




## Research Article

## Enhancing Shelf-Life of Banana Fruit Using Fermented Cabbage Extract

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ARTICLE INFO	ABSTRACT
<p><b>Article history</b> Received: 20 January 2024 Accepted: 25 June 2024 Published: 30 June 2024</p>	<p>Biochemical changes along with microbial contamination may shorten the shelf life of post harvested fruits and vegetables. Generally, various physical and chemical treatments are being used to preserve the post harvested fruits. However, those treatments are hardly good for human health as well as the environment. Interestingly, lactic acid bacteria (LAB) produce antimicrobial peptides that are harmful to food spoilage bacteria and extend shelf life by killing them. Therefore, the present study is designed to investigate spontaneous fermentation by the autochthonous LAB in the cabbage leaves and the application of fermented cabbage extract to enhance the shelf-life of banana fruits. The result showed that the fermented cabbage extract was acidic (pH = 3.6 after 40 hours) in nature and increased its acidity over time. In 24 hours, 1 mgL<sup>-1</sup> lactic acid was produced by LAB in cabbage leaves. In fermented cabbage extract, 3.85 mg per 100g vitamin-C was measured, antioxidant properties expressed by the DPPH and it was 3.02 mgml<sup>-1</sup>. Anthocyanin and flavonoids were present in cabbage extract but no tannin was observed. After 48h fermentation, the fermented cabbage extract was applied immediately to the harvested mature banana fruits. The brown surface of the banana was considered as an indicator before rotting. The brown surface in the control banana appeared on Day 7 after exposing to cabbage extract. On the other hand, such surface appeared on treated banana on Day 11. By Day 11, control banana group had already succumbed to rot. This result indicates that the treated banana took an additional 4 days to reach the state. Taken all together, it may be concluded that fermented cabbage extract possesses a promising role to extend the shelf life of banana fruits and to reduce the postharvest loss through eco-friendly natural approach without compromising quality and safety.</p>
<p><b>Keywords</b> Fermentation, Cabbage extract, LAB, Shelf life, Banana</p>	
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## Introduction

Freshly harvested fruits and vegetables are highly perishable and generally have a shorter shelf life (Hossain *et al.*, 2020). The food industry has historically relied on preservation techniques to improve the quality of food to eliminate food loss. Several chemical preservatives are used to preserve the shelf life and quality preservation; however, these chemicals are harmful to human health as well as the environment (Gupta and Yadav, 2021). Nowadays, the food industry is looking at innovative ways to increase food quality and consistency while using fewer chemicals in response to customer concerns about safe and healthy food (Alegre *et al.*, 2013; Guimarães *et al.*, 2020). The safety, sustainability and nutritional value of foods depend on several external factors (size, shape, colour, hardness, texture, and taste) and internal factors (chemical, physical, and microbiological characteristics) (Lakshmi *et al.*, 2017). These two factors have a crucial

impact on determining the degree of consumer acceptability. As fruits remain metabolically active even after harvesting and during the ripening and senescence process (Hossain *et al.*, 2020), several microorganisms contamination may occur, which cause undesirable phenomena and quality loss (Berger *et al.*, 2010; Srisamran *et al.*, 2022). Food preservation is a continuous fight against microorganisms spoiling the food or making it unsafe. Among these microorganisms, there are some GRAS (Generally Recognised as Safe) microorganisms that have been recognised as potential alternatives for preserving perishable fruits (Gorris, 1997; Kaur and Kaur, 2021). Such kinds of microorganisms are lactic acid bacteria (LAB) and/or their naturally occurring metabolites (so-called antimicrobial peptides) (Akbar *et al.*, 2016; Ayivi *et al.*, 2020; Moradi *et al.*, 2020; Smriti, 2023).

