Peer Community In Microbiology

Cutting Type as a Key Factor in shaping Microbial Dynamics during Vegetable Fermentation

Souhir Marsit *based on peer reviews by* ***Thibault Nidelet*** *and* ***Kate Howell***

Florence Valence, Romane Junker, Céline Baty, Olivier Rué, Mahendra Mariadassou, Marie-Noelle Madec, Marie-Bernadette Maillard, Anne-Sophie Bage, Victoria Chuat, Laurent Marché, Anne Thierry (2025) The cutting type of vegetables influences the spontaneous fermentation rate. HAL, ver. 2, peer-reviewed and recommended by Peer Community in Microbiology. https://hal.science/hal-04701063v2

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Fermented vegetables, traditionally consumed in Asian and Eastern countries, are gaining increasing interest in Western countries due to the growing demand for more natural, healthy, and sustainable food. Their potential health effects have only recently begun to be scientifically studied (Thierry *et al.*, 2023).

The manufacturing process of fermented vegetables consists of cutting and packing raw vegetables with salt or brine, that will draw water and nutrients out from the vegetable tissue, thus providing microorganisms with the necessary substrates to initiate spontaneous fermentation (Buckenhueskes, 2015). Various parameters, including the cutting method, which may influence the rate of solute diffusion from vegetable tissue, can affect fermentation speed and, consequently, the quality of fermented vegetables. However, the role of cutting type has rarely been addressed.

The study by Valence *et al*. (2025) used a comprehensive range of methods to investigate how cutting types and a slight reduction in salt concentration influence the spontaneous fermentation of two vegetables, carrot and cabbage. Two cutting types, finely or roughly cut, and two salt levels, 1% (the minimum concentration usually used) and a lower salt level in line with health recommendations (0.8%), were tested. Carrot and cabbage fermentations were performed under controlled conditions in duplicate, and microbiological and biochemical characteristics were monitored over one month by combining several approaches and extensive experiments in culturomics, 16S rRNA gene and gyrB metataxonomics for bacterial community analysis, and targeted metabolomics.

The study shows the sequential establishment of microbial communities during the fermentation of both vegetables. In the early stages, Enterobacteriaceae replaced the initial microbiota, but they were rapidly outcompeted by Lactic Acid Bacteria (LAB). LAB growth acidified the medium, inhibiting enterobacteria and ensuring microbial safety. Their dominance was attributed to their ability to ferment carbohydrates into lactic acid and possibly the production of antimicrobial compounds. The results of targeted metabolomic analysis show that the main fermentation byproducts are mannitol, lactic acid, and acetic acid, which is consistent with previous studies on fermented vegetables.

Most notably, this study demonstrated for the first time that the type of vegetable cutting has a major impact on fermentation dynamics by influencing the release of solutes into the brine. Finer cuts, which provide a greater surface area, facilitate nutrient diffusion, thereby promoting LAB proliferation and acidification.

The study also shows that salt addition improved solute release, though the microbial effects were less clear due to variability between replicates. Indeed, significant variability between jars was noted, affecting microbial composition, metabolite profiles, and acidification rates.

The work of Valence *et al*. (2025) highlights for the first time the crucial role of cutting type in vegetable fermentation, demonstrating that finer cuts accelerate acidification, improve microbial safety, and enhance fermentation efficiency. Their findings contribute to the optimization of fermentation processes, providing valuable insights for enhancing the quality of fermented vegetables.

References:

Buckenhueskes HJ. Quality improvement and fermentation control in vegetables. Advances in Fermented Foods and Beverages. Elsevier, 2015, 515–39. https://doi.org/10.1016/B978-1-78242-015-6.00022-0

Thierry A, Baty C, Marché L, Chuat V, Picard O, Lortal S, Valence F. Lactofermentation of vegetables: An ancient method of preservation matching new trends. *Trends Food Sci Technol.* 2023. https://doi.org/10.1016/j.tifs.2023.07.009

Valence F, Junker R, Baty C, Rué O, Mariadassou M, Madec M, Maillard M, Bage A, Chuat V, Marché L, Thierry A. The cutting type of vegetables influences the spontaneous fermentation rate. *HAL*, ver.2 2025. https://hal.science/hal-04701063v2

Reviews

Evaluation round #2

Reviewed by Kate Howell, 11 March 2025

- Nice concise title
- Abstract finishes abruptly
- Introduction reads well.
- Fig 1. Shredded not schredded
- Results read very well and I very much enjoyed the declarative subheadings
- There is no subheading for figure 8- could you highlight the key result for this figure in a subheading?
- Line 968 results not shown
- Discussion is of an appropriate length and content

Reviewed by Thibault Nidelet, 17 March 2025

The vast majority of my comments have been satisfactorily taken into account, for which I thank you. I consider the paper in its present form suitable for publication.

