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THE INFLUENCE OF STARTER CULTURES OF LACTIC ACID BACTERIA ON FERMENTATION OF WHITE CABBAGE

Mihaela MULȚESCU[1,2], Iulia-Elena SUSMAN[1,2], Alina CULEȚU[1], Medana ZAMFIR[3], Angel MARTÍNEZ SANMARTIN[4], Florentina ISRAEL-ROMING[2]

[1]National Institute of Research and Development for Food Bioresources – IBA Bucharest, 6 Dinu Vintila Street, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[3]Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independentei, Bucharest, Romania
[4]National Technological Centre for the Food and Canning Industry CTC, C. Concordia, s/n 30500 Molina de Segura, Murcia, Spain

Corresponding author email: mihaela.multescu@gmail.com

Abstract

The current investigation delineates the impact of lactic acid bacteria starter cultures of two collection strains (L. plantarum IC12353 (Lpl)) and L. paracasei CCM1837 (Lpa)) and two new isolates (L. plantarum P35 (LAB35) and L. brevis P43 (LAB43)) on the cabbage fermentation process. The content of bioactive compounds, antioxidant activity as well as the colony-forming unit counts were assessed in fermented cabbage samples. The analyzed samples revealed noteworthy quantities of phenolic compounds, ranging from 16.91 to 30.71 mg gallic acid equivalents/100 grams of fresh sample, with the highest values for LAB35 and LAB43, while Lpl had the lowest content of polyphenolics. Moreover, the vitamin C content ranged between 50.82 and 60.35 mg ascorbic acid/100 g, while DPPH values varied from 245.80 to 444.42 µmol Trolox equivalent/100g. The observed variations in the data on bioactive compounds and antioxidant activity can be attributed to the specific lactic acid bacteria strain used in the cabbage fermentation process. This highlights the importance of microbial strains in determining antioxidant properties of the final fermented product.

Key words*: white cabbage, fermentation, starter cultures, lactic acid bacteria, antioxidant capacity.*

INTRODUCTION

Fermentation, an age-old food preservation method, is acknowledged as a straightforward, natural, safe, and significant biotechnological process (Hunaefi et al., 2013). Food fermentations include four main processes: lactic fermentation, alcoholic fermentation, acetic fermentation (which is an aerobic process) and alkaline fermentation (Constantin et al., 2023). Fermented foods have been staples in human nutrition for generations, and in recent times, their popularity has surged due to their recognized health-promoting properties (Dimidi et al., 2019). Fermented foods represent a distinct category characterized by the enzymatic breakdown of carbohydrates facilitated by probiotic microorganisms (Potter & Hotchkiss, 2006). Acid-fermented vegetables showcase diverse flavors from various corners of the globe (Yuan et al., 2023).

Cabbage holds a preeminent position among the most popular fermented vegetables, also known as sauerkraut in Germany, Kimchi in Korea, Dhamuoi in Vietnam, and Cortido in Latin America (Kusznierewicz et al., 2008; Park et al., 2012). In Romania, the traditional method of preparing fermented cabbage involves whole cabbage heads, wherein microbial activity induces the conversion of sugars into lactic acid, a fundamental biological process in the fermentation (Breidt et al., 2013).

Pickled cabbage fermentation occurs due to the spontaneous production of lactic acid by lactic acid bacteria (LAB) under favorable temperature and salt conditions in an oxygen-free environment. Lactic acid bacteria represent a group of a important bacteria that are used to produce fermented foods and beverages (Zamfir et al., 2014). LABs are commonly employed as a starting culture in the fermentation of foods, leading to significant alterations in nutritional,

sensory, and physicochemical properties (Sáez et al., 2018). The prevalent LABs involved in pickle fermentation include *Leuconostoc mesenteroides*, *Enterococcus faecalis*, *Levilactobacillus brevis*, *Lactiplantibacillus pentosus, Pediococcus pentosaceus*, and *Lactiplantibacillus plantarum*. *L. plantarum*, known for its heightened acid tolerance compared to other LABs, plays a pivotal role in completing the fermentation of fruits and vegetables (di Cagno et al., 2013). The use of starter cultures containing *L. plantarum* influence the quality of pickled cabbage, particularly in environments with low salt concentrations (Zubaidah et al., 2020).

