



**HAL**  
open science

## The cutting type of spontaneously fermented vegetables impacts their fermentation rate

Florence Valence, Romane Junker, Céline Baty, Olivier Rué, Mahendra Mariadassou, Marie Noëlle Madec, Marie-Bernadette Maillard, Anne-Sophie Bage, Victoria Chuat, Laurent Marché, et al.

### ► To cite this version:

Florence Valence, Romane Junker, Céline Baty, Olivier Rué, Mahendra Mariadassou, et al.. The cutting type of spontaneously fermented vegetables impacts their fermentation rate. 2024. hal-04701063

**HAL Id: hal-04701063**

**<https://hal.inrae.fr/hal-04701063v1>**

Preprint submitted on 18 Sep 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

# The cutting type of spontaneously fermented vegetables impacts their fermentation rate

Florence Valence<sup>1\*</sup>, Romane Junker<sup>2\*</sup>, Céline Baty<sup>3</sup>, Olivier Rué<sup>2,4</sup>, Mahendra Mariadassou<sup>2</sup>, Marie-Noelle Madec<sup>1</sup>, Marie-Bernadette Maillard<sup>1</sup>, Anne-Sophie Bage<sup>1</sup>, Victoria Chuat<sup>1</sup>, Laurent Marché<sup>5</sup>, Anne Thierry<sup>1</sup>

\* co-first authors

<sup>1</sup>STLO, CIRM-BIA, INRAE, Institut Agro, Rennes, France

<sup>2</sup> Université Paris-Saclay, INRAE, MaIAGE, 78350, Jouy-en-Josas, France

<sup>3</sup>Vegenov, 29250 Saint-Pol-de-Léon, France

<sup>4</sup> Université Paris-Saclay, INRAE, BioinfOmics, MIGALE bioinformatics facility, 78350, Jouy-en-Josas, France

<sup>5</sup> UMR1014 SECALIM, INRAE, Osiris, Nantes, France

## ORCID numbers:

Florence Valence: 0000-0002-4834-086X

Romane Junker: 0009-0009-5753-2829

Céline Baty : 0000-0003-1646-0653

Olivier Rué: 0000-0001-7517-4724

Mahendra Mariadassou: 0000-0003-2986-354X

Anne-Sophie Bage : 0000-0002-8129-5503

Victoria Chuat: 0000-0003-1528-2680

Laurent Marché: 0000-0003-4666-5027

Anne Thierry: 0000-0002-9170-2889

## ABSTRACT

Fermented vegetables are essentially produced by spontaneous fermentation of raw vegetables, which are roughly or thinly cut, salted and incubated in an oxygen-free environment. Despite the variety of types of cutting and its potential role in the rate of diffusion of solutes from vegetable tissue, and thus the fermentation rate, the effect of this factor has been scarcely studied. Our aim was to investigate how the cutting and small variation of salt concentration impact the microbial and biochemical changes during the spontaneous fermentation of vegetables.

A 2 × 3 experimental design was set up with vegetable type (carrot / cabbage), cutting type (thin / rough), and salt concentration (0.8% / 1%) as factors. Vegetables were pressed down in 500 mL-jars and filled up with brine, and two independent jars used at 4 stages to characterise the microbial dynamics and biochemical changes by combining culturomics, 16S rRNA V5-V7 and gyrB metataxonomics, and targeted metabolomics.

Culturomics and metataxonomics results showed a similar succession of the main bacterial groups in both vegetables, with *Enterobacteriaceae* quickly replacing the initial microbiota,

44 further replaced within a few days by lactic acid bacteria, mainly represented by *Leuconostoc*  
45 sp. Cabbage and carrot fermentation rates quantitatively differed. Maximal *Enterobacteriaceae*  
46 counts were higher in cabbage (8 vs 7 logCFU/g), while lactic acid bacteria counts were higher  
47 in carrot (9 vs 8 logCFU/g). The acidification rate was faster in carrot (e.g. pH decreased to 3.8 in  
48 40 h vs approx. 5.0 in 86 h in cabbage). Mannitol, lactic and acetic acids were the main  
49 metabolites produced in both vegetables, but concentrations were two-fold higher in carrot.  
50 Viable *Enterobacteriaceae* were not detected anymore after two-week fermentation, except for  
51 some roughly-cut cabbage samples. No pathogenic bacteria were detected. Taxonomic profiles  
52 varied according to the marker used, e.g. *Leuconostoc* was only detected with gyrB and vice-  
53 versa for *Clostridium*. The gyrB marker provided a markedly better resolution at the species-  
54 level (for 97% of ASV vs only 20% for the 16S marker). Significant effects of cutting type, and not  
55 of NaCl concentration, were observed. Thinly-cut vegetables globally showed a quicker  
56 fermentation compared to roughly-cut ones and exhibited a higher titratable acidity, e.g. 0.8%  
57 vs 0.3%, respectively, in grated and sliced carrot at 64 h incubation. In line, a quicker production  
58 of acids and a quicker decrease of viable enterobacteria were observed in thinly-cut vegetables,  
59 in particular for cabbage, for which the surface generated by the cutting was ~20-fold greater in  
60 shredded cabbage than in leaf cabbage. Some leaf cabbage samples displayed atypical  
61 fermentations, with the presence of particular taxa and atypical metabolite profiles with high  
62 amounts of ethanol produced. These general trends were modulated by quantitative and  
63 qualitative differences between replicate jars.

64 This study confirms the highly diverse microbiota of spontaneously fermented vegetables  
65 and the tight competition between *Enterobacteriaceae* and lactic acid bacteria in their  
66 colonisation, and documents for the first time the effect of the type of cutting on the  
67 fermentation rate.

68

69 **Keywords** : Fermented carrot/ sauerkraut / cutting / lactic acid bacteria / leuconostoc /  
70 enterobacteria / natural fermentation

71

## Introduction

72 Fermented vegetables are traditionally consumed in central Europe and Asia, and have  
73 recently received a renewed interest in Western countries, for many possible reasons that  
74 include consumers' demand for more natural and sustainable foods and the growing proportion  
75 of vegetarian or vegan diets (Medina-Pradas et al., 2017; Thierry, Baty, et al., 2023). In Asian and  
76 Eastern countries, where fermented vegetables have been part of the tradition, a wide variety  
77 of vegetables are fermented and commonly consumed (Gänzle, 2022; Thierry, Baty, et al., 2023).  
78 Cabbage is the main vegetable used worldwide, either in mixture with other vegetables, as in  
79 the Korean kimchi or the Chinese paocai, or alone in sauerkraut in the East of France and in  
80 Germany, produced at industrial scale (Tamang et al., 2020). In Western countries, sauerkraut,  
81 olives, and cucumber are the main fermented vegetables commonly consumed. The other  
82 fermented vegetables are principally produced at domestic and artisanal scale (Thierry, Baty, et  
83 al., 2023). In a recent study we showed that French domestic and artisanal productions of  
84 fermented vegetables include a large variety of vegetables. Indeed, in the frame of a citizen  
85 science project, the 75 collected samples from citizens included 23 types of legumes, mainly  
86 cabbage (27%), followed by carrot (19%) and beets (12%), and 40% of them contained mixtures

87 of vegetables (Thierry, Madec, et al., 2023). The potential health effects of plant-based  
88 fermented foods have only begun to be scientifically documented, even if they make the buzz  
89 in social media without scientific support. Some vitamin concentrations can increase, or be  
90 preserved during fermentation but the effect depends on the microbial community and the  
91 conditions of production, among other factors, and contrasted results have been observed  
92 (Thierry, Baty, et al., 2023). All fermented vegetables are manufactured according to a quite  
93 simple process, which consists in cutting and tightly packing raw vegetables added with salt or  
94 brine, so that vegetables are covered with brine or with the juice released from vegetables  
95 (Buckenhueskes, 2015). Fermentation is most generally spontaneous and due to an endogenous  
96 lactic acid bacteria (LAB) community. A wide variety of recipes are used in terms of number,  
97 nature, and mixture of vegetables, and use of minor ingredients, i.e. spices and condiments (Di  
98 Cagno et al., 2013; Ashaolu & Reale, 2020).

99 Several bacterial groups succeed one another over time during the spontaneous  
100 fermentation of vegetables, and some of them are alive at the time of consumption. According  
101 to a meta-analysis on various fermented food covering 400 articles over 50 years, the average  
102 number of alive microorganisms in fermented vegetables such as sauerkraut, kimchi, pickles,  
103 and olives varies from 2 to 8 log colony-forming units (CFU)/g (Rezac et al., 2018). The  
104 environmental aerobic or facultatively anaerobic microorganisms first grow and are  
105 progressively replaced by a succession of heterofermentative and then homofermentative LAB.  
106 For example, in a study on carrot juice spontaneous fermentation, bacteria of the  
107 *Enterobacteriaceae* family first grew and reaching about 8 log CFU/g from the first hours of  
108 fermentation, then decreased to totally disappear after 10 days of fermentation (Wuyts et al.,  
109 2018). In parallel, during the first 3 days of fermentation, LAB actively grow, reaching around 9  
110 log CFU/g and are responsible for rapid decrease of pH. The first LAB that grow are typically  
111 members of the *Leuconostoc* genus, followed by the *Lactilactobacillus* and *Lactiplantibacillus*  
112 genera, at cell numbers of about 9 log CFU/g (Wuyts et al., 2018). Similar pictures were observed  
113 in other vegetables, e.g. in pepper (Li et al., 2024), sauerkraut (Müller et al., 2018), cucumbers  
114 (Stoll et al., 2020). Most kinetic studies on fermented vegetables are carried out over relatively  
115 short periods and do not exceed one month, which is generally considered as the final stage of  
116 fermentation since the pH is stabilised (Wuyts et al., 2018; Müller et al., 2018; Wang et al., 2020).  
117 In a recent study, carried out on 75 samples produced at a domestic scale, the age of samples  
118 ranged from 2 weeks to 4 years with a median value of 6 months. 84 % of analysed samples still  
119 contained alive LAB. LAB represented the majority of living microorganisms but also the  
120 majority of 16S reads recovered by 16S rRNA gene metataxonomics while bile-tolerant  
121 *Enterobacteriaceae* were detected in only four samples (Thierry, Madec, et al., 2023). Besides  
122 bacteria, yeasts and bacteriophages can also grow and survive in fermented vegetables  
123 (Tamang et al., 2016). Yeasts have been reported in various fermented vegetables (Liu et al.,  
124 2021; Wang, Chen, Tang, Ming, Huang, Li, Ye, Fan, Yin, et al., 2022) and were found in half of the  
125 75 fermented vegetable homemade analysed (Thierry, Madec, et al., 2023). Given the microbial  
126 changes over fermentation time, it is crucial to combine cultural methods with culture-  
127 independent methods such as 16S metataxonomics or shotgun metagenomics, to better  
128 understand the dynamics of the microbiota of fermented vegetables. Cultural methods allow  
129 access to the living part of the cultivable microorganisms present and therefore potentially  
130 metabolically active at the time of analysis. Culture-independent methods provide access to all

131 the microorganisms present in the sample, whether or not they are viable at the time of analysis.  
132 The complementarity of these two approaches is all the more important as each method  
133 contains numerous potential biases (Parente et al., 2022). Concerning the 16S metataxonomics  
134 method, one of these biases lies in the specificity of the 16S primers used; the combination of  
135 several primers can make it possible to overcome these biases (Poirier, 2018; Guo et al., 2022).

136 Some steps are essential for a successful fermentation, notably salting and packing. The  
137 main function of salting is to withdraw water and nutrients from vegetable tissue, thus providing  
138 microorganisms with the substrates they need for growth. The NaCl concentration generally  
139 ranges from 1% to 3% of the final product. Sliced vegetables are filled and pressed in glass jars,  
140 a tight packing being crucial to eliminate air pockets and promote an anaerobic environment to  
141 limit the growth of undesirable aerobic microbiota, potentially responsible for spoilage.  
142 Products are then let to ferment at ambient temperature for at least 3 to 4 weeks before being  
143 consumed or further stored at lower temperatures. These conditions of incubation  
144 (temperature, NaCl concentration, oxygen availability, ...) determine the start-up speed of the  
145 fermentation process and thus shape the microbial community (Thierry, Baty, et al., 2023). The  
146 rate of fermentation, in particular the time course of pH decrease, is crucial to limit the growth  
147 of undesirable microbiota. Temperature and salt concentrations influence the dynamics of LAB  
148 species. The higher the temperature of fermentation is, the faster pH decreases, the earlier the  
149 dominance of (former) lactobacilli, which have a greater acid tolerance. In contrast,  
150 leuconostocs were present at a higher abundance at temperatures < 15°C (Wang et al., 2020). As  
151 for salt concentration, early studies showed its impact on the rate of acidification and the  
152 growth dynamics within the main LAB species, e.g. the growth of *Leuconostoc mesenteroides*,  
153 which is less salt-tolerant than other, homofermentative, LAB species (Pederson & Albury, 1969).