Food fermentation is one of the oldest human-made technologies. The most common fermented products

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are different types of pickles of fruits and vegetables (Breidt *et al.*, 2012; Hui and Evranuz, 2015). Cabbage, a popular cruciferous vegetable, is highly affordable and has a wide range of health benefits. Fermented cabbage is well-known as 'Sauerkraut' in Europe and the USA or 'Kimchi' in South Korea, China, and Taiwan (Patra *et al.*, 2016; Satora *et al.*, 2021). It is found that LAB recognised as GRAS, plays an important role in cabbage fermentation (Cho *et al.*, 2006). Though many other bacteria are involved in the fermentation process, LAB is the dominant bacteria in anaerobic conditions (Park *et al.*, 2014; Penas *et al.*, 2017; Smriti, 2023). Many researchers found that several plant-derived biomolecules and antioxidants are formed during the fermentation of cabbage (Tolonen *et al.*, 2002; Hunaefi *et al.*, 2013; Anik, 2023). Traditionally, LAB are also used as bio-preservatives because of their antimicrobial properties (Schnürer and Magnusson, 2005; Reis *et al.*, 2012). Moreover, LAB is considered a potential probiotic, which means LAB's providing antimicrobial chemicals (bacteriocins) give a competitive advantage over other microbes. Even live LAB is safe and good for human health (Soomro *et al.*, 2002). In our present study, we chose cabbage as a research material because it is easily available, cheap, and consists of starchy reserved food used by the phyllosphere/leaf residential bacteria during the fermentation process. In addition, fermented cabbage extract was acidic, and contained LAB and antimicrobial peptides harmful to pathogenic and food spoilage bacteria (Smriti, 2023). This

knowledge was applied to enhance the shelf life of banana fruit in the current investigation.

Bananas are one of the most popular fruits of Bangladesh available throughout the year. But it is highly perishable and loses customer acceptability very easily. However, this study aims to quantify lactic acid formation through cabbage fermentation and to apply the fermented extract on mature banana fruits to enhance shelf-life naturally and safely.

## Materials and Methods

### Materials

The cabbage heads were purchased from Kewatkhali market, Mymensingh. Harvested mature bananas were collected from Horticulture Farm, Bangladesh Agricultural University, Mymensingh. For the fermentation process, a 60 × 180 MM Pyrex bottle was used.

### Fermentation trials

The cabbage heads were sliced into small pieces (5mm) with a knife and grater. After extracting the water from the leaf sections by hand pressing, it was placed in a glass jar, and the air was evacuated by pressing it tightly. The glass jar containing 0.5 kg of cabbage was set for spontaneous fermentation in anaerobic condition.



A



B

Figure 1. Cabbage preparation for anaerobic respiration (Fermentation): (A) cabbage head, and (B) shredded cabbage

### Preparation of cell-free extract

Fermented cabbage juice was centrifuged at 8000 rpm for 5 minutes. The supernatant was filtered and the juice was a cell-free extract (Choi *et al.*, 2018).

### pH test

The pH test was done in two ways – using litmus paper and pH meter. pH was measured using a pH meter4, 10,

18, 24, 32, and 40 hours (firstly 6h interval, then 8 h) after setting the fermentation and the values were listed carefully.

### Total titrable acidity as lactic acid of fermented cabbage

The acidity of the fermented cabbage juice was expressed as percent lactic acid. The sample of 10g fermented cabbage juice was neutralized with

0.1MNaOH until the solution reached a pH of 8.2. Since 1 ml of 0.1N lactic acid contains 0.009 g of lactic acid, the number of ml of 0.1N NaOH required to neutralize the lactic acid in the sample, multiplied by 0.9 gave the amount of lactic acid (g) in the sample. When the result was divided by the weight of the sample and multiplied by 100, the percent lactic acid was obtained (Afrin *et al.*, 2023).

$$\% \text{ Lactic acid} = \frac{\text{ml of 0.1 M NaOH} \times 0.9}{\text{sample volume}} \dots\dots\dots (1)$$

#### Calcium lactate production

The formation of lactic acid was carried out using cabbage as a carbon source. The pH of the cell-free extract was maintained at 6.2 by adding 25% (w/v) Ca(OH)<sub>2</sub>. The calcium lactate crystals were separated by filtration, crushed into small particles and dried at 50°C for 3 h to allow free water to evaporate (Li *et al.*, 2024). The crystals were then weighed using a balance.