Title and abstract

Does the title clearly reflect the content of the article? [X] Yes, [ ] No (please explain), [ ] I don't know

Does the abstract present the main findings of the study? [X] Yes, [ ] No (please explain), [ ] I don't know

Introduction

Are the research questions/hypotheses/predictions clearly presented? [X] Yes, [ ] No (please explain), [ ] I don't know

Does the introduction build on relevant research in the field? [X] Yes, [ ] No (please explain), [ ] I don't know

Materials and methods

Are the methods and analyses sufficiently detailed to allow replication by other researchers? [X] Yes, [ ] No (please explain), [ ] I don't know

Are the methods and statistical analyses appropriate and well described? [X] Yes, [ ] No (please explain), [ ] I don't know

Results

In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [X] Yes, [ ] No (please explain), [ ] I don't know

Are the results described and interpreted correctly? [X] Yes, [ ] No (please explain), [ ] I don't know

Discussion

Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [X] Yes, [ ] No (please explain), [ ] I don't know

Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [X] Yes, [ ] No (please explain), [ ] I don't know

Evaluation round #1

DOI or URL of the preprint: https://hal.science/hal-04701063v1

Version of the preprint: 1

Authors' reply, 07 February 2025

Download author's reply

Decision by Souhir Marsit, posted 21 November 2024, validated 22 November 2024

Dear Authors,

Thank you for submitting your manuscript entitled "The cutting type of spontaneously fermented vegetables impacts their fermentation rate" to PCI Microbiology.

Below, you will find comments from two expert reviewers. Both reviewers found your study highly interesting, relevant, and scientifically robust. However, they raised some concerns and made suggestions for improving the manuscript before recommendation.

We kindly ask you to provide point-by-point responses to the reviewers' comments.

We look forward to receiving the revised version of your manuscript.

Sincerely,

Souhir Marsit

Reviewed by Kate Howell, 12 November 2024

Download the review

Reviewed by Thibault Nidelet, 29 October 2024

This article is very interesting and overall well written. I recommend it for publication with minor corrections of the text and figures.

- **Title and abstract**
- Does the title clearly reflect the content of the article? [x] Yes, [ ] No (please explain), [ ] I don't know
- Does the abstract present the main findings of the study? [x] Yes, [ ] No (please explain), [ ] I don't know

- **Introduction**
- Are the research questions/hypotheses/predictions clearly presented? [x] Yes, [ ] No (please explain), [ ] I don't know
- Does the introduction build on relevant research in the field? [x] Yes, [ ] No (please explain), [ ] I don't know

- **Materials and methods**
- Are the methods and analyses sufficiently detailed to allow replication by other researchers? [x] Yes, [ ] No (please explain), [ ] I don't know
- Are the methods and statistical analyses appropriate and well described? [ ] Yes, [x] No (please explain), [ ] I don't know

Some minor justifications have to be justified. They consider the different salt concentration as equivalent replicates for other factors as there is not salt effect. That is not the most common practice but possible. It should be better justified.

- **Results**
- In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [x] Yes, [ ] No (please explain), [ ] I don't know
- Are the results described and interpreted correctly? [x] Yes, [ ] No (please explain), [ ] I don't know

- **Discussion**
- Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [x] Yes, [ ] No (please explain), [ ] I don't know
- Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [x] Yes, [ ] No (please explain), [ ] I don't know

Here is the list of my comments.

Line 45 to 47 : this phrase could be reformulated : Maximal Enterobacteriaceae counts were higher in cabbage (8 vs 7 logCFU/g), while lactic acid bacteria counts were higher in carrot (9 vs 8 logCFU/g).

85 is "legumes" the good word. For me "legumes" correspond to soybean, faba bean, lentil, broad bean, etc and not cabbage of carrots.

Line 103 to 105: this sentence "The environmental aerobic or facultatively anaerobic microorganisms first grow and are progressively replaced by a succession of heterofermentative and then homofermentative LAB" need a reference

Line 117 to 119: this sentence "In a recent study, carried out on 75 samples produced at a domestic scale, the age of samples ranged from 2 weeks to 4 years with a median value of 6 months. 84 % of analysed samples still contained alive LAB" need a reference

Last paragraph of the introduction, you speak about a slight reduction of salt but form 1% to 0,8% it is a decrease of 20% that is not a small reduction, even if the total concentration of salt is small

Methods :

Why vitamins has not been sampled à T3 ?