Numerous researchers have explored the utilization of starter cultures and identified them as advantageous for standardizing the fermentation process by regulating the microflora throughout the procedure (Gardner et al., 2001; Torres et al., 2020). Despite the ongoing progress, the application of starter cultures in cabbage fermentation remains underexplored and insufficiently investigated. Producers are actively seeking their incorporation to enhance the quality of fermented cabbage production (Draskovic Berger et al., 2020).

Therefore, the objective of this investigation was to determine the impact of lactic acid bacteria starter cultures (*L. plantarum* IC12353*, L. paracasei* CCM1837, and 2 new strains, *L. plantarum* P35 and *L. brevis* P43) on the fermentation of white cabbage, with a specific emphasis on assessing the total phenolic content (TPC), ascorbic acid (AA), and antioxidant activity using the DPPH assay in pickled cabbage. Additionally, we quantified the concentration of lactic acid using High-Performance Liquid Chromatography (HPLC) and enumerated the lactic bacteria in the fermented cabbage through incubation at 30°C to identify the optimal bacteria starter culture for cabbage's fermentation.

MATERIALS AND METHODS

Materials

White cabbage (*Brassica oleracea* L. var. *capitata*) was cultivated in the experimental fields of the Research and Development Station for Vegetables, Buzau, Romania. The selected vegetables were sown in 2019. The LAB strains were from the collection of the Bucharest Institute of Biology. Two strains, *L. plantarum* IC12353 (Lpl) and *L. paracasei* CCM1837 (Lpa) are reference strains from international collections of microorganisms, and the other two strains *L. plantarum* P35 (LAB35) and *L. brevis* P43 (LAB43) were previously isolated from borsch and were identified by molecular techniques (rep-PCR, 16S sequencing and PCR amplification with species-specific primers) (Cornea et al., 2016).

2,6-dichlorophenolindophenol; 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 6- hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox) reagent were from Sigma Chemical Co. (Switzerland). Folin Ciocalteu's phenol reagent and ascorbic acid (AA) were purchased from Merck (Germany). All reagents were of analytical grade. Standard solutions were prepared with distilled water.

Cabbage fermentation: The pickled cabbage samples were made by using the different LAB. The concentration of the inoculum was 10^8 CFU/ml. The fermented samples were inoculated with 0.5 ml LAB ($2.5x10^5$ CFU/ml) and 2.0 ml LAB ($1x10^6$ CFU/ml). The pickles were made with 9 different treatments encoded as follows: Control (salt (2.5% NaCl)), Lpl-0.5 (*L. plantarum* 0.5 ml + salt), Lpl-2.0 (*L. plantarum* 2.0 ml + salt), Lpa-0.5 (*L. paracasei* 0.5 ml + salt), Lpa-2.0 (*L. paracasei* 2.0 ml + salt), LAB35-0.5 (*L. plantarum* P35 0.5 ml + salt), LAB35-2.0 (*L. plantarum* P35 2.0 ml + salt), LAB43-0.5 (*L. brevis* P43 0.5 ml + salt), and LAB43-2.0 (*L. brevis* P43 2.0 ml + salt). The cabbages that were washed and dried were made into small pieces; and 17.5 g (2.5%) rock salt was added to 700 g cabbage, and after thoroughly blending, placed inside 800 mL pickle vessels. Pickle containers were stored for 35 days at ambient conditions (20-25°C). Several studies have presented varying fermentation durations. For instance, Touret et al. (2018) reported a fermentation period of 30 days at 15-20 °C, while Alan and Yildiz (2022) reported a longer duration of 60 days at 20-25 °C. Based on these studies we consider the fermentation process is generally complete after 35 days.

The cultivation of LAB strains was carried out in liquid MRS broth. The inoculum for

cultivation of lactobacilli strains was prepared by dissolving 52 g of powder MRS medium obtained from Carl ROTH, Germany, in one liter of distilled water following the manufacturer's instructions.

Methods

A quantity of 3.0 g of fermented cabbage was measured and immersed in 30 mL of 50% aqueous methanol. The resulting mixtures were subjected to vortexing for a duration of 3 hours at a rotational speed of 2,000 rpm using a Heidolph Instruments Multi Reax vortex. Following this step, the mixtures were subjected to centrifugation for 30 minutes at 10,000 rpm to separate and eliminate any secondary materials (Multescu et al., 2022). The extract was used for the analysis of TPC and antioxidant activity.

