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Nutritional Benefits And Health Effects Of Fermented Pomegranate Juice With *Lactobacillus Plantarum ATCC 11842*

kelloua halima dalila[1*], Benbouziane bouassria[1], Dahou abdelkader el Amine[2] and Adjoudj fatma [3]

[1*] Laboratory of bioeconomy, food security and health of the Faculty of Natural and Life Sciences of the Abdelhamid IBN BADIS University of Mostaganem, Algeria
[2] Laboratory of Animal Production Sciences and Techniques LSTPA, of the Faculty of Natural and Life Sciences of the Abdelhamid IBN BADIS University of Mostaganem. Algeria
[3]Laboratory of Applied Microbiology of the Faculty of Natural and Life Sciences of the University of Oran1, Algeria

***Corresponding author**: kelloua halima dalila
*Email :dalila.kelloua@univ-mosta.dz

Abstract:
This study investigates the fermentation of pomegranate juice from four local cultivars—**CV** (Centre Ville d'Oran, acidic**), MOS** (Mosta Sefri, non-organic), **EU** (Tlelet Oran, irrigated with wastewater), and **SP** (Saint Remy, sweet)—using *Lactobacillus plantarum ATCC 11842*. Fermentation significantly influenced cell viability, phenolic activity, antioxidant capacity, and sensory qualities over a four-week storage period. Initial cell counts of 8 log cfu/mL remained stable for three weeks before declining to values between 5.99 log cfu/mL (**SP**) and 6.10 log cfu/mL (**EU**) by week four, maintaining levels above the probiotic threshold. **MOS** exhibited the highest total phenolic and flavonoid contents (0.99 mg GAE/100 mL and 0.215 mg QEE/L, respectively). Antioxidant activity peaked at d21 for FRAP (44.49 mmol Trolox/L) and varied for DPPH across samples. Fermentation preserved sensory attributes, with fermented juices maintaining superior aroma, taste, and overall quality scores compared to non-fermented samples, which deteriorated by week four. These findings emphasize the role of Lactobacillus plantarum in enhancing both the nutritional and sensory profiles of pomegranate juice through sustained phenolic activity, bioactive compound production, and antioxidant capacity. The study highlights the potential of probiotic fermentation as a strategy for developing health-promoting beverages with extended shelf life and improved functional properties.

Keywords : *Lactobacillus plantarum* ATCC *11842*., pomegranate juice, fruit, probiotic, antioxidant activity, phenolics, functional beverage

Introduction:
Pomegranate juice (*Punica granatum L.*) is celebrated for its rich nutritional profile and health-promoting properties, primarily due to its high content of bioactive compounds, including phenolics and flavonoids. These compounds are recognized for their antioxidant activities, which are crucial in mitigating oxidative stress and reducing the risk of chronic diseases (Gil et al., 2000; Halvorsen et al., 2002; Shadab et al., 2023). The incorporation of probiotics, particularly lactic acid bacteria (LAB) such as *Lactobacillus plantarum*, into food products has gained attention for its potential to enhance both the functional properties and sensory attributes of various beverages, including fruit juices. LAB fermentation not only improves the nutritional value of these products but also contributes to their preservation and stability during storage (González et al., 2022; Shubhada et al., 2018; Kaur et al., 2023).This study focuses on the fermentation of pomegranate juice using *Lactobacillus plantarum*, specifically investigating its effects on cell viability, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities over a four-week storage period. The fermentation process was conducted under controlled conditions, allowing for the assessment of microbial growth and metabolic activity. Previous research has demonstrated that LAB can significantly enhance the antioxidant capacity of fruit juices through enzymatic hydrolysis of phenolic compounds and production of beneficial metabolites during fermentation (Xiao et al., 2020; Sharma et al., 2023).In addition to biochemical analyses, sensory evaluations were performed to assess consumer acceptability of the fermented juice compared to non-fermented controls. This comprehensive approach aims to elucidate the potential of *Lactobacillus plantarum* in transforming pomegranate juice into a functional beverage that not only retains its health benefits but also appeals to consumers' preferences. The findings from this study could provide valuable insights into optimizing fermentation conditions for enhancing the health-promoting properties of pomegranate juice and similar functional foods.

1. Materials and methods :

1.1. Microorganism :

The probiotic strain *Lactobacillus plantarum* is a lactic acid bacterium sourced from the strain collection of the **LSTPA** laboratory. It was originally isolated from a traditional Algerian cheese, J'ben de Naama. It was cultivated under anaerobic

conditions at 37°C for 48 hours in MRS broth. The wet biomass was harvested by centrifugation at 5,000 rpm for 10 minutes at 25°C using a centrifuge (50,000 rpm capacity). To achieve an initial cell density of 10^8 CFU/mL in the final juice, 15 mL of the MRS culture broth was centrifuged at 4,000 rpm for 10 minutes. The biomass was then washed twice with saline solution and introduced into 150 mL of juice. All bacterial cultures were stored frozen at -20°C in an MRS medium supplemented with 20% glycerol.
The probiotic strain *Lactobacillus plantarum ATCC 11842* was also selected and applied in fermentations for comparative purposes. It was grown under anaerobic conditions at 37°C for 48 hours in MRS broth. Wet biomass was harvested by centrifugation (Sigma 3K12, Bioblock Scientific, Lezennes, France) at 5,000 rpm for 10 minutes at 25°C.

