cell wall components comprising cellulose, hemicellulose, lignin, pectin, and structural proteins (Chen et al., 2015). These components are broken down into low molecular weight fragments during the lactic acid fermentation process, thereby releasing phenolic compounds (Filannino et al., 2015). A previous study have demonstrated

that cascara fermentation using LAB strains enhanced antioxidant activity, which shows a strong correlation with TPC over time (Oktaviani et al., 2020). Kiwi beverages fermented by *L. plantarum* increased total phenolics, total flavonoids, lactic acid, acetic acid, and succinic acid, furthermore, this beverage showed significantly higher antioxidant activity (ABTS⁺, DPPH radical scavenging activities, and hydroxyl radical scavenging activities) (Kim et al., 2024). This study found that *L. paracasei* C13 was the most effective strain for recovering TPC from coffee cascara, showing promise for industrial application. The control samples consistently maintained the lowest TPC levels, indicating the absence of specific treatments or conditions that induced the production of phenolic compounds.

The metabolite contents during cascara fermentation varied according to the microbial strains applied. CGA is the most abundant phenolic acid in coffee cascara with various health-promoting antioxidant, anti-inflammatory, and antitumor properties (Nwafor et al., 2022). PCA and CA are also bioactive phenolic acids associated with many health benefits (Bondam et al., 2022). Caffeine is a natural stimulant found in various parts of coffee beans (Faudone et al., 2021). However, excessive consumption of caffeine is not good for human health. *S. cerevisiae* SC produced ethanol during fermentation, which subsequently facilitated the liberation of CGA and PCA from coffee cascara (Table 1). Additionally, organic acids secreted by LAB strains could serve as effective solvents for extracting biomolecules from cascara. In another study, fermentation of mulberry juice with *L. plantarum*, *L. acidophilus*, and *L. paracasei* resulted in TPC ranging from 6.51 to 8.34 mg GAE/g and CGA content from 71.08 to 98.06 µg/mL, whereas the unfermented sample only reached 1.41 mg GAE/g and 61.51 µg CGA/mL (Kwaw et al., 2018). The *L. paracasei* C13 strain, demonstrating favorable characteristics and high extraction efficiency, was selected for further investigations.

4.3 Effective production of phenolic compounds by combining enzymatic treatment and fermentation processes

The combination of enzymatic pretreatment and subsequent microbial fermentation increased the production of bioactive compounds, thus enhancing product sensory characteristics (Cruz-Casas et al., 2021). The preliminary results suggested that Celluclast effectively liberated phenolic compounds from coffee cascara. These results showed the efficacy of enzyme pretreatment, which enhanced the release of phenolic components by breaking down plant cell wall structures. Enzymatic hydrolysis of carbohydrate materials showed better bioaccessibility of various bioactive compounds (Ribas-Agustí et al., 2018). Previous studies on the effect

of lactic acid bacteria fermentation also demonstrated the liberation of phenolic acids and the modification of phenolic profiles in various phenolic-rich materials (Bustos et al., 2012). Insoluble phenolics are covalently attached to plant cell wall components including in cellulose, hemicellulose, lignin, pectin, and structural proteins (Chen et al., 2015; Tang et al., 2016), which can be broken into low molecular weight sugars for LAB growth during the fermentation process (Zhang et al., 2021), thereby releasing phenolic compounds. Pretreatment of cascara with Celluclast increased TPC by 1.2 times compared to fermentation with *L. paracasei* C13 alone and by 1.9 times over the control sample. This improvement is likely due to the action of Celluclast and other enzymes from *L. paracasei* C13, which cleaved the linkages between phenolics and cell wall components, releasing bound phenolic compounds (Du et al., 2023). Enzymatic treatment and subsequent fermentation of cascara improved TPC extraction yield, leading to enhanced antioxidant activity throughout the process (Zhang et al., 2021). Results indicated the specific activity of the Celluclast enzyme in releasing phenolic compounds from the coffee cascara that subsequently improved the antioxidant capacity.

Ealier research demonstrated that chlorogenic acid was hydrolyzed into caffeic acid and quinic acid by lactic acid bacteria (Filannino et al., 2015). Lactic acid bacteria were hypothesized to use hydroxycinnamic acids (such as chlorogenic acid and caffeic acid) as substances to convert into dihydrocaffeic acid (Filannino et al., 2015). In this study, CGA, PCA, and CA levels in the fermented cascara drinks improved when applying enzymatic treatment before fermentation with *L. paracasei* C13. The release of CGA, PCA, and CA reached a peak after 12 h of enzymatic treatment and then slightly declined over the microbial fermentation period. Interestingly, the caffeine content decreased significantly as the fermentation progressed, with combined enzymatic hydrolysis and fermentation resulting in lower caffeine levels than without enzymatic treatment. However, the role of *L. paracasei* C13 in caffeine degradation requires further investigation.