154 The type of cutting markedly varies in domestic productions, as recently observed in a recent  
155 study (Thierry, Madec, et al., 2023). Vegetables can be used either thinly cut (grated, shredded),  
156 or more roughly cut (into slices, dices, or simply cut in two lengthwise, for example), or even  
157 whole in the case of some small size vegetables. For example, carrot can be thinly or more  
158 roughly grated, or cut into small dices or slides, or only roughly cut. However, to our knowledge,  
159 the role of this factor has only been rarely addressed. In an original study that explored the  
160 survival of inoculated *Escherichia coli* O157:H7 and *Listeria monocytogenes* during fermentation  
161 of whole heads and shredded cabbage, both pathogens declined faster in shredded cabbage  
162 (Niksic et al., 2005). This was explained by the significantly higher total titratable acidity in  
163 shredded cabbage, compared to whole head cabbage. In summary, a thin cutting is expected to  
164 facilitate the withdrawal of water and nutrients from vegetable tissue and thus to increase the  
165 buffering capacity of juice and accelerate the rate of (lactic acid) fermentation.

166 Our aim was to investigate the effect of two factors, the type of cutting and a slight reduction  
167 of the amount of salt added, on the microbial and biochemical changes during spontaneous  
168 fermentation of vegetables. We chose to study a root vegetable, carrot, and a leafy vegetable,  
169 cabbage, either thinly or roughly cut. We thus compared fermentation between grated carrot  
170 and sliced carrot, and between grated cabbage and whole cabbage leaves. We also studied two  
171 slightly different salt concentrations, a concentration of 1%, which is the minimum  
172 concentration of salt normally used, and, with a view to reduce salt rates to follow health  
173 recommendations, a concentration of 0.8%. We performed fermentations of carrot and cabbage  
174 under controlled conditions and the various conditions studied were carried out in duplicate.

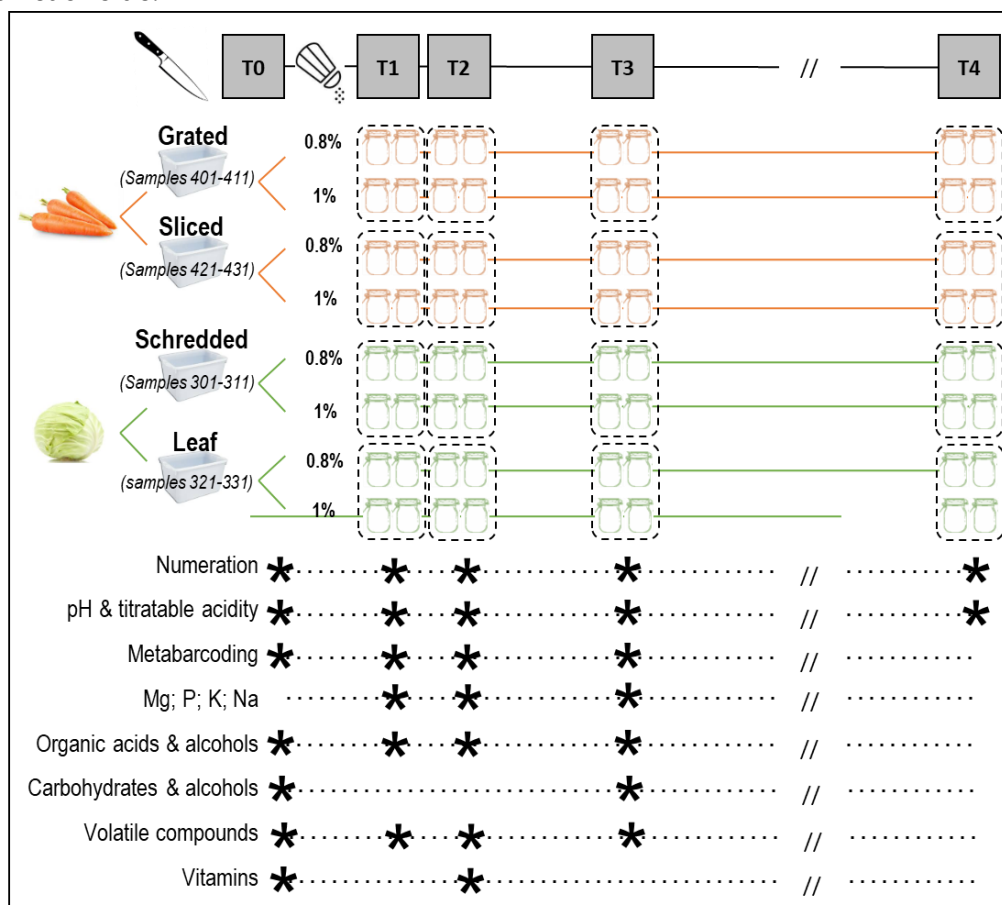
175 On these duplicates, we characterised the microbiological and biochemical changes over one  
 176 month by combining culturomics, 16S rRNA gene and *gyrB* metataxonomics analysis for  
 177 bacterial community, and targeted metabolomics.

178 **Material and methods**

179 **Ingredients and experimental design**

180 Two vegetables, carrot and white cabbage, were collectively chosen by the partners of the  
 181 FLEGME project among the most frequently used in the manufacture of fermented vegetables,  
 182 so as to include a root and an aerial vegetable. Non-washed organic carrots were provided by  
 183 the Ferme Ty Coz farm, Saint-Pol-de-Léon, France. Non-washed organic white cabbages  
 184 (*Brassica oleracea* L.), were provided by Coopérative des Producteurs Légumiers, Doué en  
 185 Anjou, France. Dry (< 0,1 % humidity) grey coarse sea salt was used. Tap water was used to  
 186 prepare the brine, water was boiled for carrot brine. 500 g-jars with glass lid and rubber seal were  
 187 used (Korken, IKEA).

188 A 2<sup>3</sup> experimental design was set up with (i) vegetable type (carrot / cabbage), (ii) cutting type  
 189 (thin / rough) and (iii) salt concentration (0.8% / 1%) as factors. The two chosen vegetables,  
 190 cabbage and carrot, were either thinly or roughly cut, firmly pressed down in 500 mL-jars and  
 191 filled up with brine to reach final NaCl concentrations of 0.8% or 1.0%, expressed as gram of raw  
 192 salt per 100 g of the mixture of vegetable and brine (**Figure 1**). More precisely, carrot was either  
 193 grated or cut into slices, and cabbage were either shredded or cut into pieces of leaf of about 6-  
 194 8 cm on each side.



195

196 **Figure 1:** Experimental design used to prepare fermented vegetables and sampling. The  
197 three factors tested were vegetable type (carrot and cabbage), cutting type (either thin or  
198 rough) and salt concentration (0.8% and 1%, expressed as g raw salt per g of preparation  
199 (vegetable and brine). Sampling was done in duplicate (two independent jars) at each  
200 sampling point, represented by a star, except for volatile analysis and vitamins, done in  
201 triplicate (three independent jars), at five points: T0, initial time, T1, 1.7 day, T2, 2.7  
202 (carrot) or 3.6 day (cabbage), T3 (4 weeks), and T4 (7 months). A few analyses were also  
203 performed at 2 weeks (stage T2b).

204 After removing the external leaves, cabbages were either shredded by using a professional  
205 Dito Sama TRS vegetable slicer, equipped with a 2 mm disk. Cabbage leaves were manually cut  
206 into ~9 cm x ~9 cm pieces of leaves. Then, 205 g shredded cabbage and 282 g of brine, or 232 g  
207 cabbage leaf and 246 g of brine, were successively weighed in each jar. As for carrots, they were  
208 washed, hand-peeled, grated in 3 mm pieces or cut in 5 mm-slices at the CTCPA pilot facility  
209 (agri-food technical centre and Oniris, Nantes, France). Then, 285 g grated carrot and 215 g of  
210 brine, or 285 g sliced carrot and 230 g of brine, were successively weighted in each jar. To ensure  
211 the final expected salt concentrations of 0.8% and 1%, preliminary tests were performed to  
212 determine the maximum amount of vegetables that could be packed in a jar, which varied  
213 between 205 g to 285 g according to the vegetable and the cutting types (**Supplementary Table**  
214 **S1**), and, consequently, the quantity of liquid (brine) that could be added (215 to 282 g), so as to  
215 calculate the salt concentration of brine in each case. The details of brine concentration are  
216 given in Supplementary Table S1.

217 Twenty jars of each type (vegetable, cutting type, salt concentration) were prepared, leading  
218 to a total of 160 jars. Jars were transported (1 h of transport between manufacturing site and  
219 laboratory) at ambient temperature (approx. 20°C) just after manufacturing to be incubated at  
220 the STLO laboratory at 19°C for 7 months. Two independent jars were characterised at each time  
221 point. One sample of raw vegetable was transported in a cooler (approx. 6°C upon arrival) to be  
222 analysed before fermentation (T0).

223 Samples were characterised for viable microorganisms, metataxonomics profiles and pH  
224 measurement at four sampling times. The first sampling was at the very beginning of  
225 fermentation, two samplings (named T1 and T2) covered the initial acidification period and the  
226 last (T3) was after four weeks incubation. Due to differences in the acidification rates, the first  
227 two sampling times were 40 h (T1) and 64 h (T2) for carrot and 40 h (T1) and 86 h (T2) for cabbage.  
228 A late sampling (T4) was performed after seven months of incubation, for microbial  
229 enumeration and pH measurement only (**Figure 1**). In addition, some intermediary sampling  
230 times were performed between T2 and T3 for volatile analysis and isolate identification (T2a,  
231 T2b, and T2c: 10, 15 and 21 days, for carrot samples and T2b and T2c: 14 and 22 days, for  
232 cabbage).

233 Sample names were coded as follows: 301 and 311 for shredded cabbage at 0.8% and 1.0%  
234 salt, respectively, 321 and 331 for cabbage leaf at 0.8% and 1.0% salt, respectively, 401 and 411  
235 for grated carrot at 0.8% and 1.0% salt, respectively, and 421 and 431 for sliced carrot at 0.8%  
236 and 1.0% salt, respectively (Figure 1). For example, the sample 331-a-T3 was the replicate 'a' of  
237 a cabbage leaf sample prepared at 1.0% salt, sampled after 4 weeks fermentation.

## 238 **Culturomics conditions**

239 Samples of 10 g of fermented vegetables (5 g of juice plus 5 g of drained vegetables) were  
240 suspended in 90 mL of a Tryptone Salt diluent (TS, sodium chloride 8.5 g/L, tryptone 1 g/L)  
241 heated at 48°C and homogenised in a filter bag (BagPage+, Interscience), in which vegetable  
242 debris was separated from the filtrate. Microbial analyses were performed on 14 different  
243 nutritive and selective media and incubated under aerobic (air atmosphere) or anaerobic  
244 conditions (Anaerocult® A, Merck, Darmstadt, Germany) at 37°C, 30°C, or 25°C depending of the  
245 medium, as previously detailed (Thierry, Madec, et al., 2023). In brief, seven media targeted the  
246 following microbial groups: LAB, total aerotolerant bacteria, halotolerant bacteria, aerotolerant  
247 Gram-negative bacteria, yeasts and filamentous fungi, bile-tolerant *Enterobacteriaceae*, and  
248 enterococci (**Supplementary Table S2**). In addition, three media targeted spore-forming  
249 bacteria (Supplementary Table S2). *Bacillus cereus*-typical colonies on BCA were further  
250 examined, by observing their aspect on the agar medium Compass Bacillus cereus (Biokar),  
251 incubated at 30°C for 24 h and 48 h, microscopical observation and *panC* gene sequencing.

252 In addition, four pathogens, namely, *Escherichia coli*, coagulase-positive staphylococci  
253 (*Staphylococcus aureus*), *Salmonella*, and *Listeria monocytogenes*, were searched by a  
254 subcontracted laboratory (LABOCEA, Fougères, France), following the ISO 16649-2, ISO 6888-2,  
255 BRD 07/11-12/05, and AES 10/03-09/00 standards, respectively.