Determination of total phenolic content (TPC)

The determination of TPC in fermented white cabbage was conducted using the Folin-Ciocalteau reagent, following adaptations made for laboratory conditions (Singleton et al., 1999). In brief, 1 mL of the extract was combined with 5 mL of Folin-Ciocalteau reagent and 4 mL of 20% sodium carbonate solution. After 20 minutes of incubation at room temperature, the absorbance was measured at 752 nm using a Specord 210 UV-VIS spectrophotometer (Analytic Jena, Bremen, Germany). To create a standard curve, various concentrations (ranging from 10 to 50 µg/mL) of gallic acid were utilized under the same conditions as the samples (R^2= 0.9990). The total phenolic content was then quantified and expressed as milligrams of gallic acid equivalent per 100 grams of fresh weight (mg GAE/100g FW).

Determination of Antioxidant Activity through DPPH

The evaluation of DPPH radical scavenging activity was conducted based on the reduction of the DPPH radical, following the method outlined by Culetu et al. (2016) with slight modifications. The reaction mixture comprised 1 mL of the methanolic extract and 6 mL of the DPPH radical solution, which underwent incubation for 20 minutes in the absence of light. Then, the absorbance was measured at 517 nm using a Specord 210 UV-VIS spectrophotometer (Analytic Jena, Bremen, Germany). The antioxidant activity was calculated utilizing a calibration curve (ranging from 0.05 to 0.6 mmol/L) established with Trolox as a standard (R^2 = 0.9995). The results were expressed in milligrams of Trolox equivalent per 100 grams of fresh weight (µmol TE/100 g FW).

Determination of pH value

The results were acquired utilizing a WTW Inolab 7110 pH-meter. The measurements were conducted at a controlled temperature of 20 °C, whereby the pH electrode was immersed in the juice of the cabbage samples. The recorded pH values were taken after reaching equilibrium.

Determination of total titratable acidity (TTA)

Titratable acidity was determined using 942.15 Association of Official Analytical Chemists (AOAC) official method (AOAC, 2000). 20 mL of fermented cabbage juice was titrated with 0.1 N alkali in the presence of phenolphthalein until it reached a pH of 8.3. The results were recorded as the percent of lactic acid.

Detrmination of lactic acid

A supelcogel H column with 9 µm spherical particles was used for the separation, with a column inner diameter of 4.6 mm and a column length of 25 cm. The mobile phase utilized consisted of a 0.1% phosphoric acid solution, with the elution conducted in an isocratic manner at 1ml/min rate. The separation was performed at 30°C, with an injection duration of 50 minutes and 5 minutes of equilibration time between samples. The identification of lactic acid was based on the recorded retention time for the analyte in the standard solutions, with the retention time for lactic acid being 14.85 minutes. The examination of lactic acid levels in fermented cabbage juice was conducted at the following time points: 7 days, 16 days, and 28 days following the introduction of lactic acid bacteria.

Determination of vitamina C

To quantify the AA content, the dye-titration method was employed, following the guidelines outlined in the AOAC procedure of 2000,

AOAC, 967.21 (AOAC, 2000). An amount of 3.0 g of samples was mixed with 30 mL metaphosphoric acid for 3 hours. The extracts of fermented cabbage were subjected to titration with 2,6-dichlorophenolindophenol. The endpoint of the titration was determined when excess unreduced dye produced a rose-pink color in an acidic environment. Notably, dehydroascorbic acid was not analyzed in this study. The results were reported in milligrams of ascorbic acid per 100 grams of fresh weight (mg AA/100 g FW).

Determination of lactic acid bacteria

Determination of mesophilic lactic acid bacteria was performed according to standard (SR ISO 15214:2001) by plating 0.1 mL of each sample on Plate Count Agar (PCA-Oxoid, UK) using a Drigalski spatula, followed by incubation at 30°C for three days. After the incubation period, the colonies from the plates were numbered. Interpretation of results was performed using the following Formula (1):

$$CFU = (\sum C)/((n_1 + 0.1n_2) \times d) \quad (1)$$

where: CFU = average no. of colony forming units from two serial dilutions; ΣC = sum of colonies counted in all retained plates; n_1 = number of plates retained at first dilution; n_2 = number of plates retained at the second dilution; d = dilution from which the first counts were made.

