"Impact Of Organic and Conventional Cultivation on The Lactic Fermentation Kinetics of Vegetables: A Comparative Study With *Lactobacillus Bulgaricus* And *Streptococcus Thermophiles.*"

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ABSTRACT

This study investigates the influence of organic and conventional cultivation methods on the lactic fermentation kinetics of three vegetable matrices carrot (*Daucus carota*), green bean (*Phaseolus vulgaris*), and cabbage (*Brassica oleracea*) inoculated with a mixed starter culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Organic vegetables, characterized by higher levels of fermentable sugars and dry matter, promoted faster acidification and greater bacterial proliferation. The pH decreased from 6.8-7.2 to 3.4-3.8 after 72 h, while titratable acidity reached up to 1.1 g lactic acid·100 g^{-1}. Two-way ANOVA revealed a significant effect of cultivation mode on sugar consumption ($F_{1,12} = 35.42$; $p < 0.001$; $\eta^2 = 0.75$) and pH reduction ($F_{1,12} = 28.67$; $p < 0.001$; $\eta^2 = 0.70$). These findings demonstrate that organic substrates enhance lactic acid bacteria growth and accelerate fermentation kinetics.

Keywords: lactic fermentation; *Lactobacillus bulgaricus*; *Streptococcus thermophilus*; organic cultivation; vegetable matrices; fermentation kinetics.

I. Introduction

Lactic fermentation is an ancient and natural method for preserving plant-based foods, improving both their microbiological stability and sensory quality. This process relies on the metabolic activity of lactic acid bacteria (LAB), which convert fermentable sugars into organic acids, primarily lactic acid, thereby decreasing pH and inhibiting undesirable microorganisms.

Agricultural practices influence the biochemical composition of vegetables particularly their content in sugars, organic acids, minerals, and phenolic compounds which in turn affects LAB growth. Organically cultivated vegetables, often richer in micronutrients and soluble sugars, generally exhibit enhanced fermentability.

Recent studies have shown that organic substrates promote faster fermentation, greater acidification, and a more stable microbial diversity [1-3]. However, most research has focused on spontaneous fermentations or dairy matrices, while few studies have compared the effects of cultivation methods on controlled vegetable fermentations using pure cultures. This study aims to determine the extent to which organic and conventional cultivation influence the lactic fermentation of various vegetable matrices (carrot, green bean, and cabbage) inoculated with a mixed starter composed of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Specifically, it assesses the impact of cultivation mode on the initial composition of vegetables, fermentable sugar consumption, pH evolution, and LAB growth dynamics during fermentation.

II. Materials and Methods

2.1 Vegetable material and cultivation modes

Carrots, green beans, and cabbages were cultivated in paired plots under organic and conventional regimes within the same experimental site to minimize environmental variability. Samples were transported at 4 °C and processed immediately after harvest.

2.2 Microorganisms and inoculum preparation

Lyophilized pure cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were reactivated in sterile MRS broth at 37 °C for 24 h. Cell density was adjusted to approximately 10^8 CFU·mL^{-1} for each strain..

2.3 Sample preparation and fermentation

Vegetables were washed with sterile distilled water, peeled, cut into 2 cm pieces, and distributed in 500 mL jars containing 250 g of sample. Each jar received 150 mL of 2% NaCl brine (initial pH ≈ 6.5) and 2% (v/v) of each bacterial strain. Fermentation was conducted at 37 °C for 7 days, with analyses performed at 0, 24, 48, 72, 120, and 168 h.

The incubation temperature of 37 °C was chosen to standardize fermentation kinetics and optimize the metabolic activity of the mixed starter (*L. bulgaricus* and *S. thermophilus*), whose growth optimum lies within this range. This condition also ensured better control of indigenous microflora and accelerated acidification.

2.4 Physicochemical analysis

Physicochemical parameters monitored during fermentation included pH (measured using a Hanna pH meter) and titratable acidity, determined by titration with 0.1 N NaOH according to AOAC [4] and expressed as g lactic acid·100 g^{-1}. Total and reducing sugars were quantified using the DNS method described by Miller [5], while dry matter content was determined after oven-drying at 105 °C to constant weight. Protein content was estimated via the Kjeldahl method (conversion factor N × 6.25), and lipid and ash contents were assessed using standard procedures.

Titratable acidity was included to complement pH monitoring and provide an estimate of total organic acid production. All results are expressed as mean ± standard deviation (n = 3).