## 256 **Microbial isolation and identification**

257 To collect LAB strains, 1 to 3 isolates were picked up on the several culture media used for  
258 agar plate containing 20 to 100 colonies, according to visual aspect of the colonies (size, colour,  
259 morphology), to favour the diversity of the isolates collected. Yeast isolates were collected  
260 following the same methodology. The isolates were collected from T0 to T4 with an  
261 intermediate collection stage at 15 days which corresponds to the stabilisation of the pH.  
262 Bacteria and yeast clones were identified by 16S rRNA gene and D1/D2 domain of 26S rRNA gene  
263 sequencing, respectively. Bacterial and yeast identification were performed following the  
264 procedures previously described (Thierry, Madec, et al., 2023).

## 265 **16S rRNA gene and gyrB metataxonomic analysis**

266 DNA was extracted from the samples, using the Nucleospin Tissue kit (Macherey-Nagel,  
267 Düren, Germany) as previously described (Thierry, 2024). DNA sequences were amplified in 16S  
268 rRNA gene V5-V7 region for bacteria using primers 799F/1193R (Forward-  
269 AACMGGATTAGATACCCCKG, Reverse-ACGTCATCCCCACCTTCC) and PCR conditions as previously  
270 described (Beckers et al., 2016). In parallel, the degenerate primers F64 (5'-  
271 MGNCCNGSNATGTAYATHGG-3') and R353 (5'-CNC CRTGNARDCCDCCNGA-3') were used to  
272 amplify a ~280-bp region of *gyrB* (Poirier et al., 2018). The 16S rRNA and *gyrB* amplicons were  
273 sequenced at Génome Quebec sequencing platform (Montreal, Quebec) using Illumina MiSeq  
274 PE250 technology, which generated 2 x 250 bp reads and a total of 2.45 Gb of data for amplicons.

## 275 **Bioinformatic analyses**

276 The raw sequences of 16S rRNA gene sequencing were processed as previously described  
277 (Thierry, Madec, et al., 2023). The raw sequences of *gyrB* gene sequencing were also processed  
278 using DADA2 package v 1.20.0 (Callahan et al., 2016), following the authors guidelines: we



279 successively applied the functions filterAndTrim, learnErrors, dada, mergePairs,  
280 makeSequenceTable, removeBimeraDenovo, and assignTaxonomy. The gyrB database from  
281 (Poirier et al., 2018) was used for the taxonomic affiliation. The amplicon sequence variants  
282 (ASV) count table, the ASV taxonomy table, and the sample metadata were combined into one  
283 phyloseq object (McMurdie & Holmes, 2013; Canon et al., 2020) for each target gene. The  
284 phyloseq (v1.44) R package was used to visualise barplots. Data were transformed into relative  
285 abundances before computing beta diversity (Bray-Curtis dissimilarity). The ComplexHeatmap  
286 (v2.16.0) R package was used to visualise the relative abundance of the different genera on a  
287 heatmap with a complete clustering based on Bray-Curtis dissimilarity computed after depth  
288 normalisation. Principal coordinate analyses (PCoAs) based on Bray-Curtis dissimilarity,  
289 calculated from the relative abundances of different genera, were conducted to evaluate the  
290 beta diversity of the samples using gyrB and 16S markers.

## 291 **Biochemical analyses**

292 The pH of juice samples was measured with a pH-metre (Hanna Instruments HI 2020-02).

293 Total titratable acidity (TTA), was determined on centrifuged (18000 g for 10 min at 20°C)  
294 juice samples, by titrating approximately 10 mL juice with 0.1 M NaOH to pH 8.3. It was estimated  
295 as follows: total acidity (%) =  $V_{\text{NaOH}} \times 0.1 \times m / S / 10$  and expressed as percentage (w/w) of lactic  
296 acid, with  $V_{\text{NaOH}}$ , volume of 0.1 M NaOH (mL); 0.1, factor corresponding to NaOH normality;  $m =$   
297 90, molar mass of lactic acid,  $S$ , mass of sample used (g), and was

### 298 *Sample preparation*

299 Before metabolite analysis except volatiles and vitamins, aliquots of juices were first  
300 centrifuged at 8000 g for 10 min at 4°C to eliminate vegetal debris, and the supernatant  
301 deproteinized by ultrafiltration on Vivaspin 2 centrifugal concentrator columns  
302 (polyethersulfone, 10 kDa cutoff, Sartorius) at 8000 g for 15 to 30 min at 4°C. Two  
303 chromatographic systems, High-Performance Liquid Chromatography (HPLC) coupled to UV  
304 and refractometry detection, and high-performance anion-exchange chromatography coupled  
305 to pulsed amperometric detection (HPAEC-PAD) were combined to analyse a range of organic  
306 acids, carbohydrates, and alcohols.

307 Before mineral analysis, juice samples were centrifuged at 18000 g for 10 min at 4°C, and the  
308 supernatant 40-fold (for Mg and P analysis) to 1000-fold diluted (for Na and K analysis) in a 2%  
309 v/v HNO<sub>3</sub> (Thermo fisher scientific, Waltham, MA, USA).

310 For volatile metabolites, triplicate samples, taken from three independent jars, were directly  
311 analysed from juice, using headspace (HS) gas chromatography- mass spectrometry (GC-MS).  
312 Juice aliquots (2.5 mL) were placed in Perkin-Elmer 22 mL vials (B0104236, 20 mm),  
313 hermetically sealed, and the vials stored at -80°C until analysis.

314 Vitamin analysis was performed in drained vegetable samples stored at -20°C before  
315 analysis.

### 316 *Acids and alcohol analysis by high-performance liquid chromatography*

317 Supernatants were 2- to 4-fold diluted in a 0.005 mol-L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> and stored at -20 °C until  
318 analysis. Lactic, acetic, citric, succinic, oxalic, and pyruvic acids, ethanol, and butanediol were  
319 quantified by High-Performance Liquid Chromatography (HPLC, Ultimate 3000, Thermo Fisher  
320 Scientific 91941 Courtaboeuf), using a Rezek ROA organic acid H + column (300\*7.8 mm,

321 Phenomenex, California), with H<sub>2</sub>SO<sub>4</sub> 0.005 M as the mobile phase at a flow rate of 0.4 mL/min  
322 at 60 °C. Two detectors were used: UV detector (DIONEX-UVD 1704) operated at 210 nm and  
323 refractometer (RI 2031 Plus Jasco).

324 Data was processed with Chromeleon™ software. Quantification was performed using multi-  
325 standard external calibration. Standards of ethanol, butanediol, oxalic, lactic, citric, propionic,  
326 butyric, succinic, and pyruvic acids were from Merck, St. Quentin Fallavier, France, and acetic  
327 acid from PanReac, Lyon, France. Mannitol, fructose and glucose can also be analysed by this  
328 method but in the present study were not quantified because the sucrose of vegetables  
329 hydrolyses in glucose and fructose during analysis, and fructose is co-eluted with mannitol.

#### 330 *Carbohydrate analysis by high-performance anion-exchange chromatography*

331 Supernatants were diluted by 400-fold in milli-Q® water (Merck, Darmstadt, Germany) and  
332 kept frozen at -20°C until analysis. Carbohydrates (sucrose, glucose, fructose, galactose,  
333 raffinose, xylose, arabinose, mannose, and mannitol) were quantified by high-performance  
334 anion-exchange chromatography (HPAEC) and pulsed amperometric detection (PAD) on an ICS-  
335 5000+ Dionex system (Thermo Electron SAS, Courtaboeuf France), as previously described  
336 (Canon et al., 2020). The system was equipped with a Dionex CarboPac PA210-Fast-4 µm column  
337 preceded by a CarboPac PA210-4 µm guard column (2\*30 mm). Metabolites were eluted with  
338 KOH as eluent, at a flow rate of 0.2 ml/min with the following gradient: 0 to 32 min 13 mM, 32  
339 min to 55 min 100 mM then return to 13 mM from 55 to 65 min. Data were acquired and  
340 processed by using the Chromeleon7™ software (Thermo Scientific). Metabolites were  
341 quantified using multi-standard external calibration (prepared at 0.1 mg/L to 40 mg/L (Merck,  
342 St. Quentin Fallavier, France).

#### 343 *Minerals analysis using inductively coupled plasma-optical emission spectrometer (ICP-OES)*

344 Minerals were quantified in the initial brine and in juices during incubation using an  
345 inductively coupled plasma-optical emission spectrometer (ICP-OES) (iCAP 7200, Thermo Fisher  
346 Scientific, Courtaboeuf, France), as previously described (Martin et al., 2022). Sodium,  
347 potassium, magnesium, phosphorus, and selenium were quantified using standard external  
348 calibration. Na, Mg, K, and P standards were prepared from 100 ppm standard solutions  
349 (Reagecon, Shannon, Ireland) at 0.5 to 10 ppm in a 2% v/v HNO<sub>3</sub> solution, and Se at 0.01 to 1  
350 ppm in a 2% v/v HNO<sub>3</sub> solution. NaCl concentrations were calculated from Na concentrations.

#### 351 *Volatiles analysis by headspace - gas chromatography - mass spectrometry*

352 Volatile compounds were extracted using a Turbomatrix HS-40 trap automatic headspace  
353 sampler and analysed using a Clarus 680 gas chromatograph coupled to a Clarus 600T  
354 quadrupole mass spectrometer, operated within a mass range of m/z 29 to m/z 206 and  
355 ionisation impact of 70 eV (Perkin Elmer, Courtaboeuf, France) as previously detailed (Pogačić,  
356 2015). Volatiles were eluted on an Elite WAX ETR column (30 m by 0.25 mm by 0.25 mm; Perkin  
357 Elmer, Waltham, MA), with helium as the mobile phase, under the following conditions: initial  
358 temperature 35°C maintained for 10 min, then increased t 5°C/min up to 230°C. Volatiles were  
359 identified by comparing their mass spectra and retention index with data from the NIST 2008  
360 Mass Spectral Library data (Scientific Instrument Services, Ringoes, NJ, USA), from literature  
361 and from standard injection, when available. Volatiles were semi-quantified from the  
362 abundance of one specific mass fragment (m/z), in arbitrary units. Mass spectrometry (MS) data

363 were processed using XCMS on R software (R Core Team. 2013. R: a language and environment  
364 for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). The full  
365 width at half maximum was set to 5, the maximum number of peaks per ion to 1000, the interval  
366 of m/z value for peak picking to 0.4, the signal-to-noise ratio threshold to 6, the group bandwidth  
367 to 3, and the minimum to 0.4. The other parameters were those by default.

### 368 *Vitamins*

369 Vitamins C, K1, B9 and for carrot, beta-carotene, were quantified in vegetable samples  
370 (without juice) sampled at T0 and T3, while vitamins K2 and B12, which are not present in raw  
371 vegetables but potentially produced by bacteria, were analysed only in T3 samples. Vitamin C  
372 was analysed at Vegenov laboratory (Saint-Pol-de-Léon, France), vitamins B9 et K1 by a  
373 subcontractor laboratory, Labexia (Quimper, France), and beta-carotene and vitamins B12 and  
374 K2 were determined by another subcontractor laboratory, Agrobio (Bruz, France), using HPLC  
375 and LC-fluo internal methods.

376 Vitamin C was determined following NF V03-135 standard. Briefly, vitamin C was extracted  
377 from samples using a 20 g/l metaphosphoric acid solution. L(+)-dehydroascorbic acid was  
378 reduced in L(+)-ascorbic acid by using a 40 g/l L-cystein solution. L(+)-ascorbic acid was  
379 quantified by HPLC (Agilent, Les Ulis, France) with photo diode array at 265 nm as detector.

380 Vitamin B9 was extracted from samples, and the diluted extracts and test broth medium  
381 were put in the wells of a Vitafast B9 microplate kit, in the presence of a *Lacticaseibacillus*  
382 *rhamnosus* strain, and incubated in the dark at 37°C for 44 to 48 h. The intensity of metabolism  
383 of *L. rhamnosus* due to the vitamin B9 brought by the extract was measured by turbidity using a  
384 microplate reader at 620 nm and compared to a standard curve.

385 Vitamin K1 was determined following the NF EN 14148 standard. Fat was first eliminated by  
386 an enzymatic treatment, and vitamin K1 was measured by HPLC with fluorescent detection,  
387 after post-column reduction.