1.2. Fermentation of Pomegranate Juice:

Pomegranates (*Punica granatum L.*): The plant material evaluated consisted of four local cultivars obtained from the local market:**CV** (Centre Ville d'Oran, Bio Chergui – acidic),**MOS** (Mosta Sefri – non-organic), **EU** (Tlelet Oran – non-organic, irrigated with wastewater),**SP** (Saint Remy, Bio Sefri – sweet).1 g (wet weight) of harvested *Lactobacillus plantarum ATCC 11842* was added to 100 mL of pomegranate juice after adjusting the pH and sugar levels. The pH was adjusted to 3.5 using a digital pH meter (MI150, Milwaukee, Italy), and the sugar concentration was adjusted to 90 g/L (9° Brix) using a refractometer (Rifrattometro Mod MR32ATC, Italy). The juice was then fermented at 30°C for 24 hours. The initial cell viability was determined to be 8 log CFU/mL of juice.
After fermentation, the flasks were stored at 4°C for 28 days (4 weeks). All fermentations were performed in triplicate.

1.3. Microbiological Analysis:

Aliquots of 10 mL were collected from each pomegranate juice sample (after thorough homogenization by shaking) at various time intervals during fermentation and storage. The samples were mixed with 90 mL of sterile 1/4 strength Ringer's solution (Sigma-Aldrich) and blended using a stomacher blender. Serial decimal dilutions were then prepared in 1/4 strength Ringer's solution. Viable counts of *Lactobacillus plantarum* were enumerated on acidified MRS agar (Merck, Darmstadt, Germany) after incubation at 37°C for 72 hours under anaerobic conditions (using an anaerobic jar with Anerocult C, Merck, Darmstadt, Germany). Coliforms were enumerated on Violet Red Bile Agar (Lab M, Lancashire, UK) after incubation at 30°C for 24 hours. Yeasts and fungi were quantified by plating on Sabouraud Chloramphenicol Agar (Merck, Germany) and incubating at 30°C for 72 hours.
All cell counts were expressed as the logarithm of the mean colony-forming units (CFU) per milliliter of pomegranate juice. Results are presented as the means of three repetitions, along with their standard deviations.

1.4. Total Phenolic Content :

The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent (Sigma, St. Louis, MO, USA) based on a colorimetric reduction method. Phenolic compounds are oxidized to phenolates by the reagent in a saturated sodium carbonate solution at alkaline pH, leading to the formation of a blue complex. Approximately 1 mL of Folin-Ciocalteu reagent (10%, w/v) was added to 0.2 mL of prepared pomegranate juice, followed by the addition of 1.2 mL of aqueous Na_2CO_3 (7.5%, w/v). The mixture was then kept in the dark for 90 minutes. The absorbance of the blue-colored solution was measured at 760 nm using a UV-visible spectrophotometer (Shimadzu, Kyoto, Japan), with distilled water used as the blank. The total phenolic content was quantified by plotting a gallic acid calibration curve and expressed as milligrams of gallic acid equivalents (GAE) per 100 mL of juice. (Singleton et al., 1999; González et al., 2022).

1.5. Total flavonoids content (TFC) :

The Total Flavonoids Content (TFC) of the pomegranate juices was determined using the aluminum chloride (AlCl3) colorimetric method, which is a widely accepted technique for quantifying flavonoids in various plant extracts. The absorbance of the reaction mixture was measured at 510 nm using a UV-VIS spectrophotometer, and results were expressed as quercetin equivalents (QRE) in mg QEE/L. This method relies on the formation of stable complexes between aluminum ions and flavonoids, which results in a color change that can be quantified spectrophotometrically (Shraim et al., 2021). This approach not only enhances our understanding of flavonoid profiles in pomegranate juice but also contributes to the broader field of food science by providing a reliable means for assessing antioxidant capacity (Xiao et al., 2020).

1.6. Antioxidant activities analysis :

The antioxidant activities of the lactic acid bacteria (LAB) fermented and control samples of pomegranate juice were evaluated using two widely recognized methods: the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the FRAP (Ferric Reducing Antioxidant Power) assay.

1.6.1. Determination of DPPH radical scavenging activity:

DPPH radical scavenging was determined by previously reported method (Xiao et al., 2020). The absorbance of the reaction mixture was measured at 517 nm using UV-VIS spectrophotometer Results were expressed as % scavenging of DPPH following the equation:

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Inhibition ratio (%) = (A1 - A2) × 100⁄A1
Where A1 is the absorbance of the addition of ethanol instead of testing sample and A2 is the absorbance of testing sample solution.