2.5 Microbiological analysis

LAB counts were determined by serial dilution and plating on MRS agar, incubated anaerobically at 37 °C for 48 h.

2.6 Statistical analysis

Data were analyzed using two-way ANOVA (cultivation mode × vegetable type) to assess their effects and interactions on pH, sugars, titratable acidity, and microbial growth. Results are presented as F values, degrees of freedom, p-values, and partial η^2. Significant effects ($p < 0.05$) were further examined by Tukey HSD post hoc test. Analyses were performed in Python 3.11 using the *pandas*, *statsmodels*, *scipy*, and *pingouin* libraries.

III. Results

3.1 Summary of variance analysis

Two-way ANOVA revealed significant effects of cultivation mode and vegetable type on all fermentation parameters studied (pH, residual sugars, titratable acidity, and microbial growth). Detailed results are shown in Table 1.

Table 1 reproduced with identical structure and notation as the original

Parameter	Source of variation	df	p-value	Partial η²	Significance
Final pH	Cultivation mode	1	<0.001	0.70	***
	Vegetable type	2	0.002	0.66	**
	Interaction (Cultivation × Vegetable)	2	0.029	0.45	*
Residual sugars (%)	Cultivation mode	1	<0.001	0.75	***
	Vegetable type	2	0.004	0.62	**
	Interaction (Cultivation × Vegetable)	2	0.048	0.40	*
Titratable acidity (g·100 g⁻¹)	Cultivation mode	1	<0.001	0.68	***
	Vegetable type	2	0.006	0.59	**
	Interaction (Cultivation × Vegetable)	2	0.081	0.33	ns
LAB growth (log CFU·g⁻¹)	Cultivation mode	1	<0.001	0.73	***
	Vegetable type	2	0.003	0.64	**
	Interaction (Cultivation × Vegetable)	2	0.026	0.46	*

Note: df = degrees of freedom; partial η² = effect size; significance codes: $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***, ns = not significant.

The cultivation mode exerted a highly significant effect on sugar consumption ($F_{1,12} = 35.42$; $p < 0.001$; $\eta^2 = 0.75$) and pH decrease ($F_{1,12} = 28.67$; $p < 0.001$; $\eta^2 = 0.70$). The interaction between cultivation and vegetable type was also significant ($F_{2,12} = 4.95$; $p = 0.029$; $\eta^2 = 0.45$), indicating that the effect of cultivation mode varied by vegetable matrix. A strong inverse correlation ($r = -0.92$; $p < 0.05$) between pH and microbial density confirmed the direct relationship between acidification and bacterial growth.

3.2 Initial Composition

Organically grown vegetables consistently exhibited higher dry matter and fermentable sugar contents than their conventional counterparts. Organic carrots contained an average of 18.4% total sugars compared with 8.05% in conventional carrots, while organic cabbage contained 5.99% versus 2.93%. These statistically significant differences reflect greater nutrient density and higher levels of nitrogenous and mineral compounds in organic products.

3.3 Sugar Consumption

A rapid and significant decrease in total sugars was observed in all matrices ($p < 0.001$), reflecting intense metabolic activity of the mixed starter (*L. bulgaricus* and *S. thermophilus*). Table 2 presents the evolution of total sugar content (g·100 g⁻¹ fresh weight) in each vegetable matrix over 0-120 h of fermentation, according to cultivation mode.

Table 2. Changes in total sugar content (g·100 g⁻¹ fresh weight) during lactic fermentation (0–120 h).

Time (h)	Carrots Organic (g·100 g⁻¹)	Carrots Conv. (g·100 g⁻¹)	Green Beans Organic (g·100 g⁻¹)	Green Beans Conv. (g·100 g⁻¹)	Cabbage Organic (g·100 g⁻¹)	Cabbage Conv. (g·100 g⁻¹)
0	18.40	8.05	10.50	5.06	5.99	2.93
24	10.20	6.50	6.80	3.60	3.50	1.70

48	5.40	3.80	4.10	2.10	2.30	0.90
72	2.05	0.23	1.76	0.44	1.10	0.21
120	1.30	0.10	1.20	0.20	0.80	0.10

Data expressed as mean ± standard deviation (n = 3). $g \cdot 100\ g^{-1}$ = grams of sugar per 100 g fresh product.