#### Phytochemical properties of fermented cabbage

Cabbage extract was added with 95% methanol and then filtered to get the expected sample. Sample extraction was added with NaOH and the colour change was observed, which denotes the presence of Anthocyanin. The sample extract was added with FeCl<sub>3</sub> and the result was observed. In the presence of Flavonoids, acidified methanol extract will show a brown colour, and dirty green precipitation indicates the presence of tannins (Shaikh and Patil, 2020).

#### Vitamin-C analysis

A sample of 10 ml was added with 5% oxalic acid. From this mixture, 5 ml was taken and this process was repeated three times. It was taken for titration using 2,6dichlorophenol indophenols. The starting point and ending point (pink colour) were recorded. The standard vitamin-C was 3.9 mg per 100 g (Parvin *et al.*, 2023)

#### DPPH radical scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay is carried out with some modifications (Sanja *et al.*, 2009). Different concentration of methanolic extracts was taken in different test tubes. The volume was adjusted to 100 µL by adding methanol, 3 ml of a 0.1 mM methanolic

solution of a DPPH was added to those tubes and shaken vigorously. The tubes were allowed to stand in the dark at room temperature for 30 minutes. The control was reported as above without any extract. DPPH radical scavenging activity is measured by a reduction in intensity of purple colour and quantified by a decrease in absorbance at wavelength 517 nm. Radical scavenging activity was calculated using the following formula –

$$\% \text{ radical scavenging activity} = \frac{\text{Control O.D} - \text{Sample O.D}}{\text{Control O.D}} \times 100$$

Here, O.D represents Optical Density.

#### Application of cabbage extract on mature banana surface

Mature and alike age group of banana fruits were collected from the same bunch. Then fruits were categorized into two groups for treatments: (i) control group (without LAB) and (ii) fermented cabbage extract (LAB), each group consisting of two bananas with four replications. The treated banana was sunk into the cabbage extract rich in lactic acid bacteria for 2 minutes. Then changes are observed day by day.

#### Statistical analysis

T-test, arithmetic mean and standard deviation were used on the programme Excel (Microsoft) to determine statistical differences.

### Results and Discussion

#### Anaerobic respiration or fermentation of cabbage leaf

After placing the shredded cabbage into the jar, the condition was observed very minutely and noticed some bubbles after 24 hours (Figure 2B). These bubbles may be the results of anaerobic respiration of cabbage and a large amount of extract was collected after 48 hrs in a bowl for further analysis. Under anaerobic condition, metabolic activities of LAB generate CO<sub>2</sub> and consequently huge bubbles were formed. It was harmonized with the scientific report of Enwa (2014), who stated that there was a keen competition for food among microbes at the beginning of the fermentation process which creates bubble.

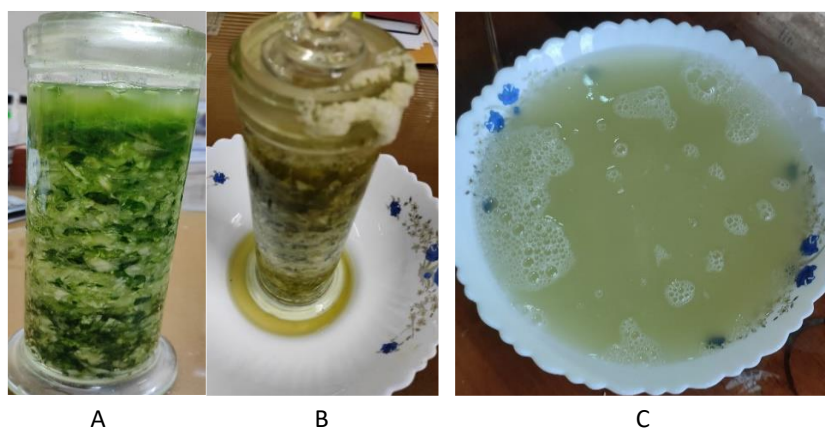


Figure 2. Anaerobic respiration or fermentation of cabbage leaf and its extract: (A) shredded cabbage, (B) cabbage after fermentation, and (C) fermented juice