### 388 **Statistical analyses**

389 In figures 2 and 4, means and 95% confidence intervals were calculated by using the excel  
390 functions AVERAGE and CONFIDENCE.NORM, respectively.

391 Four-way ANOVAs were performed to determine whether the microbial and biochemical  
392 variables differed according to the vegetable, the fermentation stage, the cutting type and the  
393 NaCl concentration and the 2-way interactions, by using the R function *aov*. Means were then  
394 compared using the sidak posthoc test from the R package *emmeans*. The factors and  
395 interactions that were not significant ( $p$ -value > 0.05) were further removed from the model.  
396 When the interactions were significant, the corresponding single effects were studied on a case-  
397 by-case basis, for example the effect of the fermentation stage and the cutting type were  
398 separately tested for each vegetable. The model was also adapted for the compounds detected  
399 in only one of the two vegetables studied.

400 A Principal component analysis (PCA) was performed by using the PCA function of the  
401 *FactoMineR* R package to illustrate the global biochemical and microbiological composition of  
402 the fermented vegetables and the relationships between the different variables. A PCA was also  
403 done on the volatile profile for each vegetable, using the *FactoMineR* R package. Hierarchical  
404 clustering was performed on PCA data. The association of qualitative variables with sample

405 clusters was investigated by using v-tests and was considered significant for v-test values of <-2  
406 or > 2.

407 A multi-block Partial Least Squares-Discriminant Analysis (PLS-DA) was performed to  
408 determine if samples exhibited different signatures at T3 regarding metataxonomics (16S and  
409 *gyrB*), metabolites (lactic acid, acetic acid, mannitol, ethanol, butanediol), volatiles and  
410 enumeration results, given their cutting type or salt concentration. The relative abundance  
411 values of a genus were retained only for the marker in which they were the highest. The function  
412 *block.plsda* of the R package *mixOmics* was used with two components ( $ncomp = 2$ ). Outputs  
413 were visualised with the *plotIndiv* and *plotVar* R functions, and the discriminating power of the  
414 model was interpreted thanks to the *auroc* R functions, giving the AUC and Wilcoxon test p-value  
415 for each class comparison performed.

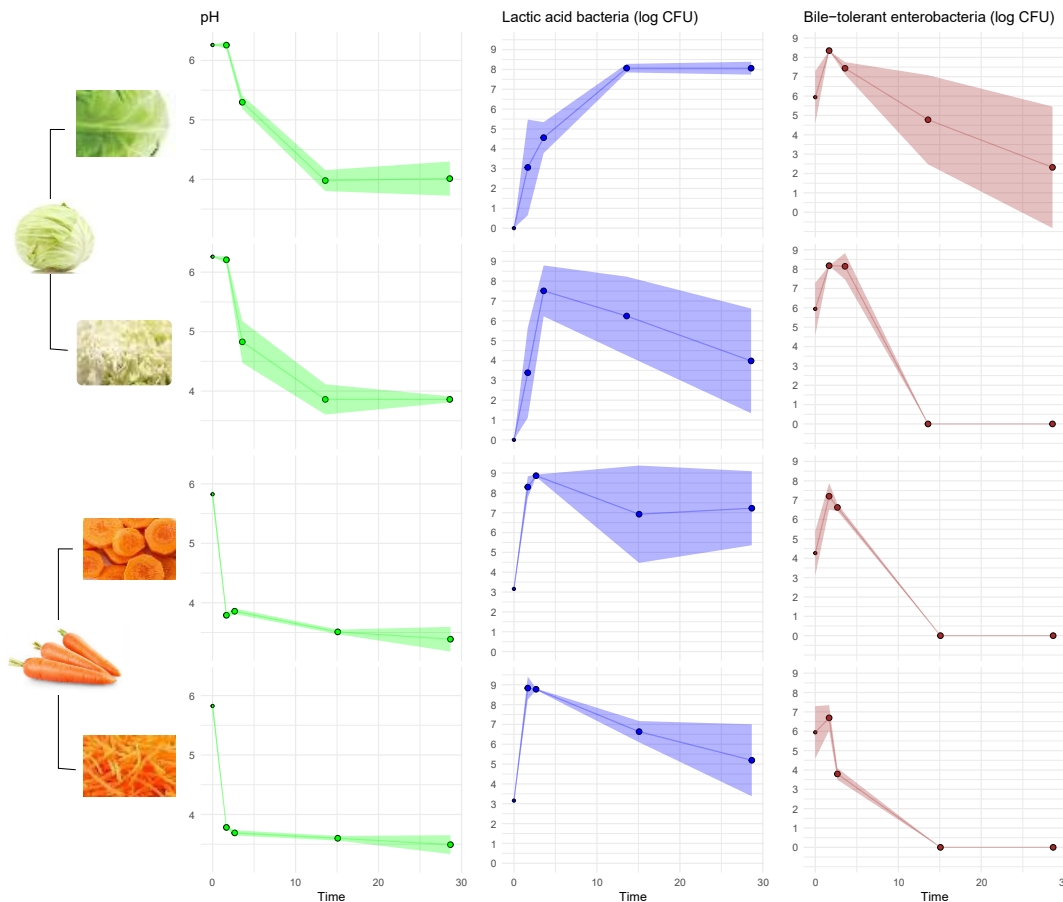
## 416 Results

### 417 Time course of microbial growth and acidification

418 The time-course of pH decrease and growth of the two main bacterial groups, i.e. bile-  
419 tolerant *Enterobacteriaceae* and LAB, is depicted on **Figure 2**. These three variables, as well as  
420 TTA, were significantly impacted by the vegetable studied, the fermentation stage, and, to a  
421 lesser extent, the cutting type. In contrast, no effect of the NaCl content was observed at the  
422 concentrations studied. Therefore, four replicates instead of two were available at each  
423 sampling point for statistical analyses. Marked differences of microbial counts and pH values  
424 were observed between the replicate jars sampled at each time point, but some significant  
425 trends were however highlighted concerning the time-course of fermentation.

426 The first bacterial group that grew was bile-tolerant *Enterobacteriaceae*, enumerated on  
427 VRBG, referred to as 'enterobacteria' below. Raw carrot and cabbage contained about 4.3 and  
428 6.0 log enterobacteria CFU/g, respectively. Their number increased rapidly during the first days  
429 of fermentation for both vegetables. They reached 6.9 +/- 0.7 log CFU/g within 40 h in carrot  
430 samples and 8.3 log CFU/g in 40 h in cabbage samples (**Figure 2**). Then they decreased, at a  
431 faster rate in carrot than in cabbage, and in thinly-cut vegetables than in roughly-cut ones. The  
432 enterobacteria counts were 6.6 vs 3.8 log CFU/g at 64h in sliced and grated carrot, respectively,  
433 and 8.1 vs 7.4 log CFU/g at 86h in leaf and shredded cabbage, respectively. For carrot and  
434 shredded cabbage, no enterobacteria were detected after 14 days fermentation, while for leaf  
435 cabbage, they were still detected in half samples after one-month fermentation, despite the pH  
436 decrease to 3.2 to 4.4 (**Figure 2**). At that sampling point, 3 out of the 4 isolates from VRBG  
437 medium were identified as *Hafnia alvei*. Enterobacteria were however no more enumerable in  
438 7-month old cabbage, in which the pH was 3.1 to 3.7 (data not shown).

439 LAB were enumerated on MRS, on which the 58 isolates collected were effectively LAB. LAB  
440 were present at low levels in raw carrot (3.16 +/- 0.06 log CFU/g), whereas they were below  
441 detection level in cabbage. LAB counts significantly increased over time ( $p\text{-value} < 0.005$ ). Like  
442 for enterobacteria the number of LAB rapidly increased during the three first days of  
443 fermentation and reached 8.8 log in 65 h-fermented carrot regardless of the cutting type. LAB  
444 grew more slowly in cabbage than in carrot, with marked differences according to the cutting  
445 type. LAB mean counts were 7.51 and 4.56 log CFU/g in 86 h in shredded and leaf cabbage,  
446 respectively, in which they reached 8 log CFU/g only after ~5 and 13.6 days, respectively. At one



448

449

450

451

452

453

454

455

**Figure 2** Time-course of fermentation in cabbage and carrot for each cutting type, illustrated by the change in pH, and the viable counts of lactic acid bacteria enumerated on MRS and bile-tolerant *Enterobacteriaceae*, enumerated on VRBG, expressed as logCFU/g. Values are means of the results observed on four independent jars, and the size of symbols proportional to the number of biological replicates (n=4, except at T0 and 15 days where n=2). The coloured areas show the 95% confidence intervals. Panels, from top to bottom: leaf cabbage, shredded cabbage, sliced carrot, grated carrot.

456

457

458

459

460

461

462

463

464

465

466

467

468

469

month (T3), LAB showed higher counts in roughly-cut vegetables than in thinly-cut ones (8.1 vs 4.0 log CFU/g in leaf and shredded cabbage, and 7.2 vs 5.2 log CFU/g in sliced and grated carrot). After 7 months LAB were still viable both in all samples with mean value of 4.3 +/- 1.1 log CFU/g. Among BL, enterococci, selectively enumerated on KF medium, were detected at low counts (< 4 log CFU/g, i.e. less than 0.04% of the total lactic acid bacteria) at the beginning of fermentation in both vegetables. They disappeared after 2 weeks fermentation, except in leaf cabbage samples, which all contained viable enterococci, ranging from 1.8 to 8.3 log CFU/g at one month. The 12 isolates collected from cabbage samples on KF were all identified as *Enterococcus faecalis*, while for carrot 7 of the 10 isolates collected were identified as *Enterococcus sp.* and 3 as other LAB genera (*Leuconostoc mesenteroides* and *Lactiplantibacillus sp.*).

The initial pH for raw material was 5.83 and 5.94 for carrot and cabbage, respectively. For carrot, the pH markedly decreased during the first two days of fermentation, with a mean value of 3.79 +/- 0.04 in 40 h regardless of the cutting type. TTA, in contrast, was highly significantly

470 ( $p < 0.0001$ ) influenced by the stage and by the interaction stage\*cutting ( $p < 0.01$ ). TTA increased  
471 faster in grated than in sliced carrot, with values of 0.30% vs 0.04%, respectively, at T1 (40h) and  
472 76% vs 0.32% at T2 (64h). TTA was 1.06 +/- 0.35 % in carrot at T3 (1 month). Afterwards, the pH  
473 only very slightly decreased, to reach 3.44 +/- 0.18 and 3.39 +/-0.30 after 1 and 7 months of  
474 fermentation, respectively, in all carrot samples. For cabbage, the pH decreased faster in  
475 shredded than in leaf cabbage during the first days of fermentation and reached 4.8 and 5.3 at  
476 T2 (86 h) in shredded and leaf cabbage, respectively. However, at this stage TTA was still very  
477 low ( $< 0.08\%$ ) regardless the cutting type. TTA was 0.63 +/- 0.11 % in cabbage at T3. The pH  
478 continued to decrease until about 15 days of fermentation, then stabilised to 3.94 +/- 0.21 and  
479 3.55 +/-0.13 after 3 and 7 months, respectively, in all cabbage samples.

480 Regarding the other media used to enumerate bacteria, the counts enumerated on BHI-  
481 YEnp, a medium used to enumerate Gram-negative aerotolerant bacteria, were very similar or a  
482 bit lower compared to the counts on VRBG, suggesting that the same bacteria grew on both  
483 these media. Accordingly, 28 out of the 30 collected isolates from BHI-YEnp medium at T1, T2 or  
484 T2b were members of the *Enterobacteriales* order (*Enterobacteriaceae*, *Hafniaceae*, *Erwiniaceae*,  
485 *Yersiniaceae* family) (**Supplementary Table S3**). Only a few carrot samples at the very beginning  
486 of fermentation showed higher counts on BHI-YEnp than on VRBG. They probably corresponded  
487 to the initial plant microbiota such as *Pseudomonadaceae* and *Erwiniaceae* members, the main  
488 taxa isolated from raw carrot on this medium. On the BHI-YEn medium chosen to enumerate  
489 total aerobic bacteria, *Pseudomonas sp.* and *Janthinobacterium sp.* isolates were identified at  
490 T0, while only LAB were isolated from T2 (Supplementary Table S3). Likewise, on the TSA-NaCl  
491 medium that targeted halophilic bacteria, 11 out of the 14 isolates collected at T3 were  
492 identified as LAB, the major part belonging to the species *Leuconostoc mesenteroides*. On the  
493 basis of the isolated and identified clones, the selectivity of KF, and VRBG media was confirmed  
494 with, respectively, 19 enterococci out of the 22 collected isolates, and 4 bile-tolerant  
495 *Enterobacteriaceae* out of the 4 collected isolates (Supplementary Table S3).