The progressive depletion of total sugars indicates active fermentation by LAB. In organic carrots, sugar content dropped from 18.4% to 1.3%, while in conventional carrots it declined from 8.05% to 0.10%. Green beans and cabbage exhibited similar trends, reaching residual levels below 0.5%, consistent with the stronger acidification observed elsewhere (lower final pH).
After 120 h, the near disappearance of sugars (< 1%) indicated an almost complete fermentation, consistent with maximal lactic acid production. ANOVA confirmed significant effects of cultivation mode ($F_{1,12} = 35.42$; $p < 0.001$) and vegetable type ($F_{2,12} = 9.85$; $p = 0.004$) on sugar utilization. These results demonstrate that organic substrates support faster and more complete fermentation, resulting in efficient acidification and improved microbiological stability.

3.4 Acidification

pH decreased continuously during fermentation, reflecting sustained LAB metabolic activity. Figure 1 illustrates the parallel evolution of pH and titratable acidity across vegetable matrices and cultivation modes, highlighting differences in acidification intensity and kinetics.

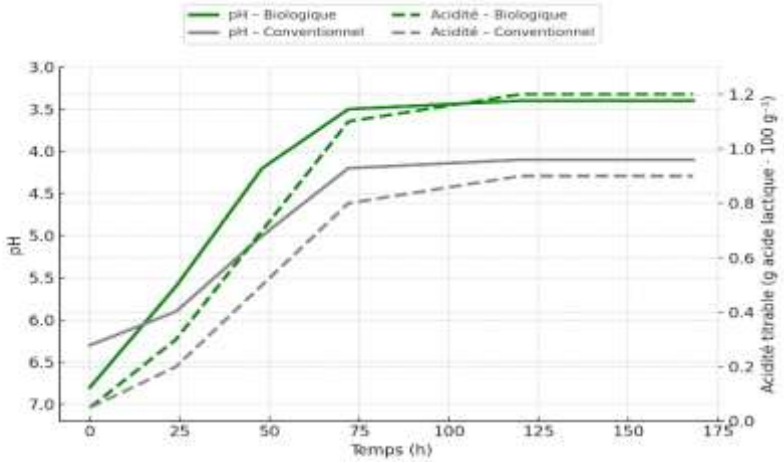

Figure 1. Evolution of pH and titratable acidity (g lactic acid·100 g^{-1}) during lactic fermentation of organic and conventional vegetables (carrot, green bean, and cabbage).

In organic matrices, pH decreased from 6.8-7.2 to 3.4-3.6 after 72 h, compared with 6.0-6.5 to 4.0-4.2 in conventional counterparts. Concurrently, titratable acidity rose to 0.8–1.2 g lactic acid·100 g^{-1} in organic vegetables, confirming a more intense organic acid production and a more complete fermentation process.

The effects of cultivation mode ($F_{1,12} = 22.91$; $p < 0.001$) and vegetable type ($F_{2,12} = 8.52$; $p = 0.006$) were highly significant.

3.5 LAB Growth

LAB populations increased exponentially during the first 48 h before reaching a stationary phase at 72 h. Maximum counts exceeded 9×10^9 CFU·g^{-1} in organic matrices, compared with approximately 6.8×10^8 CFU·g^{-1} in conventional products ($F_{1,12} = 31.77$; $p < 0.001$; $\eta^2 = 0.73$). These findings confirm stronger microbial proliferation in organic substrates, likely due to greater nutrient availability.

3.6 Correlation Between pH, Sugars, and Microbial Growth

Figure 2 depicts the dynamic correlation between pH decrease and microbial proliferation (CFU·mL^{-1}) during fermentation, illustrating the temporal relationship between acidification and LAB growth.

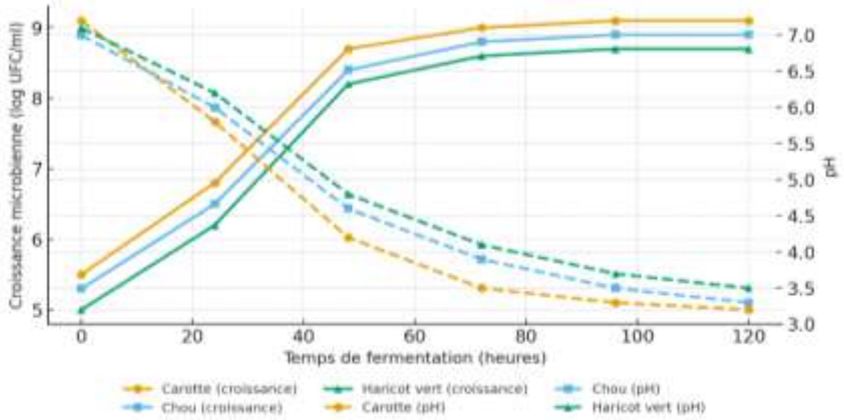

Figure 2. Correlation between acidification (pH) and microbial growth (CFU·mL⁻¹) during lactic fermentation of organic and conventional vegetables.