Statistical analysis

All the analyses were performed in at least three repetitions. Results are presented as means ± standard deviation (SD). To determine the relation between results of total phenolics and antioxidant assays, the Pearson correlation was used. The data were analyzed through an analysis of variance (ANOVA) using Minitab software (version 19, Minitab Inc., Coventry, UK) to observe if there were significant differences between the results of the analyses. In order to compare the means of each analysis, Tukey's test was used, and the level of significance was considered to be less than 0.05 ($p < 0.05$). Besides, the data were subjected to the principal component analysis (PCA) to identify variations among the samples and correlations between the studied parameters.

RESULTS AND DISCUSSIONS

1. pH values and titratable acidity

One crucial parameter in fermentation process is the pH value. On day 0 of fermentation, fresh cabbage had a pH value of 6.25. After 35 days of fermentation, the pH levels decrease in all pickled samples, ranging from 3.15 to 3.39. The reduced pH levels may be attributed to the addition of different concentrations of *L. plantarum* and *L. brevis.* The production of lactic acid by LAB leads to a reduction in the environmental pH. Fermentation is initiated by heterofermentative LAB, mainly *Leuconostoc mesenteroides* (Peñas et al., 2017). These bacteria dominate the microbiota at the beginning of fermentation since it possesses less acid tolerance and microaerophilic properties, and has a shorter generation time than other LAB over temperatures of 18–20°C and NaCl concentrations up to 5% (Peñas et al., 2017). Other LAB, such as *Leuconostoc fallax* seem to be also involved in the early fermentation period. These bacterial communities produce significant amounts of organic acids that lead to a decrease of the pH, as well as carbon dioxide that provides an anaerobic environment (Peñas et al., 2017). When the acid content increases to 0.7-1% and the pH decreases below 4.5, *Leuconostoc* species are replaced by more acid-tolerant LAB, such as *Lactobacillus plantarum* and *Lactobacillus brevis*. These populations produce lactic acid almost exclusively and dominate the late stage of the fermentation, when the pH reaches values ranging from 3.4 to 3.7 (Peñas et al., 2017). Zamfir et al. (2022) used three strains in the fermentation process of wheat bran combined with root vegetables, which led to a notable reduction in pH, reaching pH values between 3.1 and 3.6. These lower pH values depend on the strain and on the substrate, with a concomitant increase in the viable cell numbers and in the lactic acid production, as it has been previously reported for LAB grown in various plant-based substrates (Gupta et al., 2011).

The total acid content in the fermented brine was subjected to analysis. At the beginning of fermentation (day 0), fresh cabbage presented a TTA value of 0.33%, significantly lower compared with the fermented samples ($p< 0.05$).

All fermented samples had an increased TTA during the fermentation period (Table 1). Fresh cabbage presented a TTA value of 0.33%. After 35 days of fermentation, the concentration of total titratable acidity fell within the range of 0.54-0.77%.

Table 1. pH value and total titratable acidity (TTA) of fermented cabbage after 35 days

Sample	pH	TTA (%)
Fresh cabbage	6.25±0.01 ^{a}	0.33±0.02 ^{d}
Control	3.16±0.01 ^{d}	0.65±0.01 ^{b}
Lpa-0.5	3.15±0.01 ^{d}	0.77±0.01 ^{a}
Lpa-2.0	3.16±0.01 ^{d}	0.65±0.01 ^{b}
Lpl-0.5	3.19±0.01 ^{d}	0.59±0.02 ^{bc}
Lpl-2.0	3.17±0.02 ^{d}	0.64±0.02 ^{b}
LAB35-0.5	3.29±0.01 ^{c}	0.54±0.01 ^{c}
LAB35-2.0	3.39±0.02 ^{b}	0.58±0.02 ^{bc}
LAB43-0.5	3.18±0.02 ^{d}	0.59±0.01 ^{bc}
LAB43-2.0	3.17±0.01 ^{d}	0.62±0.02 ^{bc}

The results are presented as mean ± standard deviation. Means with different letters in a column are significantly different ($p < 0.05$).
Control: natural fermentation; Lpl: *L. plantarum* IC12353; Lpa: *L. paracasei* CCM1837; LAB35: *L. plantarum* P35; LAB43: *L. brevis* P43.

Among the samples, Lpa-0.5 exhibited the highest acidity value (0.77%, $p< 0.05$), followed by Lpa-2.0 and Lpl-2.0, whereas LAB35-0.5 displayed the lowest value (0.54%). Prior research has indicated that the most favorable fermentation conditions are achieved when the acidity concentration is maintained at 0.6% (Ku et al., 1998). Similar to pH values, the acidity levels achieved at the end of fermentations play an important role in determining the success of fermented products (Pires-Cabral et al., 2022).