#### *pH over time*

Just after keeping shredded cabbage under water, the liquid was taken for acidity test with litmus paper. No change in the colour of litmus paper (blue) was observed just after setting the fermentation (Figure 3A). The blue colour litmus paper turned into red which indicated that acid fermentation had started (Figure 3B). It meant that fermentation was running. Again, the acidity test was done by pH meter at 4, 10, 18, 24, 32, and 40 hours after setting the fermentation. After 4, 10, 18, 24, 32 and 40 hours, the pH was measured and 5.8,

4.8, 4.7, 4.1, 3.8 and 3.6 were found respectively (Figure 4). The results are in agreement with the findings of Drašković *et al.* (2018), which reported that pH changed during the fermentation process. Another report also argued that pH changed in Sauerkraut, where no pH was changed at initial (beginning) and became acidic over time (Enwa, 2014). In anaerobic conditions, many microbes, especially pathogenic bacteria, cannot survive due to lack of oxygen and low pH as well. Interestingly, only LAB can survive and grow such harsh condition (Smriti, 2023).

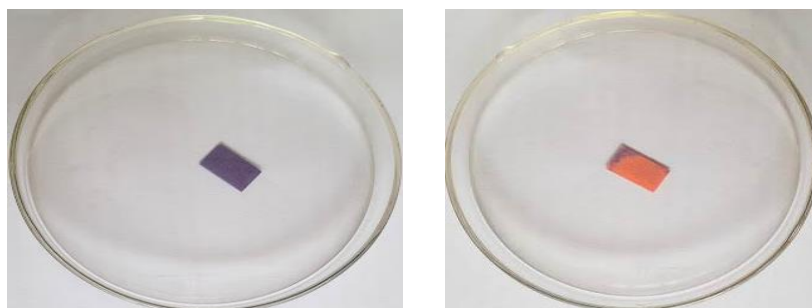


Figure 3. Litmus paper test: (A) just after the fermentation setting (no colour change), and (B) fermentation beginning (blue litmus turn into red)

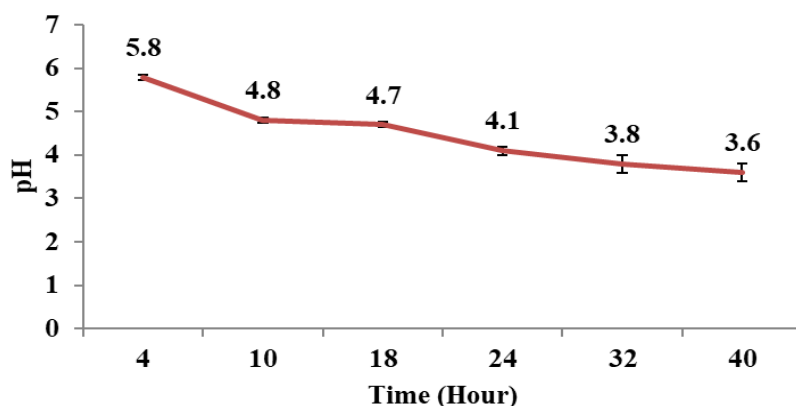


Figure 4. Changes of pH during anaerobic respiration of shredded cabbage

*Lactic acid production over time*

At the beginning of fermentation, cabbage had relatively low TTA % (as lactic acid)  $0.50 \pm 0.02$ . Afterwards, fermented cabbage had an increased TTA during the fermentation period and TTA was 4 times greater within 40 hrs than the beginner (4hrs) level (Figure 5). It depends on the conversion of sugars from shredded vegetables into lactic acid primarily by LAB. According to Enwa (2014), lactic acid bacteria are present in cabbage and start to produce lactic acid

slowly. As most pathogenic bacteria are acidophobic, that's why pH change becomes unbearable for their survival and growth. Only the lactic acid bacteria (LAB) could survive in this environment. A final acidity level of 2.0-2.5% can be reached through fermentation process (Wilson, 1988). According to Wilson (1988), a significant amount of lactic acid, a small amount of acetic acid, propanoic acid, CO<sub>2</sub>, alcohol, and aromatic esters produce at the end of cabbage fermentation.