1.6.2. Determination of Ferric Reducing Antioxidant Power (FRAP):

The Ferric Reducing Antioxidant Power (FRAP) of the pomegranate juices was evaluated using the method described by Benzie and Strain (1996). The absorbance of the reaction mixture was measured at 593 nm using a UV-VIS spectrophotometer. In this assay, the FRAP reagent was prepared by mixing 2.5 mL of 10 mM tripyridyltriazine (TPTZ) in 40 mM HCl, 2.5 mL of 20 mM FeCl3·6H2O, and 25 mL of 0.3 M acetate buffer at pH 3.6. The mixture was incubated at 37°C for 30 minutes before measuring the absorbance. The results were expressed as mmol Trolox/L, using Trolox as a standard to quantify the antioxidant capacity (Widyastuti, 2010; Ou et al., 2002). This method is widely utilized for determining the antioxidant activity of various food extracts, as it provides a reliable measure of the reducing power of antioxidants present in the samples (Xiao et al., 2020; Thaipong et al., 2006).

1.7. Sensory Evaluation:

The sensory evaluation of fermented pomegranate juice (FPJ) was conducted with 30 non-trained panelists, evenly distributed by gender and aged between 25 and 35 years. The panelists assessed various sensory attributes, including taste, color, aroma, and overall acceptability, using a structured scoring system based on established methodologies (Stone & Sidel, 2004). The results are presented as average scores with standard deviations to reflect the panelists' perceptions of the juice's sensory characteristics over time.

1.8. Statistical Analysis:

The data collected on the physicochemical characteristics, antioxidant activity, total phenolic content, and cell viability of both non-fermented and fermented pomegranate juice were subjected to statistical analysis to assess mean differences. This analysis was performed using the Analysis of Variance (ANOVA) procedure, followed by Duncan's post hoc multiple range test to identify specific differences among the various treatment groups.

2. Results and discussion :

2.1. Cell Viability:

The viability of *Lactobacillus plantarum ATCC 11842* in fermented pomegranate juice was evaluated after fermentation at 30°C for 24 hours and during four weeks of storage at 4°C . Initial cell counts across all treatment groups (**CV, MOS, EU, and SP**) were approximately 8 log cfu/mL.After fermentation, cell viability held steady for the first three weeks of storage before declining by the fourth week. Viability levels in the EU were 6.10 log cfu/mL, while in SP they were 5.99 log cfu/mL. Despite this reduction, the cell counts remained above the recommended probiotic threshold of 6–7 log cfu/mL, ensuring the retention of probiotic functionality (Saarela et al., 2006).

Temperature (^{0}C)	**Time**	**Viability of *Lactobacillus Plantarum* ATCC 11842 (log cfu/mL)**					
		CV	**MOS**	**EU**	**SP**	**Yeasts & Fungi**	**Coliforms**
30	0	8 ± 0,57^a	8 ± 0,45^a	8± 0,74^a	8± 0,85^a	0	0
30	24 h	7,5± 0,53^a	7,65 ± 0,52^a	7,36 ± 0,52^a	7,47 ± 0,52^{ab}	0	0
4	Week 1	7,27± 0,51^a	7,38± 0,51^a	7,16 ± 0,51^a	7,25± 0,51^{ab}	0	0
4	Week 2	7,29± 0,55^a	7,20 ± 0,52^a	7,38 ± 0,53^a	7,26± 0,53^{ab}	0	0
4	Week 3	7,02± 0,51^a	6,90 ± 0,51^a	7,19± 0,51^a	7,03± 0,51^{ab}	0	0
4	Week 4	6,O1± 0,48^a	6,00 ± 0,54^a	6,1± 0,48^a	8± 0,85^a	0	0

Table 1. Viability of the *Lactobacillus plantarum* ATCC 11842 cells in the fermented pomegranate juices after fermentation (24 h in 30 °C) and over 4 weeks of storage at 4 °C.
Different superscript letters in columns indicate significant differences at an alpha = 0.05 (ANOVA, Duncan Post Hoc Multiple Comparisons).

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The sustained viability of *Lactobacillus plantarum ATCC 11842* throughout storage can be attributed to multiple factors. One significant aspect is the potential prebiotic effect of bioaccessible phenolic compounds in pomegranate juice, which may have promoted probiotic growth (Filannino et al., 2018). Additionally, the well-documented acid resistance and adaptability of *Lactobacillus plantarum* strains make them well-suited for survival in fruit-based matrices with low pH (Coda et al., 2012). The pH adjustment of pomegranate juice to 3.5 before fermentation further supported the probiotic's viability.

Furthermore, no growth of spoilage microorganisms, including yeasts, fungi, and coliforms, was observed throughout the storage period. This finding underscores the microbiological stability of the fermented product, likely due to the antimicrobial effects of organic acids and phenolic compounds formed during fermentation (Valero-Cases & Frutos, 2015). The lactic acid fermentation process likely provided an inhibitory environment for spoilage microorganisms, contributing to the product's safety and quality.

2.2. Phenolic activity :

The results from the fermentation study of pomegranate juice samples with *Lactobacillus plantarum ATCC 11842* over 28 days—**CV** (Centre Ville d'Oran), **MOS** (Mosta Sefri), **EU** (Tlelet Oran), and **SP** (Saint Remy)—reveal significant differences in total phenolic content (TPC) over four weeks. Initially, the TPC values varied, with **EU** showing the highest average at approximately 0.27 mg GAE/100 mL, while **SP** had the lowest at 0.14 mg GAE/100 mL. After 24 hours of fermentation, **MOS** exhibited a remarkable increase in TPC, reaching 0.99 mg GAE/100 mL by week four, indicating its superior capacity for phenolic retention compared to **CV** and **EU**, which maintained lower levels of 0.67 mg GAE/100 mL and 0.64 mg GAE/100 mL, respectively. **SP** also improved significantly, reaching 0.96 mg GAE/100 mL by the end of the study.