2. Phenolic compounds and vitamin C content

Table 2 illustrates the impact of fermentation using different lactic acid bacterial cultures on the concentration of phenolic compounds and vitamin C content in white fermented cabbage samples. Fresh cabbage presented a TPC of 20.87 mg GAE/100 g FW. After 35 days of fermentation, the analyzed samples exhibited quantities of phenolic compounds, ranging from 16.91 to 30.71 mg GAE/100g FW. With the exception of samples Lpl-0.5 and Lpl-2.0, which demonstrated lower levels of total polyphenols at 16.91 mg GAE/100g FW and 18.29 mg GAE/100 g FW, respectively, all other samples exhibited higher phenolic contents compared to control sample (24.53 mg GAE/100 g FW).

Notably, LAB35-0.5 and LAB43-0.5 samples exhibited the highest concentration of phenolic compounds. There were statistically significant variations for TPC among all the samples ($p<0.05$), except for samples LAB35-0.5 and LAB43-0.5, as well as LAB35-2.0 and LAB43-2.0, where no significant differences were observed ($p>0.05$). In case of Lpa, LAB35 and LAB43, it was observed that a lower volume of lactic acid bacteria (0.5 mL) used for fermentation led to a significant increase of TPC compared to the volume of 2 mL ($p<0.05$).

Following fermentation, samples inoculated with *L. plantarum* P35 and *L. brevis* P43 exhibited the highest concentrations of total polyphenols. The increase in phenolic compounds is attributed to the enzymatic action of microorganisms that break down the cell wall matrix (Hole et al., 2012). Consistent with our study's findings, previous research has shown that the total polyphenol content in vegetables rises after fermentation with LAB. This occurs as glucosides are converted to their aglyconic form, leading to an enhanced total polyphenol content in vegetables and fruits (Sayin et al., 2015).

Ciska et al. (2005) reported concentrations of phenolic compounds in white cabbage and fermented cabbage samples as 575 mg GAE/100g FW and 825 mg GAE/100g FW, respectively. Chun et al. (2004) analyzed various naturally fermented cabbage samples and found a total polyphenol content ranging from 57.3 to 85.5 mg GAE/100 g FW. Notably, the outcomes from our research indicate that the levels of polyphenols in fermented cabbage are lower compared to the values reported in the aforementioned studies.

The impact of fermentation on food phenolics has been explored in various studies, yielding divergent findings. This variability could be due to the complexity of biotransformation of phenolic compounds by the different microorganisms, and different fermentation conditions and food matrix (Zhao et al., 2006).

At day 0, raw cabbage had a vitamin C concentration of 38.25 mg AA/100 g FW. After fermentation period, the vitamin C content ranged from 50.82 to 60.35 mg as AA/100 g FW (Table 2). The fermented extracts inoculated with LAB35 and LAB43 presented the highest levels of vitamin C, with samples LAB35-2.0 and LAB43-0.5 recording concentrations of 60.35 mg AA/100g FW and 60.32 mg AA/100 g FW, respectively ($p>0.05$). The control sample

demonstrated the lowest level of vitamin C, measuring 50.82 mg AA/100 g FW.

Table 2. Phenolic content and vitamin C concentration in fermented cabbage

Sample	TPC (mg GAE/100g FW)	Vitamin C (mg AA/100g FW)
Fresh cabbage	20.87±0.02 ^{f}	38.25±0.02 ^{h}
Control	24.53±0.08 ^{e}	50.82±0.04 ^{g}
Lpa-0.5	26.87±0.02 ^{c}	51.13±0.05 ^{f}
Lpa-2.0	25.35±0.07 ^{d}	57.02±0.03 ^{c}
Lpl-0.5	16.91±0.04 ^{h}	55.61±0.03 ^{d}
Lpl-2.0	18.29±0.06 ^{g}	54.15±0.06 ^{e}
LAB35-0.5	30.71±0.1 ^{a}	51.09±0.04 ^{f}
LAB35-2.0	30.25±0.06 ^{b}	60.35±0.06 ^{a}
LAB43-0.5	30.69±0.04 ^{a}	60.32±0.03 ^{a}
LAB43-2.0	30.38±0.04 ^{b}	59.77±0.04 ^{b}

The results are presented as mean ± standard deviation. Means with different letters in a column are significantly different ($p < 0.05$).
Control: natural fermentation; Lpl: *L. plantarum* IC12353; Lpa: *L. paracasei* CCM1837; LAB35: *L. plantarum* P35; LAB43: *L. brevis* P43.