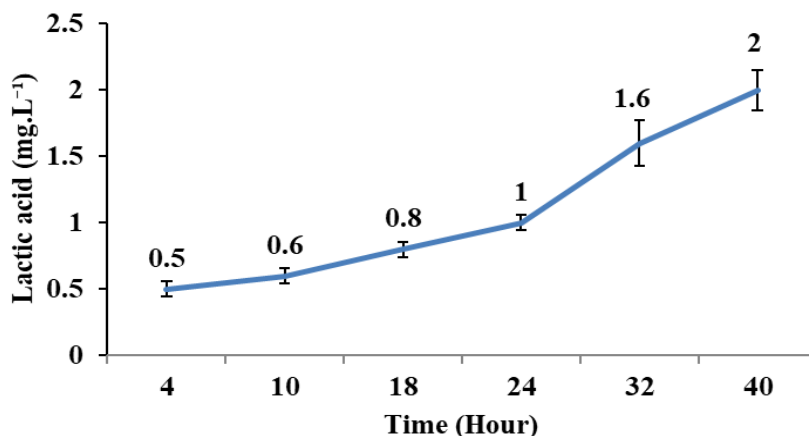


Figure 5. Lactic acid production by lactic acid bacteria (LAB)-mediated fermentation of shredded cabbage

*Calcium lactate production*

Lactic acid was reacted with calcium hydroxide at a 2:1 ratio. 12.5 ml lactic acid and 25ml calcium hydroxide produced water-soluble calcium lactate. Five grams Ca(OH)<sub>2</sub> was added with 25 ml fermented cabbage extract. This time similar precipitation of Ca-lactate was found (Figure 6). Lactic acid formed calcium lactate

(Figure 6) in the presence of Ca(OH)<sub>2</sub> according to the following reaction. However, Ca-lactate also produced by using CaO (Son *et al.*, 2021). The reaction with Ca(OH)<sub>2</sub> was as followed:



A



B

Figure 6. Calcium lactate production: (A) pure Ca-Lactate crystal, and (B) Ca-Lactate from cabbage extract.

*Phytochemical properties of fermented cabbage extract*

The qualitative estimation of cabbage extract was performed and the results showed the availability of various phytochemicals, which are presented in (Table

1). Phytochemical screening of freshly prepared cabbage extract was carried out using simple chemical tests to identify the presence of active phytoconstituents i.e., anthocyanin, flavonoids, tannins,



etc. in the sample. Phytochemical analysis exhibits positive results for flavonoids and anthocyanin in the cabbage extract inferring its significant properties and shows negative results for tannins. Similarly, flavonoid content in fermented cabbage (Sun *et al.*, 2009, Tolonen *et al.*, 2002) and the presence of anthocyanin (Wiczowski *et al.*, 2015) were observed in fermented cabbage.

**Table 1. Phytochemical test (Flavonoids, Tannins, Anthocyanin) in cabbage extract**

Phytochemicals	Results
Flavonoids	+
Tannins	-
Anthocyanin	+

#### Vitamin C content

Table 2 shows the vitamin C content in fermented cabbage. In 100 g fermented cabbage, 3.85 mg of vitamin-C was determined. It meant that fermented cabbage may be an important source of potent antioxidant, vitamin-C. However, Jansone and Kampuse (2019) found 26.66 mg. 100g<sup>-1</sup> vitamin C in fermented cabbage juice. The presence of vitamin-C was also reported by Martinez-Villaluenga *et al.* (2009).

#### DPPH radical scavenging activity

Table 2 shows the DPPH radical scavenging activity of fermented cabbage extract. The IC<sub>50</sub> value of fermented cabbage extract was 3.02 mg mL<sup>-1</sup>. The DPPH radical scavenging activity in fermented cabbage was reported by Kusznierevicz *et al.* (2008). Recently, Jansone and Kampuse (2019) also reported that 350.23 mg TE 100g<sup>-1</sup> DPPH in fermented cabbage juice.