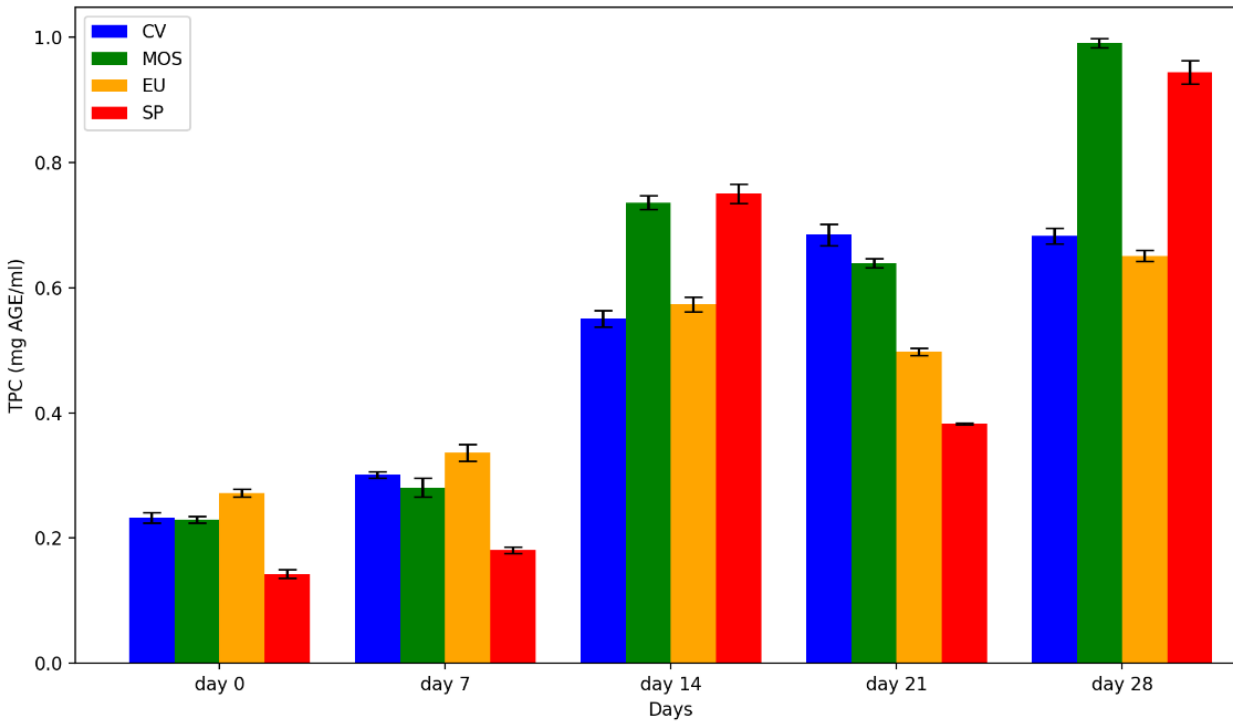

Figure 1: Total phenolics of fermented with *Lactobacillus plantarum ATCC 11842* and non-fermented pomegranate juice during storage at 4 °C for 4 weeks.

These findings align with previous research indicating that lactic acid fermentation enhances TPC in pomegranate juice due to microbial metabolic activities that convert complex phenolic compounds into more bioavailable forms (Mousavi et al., 2011; Sharma et al., 2022). Furthermore, studies have shown that different cultivars can exhibit varying responses to fermentation, with sweeter varieties often yielding higher phenolic content post-fermentation (Zhuang et al., 2015). The distinct performance of **MOS** in this study suggests that cultivar selection is critical for maximizing the health benefits associated with fermented pomegranate juice, reinforcing the idea that fermentation can significantly alter both the chemical composition and sensory attributes of fruit juices, making them more appealing to health-conscious consumers.

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2.3. Total flavonoids content (TFC) :

The research on permanganate fermented juice flavonoid content throughout 28 days demonstrates different patterns for the **CV, MOS, EU**, and **SP** samples. The metabolite activity of **CV** remained consistent by showing a continuous increase from day 0 (0.029 mg QEE/L) to day 28 (0.155 mg QEE/L). However, **MOS** showed peak activity at day 14 (0.213 mg QEE/L) followed by a decrease on day 21 (0.108 mg QEE/L) before returning to the initial level at day 28 (0.215 mg QEE/L), which indicates variations in microbial activities impact flavonoid stability. QEE levels in the **EU** sample began moderately increasing until day 14 (0.166 mg QEE/L) when they reached their maximum point and then stabilized while the **SP** sample displayed identical patterns to **MOS** with its peak (0.156 mg QEE/L) on day 14 followed by a decline (0.103 mg QEE/L) on day 21 before regaining strength to reach 0.202 mg QEE/L at day 28.