Figure 1 the corresponding time of the sample could be added to the figure and not only indicated in the legend

Line 204 ”either” implices a second option

Line 284 ”to visualise barplots” which barbarplot do you speak off ? I think Phyloseq has also been used to calculate the abundances

Line 369 as the different vitamins were not measure at the same time, it will be important to précise it in the Fig 1

Lines 388 the used packages have to be cited with a specific reference

Line 395 why do you take out the factors that were not significant from your anova ?

Line 400 it will be interesting to add the total number of variables used in the PCA, its help to analyse de percentage of variance explained by the PCA’s axes

Results

Line 420 ”were significantly impacted by the vegetable studied, the fermentation stage, and, to a lesser extent, the cutting type.” Add the corresponding pvalues of these tests in the text of in a table

Line 422 ”Therefore, four replicates instead of two were available at each sampling point for statistical analyses.” This is due to the suppression from the model of the NACL effect and considering the different NACL concentrations as equivalent replicates. It have to be justified or at least specified here or in material and methods

Figure 2 : adding the indicating of sampling numbers T1, T2, T3, etc. in the graph will be interesting as there as used in the text. In the texte you explained that the sampling times were not the same in the different medium as the speed of fermentation was different. However in the graph they appear identical ? The time unity is not indicated. The order of the panels don’t follow the order of the text that is enterobacteria, LAB and pH. It will be better to have the same order in both the text and the graph.

Line 458 : add the fact that 7 months is not represented in figure 2

Lines 469 : remind what is TTA

Lines 496 to 500 : as for yeast there is no statistical information indicating any effect

Figure 3 : as you have tow jars you can calculate averages and variances of concentrations and therefore add error bars on the graph

Line 518 to 532 no indication of statistic tests

Line 539 figure 4 is not in bold

Figure 3 : in the same manner that the type of cutting the salt concentration could be add in the upper tree

Figure 4 as you compare the different type of fermentation more that the time of fermentation inverting the lines and the column will help to the main comparison. You could also superpose the four graphs with different colors. That is also possible for Figure 2. Time unity is not indicated

Figure 5 : is often better to do the PCA on the averages and not on the replicates, in particular for reasons of legibility. You have to add indicator for the three different panels like A) individuals graph, B) variables graph and C) confidence zones for different factors. In the las panel it will be better to have different color pallets for the type of factors. Here green been T2, S1 or cabbage_thin depending of the three graphs.

Line 575 yo 578 : it will be interesting to make the link between these separation and the statistical effects of the different factors previously shown

Line 596 to 597 : add the fact that this minor metabolites have been not used to calculate the PCA’s axes, supplementary variables is not very clear by itself

Line 607 ”27, 15, and 10 volatiles” : it not clear at what the numbers make reference to

Figure 6 : the graph of the variable is important to interpret the PCA, if there is to much variables you

can plot only the 20 most important variables for the PCA construction. You can make filter for that and the FactoMineR package. Add indication of the panel A and B for more clarity

Line 630 : same remark than for the line 607, it could be rephrased for clarity

Lines 667 to 669 : add the corresponding pvalues

Table 1 add the standard deviation to the average in the table as well as * indicating the significant difference between samples

Figure 7 : increase it size of the size of the label especially for the sample of the first heatmap. I will split the two heatmaps in two separate figures. Here the A and B indicating the two panels are absent

Lines 767 yo 769 : can you give examples of the differences between duplicate jars

Line 796 figure 8 is not in bold

Figure 8 : the labels are too small to be read. Use A and B ton indicate the two panels. The legend is incomplete as you only speak of the variable in brown and not the others one.

Line 785 to 833 it will be interesting to put more in light the new results shown by the Partial Least Squares-Discriminant Analysis (PLS-DA) compared with previous analyses. Why this additional analysis?

Discussion

Lien 842 ”slight reduction” 20% is not slight

Lines 856 to 858 ”As a direct consequence, quadruplicate samples instead of duplicate samples were available at each stage to investigate the effect of the other factors examined, i.e. the cutting and the fermentation stage.” That way of using statistic is very specific and has to be better justified

Line 861 the intra-jar variation is not specifically shown by any of this graph. A specific graph in supplementary information could be interesting showing the value for each sample rather than the average and confidence interval

Line 1173 ”DOI of the webpage hosting the supplementary information” has to be removed.