496 Yeasts were present on the raw vegetables, at counts ranging from 1.7 to 2.7 log CFU/g,  
497 depending on the samples. Yeasts did not develop in cabbage and disappeared fairly quickly  
498 after 4 days of fermentation. In contrast, for carrot, alive yeasts were enumerated after 7 months  
499 regardless of the cutting type. Strong variations between replicate jars were observed, with, for  
500 example at time T3 for sliced carrot, values ranging from 0 to 7.9 log CFU/g.

501 Regarding pathogenic and undesirable bacteria, none of the four pathogenic bacteria were  
502 detected. Some spore-forming bacteria were present at low counts in carrot samples (median  
503 value of 75 CFU/g enumerated on a rich medium, BHI-YE) and absent from cabbage except for  
504 one sample, a one-month-old cabbage sample (331-a-T3). This specific sample was also the sole  
505 that contained clostridia - 4 log CFU/g, enumerated on the selective TSN medium, and 2.44 log  
506 CFU/g on BCA medium.

507 A total of 191 bacterial strains (126 from carrot and 65 from cabbage) were isolated and  
508 identified to the species level by 16S rRNA gene sequencing. 97 strains were isolated during the  
509 first stages of fermentation (from T0 to T2) and 94 strains were isolated after 2 to 4 weeks of  
510 fermentation. Concerning LAB, the *Leuconostoc* genus was dominant and represented 52% of  
511 the isolates collected during the first 15 days of fermentation, followed by the *Enterococcus*  
512 (22%) and *Lactiplantibacillus* (10%) genera (Supplementary Table S3). Concerning non-lactic  
513 acid bacteria, the *Bacillus* genus dominated, followed by *Hafnia*, *Pantoea*, *Rhanella*, and

514 *Pseudomonas* genera. Concerning yeasts, 12 strains (10 from carrot and 2 from cabbage) were  
515 isolated and identified as members of the genera *Kazachstania*, *Rhodotorula*, *Saccharomyces*,  
516 *Candida*, *Pichia*, and *Debaryomyces*(Supplementary Table S3).

### 517 **Changes in metabolite concentration**

518 The targeted compounds, which included carbohydrates, organic acids, and alcohols, were  
519 analysed in sample juices. Concerning carbohydrates, saccharose, glucose, and fructose were  
520 the main compounds detected at the beginning of fermentation (T0). After one-month  
521 fermentation (at stage T3), the main vegetable carbohydrate detected in both vegetables was  
522 glucose, with large variation in concentrations (0 to 4.8 g/kg juice), followed by saccharose in  
523 carrot (0.5-1 g/kg juice) and fructose (0 to 0.4 and 0.1 to 1.6 g/kg juice in carrot and cabbage,  
524 respectively). Low amounts of galactose (~250 mg/L) were also detected at T3 in both  
525 vegetables and traces (< 30 mg/L) of xylose, mannose, and arabinose, and, in cabbage only, of  
526 raffinose. Concerning bacterial metabolites, five organic acids (lactic, acetic, succinic, citric and  
527 pyruvic acids) and mannitol, ethanol, and 2,3-butanediol were identified in vegetable juices, the  
528 main ones being mannitol and lactic acid, which accounted for 67 to 80% of total metabolites  
529 at T3, except in roughly-cut cabbage, which also contained high amounts of ethanol (**Figure 3**).  
530 Two other, unidentified, compounds were detected by using HPLC-UV, at retention times 26 min  
531 and 28 min, named RT26 and RT28. In addition, oxalic acid was also detected in carrot samples.  
532 Its concentration increased from 30 mg/kg at T0 to 70 mg/kg juice at 3-month fermentation.

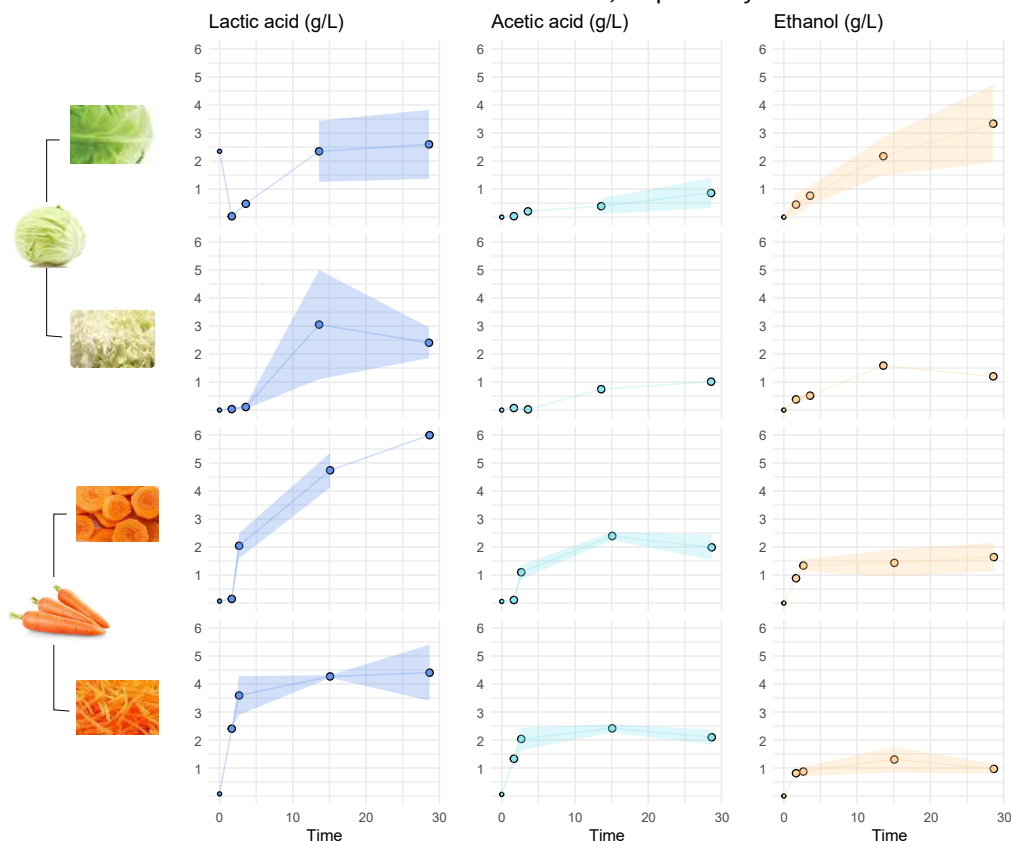
533 The concentrations of all metabolites except butanediol significantly ( $p$ -value < 0.001)  
534 increased over time, with different rates depending on the vegetable and, for some metabolites,  
535 on the cutting type. Their concentrations stabilised in juice after ~3 days in grated carrot, ~2  
536 weeks in sliced carrot and shredded cabbage, and ~30 days in cabbage leaf, as illustrated in  
537 **Figure 4** for lactic acid, acetic acid and ethanol. Lactic acid concentrations were globally 2.5-  
538 fold higher in carrot compared to cabbage, and similar values about 2.8 g/kg were observed in  
539 15 days-cabbage and 3-days-carrot juice samples (Figure 4). At T3 lactic acid concentrations  
540 were 2.5-fold higher in carrot compared to cabbage (5.2 versus 2.5 g/kg, respectively). Similarly,  
541 acetic acid concentration also increased over time and was higher in carrot than in cabbage  
542 (Figure 4), but it showed complex variations with significant effects of the interactions between  
543 the factors stage, vegetable (globally four-fold higher in carrot than in cabbage) and cutting type  
544 (1.4-fold higher in thinly-cut vegetables). In contrast, ethanol concentration increased with  
545 fermentation time but was mainly affected by the cutting type, with, globally 1.6-fold higher  
546 values in roughly-cut than in thinly-cut vegetables (Figure 4). For example, at T3, ethanol was  
547 two-fold more concentrated in leaf-cabbage than in shredded cabbage (3.0 vs 1.6 g/kg,  
548 respectively), and threefold more concentrated in sliced carrot than in grated carrot (2.0 vs 0.6  
549 g/kg, respectively) (Figure 3). Mannitol was quantified only after one month of fermentation (see  
550 material and methods). At this stage, mannitol concentration was about two-fold higher in  
551 carrot compared to cabbage (9.1 versus 3.9 g/kg, respectively) (Figure 3).



552

553  
554  
555  
556  
557  
558

**Figure 3** Concentration of the metabolites in fermented cabbage and carrot juice, expressed in g/kg, after one month of fermentation (stage T3), for each cutting type, and two salt concentrations, for two replicate jars. Sample codes: 301 and 311 for shredded cabbage at 0.8% and 1.0% salt, respectively, 321 and 331 for cabbage leaf at 0.8% and 1.0% salt, respectively, 401 and 411 for grated carrot at 0.8% and 1.0% salt, respectively, and 421 and 431 for sliced carrot at 0.8% and 1.0% salt, respectively.



559  
560  
561  
562  
563  
564  
565

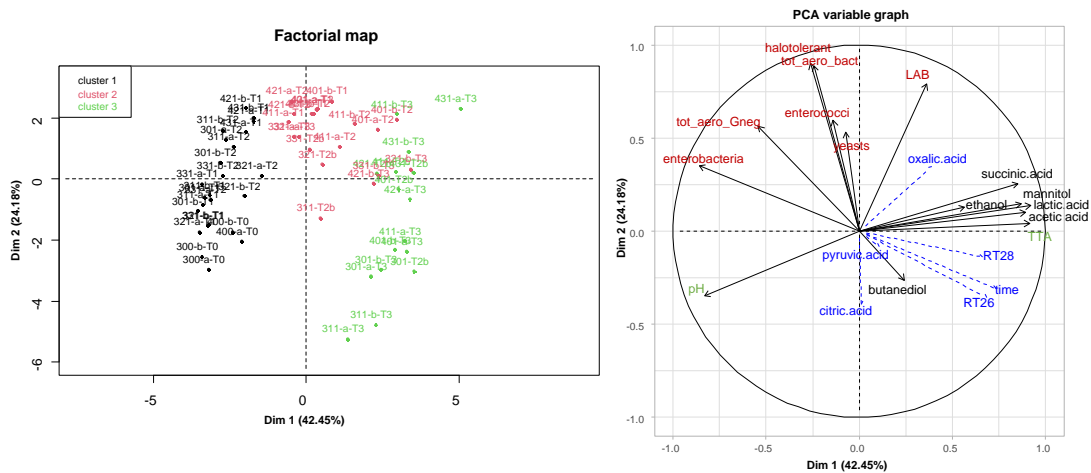
**Figure 4** Time-course (day) of fermentation in cabbage and carrot for each cutting type, illustrated by the change in concentrations in lactic acid, acetic acid and ethanol, in g/kg juice. Values are means of the results observed on four independent jars, and the size of symbols proportional to the number of biological replicates. The coloured areas show the 95% confidence intervals. Panels, from top to bottom: leaf cabbage, shredded cabbage, sliced carrot, grated carrot.