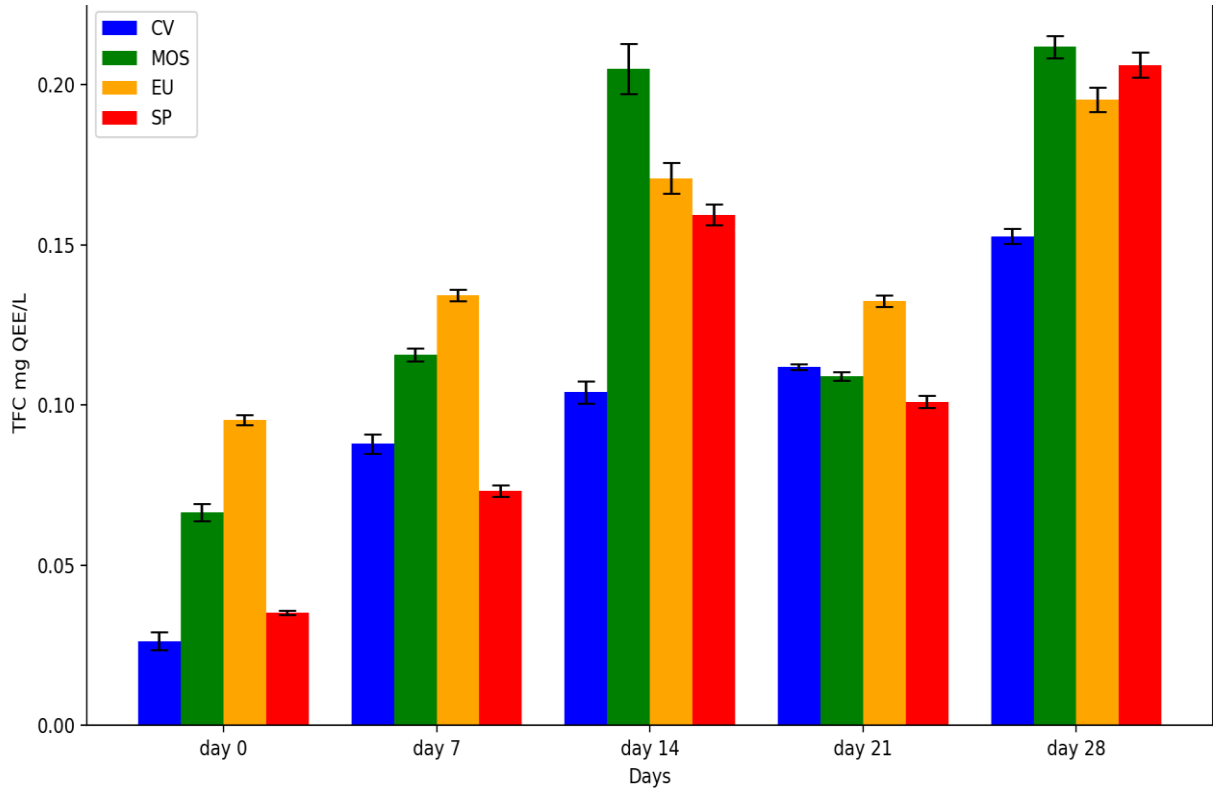

Figure 2 : Total flavonoids content of fermented with *Lactobacillus plantarum ATCC 11842* and non-fermented pomegranate juice during storage at 4 °C for 4 weeks.

Previous studies using *Lactiplantibacillus plantarum* to ferment pomegranate juice yielded higher phenolic and flavonoid levels, which prove that LAB enhances bioactive compounds, as reported by Sarıtaş et al. in 2024. Research on grape juice fermentation detected a 26.83% rise in flavonoids after *L. plantarum* started working, which proves that LAB enhances fruit juice nutrition during fermentation processes (Baimin Wu et al., 2021).

2.4. Antioxidant activity:

The investigation of antioxidant activity in fermented pomegranate juice using DPPH and FRAP methodologies provides valuable insights into the efficacy of lactic acid bacteria (LAB). fermentation in increasing the juice's radical scavenging properties.

2.4.1. DPPH :

The results indicate varying DPPH radical scavenging activity levels across different time points (d0, d7, d14, d21, d28) and sample conditions (**CV, MOS, EU, SP**). At d0, the control samples without LAB showed a baseline scavenging activity, with the **SP** group exhibiting the highest percentage (84.31%), suggesting inherent antioxidant properties even before fermentation. As fermentation progressed to d7, the **CV** group demonstrated a notable increase in scavenging activity (64.92%). In comparison, the **MOS** group showed a decline (35.38%), indicating that initial fermentation may alter the antioxidant profile differently among samples.

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By d14, the **CV** group peaked at 82.46%, suggesting that LAB fermentation significantly enhances antioxidant capacity during this phase. The **SP** group maintained high activity (79.08%), reinforcing its potential as a robust source of antioxidants. Further analysis at d21 revealed that all groups exhibited high scavenging percentages, with **CV** again leading at 83.54%. However, by d28, there was a decline in activity for most groups except for the **EU**, which reached 85.29%.