Only in the case of Lpa and LAB35, a higher volume of lactic acid bacteria used in the cabbage fermentation contributed to a significant higher content of vitamin C ($p<0.05$). Zhao et al. (2006) demonstrated that the level of vitamin C in fermented foods is susceptible to degradation due to oxidation. Additionally, ascorbic acid is an unstable component and can rapidly decompose due to storage conditions (Hashemi et al., 2017). The findings from the present study indicate lower levels of vitamin C compared to previous research, where fermented cabbage contained 109.89 mg AA/100 g FW, and fermented cabbage juice contained 208.14 mg AA/100g FW (Őzer et al., 2019). In contrast to the data presented in this study, some authors reported that after the fermentation process of cabbage, the vitamin C content decreases, resulting in white cabbage having a higher vitamin C level than fermented cabbage products (Ciska et al., 2005; Martinez-Villaluenga et al., 2012; Martinez-Villaluenga et al., 2009; Kapusta-Duch et al., 2017).

3.Comparative analysis of lactic acid synthesis from fermented white cabbage

The analysis of lactic acid in fermented white cabbage was performed at 7, 16, and 28 days after inoculation with LAB (Figure 1). Inoculation with lactic acid bacteria resulted in an increase in the level of lactic acid. After 7 days of fermentation, the lactic acid concentration varied between 2.42 and 4.62 mg/ml.

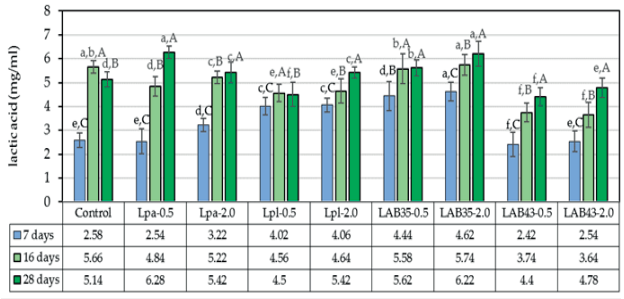

Figure 1. Lactic acid content in fermented cabbage samples

Bar represents standard deviation of mean. Means with different lowercase letters are significantly different within the same period studied ($p < 0.05$). Means with different uppercase letters are significantly different between the 3 intervals studied within the same sample ($p < 0.05$).
Control: natural fermentation; Lpl: *Lactobacillus plantarum* IC12353; Lpa: *Lactobacillus paracasei* CCM1837; LAB35: *L. plantarum* P35; LAB43: *L. brevis* P43.

Compared with the control, all the samples exhibited a high lactic acid content except Lpa-0.5, LAB43-0.5 and LAB43-2.0 samples. LAB35-2.0 presented the highest concentration of lactic acid, while LAB43-0.5 had the lowest content of lactic acid. During this period, the lactic acid bacteria level was notably lower compared to the other two intervals examined (16 and 28 days). At the 16 days, LAB35 and Lpa strains exhibited the highest lactic acid concentrations. Consequently, after 16 days LAB35-0.5, LAB35-2.0 and Lpa-2.0 samples had concentrations of 5.58 mg/ml, 5.74 mg/ml and 5.22 mg/ml, respectively. Subsequently, over the 28-day period, cabbage fermented with the LAB35 and Lpa strains exhibited the highest lactic acid concentration, while LAB43 strain had the lowest acid lactic content.

3.4. Antioxidant activity of fermented cabbage samples

The inoculation of lactic acid bacteria had a substantial effect compared to the natural fermentation of white cabbage, resulting in increased antioxidant activity levels. Overall, following fermentation treatments, there was an increase in antioxidant activity in all types of fermentation (natural or inoculated with LAB cultures) compared to day 0 (Hunaefi et al., 2013). The antioxidant activity ranged between 245.80-444.42 µmol TE/100g FW (Figure 2). The highest antioxidant activity was found in the samples inoculated with LAB35 and LAB43. This enhancement could be attributed to the specific lactic acid bacteria used, which might have the capacity to degrade certain

polyphenolic compounds, resulting in compounds that influence the food's flavor and exhibit higher antioxidant activity (Rodriguez et al., 2009).

A significant correlation (r=0.9333) was observed between the antioxidant activity by DPPH method and TPC.