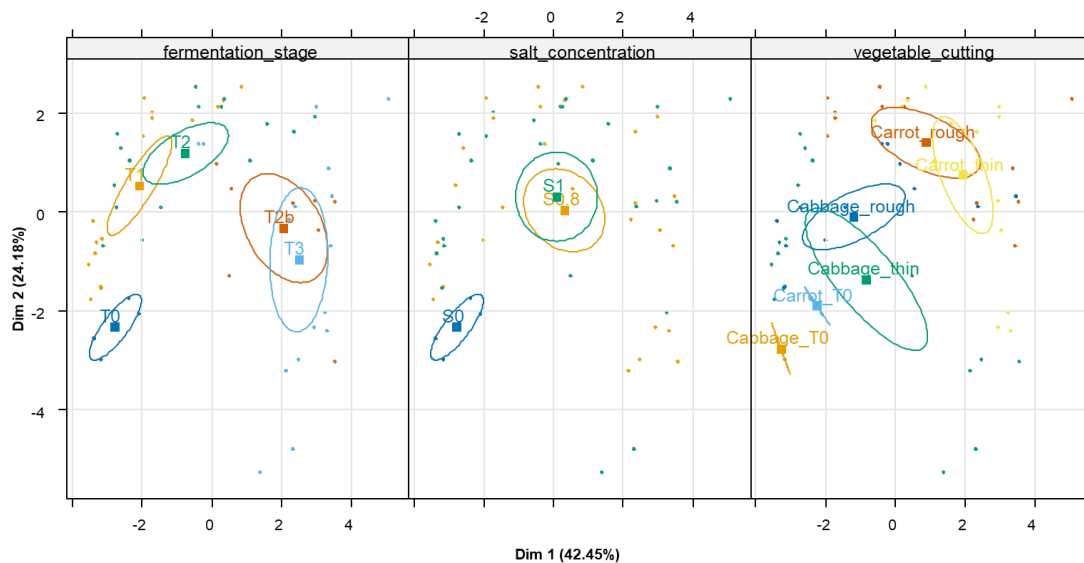
## 566 Global representation of microbial and biochemical changes using PCA

567 A PCA was done to draw a global picture of the changes during fermentation, using 14  
568 variables, namely the viable counts of microorganisms (n=7), the amounts of the main  
569 metabolites (n=6) and the pH values, for all cabbage and carrot samples analysed over time  
570 **(Figure 5)**. The first axis, which explains 42.4% of total variability, separates samples on the  
571 basis of pH value, negatively associated with PC1 and the concentrations in the three main  
572 metabolites, mannitol, lactic acid and acetic acid, and of succinic acid, all positively associated  
573 with PC1. PC1 was also negatively associated with enterobacteria counts. The second axis,  
574 which explains 24.2% of total variability, separates samples on the basis of high viable counts in  
575 Gram-positive bacteria, including LAB (Figure 5). Samples were clearly differentiated according  
576 to the stage of fermentation (bottom 1<sup>st</sup> panel). They were also clearly differentiated according  
577 to the vegetable and cutting type (bottom 3<sup>rd</sup> panel) but not to the salt concentration (bottom  
578 middle panel). These results show that carrot fermentation was quicker than cabbage  
579 fermentation.

580 Three clusters of samples were distinguished based on the hierarchical clustering performed  
581 on PCA data (Figure 5). Cluster 1, shown in black, consisted of samples at the beginning of  
582 fermentation. It was significantly ( $v\text{-test} > 2$ ) associated with stages T0 and T1, a high pH, a low  
583 titratable acidity, and high enterobacteria and Gram-negative bacteria counts. Cluster 2, shown  
584 in red, characterised intermediary stage. It was significantly ( $v\text{-test} > 2$ ) associated with high  
585 microbial counts on varied culture media that targeted total anaerobic bacteria, halotolerant  
586 bacteria, LAB, enterococci, and yeasts. It contained many thinly-cut carrot samples. Finally,  
587 cluster 3, shown in green, consisted of samples at the end of fermentation. It was significantly  
588 ( $v\text{-test} > 2$ ) associated with stages T2b and T3, a low pH, a high titratable acidity and high  
589 concentrations in metabolites, e.g. lactic and acetic acids and mannitol.



590



591

592 **Figure 5** Principal component analysis made from the data of enumeration of viable  
 593 microorganisms (n=7, shown in brown), the amounts of main metabolites (n=6, shown in  
 594 black) and pH and total titratable acidity (TTA) (shown in green) for all samples (cabbage  
 595 and carrot) analysed at five fermentation stages: T0, initial time, T1, 1.7 day, T2, 2.7  
 596 (carrot) or 3.6 day (cabbage); T2b (2 weeks) and T3 (4 weeks). Some minor metabolites  
 597 and unknown metabolites are projected as supplementary variables (blue, dashed lines).  
 598 For culture media, see Table S1; LAB, lactic acid bacteria, enumerated on MRS,  
 599 enterobacteria refers to the bile-tolerant *Enterobacteriaceae* enumerated on VRBG. The  
 600 individual maps (bottom panels) show the 95% confidence ellipses according to the stage  
 601 of fermentation (1<sup>st</sup> panel) the salt concentration (S0.8: 0.8% or S1:1%, 2<sup>nd</sup> panel), and the  
 602 type of vegetable (carrot or cabbage) and its cutting (thin or rough, 3<sup>rd</sup> panel).

### 603 Volatile compounds

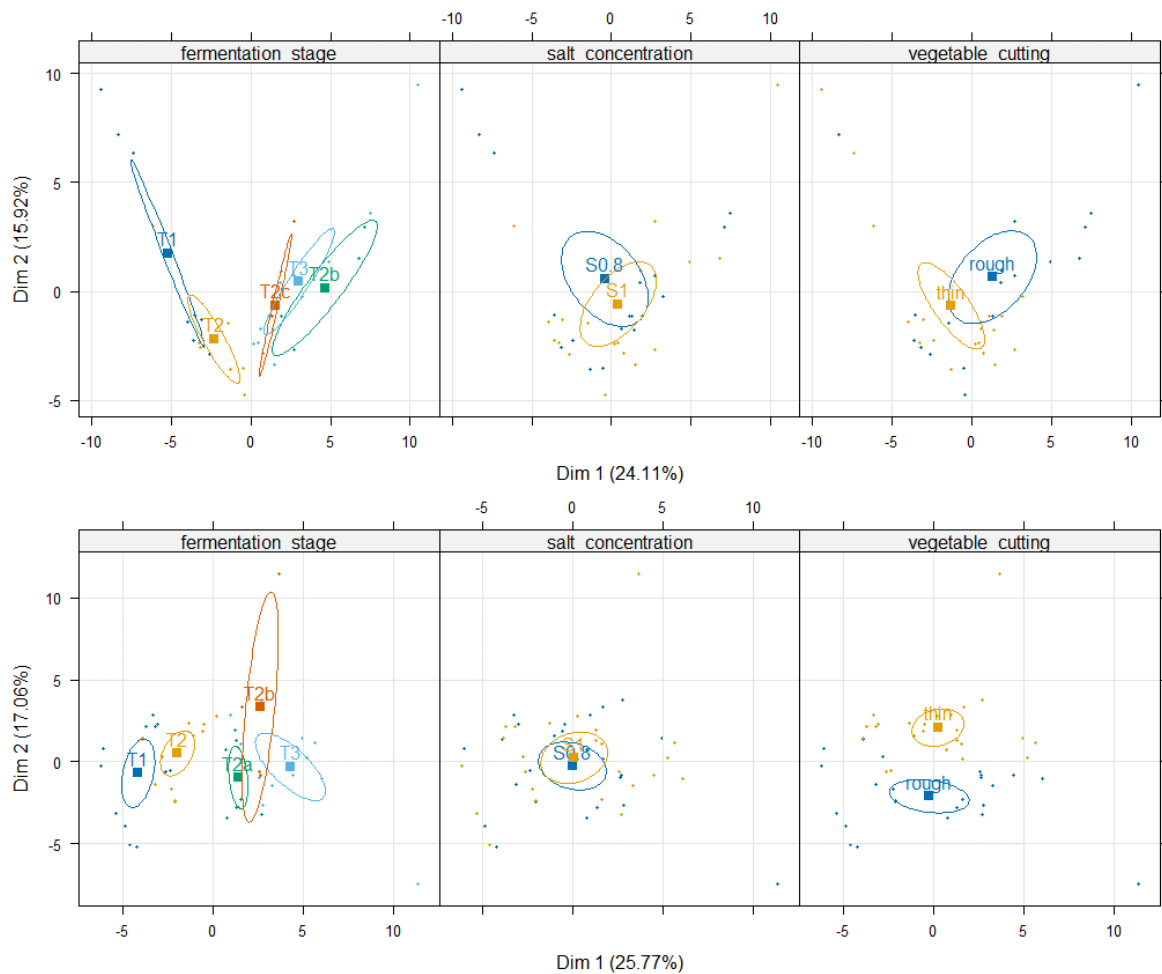
604 In fermented cabbage, 78 volatile compounds were identified. They contained 23 sulphur-  
 605 containing compounds that included 9 (iso)thiocyanates and 4 nitriles, 23 esters, 8 acids, 7  
 606 alcohols, 7 aldehydes, 6 ketones, and 4 other compounds (**Supplementary Table S4**). The  
 607 abundance of 27, 15, and 10 volatiles were impacted by the stage of fermentation, the type of  
 608 cutting, and the salt concentration, respectively, but in they were also interactions between  
 609 these factors. The effect of the salt treatment, in particular, was always associated with  
 610 interactions with the effect of stage and/or of cutting. The abundance of one third of cabbage  
 611 volatiles significantly (p-value<0.01) varied during fermentation, with 27 that increased (fold  
 612 change stage T3/stage T1 >2), and 9 that decreased (fold change stage T1/stage T3 >2). A PCA  
 613 made to illustrate the global results on cabbage volatile profiles, showed that cabbage samples  
 614 were separated on the first axis (24.1% of the total variability) according to the stage of  
 615 fermentation, and on the 2<sup>nd</sup> axis (15.9% of the total variability) according to their cutting type  
 616 and salt concentration (**Figure 6**). Four clusters of samples were distinguished based on the  
 617 hierarchical clustering performed on PCA data (**Supplementary Figure S1**). Clusters 1, 2 and 3  
 618 were significantly associated with stages T1, T1/T2, and T3, respectively, while cluster 4  
 619 contained only one sample of roughly-cut cabbage (Supplementary Figure S1). Samples at the  
 620 beginning of fermentation (cluster 1) showed high abundances of many esters, while one-month  
 621 aged samples (cluster 3) contained more ethanol, butan-1-ol, acetic and butanoic acids, and

622 sulphur-containing compounds that originate from cabbage, such as isothiocyanates and  
623 nitriles (Supplementary Figure S1).

624 In fermented carrot, 52 volatiles were identified (**Supplementary Table S5**). They included  
625 9 ketones, 9 aldehydes, 8 alcohols, 7 esters, 5 acids, 8 terpenes, 2 sulphur-containing  
626 compounds and 4 other compounds. In addition, many other terpenes and terpenoids were  
627 tentatively identified, e.g.  $\gamma$ -terpinene, terpinolene, 1,3,8-p-menthatriene,  $\alpha$ -bergamotene,  
628 caryophyllene, terpinen-4-ol,  $\beta$ -cyclocitral, (E)- $\gamma$ -bisabolene, zingiberene, cis- $\beta$ -farnesene,  $\beta$ -  
629 curcumene, and  $\beta$ -sesquiphellandrene. These compounds come from carrot and were not  
630 further considered in this stud. The abundance of 32, 22, and 3 volatiles were significantly (p-  
631 value<0.01) impacted by the stage of fermentation, the type of cutting, and the salt  
632 concentration, respectively, but there were some interactions between these factors. One third  
633 of the volatiles (18 of 52) increased in concentration (p-value< 0.01; fold change T3/T0 >2). The  
634 compound with the highest fold-change was ethyl lactate (> 6000). Nine volatiles, decreased in  
635 concentration over time (p-value< 0.01; fold change T0/T3 >2), of which four aldehydes. The type  
636 of cutting also significantly (p-value< 0.01, fold-change >2) impacted the amount of 6 volatile  
637 compounds, which were all more abundant in grated carrot compared to sliced carrot. The  
638 greatest fold-changes were for two terpenes, beta-myrcene and D-limonene, suggesting that  
639 grating facilitated their release in brine compared to slicing. A PCA made to illustrate the global  
640 results on volatile profiles showed that, as observed in fermented cabbage, the samples were  
641 first separated according to their stage of fermentation on the first axis (25.8% of the total  
642 variability), and on the 2<sup>nd</sup> axis (17.1% of the total variability) according to the cutting type, and  
643 not differentiated depending on their salt concentration (Figure 6). Five clusters of samples were  
644 distinguished based on the hierarchical clustering performed on PCA data (**Supplementary**  
645 **Figure S2**). Cluster 1 was significantly (v-test > 2) associated with stage T1, rough cutting, and  
646 high abundance of diacetyl, cluster 2 with thin cutting and high abundance of several terpenes,  
647 such as beta-myrcene and D-limonene, cluster 3 with last stages of fermentation (T2a and T3),  
648 and many volatiles (e.g. ethyl lactate, benzaldehyde, hydroxypropanone, and dimethyl  
649 trisulfide). Clusters 4 and 5 each contained only one sample (a grated carrot at stage T2b and a  
650 sliced carrot at stage T3), characterized by peculiar volatile profiles (Supplementary Figure S2).