5 CONCLUSIONS

The cascara treated with Celluclast and followed by fermentation with *L. paracasei* C13 yielded the highest TPC at 29.56 mg GAE/g and the antioxidant activity of 15.40 mg AAE/g. The combined process achieved CGA, PCA, and CA levels of 151.95, 27.99, and 22.58 mg/100 g at 36 h, respectively, which were higher than those obtained through individual microbial fermentation. This approach shows potential for developing coffee cascara-based products, minimizing by-products from coffee processing and promoting ecological sustainability.

6 AUTHORS' CONTRIBUTION

Conceptual Idea: Ly, T.P.T; Trang, N.P.H; Thao, T.T.N; Methodology design: Trang, N.P.H; Thao, T.T.N; Data collection: Vinh, D.H.N.; Hai, H.H.; Data analysis and interpretation: Trang, N.P.H.; Thao, T.T.N, and Writing and editing: Trang, N.P.H; Thao, T.T.N.; Ly, T.P.T; Anh, Q.N.

7 REFERENCES

- ACOSTA-ESTRADA, B. A. et al. Bound phenolics in foods, a review. **Food Chemistry**, 152:46-55, 2014.
- ALRAHMANY, R. et al. Treatment of oat bran with carbohydrases increases soluble phenolic acid content and influences antioxidant and antimicrobial activities. **Food Research International**, 52(2):568-574, 2013.
- AMADI, O. C. et al. Process optimization for simultaneous production of cellulase, xylanase and ligninase by *Saccharomyces cerevisiae* SCPW 17 under solid state fermentation using Box-Behnken experimental design. **Heliyon**, 6(7):e04566, 2020.
- BABBAR, N. et al. Effect of extraction conditions on the saccharide (Neutral and Acidic) composition of the crude pectic extract from various agro-industrial residues. **Journal of Agricultural and Food Chemistry**, 64:268-276, 2016.
- BONDAM, A. F. et al. Phenolic compounds from coffee by-products: Extraction and application in the food and pharmaceutical industries. **Trends in Food Science & Technology**, 123:172-186, 2022.
- BUSTOS, I. et al. Effect of flavan-3-ols on the adhesion of potential probiotic Lactobacilli to intestinal cells. **Journal of Agricultural and Food Chemistry**, 60(36):9082-9088, 2012.
- CHEN, P. X. et al. Characterization of free, conjugated and bound phenolics and lipophilic antioxidants in regular- and non-darkening cranberry beans (*Phaseolus vulgaris* L.). **Food Chemistry**, 185:298-308, 2015.
- CRUZ-CASAS, D. E. et al. Enzymatic hydrolysis and microbial fermentation: The most favorable biotechnological methods for the release of bioactive peptides. **Food Chemistry: Molecular Sciences**, 3:100047, 2021.
- DE MELO PEREIRA, G. V. et al. Chemical composition and health properties of coffee and coffee by-products. **Advances in Food and Nutrition Research**, 91:65-96, 2020.
- DEGRAIN, A. et al. Effect of Lactic acid fermentation on color, phenolic compounds and antioxidant activity in African nightshade. **Microorganisms**, 8(9):1324, 2020.