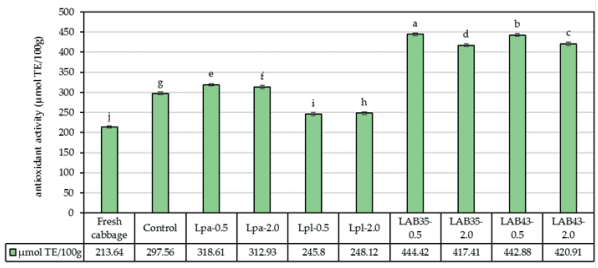

Figure 2. The antioxidant activity in the fermented cabbage

Bar represents standard deviation of mean and means with different letters are significantly different ($p < 0.05$).
Control: natural fermentation; Lpl: *Lactobacillus plantarum* IC12353; Lpa: *Lactobacillus paracasei* CCM1837; LAB35: *L. plantarum* P35; LAB43: *L. brevis* P43.

Samples Lpl-0.5 and Lpl-2.0 presented the lowest values of antioxidant activity, 245.80 µmol TE/100g FW and 248.12 µmol TE/100g FW, respectively. It is observed that the Lpl strain used in the fermentation were not beneficial, as their addition led to a decrease in antioxidant activity. In a study conducted by Kapusta-Duch et al. (2017), the antioxidant activity of fermented cabbage was determined to be 20.0 µmol TE/g FW, which was higher than the results obtained in our study.

Previous studies have demonstrated that fermentation increased the antioxidant capacity of vegetables like cabbage (Sayin and Alkan, 2015) but some plant foods showed decrease in antioxidant capacity like olive (Othman et al., 2009). The activity of lactic bacteria during cabbage fermentation enhances the antioxidative properties of this vegetable (Kusznierewics et al., 2008). The enhanced antioxidant activity observed in fermented cabbage is associated with the capability of lactic acid bacteria to break down polyphenols (Peñas et al., 2015). Degrain et al. (2020) investigated the impact of fermentation using different strains of LAB (LAB17a, LAB56, LAB64, LAB21, and LAB75) on the antioxidant activity in various African nightshade plant species. Fermentation with LAB75 and LAB17a lactic cultures resulted in an enhancement of antioxidant activity by 11.9% and 7.1%, respectively, compared to the fresh sample. Conversely, the African nightshade (*Solanum retroflexum* Dun.) fermented with LAB56 exhibited the lowest antioxidant activity. The observed increase in antioxidant activity was attributed to the release of phenolic compounds during the fermentation process. Hur et al. (2014) reported that the increase in antioxidant activity observed in fermented foods is attributed to the accumulation of antioxidant compounds. Furthermore, certain lactic acid bacteria possess the capability to synthesize superoxide dismutase enzyme, consequently leading to an increased antioxidant activity within the fermentation system (Hur et al., 2014).

3.5. Comparative analysis of lactic acid bacteria number in fermented white cabbage

Fresh cabbage presented a LAB population of $4*10^2$ CFU/g. Peñas et al. (2017) have found that in fresh cabbage the populations of aerobic mesophilic microorganisms range between 10^2 - 10^3 CFU/g. After the fermentation time, the colony-forming units (CFU) of lactic acid bacteria were determined. The CFU count in the fermented cabbage ranged between $5.0*10^3$-$1.3*10^5$ CFU/g (Figure 3).

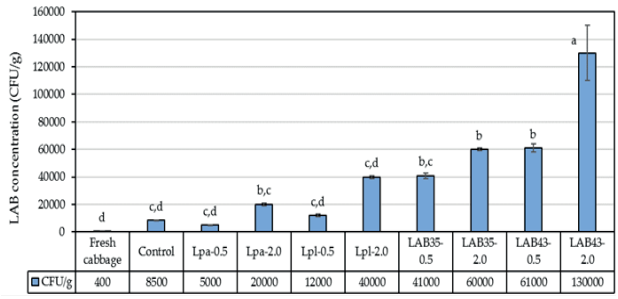

Figure 3. Number of lactic acid bacteria in fermented cabbage

Bar represents standard deviation of mean and means with different letters are significantly different ($p < 0.05$).
Control: natural fermentation; Lpl: *Lactobacillus plantarum* IC12353; Lpa: *Lactobacillus paracasei* CCM1837; LAB35: *L. plantarum* P35; LAB43: *L. brevis* P43.