651 Only 21 volatiles were shared by the two vegetables, including 7 aldehydes (pentanal,  
652 hexanal, heptanal, octanal, hepten-2-al(Z), nonanal, and benzaldehyde), 4 esters (methyl  
653 acetate, ethyl acetate, ethyl butanoate, and ethyl hexanoate), 3 alcohols (ethanol,  
654 methylbutanol, and hexan-1-ol), 3 ketones (pentan-2-one, diacetyl, and acetoin), 3 acids (acetic,  
655 octanoic, and nonanoic acids) and one sulphur-containing compound (dimethyl trisulfide).

656



657

658

659 **Figure 6** PCA of volatiles identified in fermented cabbage (upper row) and fermented  
 660 carrot (bottom row). The three maps of individuals are coloured and show the 95%  
 661 confidence ellipses according to, first panel, the fermentation stage: T1 (40 h), T2 (2.7 day  
 662 for carrot or 3.6 day for cabbage), T2a (9.7 days), T2b (15 days), T2c (22 days), T3 (one  
 663 month), 2nd panel, the salt concentration : S0.8: 0.8% or S1: 1%, and 3rd panel, the type  
 664 of cutting: thin or rough

665

## 666 Mineral and vitamin concentrations

667 NaCl concentrations in juices, calculated from Na concentrations, significantly varied  
 668 according to the amount of added salt with, on average, 9.06 g/l and 7.08 g/l in 1%-salted and  
 669 0.8%-salted samples, respectively. The differences in salt concentrations between the salt  
 670 concentrations observed in juices and the targeted concentrations (0.8 and 1%) results from the  
 671 composition of the coarse grey salt used to prepare the brines. Our results are consistent with  
 672 the composition of coarse grey salt (e.g. 34 +/- 3 g Na per 100 g of product, i.e. 86.4 g NaCl per  
 673 100 g of product). The differences between targeted and observed salt concentrations could  
 674 also result from Na migration from the brine to the vegetables during incubation, thus  
 675 decreasing its concentration in the juice. Significant concentrations of Mg were also brought in  
 676 the initial brine by the coarse salt: 1 g of Mg for 78 g of Na. By comparing the total concentrations  
 677 in juice and the concentration brought by the salt added, we calculated that the proportion of  
 678 Mg coming from salt accounted for more than half of the total Mg content in juices (59%, and

679 64% in thinly- and roughly-cut carrot, respectively, and 51% and 59% in thinly- and roughly-cut  
680 cabbages, respectively). We thus calculated the Mg concentration that originated from  
681 vegetables only, called 'Mg\_veg'. In contrast, the coarse salt used did not bring P and K, and  
682 their proportions coming from the salt in juice were negligible (< 0.01% and < 0.09%,  
683 respectively).

684 The mineral content in P, K, and Mg\_veg (Mg coming from the vegetable, see above)  
685 significantly (p-value < 0.001) differ in the two vegetables. Carrot juice contained 1.9 times more  
686 P, 1.7 times more K, and 20% less Mg\_veg compared to cabbage juice, with average  
687 concentrations of P, K, and Mg\_veg of 1793, 146, and 27 mg/L, respectively, in carrot, and 1068,  
688 78 and 34 mg/L, respectively, in cabbage (**Supplementary Table S6**). During fermentation, the  
689 concentrations of P and Mg\_veg significantly (p-value < 0.01) increased in cabbage and carrot  
690 juice, with 32% more P and 52% more Mg\_veg after a 1-month incubation (T3) than at the  
691 beginning of fermentation (stages T1-T2), showing a gradual migration of minerals from the  
692 vegetable tissue to the juice. The contents in P, K, and Mg\_veg were significantly (p-value <  
693 0.001) higher in the juice of thinly-cut vegetables, with a greater effect in cabbage than in carrot.  
694 Juices from shredded cabbage contained, on average, 32%, 18% and 20% more P, K, and  
695 Mg\_veg, respectively, compared to juices from leaf cabbage, while grated carrot contained 16%,  
696 10% and 13% more P, K, and Mg\_veg, respectively, than sliced carrot. The contents in P and K  
697 were also globally slightly but borderline significantly (p-value < 0.06) higher in the more salted  
698 samples, with on average, 8% and 16% more P in 1% salted carrot and cabbage juice samples,  
699 respectively, and 5% and 9% more K in 1% salted carrot and cabbage juice samples,  
700 respectively. Trace amounts of Se were also detected in carrot only, at concentrations < 0.040  
701 mg/L.

702 The concentrations of the six vitamins analysed, expressed in mg or µg per 100 g of fresh  
703 drained vegetable, and the content in dry matter are given in **Table 1**.

704 Cabbage globally contained higher amounts of vitamins. Concentrations in vitamins C and  
705 B9 were 12-fold and 2.8-fold higher in cabbage than in carrot, on average, while vitamin K1 was  
706 detected at ~4 µg/100g in cabbage and was under the detection level (< 3 µg/100g) in carrot.  
707 Compared to raw cabbage, fermented cabbage contained about twice more vitamin C after 1  
708 month, while in carrot, a slight but not statistically significant increase of vitamin C was  
709 observed. In contrast, vitamin B9 and beta-carotene tended (p-value = 0.010) to decrease over  
710 time. Regarding vitamins B12 and K2, which are not present in raw vegetables, they were not  
711 produced or at too low amounts to be detected in fermented vegetables. Concerning the impact  
712 of cutting size, roughly-cut vegetables were slightly, but significantly (p-value<0.03) more  
713 enriched in vitamin C than thinly-cut ones (+19% and +43% in cabbage and carrot, respectively).  
714 As for the effect of salt concentration, the content in vitamin C was slightly higher (+20%) in the  
715 less salty carrot samples, while it was not significantly impacted by salt in cabbage. The vitamin  
716 B9 decreased more in the 0.8%-salted than in the 1%-salted samples.

717  
718  
719  
720  
721

722  
723  
724  
725

**Table 1:** concentrations of vitamins C, B9, B12, K1, K2, of beta-carotene and content in dry matter in vegetables before (T0) and after one-month fermentation (T3). Values are mean and standard deviation of duplicate analyses (except for vitamins K1, K2 and B12 for which one analysis was done)

Sample	Code	Vitamin C mg/100g		Vitamin B9 µg/100g		β-caroten mg/100g		Vit K1	Vit K2	Vit B12	Dry matter, %	
		mean	sd	mean	sd	mean	sd	µg /100g	mg /100g	µg /100g	mean	sd
cabbage T0	300-T0	10.17	0.39	16.5	2.12	nd		3.9			10.3	0.2
cabbage T3 thin salt 0.8%	301-T3	19.13	1.01	14.0	2.83	nd		<3.0	<5	<0.1	18.3	1.2
cabbage T3 thin salt 1%	311-T3	20.44	0.67	17.0	1.41	nd		3.4	<5	<0.1	17.0	1.6
cabbage T3 rough salt 0.8%	321-T3	22.25	0.53	11.5	0.71	nd		4.3	<5	<0.1	17.6	0.8
cabbage T3 rough salt 1%	331-T3	24.79	2.63	17.5	0.71	nd		6.7	<5	<0.1	17.7	1.9
carrot T0	400-T0	1.05	0.11	7.65	0.07	11.3	0.3	<3.0			9.2	0.1
carrot T3 thin salt 0.8%	401-T3	1.31	0.22	2.35	0.07	8.0	2.4	<3.0	<5	<0.1	12.6	0.5
carrot T3 thin salt 1%	411-T3	1.20	0.16	8.65	1.77	9.8	1.5	<3.0	<5	<0.1	12.9	0.1
carrot T3 rough salt 0.8%	421-T3	2.04	0.02	1.35	0.21	9.3	1.5	<3.0	<5	<0.1	13.8	0.1
carrot T3 rough salt 1%	431-T3	1.54	0.02	1.65	0.07	0.4	1.3	<3.0	<5	<0.1	12.6	0.3

726  
727

vitamin concentrations are expressed in mg or µg per 100 g of fresh drained vegetable; sd: standard deviation; nd: not determined;

## 728 Metataxonomics results

729 The read numbers of sequenced samples ranged from 2,617 to 186,755, with a median value  
730 of 29,203 and a mean of 43,484. All samples sequenced after T0 had more than 10,000 reads. A  
731 total of 314 ASVs were obtained after 16S rRNA gene sequencing, and 2,640 ASVs after gyrB  
732 sequencing, each with an abundance exceeding 0.005% of the total. In total, ASVs belonged to  
733 98 different genera, of which 62 detected using gyrB only, 12 using 16S only, and 24 common to  
734 both markers. The ASVs derived from gyrB sequencing enabled a higher taxonomic resolution,  
735 with 132 species identified.

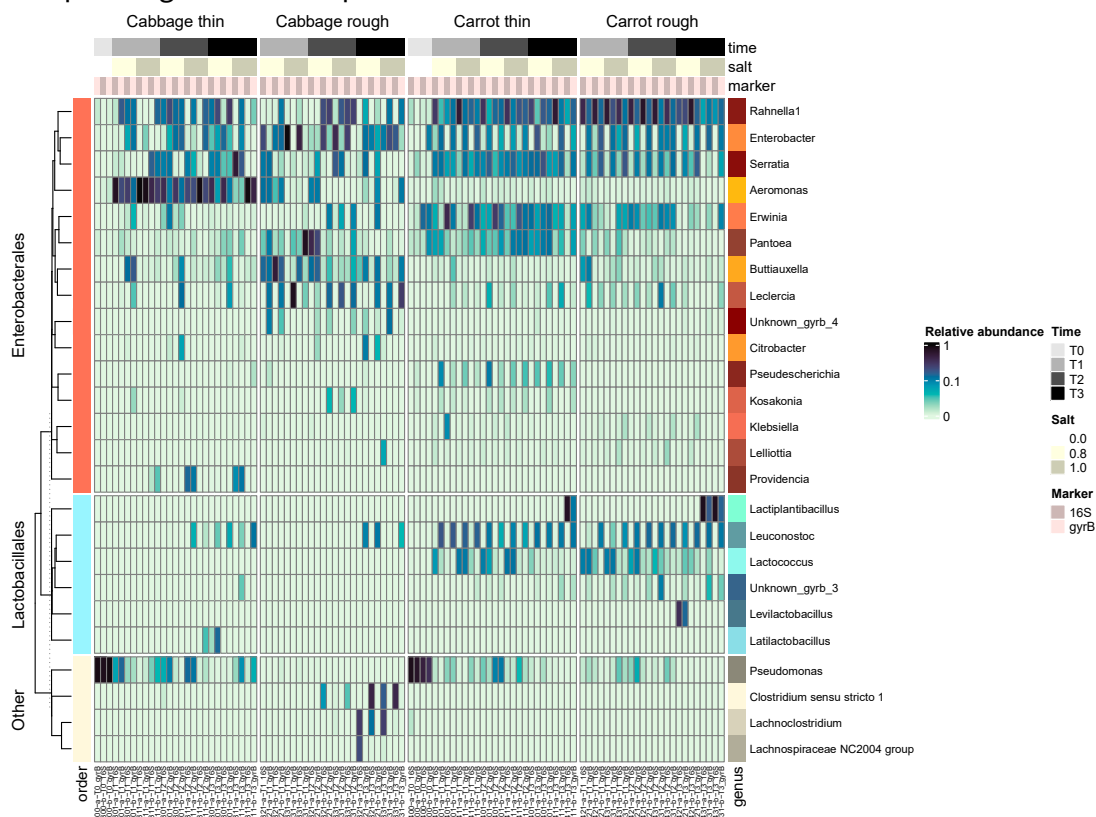
736 The two markers were used in parallel to define the taxonomic profile of the samples at the  
737 genus level (**Figure 7**).