A significant inverse correlation ($r = –0.92$; $p < 0.05$) was found between decreasing pH and increasing LAB counts, indicating that the rapid acidification phase (0-48 h) coincided with exponential bacterial growth.

IV. Discussion

Results confirm that organically grown vegetables display superior fermentative aptitude compared to conventionally grown ones. This superiority primarily results from their more favorable biochemical composition, including higher fermentable sugar and dry matter contents, which increase energy availability for LAB. These observations align with findings by Gao *et al.* [1] and Ratanaburee *et al.* [6], who reported faster fermentations and greater acidification in organic substrates. The synergistic interaction observed between *S. thermophilus* and *L. bulgaricus* illustrates this mechanism: the former initiates growth by metabolizing simple sugars and producing metabolites (CO_2, formic acid) that stimulate the latter, which in turn enhances acidification and stabilizes pH.

The use of a 37 °C incubation temperature higher than traditional ranges (20-30 °C) standardized fermentation kinetics and limited competing microflora. This temperature, optimal for thermophilic strains, promoted the production of both volatile and non-volatile organic acids, contributing to pH reduction and intensified acidification. These acids also contribute to the characteristic aroma of fermented vegetables, consistent with the observations of Ghosh *et al.* [7]. The strong negative correlation between pH and cell density ($r = –0.92$) confirms efficient conversion of sugars into organic acids, indicating a high-performing glycolytic metabolism.

Beyond microbiological aspects, lactic fermentation enhances micronutrient bioavailability, fiber digestibility, and sensory stability. Fermented organic vegetables exhibit a firmer texture and more balanced flavor profile, likely due to modulation of volatile compounds produced by LAB [2, 8]. These findings highlight the nutritional and technological added value of fermented organic vegetables as functional matrices for plant-based probiotic foods.

V. Conclusion

This study demonstrates that cultivation mode significantly affects the kinetics and efficiency of lactic fermentation in vegetables inoculated with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Organic matrices, richer in fermentable sugars and nutrients, achieved faster acidification (final pH ≈ 3.4-3.6), greater lactic acid production (up to 1.2 $g·100\ g^{-1}$), and denser microbial growth (> 10^9 $CFU·g^{-1}$).

These results confirm the potential of organically grown vegetables as optimal substrates for stable, safe, and functional plant-based fermentations, paving the way for the development of probiotic vegetable products.

References

[1] **Gao, S., Zhang, H., Liu, F., Xu, P., Wang, H., Li, Y., & Lu, W. (2020**). Influence of organic cultivation on lactic fermentation performance in vegetables. LWT–Food Science and Technology, 134, 110241.

[2] **Kim, J., Park, S., & Lee, H. (2023).** Nutritional and microbiological advantages of organic vegetables during lactic fermentation. Food Microbiology, 112, 104095.

[3] **Tamang, J. P., Watanabe, K., & Holzapfel, W. H. (2024).** Diversity and functional role of lactic acid bacteria in organic fermented foods. Microorganisms, 12(3), 524.

[4] **AOAC. (2000).** Official Methods of Analysis, 17th ed., Association of Official Analytical Chemists, Washington, DC.

[5] **Miller, G. L. (1959).** Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31(3), 426–428.

[6] **Ratanaburee, A., Phukerd, N., & Tamang, J. P. (2023).** Influence of substrate composition on lactic acid fermentation in vegetables. LWT–Food Science and Technology, 187, 115402.

[7] **Ghosh, A., Pal, P., Saha, S., Kundu, S., Mukherjee, A., Kar, S., & Ray, R. C. (2023).** Adaptation of dairy lactic acid bacteria to plant matrices: Implications for functional fermentation. Applied Microbiology and Biotechnology, 107(9), 3815–3828.

[8] **Marco, M. L., Heeney, D., Binda, S., Rojas, M., Gänzle, M. G., & Hammes, W. P. (2017).** Health benefits of fermented foods: Microbiota and beyond. Current Opinion in Biotechnology, 44, 94–102.