**Table 2. Vitamin C and antioxidant in fermented cabbage juice**

Antioxidant properties	Results
Vitamin C (mg 100 g <sup>-1</sup> )	3.85 ± 0.06
DPPH (mgml <sup>-1</sup> )	3.02 ± 0.08

#### Impact of fermented cabbage extraction mature banana fruits

Control and treated banana fruits were observed on a regular basis and a significant change was noticed. On Day 7 the control banana started ripening but the treated banana was unripe (Figure 7). On Day 9, a brown surface was observed in the ripened banana of the control. On Day 10, the treated banana started ripening, but the controlled banana started rotting. The extended shelf-life of banana fruits in the current study could be explained in the following ways. The lactic acid bacteria (LAB) induced low pH which inhibits the growth of pathogenic bacteria and enhances the shelf life of fruits and vegetables (Agriopoulou *et al.*, 2020). LAB may produce antimicrobial peptides (bacteriocine) which also suppress the growth of pathogenic bacteria (Nebbia *et al.*, 2020; Dhundale *et al.*, 2018; Deegan *et al.*, 2006). Recently, Ibrahim *et al.* (2021) showed that LAB had a strong influence on pathogenic bacteria and increased the shelf-life of different fruits such as apples, pears and fig fruits. In addition, LAB acts as antifungal, which was collected from fresh vegetables and applied it to fresh vegetables (Sathe *et al.*, 2007). Besides, LAB acts not only as probiotic but generates LAB-induced metabolites which are promising for food preservation (Badea *et al.*, 2022).

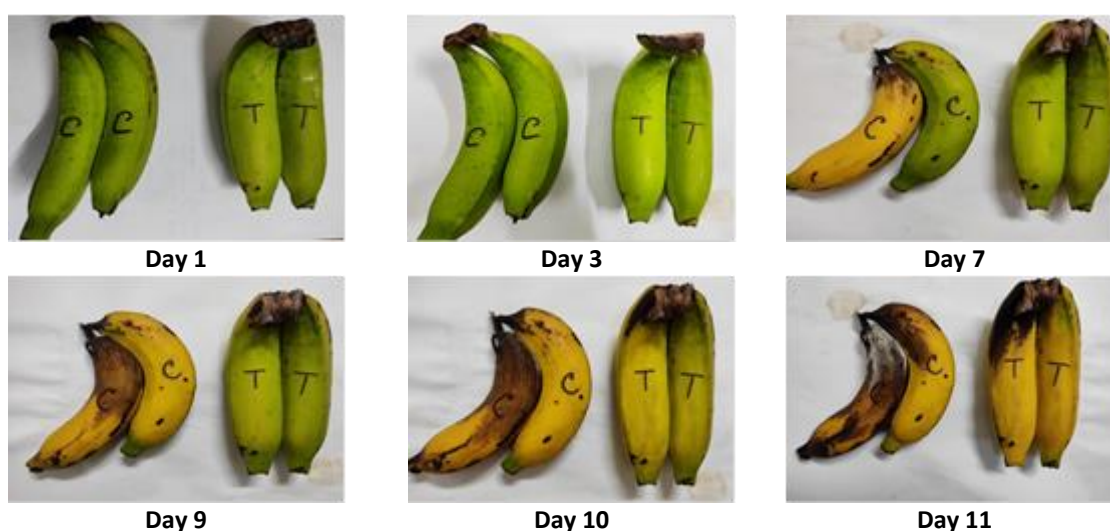


Figure 7. Changes of the colour of banana fruits after applying cabbage extract (Treatment) and without cabbage extract (Control) in exposing different duration (Days). Here, C= Control without cabbage extract, T=Treatment with cabbage extract.

## Conclusion

Under anaerobic (submerged) conditions, anaerobic respiration was accomplished by the autochthonous bacteria of cabbage leaves. Litmus paper (red) reaction proved that lactic acid fermentation occurred causing low pH (acidity) and the TTA method quantifies the amount of lactic acid formed during anaerobic respiration. Lactic acid content increased with decreasing pH in anaerobic conditions. Finally, fermented cabbage extract significantly enhanced the shelf life of matured banana fruits with quality and safety. However, it is preliminary research finding; this needs further investigation for better understanding of the mechanisms involved in enhancing shelf life of fruits.

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