- DOO, H. et al. Lactic acid bacteria in Asian fermented foods and their beneficial roles in human health. **Food Science and Biotechnology**, 33:2021-2033, 2024.
- DU, G. et al. Effects of Tibetan kefir grain fermentation on the physicochemical properties, phenolics, enzyme activity, and antioxidant activity of Lycium barbarum (Goji berry) juice. **Food Bioscience**, 53:102555, 2023.
- ESQUIVEL, P.; JIMÉNEZ, V. M. Functional properties of coffee and coffee by-products. **Food Research International**, 46(2):488-495, 2012.
- FAUDONE, G. et al. The Medicinal chemistry of caffeine. **Journal of Medicinal Chemistry**, 64(11):7156-7178, 2021.
- FILANNINO, P. et al. Metabolism of phenolic compounds by *Lactobacillus* spp. during fermentation of cherry juice and broccoli puree. **Food Microbiology**, 46:272-279, 2015.
- FILANNINO, P. et al. Metabolic and functional paths of lactic acid bacteria in plant foods: get out of the labyrinth. **Current Opinion in Biotechnology**, 49:64-72, 2018.
- GAO, L. et al. Customized optimization of lignocellulolytic enzyme cocktails for efficient conversion of pectin-rich biomass residues. **Carbohydrate Polymers**, 297:120025, 2022.
- GLIGOR, O et al. Enzyme-assisted extractions of polyphenols: A comprehensive review. **Trends in Food Science & Technology**, 88:302-315, 2019.
- GÓMEZ-GARCÍA, R.; MARTÍNEZ-ÁVILA, G. C. G.; AGUILAR, C. N. Enzyme-assisted extraction of antioxidative phenolics from grape (*Vitis vinifera* L.) residues. **3 Biotech**, 2:297-300, 2012.
- HU, X. et al. Citrus pomace fermentation with autochthonous probiotics improves its nutrient composition and antioxidant activities. **LWT**, 157:113076, 2022.
- HUANG, R. C et al. Enhanced ethanol production from pomelo peel waste by integrated hydrothermal treatment, multienzyme formulation, and fed-batch operation. **Journal of agricultural and food chemistry**, 62(20):4643-4651, 2014.
- KEDARE, S. B.; SINGH, R. P. Genesis and development of DPPH method of antioxidant assay. **Journal of Food Science and Technology**, 48:412-422, 2011.
- KIM, H. G. et al. Antioxidant and anticancer effects of kiwi (*Actinidia deliciosa*) fermented beverage using *Lactobacillus plantarum*. **Food Science and Biotechnology**, 34:207-216, 2024.
- KWAW, E. et al. Effect of *Lactobacillus* strains on phenolic profile, color attributes and antioxidant activities of lactic-acid-fermented mulberry juice. **Food chemistry**, 250:148-154, 2018.
- MARTILLANES, S. et al. Cellulase-assisted extraction of phenolic compounds from rice bran (*Oryza sativa* L.): Process optimization and characterization. **Journal of Food Measurement and Characterization**, 15:1719-1726, 2021.
- MYO, H.; NANTARAT, N.; KHAT-UDOMKIRI, N. Changes in bioactive compounds of coffee pulp through fermentation-based biotransformation using *Lactobacillus plantarum* TISTR 543 and its antioxidant activities. **Fermentation**, 7(4):292, 2021.
- NWAFOR, E.-O. et al. Chlorogenic acid: Potential source of natural drugs for the therapeutics of fibrosis and cancer. **Translational Oncology**, 15(1):101294, 2022.
- OKTAVIANI, L. et al. Fermentation of coffee pulp using indigenous lactic acid bacteria with simultaneous aeration to produce cascara with a high antioxidant activity. **Heliyon**, 6(7):e04462, 2020.
- POLÁKOVÁ, K. et al. Quality attributes and sensory acceptance of different botanical coffee co-products. **Foods**, 12(14):2675, 2023.
- RIBAS-AGUSTÍ, A. et al. Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods. **Critical Reviews in Food Science and Nutrition**, 58(15):2531-2548, 2018.
- ROBERTS, A. Caffeine: An evaluation of the safety database. In: RAMESH, C. et al. **Nutraceuticals efficacy, safety and toxicity**. London: Nutraceuticals, p. 501-508, 2021.
- SZUTOWSKA, J. Functional properties of lactic acid bacteria in fermented fruit and vegetable juices: A systematic literature review. **European Food Research and Technology**, 246(3):357-372, 2020.
- SCULLY, D. S.; JAISWAL, A. K.; ABU-GHANNAM, N. An investigation into spent coffee waste as a renewable source of bioactive compounds and industrially important sugars. **Bioengineering**, 3(4):33, 2016.
- TANG, Y. et al. Bound phenolics of quinoa seeds released by acid, alkaline, and enzymatic treatments and their antioxidant and α -glucosidase and pancreatic lipase inhibitory effects. **Journal of Agricultural and Food Chemistry**, 64:1712-1719, 2016.
- TRINH, L. T. P. et al. Production of phenolic compounds and biosugars from flower resources via several extraction

- processes. **Industrial Crops and Products**, 125:261-268, 2018.
- WANG, H. et al. Isocratic elution system for the determination of catechins, caffeine and gallic acid in green tea using HPLC. **Food Chemistry**, 68:115-121, 2000.
- XU, Y. et al. Fermentation by probiotic *Lactobacillus gasseri* strains enhances the carotenoid and fibre contents of carrot juice. **Foods**, 9(12):1803, 2020.
- ZHANG, J. et al. Viscozyme L hydrolysis and *Lactobacillus* fermentation increase the phenolic compound content and antioxidant properties of aqueous solutions of quinoa pretreated by steaming with α -amylase. **Journal of Food Science**, 86(5):1726-1736, 2021.
- ZHU, W. et al. Functional efficacy of probiotic *lactobacillus sanfranciscensis* in apple, orange and tomato juices with special reference to storage stability and in vitro gastrointestinal survival. **Beverages**, 6(1):13, 2020.