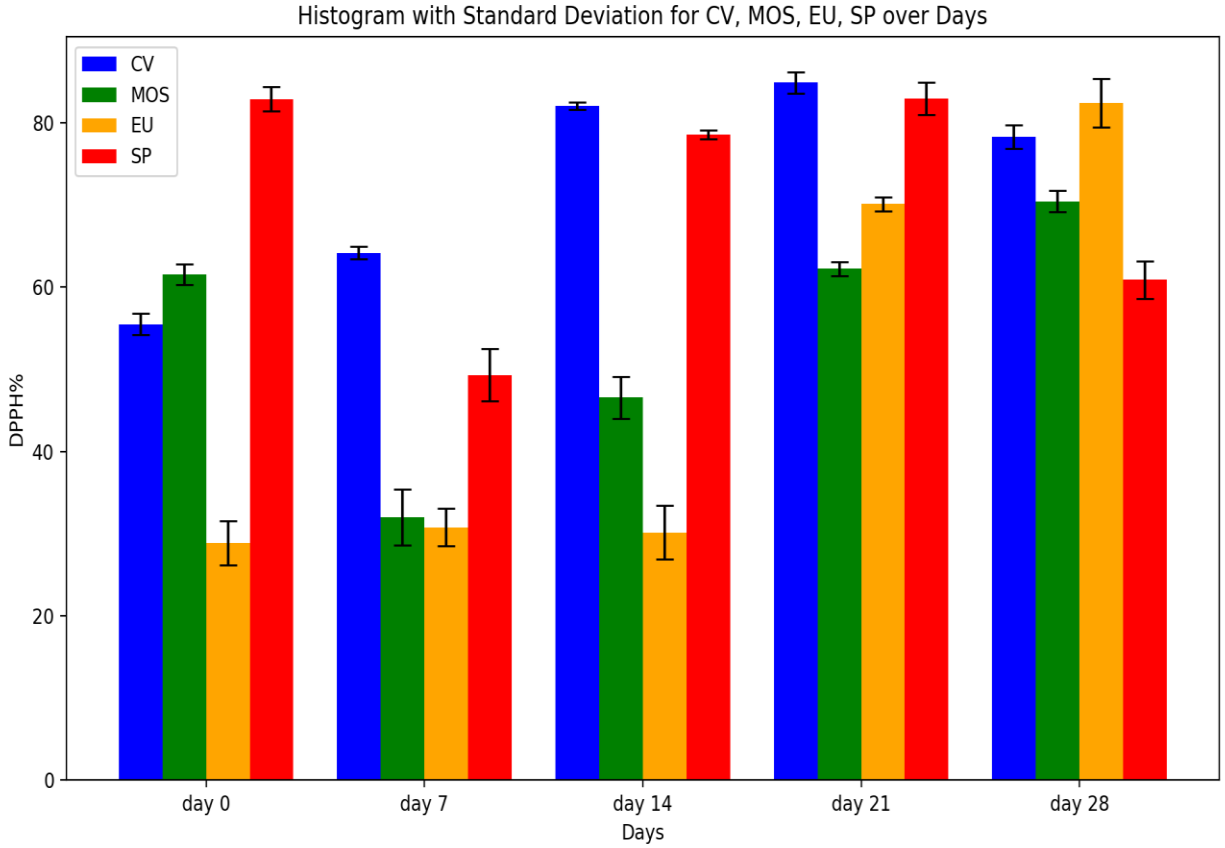

Figure 3 : DPPH activity of fermented with *Lactobacillus plantarum ATCC 11842* and non-fermented pomegranate juice during storage at 4 °C for 4 weeks.

This fluctuation suggests that while LAB fermentation can enhance antioxidant properties initially, prolonged fermentation may lead to the degradation of certain compounds or changes in their bioavailability. These findings are consistent with previous studies that have reported enhanced antioxidant activities in pomegranate juice through fermentation processes. For instance, Xiao et al. (2020) observed similar increases in DPPH scavenging ability in LAB-fermented juices compared to controls.

Furthermore, research has shown that pomegranate juices generally exhibit higher antioxidant capacities than many other beverages, including red wine and green tea (Gil et al., 2000; Halvorsen et al., 2002). The mechanisms underlying these enhancements are often attributed to the breakdown of phenolic compounds and the production of bioactive metabolites during fermentation (González et al., 2022).

2.4.2. The Ferric Reducing Antioxidant Power (FRAP) :

The Ferric Reducing Antioxidant Power (FRAP) assay reveals significant changes in the antioxidant capacity of fermented pomegranate juice over a 28-day fermentation period, measured in mmol Trolox/L using Trolox as a standard reference. Initial measurements (J0) show inherent antioxidant properties in the unfermented juice, with **MOS** exhibiting the highest FRAP value (12.43 mmol Trolox/L) and **SP** the lowest (5.31 mmol Trolox/L). By d7, a decline in FRAP values across all groups suggests a temporary reduction in antioxidant capacity, possibly due to early microbial activity.