It is observed that the inoculation of lactic acid bacteria resulted in higher total CFU levels in the fermentation medium compared to natural fermentation. Specifically, the LAB43 strain displayed the highest number of colonies ($1.3*10^5$ CFU/g), followed by LAB35 ($4.1*10^4$ CFU/g). In contrast, the addition of Lpl and Lpa strains to the fermentation medium led to the lowest amounts of lactic acid bacteria.

LAB43 strain multiplied its lactic acid bacteria rapidly but did not produce lactic acid. In contrast, LAB35 strain, though not having a significant increase in lactic bacteria, exhibited a superior capacity for biosynthesis by producing a high concentration of lactic acid.

3.6. Principal Component Analysis

Principal component analysis (PCA) was employed to show the overall variability among the fermented cabbage samples and find correlations between the analyzed parameters: antioxidant activity, number of lactic acid bacteria, lactic acid content at 7 days, lactic acid content at 16 days, lactic acid content at 28 days, total phenolic content, titratable acidity, pH, vitamin C (Figure 4).

The first two principal components (PC) described 37.6% and 31.4% of variance, respectively. The PCA map showed the similarity between LAB43-0.5 and LAB43-2.0 as well as between LAB35-0.5 and LAB35-2.0 as the groups are located in the same area of the plot (upper right vs. bottom right part of the plot). It was also observed that all the other samples were clustered together on the left part of the PCA plot, opposite to the LABs samples. PCA showed that vitamin C, the number of lactic acid bacteria, TPC and antioxidant activity were located close to one another in the PCA plot, indicating significant positive correlations between them. Lactic acid content at 16 days and 28 days were also located close together, indicating a high positive correlation.

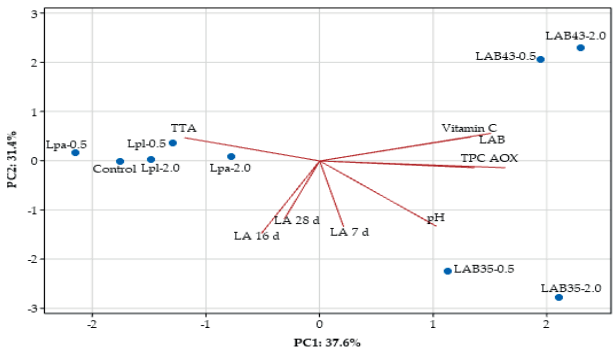

Figure 4. Principal component analysis
AOX: antioxidant activity; LAB: number of lactic acid bacteria; LA 7 d: lactic acid content at 7 days; LA 16 d: lactic acid content at 16 days; LA 28 d: lactic acid content at 28 days, TPC: total phenolic content; TTA: titratable acidity

On the other hand, negative correlations between variables are represented by vectors located opposite to each other. Negative correlations between TTA and pH, vitamin C, the number of lactic acid bacteria, TPC and antioxidant activity were observed.

CONCLUSIONS

The fermented cabbage samples were prepared using four lactic acid bacteria starter cultures: *L. plantarum* (Lpl), *L. paracasei* (Lpa), *L. plantarum* P35 (LAB35), and *L. brevis* P43 (LAB43). Remarkably, the highest total phenolic content was observed in LAB35-0.5, followed by LAB43-0.5, LAB43-2.0, and LAB35-0.5. A decrease of 25.44% (Lpl-2.0) to 31.06% (Lpl-0.5) was obtained in the polyphenolic content of fermented cabbage when *L. plantarum* was used**.** Furthermore, the study revealed that the highest levels of vitamin C were present in LAB35-2.0 and LAB43-0.5. The addition of lactic acid bacteria resulted in heightened antioxidant properties in the fermented samples, except for Lpl-0.5 and Lpl-2.0, which exhibited lower DPPH values than the control. Moreover, the inoculation of LAB35 and LAB43 led to higher total colony-forming unit (CFU) levels in the fermentation medium compared to the natural fermentation sample. In contrast, the addition of Lpl and Lpa strains resulted in the lowest amounts of lactic acid bacteria. Additionally, an increase in lactic acid levels was observed with the inoculation of lactic acid bacteria. PCA analysis concluded that there were similarities between control, Lpa and Lpl samples, while LAB 35 and LAB43 were distinct, highlighted by their opposite position in the PCA figure.

Overall, this investigation supports the utilization of different lactic acid bacteria starter cultures in the fermentation process, demonstrating their potential to enhance antioxidant properties, polyphenolic and vitamin C content.

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