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Recovery is evident at d14, particularly for the **EU** group (12.16 mmol Trolox/L), though the **CV** group shows only a slight decrease from d0. A significant enhancement occurs by d21, with the **EU** reaching its highest value (22.38 mmol Trolox/L) and **CV** showing substantial improvement (20.58 mmol Trolox/L). At d28, FRAP values peak for **CV** and **MOS** (40.15 and 44.49 mmol Trolox/L, respectively), while **EU** remains high (33.95 mmol Trolox/L) and **SP** shows a notable but comparatively lower increase (26.40 mmol Trolox/L).

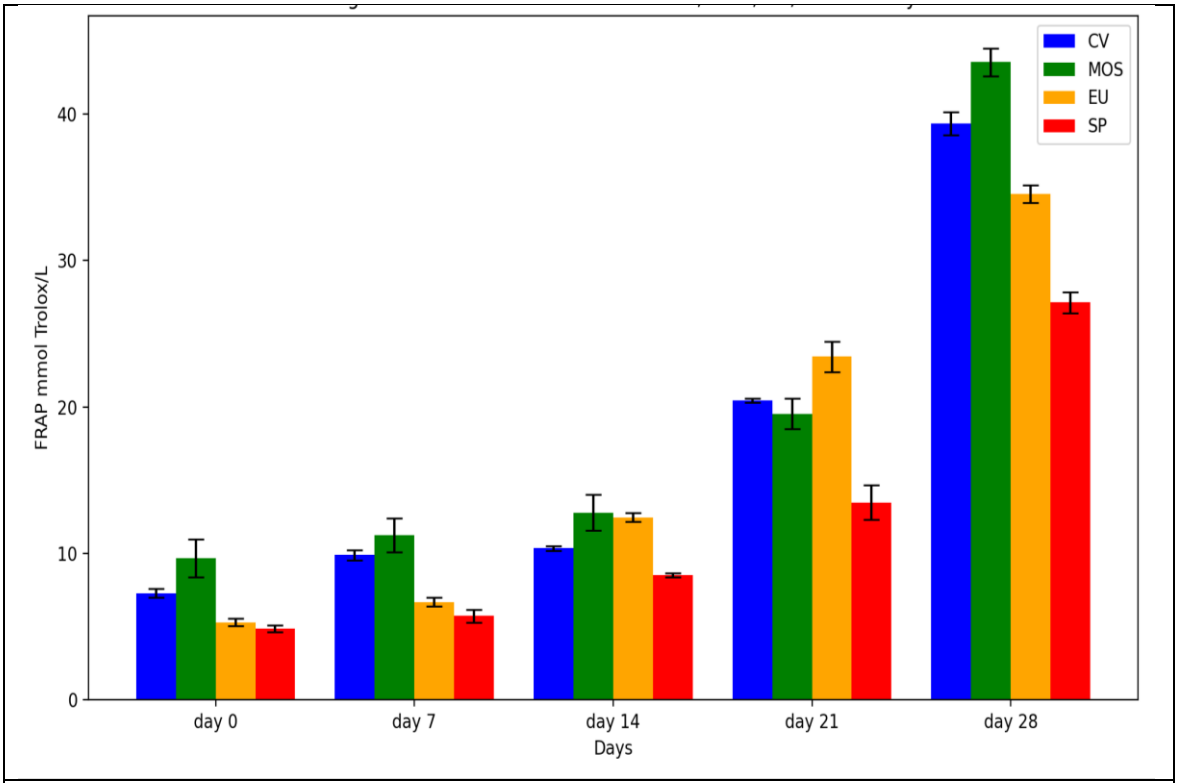

Figure 4 : FRAP activity of fermented with *Lactobacillus plantarum ATCC 11842* and non-fermented pomegranate juice during storage at 4 °C for 4 weeks.

These findings align with previous studies that emphasize the role of lactic acid bacteria in enhancing antioxidant capacity by breaking down complex phenolic compounds and generating new antioxidants (Xiao et al., 2020; González et al., 2022). The overall trend suggests that prolonged fermentation optimizes antioxidant profiles, underscoring the potential of fermented pomegranate juice as a functional beverage. Further investigations into specific phenolic transformations and their contributions to antioxidant activity could refine fermentation strategies to maximize health benefits.

2.5. The sensory evaluation:

of fermented pomegranate juice (FPJ) using Lactobacillus plantarum over a four-week cold storage period revealed that fermentation positively influenced the aroma, taste, and overall quality of the juice. Initially, both non-fermented and fermented samples received comparable high scores (aroma: 8.6, taste: 8.5, overall quality: 8.1), indicating that fermentation did not negatively impact the sensory properties within 24 hours. However, as storage progressed, non-fermented samples exhibited a sharper decline in sensory attributes, with aroma, taste, and overall quality dropping to 5.6, 5.3, and 5.2, respectively, by week four. In contrast, fermented samples retained higher scores (aroma: 6.2, taste: 6.2, overall quality: 6.2), suggesting that fermentation provided a protective effect against sensory degradation. The stability and slight enhancement in sensory qualities over time may be attributed to the metabolic activities of *L. plantarum*, which potentially contributed to flavor development and preservation. These findings align with previous research demonstrating that lactic acid fermentation can maintain or enhance the organoleptic properties of fruit juices by generating desirable volatile compounds and inhibiting spoilage due to lower pH levels (Xiao et al., 2020; González et al., 2022). The study underscores the potential of fermentation as a valuable process for improving the shelf life and sensory appeal of functional beverages.

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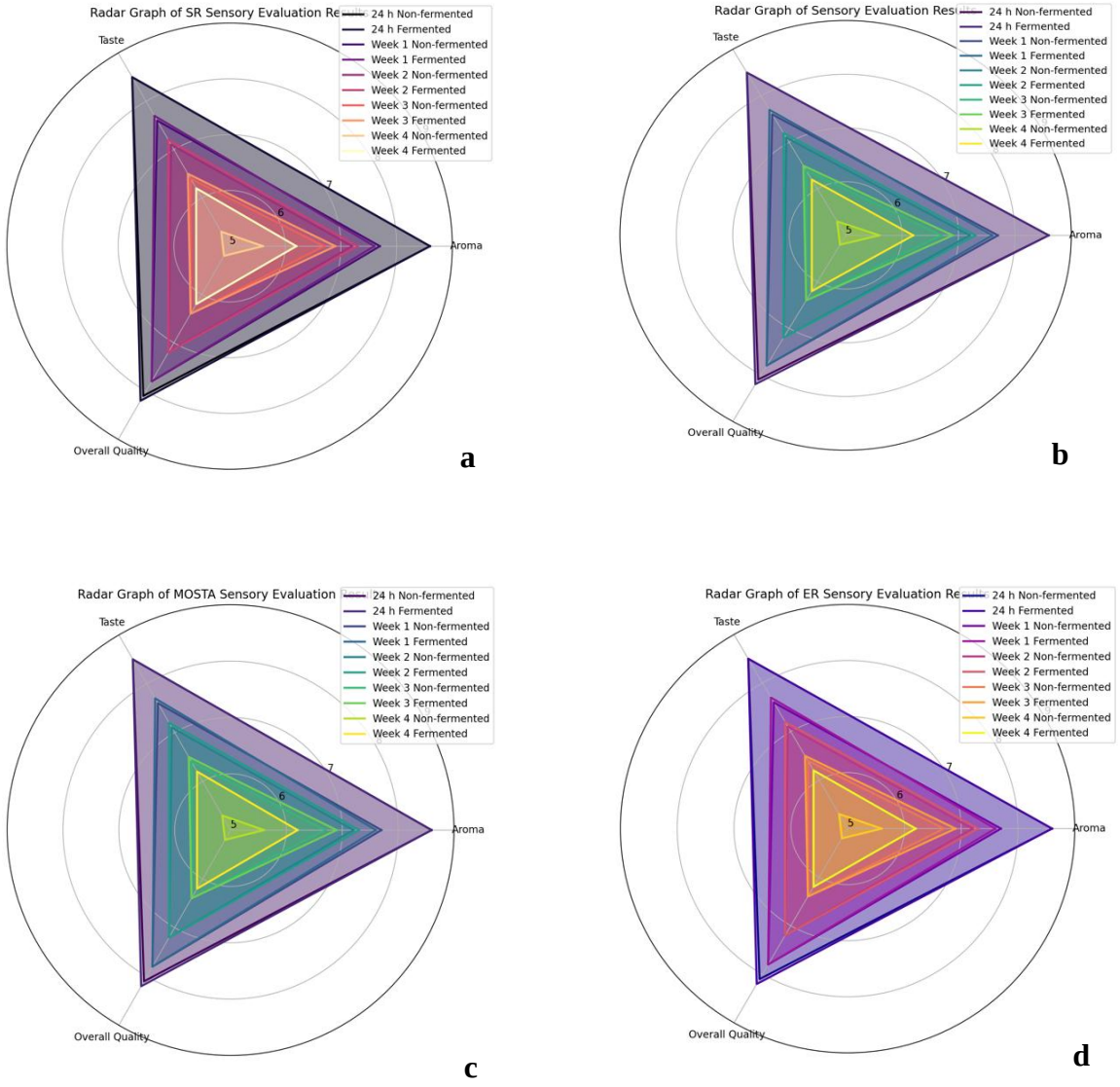

Figure 5: Radar Graph of Sensorial Evaluation for Fermented and Non-Fermented Pomegranate Juice Over 4 Weeks
a: CV ,b:EU, c:MOS , d:SP

3. Conclusion:

the results of this study demonstrate the significant potential of *Lactobacillus plantarum ATTC 1842* fermentation to enhance both the phenolic activity and antioxidant capacity of pomegranate juice over time. The variations observed among different treatment groups indicate that specific fermentation conditions can optimize the extraction and stability of beneficial phenolic compounds, transforming pomegranate juice into a functional beverage rich in antioxidants and health-promoting properties. Notably, the MOS group emerged as particularly effective in maximizing phenolic content, while all groups demonstrated varying degrees of improvement throughout the fermentation process. Additionally, the sensory evaluation revealed that fermentation not only preserves desirable characteristics but may also enhance the overall acceptability of pomegranate juice, suggesting its viability as a marketable functional food. These findings underscore the importance of optimizing fermentation conditions to maximize health benefits and consumer appeal.

Conflict of interest statement

The authors declare that they have no conflict of interest. All authors have given their final consent for publication.

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