738 At T0, *Pseudomonas* largely dominated the bacterial community of both carrot and cabbage  
739 samples. Then, taxonomic profiles shifted, and *Enterobacteriaceae* became predominant  
740 (**Supplementary Figure S3**). Some genera were preferentially observed in shredded cabbage  
741 (*Aeromonas*), leaf cabbage (*Buttiauxella*), or carrot (*Erwinia*) samples, while others were present  
742 regardless of the vegetable (*Rahnella*, *Enterobacter*, *Serratia*). LAB appeared from T1  
743 (*Leuconostoc*, *Lactococcus*) with *Lactiplantibacillus* or *Levilactobacillus* dominant at T3 in grated  
744 and sliced carrot (except for sample 411-a-T3). In cabbage samples, the profile greatly differed,  
745 depending on the type of cutting. *Leuconostoc* and *Latilactobacillus* were detected at T2 in  
746 shredded cabbage, while *Clostridium* developed and became the main genus in leaf cabbage  
747 (even if *Leuconostoc* was detected at T2). Therefore, the taxonomic profiles differed according  
748 to the vegetable and the cutting type, especially for cabbage, but they did not appear as  
749 impacted by the salt concentration.

750 The taxonomic profiles also varied according to the marker used (Figure 7, **Supplementary**  
751 **Figure S3** and **Figure S4**). Some genera were similarly detected by both 16S and gyrB markers  
752 (e.g., *Lactococcus*, *Aeromonas*, *Erwinia*, *Pseudomonas*), while others exhibited differential

753 detection patterns according to the marker. For example, the genus *Leuconostoc* was almost  
 754 undetectable with the 16S marker but which was detected at a high abundance with the gyrB  
 755 marker. Conversely, the genus *Clostridium* was scarcely detectable with the gyrB marker but  
 756 detected with the 16S marker.

757 The gyrB marker higher resolution compared to the 16S marker made it possible to refine  
 758 taxonomic profiles by identifying species (Figure 7), notably those relating to *Lactobacillales* and  
 759 *Enterobacteriales*. Regarding *Enterobacteriales*, the species *Rahnella aquatilis*, *Enterobacter sp.*  
 760 638, *Serratia sp. Leaf51* and *Pantoea agglomerans* were found in both cabbage and carrot  
 761 samples. For LAB, *Leuconostoc mesenteroides*, *Lactococcus piscium*, and *Leuconostoc gelidum*  
 762 were present in carrot samples from T1 onwards. At T3, *Lactiplantibacillus plantarum* and  
 763 *Levilactobacillus brevis* were mainly observed. For shredded cabbage samples, *L. mesenteroides*  
 764 and *Leuconostoc carnosum* were present from T2 onwards. *L. carnosum* and *Enterococcus*  
 765 *faecalis* were observed for leaf cabbage samples at T3. Therefore, the taxonomic profiles at the  
 766 species level also differed according to vegetable and cutting type, but they did not seem to be  
 767 impacted by the salt concentration. Furthermore, it is worth noting that there were pronounced  
 768 differences in taxonomic profiles between the duplicate jars of the carrot and cabbage samples  
 769 at T0 (coded a and b). Among the 17 *Lactobacillales* species identified by gyrB sequencing, 10  
 770 were common to the isolated bacterial strains: *L. mesenteroides*, *Lactiplantibacillus pentosus*,  
 771 *Enterococcus faecium*, *L. brevis*, *L. carnosum*, *Latilactobacillus curvatus*, *E. faecalis*, *L. plantarum*,  
 772 *Lactococcus lactis*, and *Enterococcus casseliflavus*. For instance, strains of *L. curvatus* were  
 773 isolated from the samples 311-b-T2, 331-a-T2 and 301-a-T3 and were effectively detected with  
 774 gyrB sequencing in those samples.



775



776

777

778

779

780

781

782

783

784

**Figure 7:** Heatmaps showing (A): the relative abundance of the 25 most frequently observed genera using both 16S rRNA and *gyrB* marker and (B) the relative abundance of the species detected with the *gyrB* marker. Taxa are coloured (*Lactobacillales* shown in different shades of blue, *Enterobacterales* in different shades of orange, and “other” in different shades of grey) and split according to their taxonomic order (*Lactobacillales*, *Enterobacterales* and “other”) and then clustered based on the Bray-Curtis dissimilarity. Samples are organised by vegetable and cutting types. Stage and salt level are indicated by an annotation at the side of the heatmap.

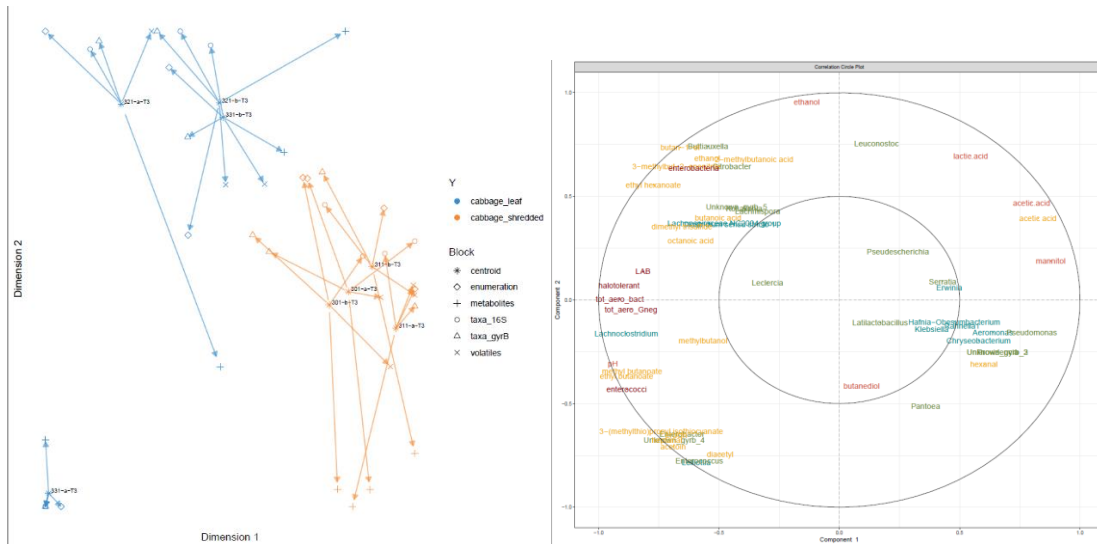


785 A multi-block Partial Least Squares-Discriminant Analysis (PLS-DA) was performed to  
786 determine if samples exhibited different signatures after one-month fermentation (stage T3),  
787 given their cutting type or salt concentration. The datasets included gyrB and 16S  
788 metataxonomics profiles, concentration of the main metabolites from central metabolism  
789 (lactic acid, ethanol, acetic acid, mannitol, butanediol), viable counts for six bacterial groups  
790 and yeasts and some volatile compounds, selected because they result from different synthesis  
791 pathways and showed marked changes in abundance during fermentation. Models based only  
792 on cutting type did allow for the identification of a discriminant signature among the samples,  
793 unlike the models based solely on salt concentration or on both salt concentration and cutting  
794 type. The results of the PLS-DA on carrot and cabbage samples are shown in **Figure 8**. The model  
795 discriminated the samples based on the cutting type according to variables belonging to the  
796 different datasets (Figure 8).

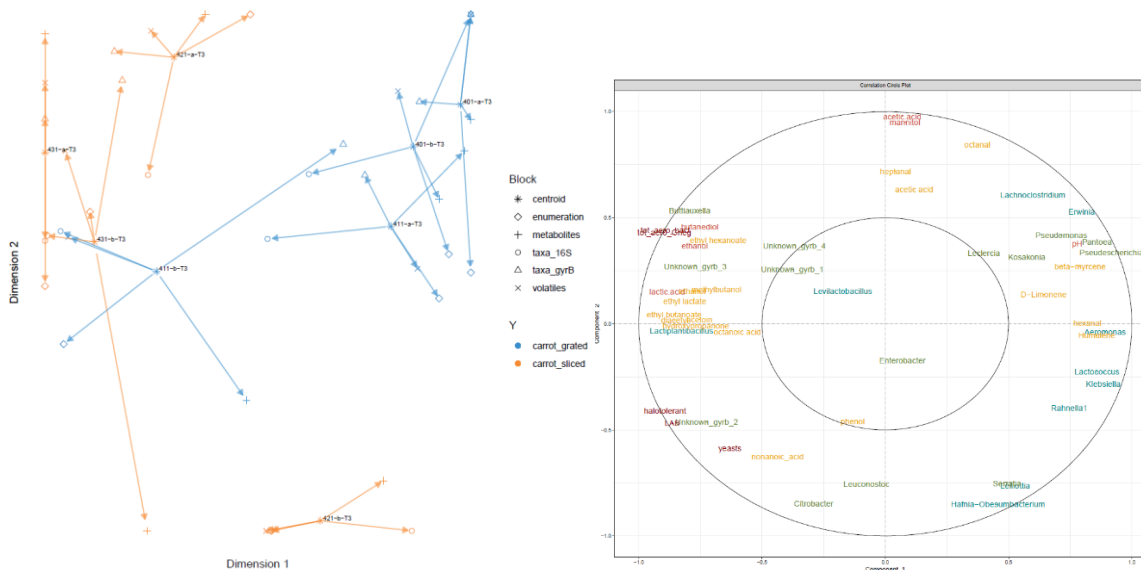
797 Regarding cabbage samples, the first dimension distinguishes the samples based on cutting  
798 type across all datasets (p-value < 0.05 for all datasets, Wilcoxon test). Thinly-cut samples  
799 exhibited a relatively homogeneous signature, characterised by high concentrations of acetic  
800 acid, lactic acid and mannitol, a low pH, a higher abundance of *Leuconostoc*, and overall lower  
801 bacterial viable counts. Roughly-cut cabbage samples showed a signature marked by high  
802 ethanol concentrations, the presence of *Clostridium*, *Lachnoclostridium*, *Lachnospiraceae*, and  
803 a higher abundance of *Buttiauxella*. One of the replicates of rough cabbage, 331-a-T3, is a  
804 particular case with a higher abundance of *Enterococcus* and *Enterobacter*, and higher amounts  
805 of acetoin and diacetyl compared with the three other roughly cut cabbage samples and lower  
806 amounts of the main metabolites (mannitol, acetic and lactic acids).

807 For carrot, the first dimension distinguished samples based on cutting type only for the gyrB  
808 and metabolite datasets, while the 16S, enumeration and volatile datasets failed in  
809 discriminating the type of cutting of carrot samples at T3. Thinly-cut carrot samples had a  
810 signature characterised by higher abundance of *Pseudodescherichia* while roughly-cut carrot  
811 samples having a signature characterised by a higher abundance of *Buttiauxella* and higher  
812 concentrations of lactic acid, ethanol and butanediol and a lower pH. The fermentation profiles  
813 differed between carrot samples independently of their cutting type. For example, one thinly-  
814 cut sample, 411-b-T3, showed a profile similar to that of two roughly cut samples (431-a-T3 and  
815 431-b-T3) characterised by a lower pH, a higher abundance of *Lactiplantibacillus* and higher  
816 concentrations of lactic acid, ethanol, acetoin, and diacetyl.

817  
818



819  
820



821  
822  
823  
824  
825  
826  
827  
828  
829  
830  
831

**Figure 8:** Multiblock PLS-DA result for cabbage (upper) and carrot (bottom) samples at T3 (one-month fermentation). The left panels represent samples from multiple coordinates to assess the alignment in the latent space. The start of the arrow indicates the centroid between all data sets for a given sample and the tips of the arrows the location of that sample in each block. The right panels represent the correlation circle between each variable and the discriminant axes as a scatter plot. The variables corresponding to the microbial viable counts for the seven following targeted microbial groups are shown in brown: lactic acid bacteria (LAB), total aerotolerant bacteria (tot\_aero\_bact), halotolerant bacteria, aerotolerant Gram-negative bacteria (tot\_aero\_Gneg), yeasts, bile-tolerant *Enterobacteriaceae* (enterobacteria), and enterococci.

832

833

## Discussion

834

835

836 **The experimental design allowed us to highlight a role of vegetable cutting despite a great**  
837 **variability from jar-to-jar**

838

839

840

841

842

843

844

845

846

847

848

849

850

851

852

853

854

855

856

857

858

859

860

861

862

863

864

865

866

867

868

869

870

871

872

873

874

875

876

The objective of the present study was to better understand the microbial dynamics and biochemical changes of spontaneous fermentation of vegetables by combining several omics approaches: culturomics, 16S rRNA gene and *gyrB* metataxonomics, and targeted metabolomics. More specifically, we aimed to investigate the effect of two factors, the type of cutting and a slight reduction of the amount of salt added, on the microbial and biochemical changes during fermentation. We chose both a root and a leafy vegetable, carrot and cabbage, which are commonly used for making fermented vegetables in France (Thierry, Madec, et al., 2023). However, the experiment was not designed to address the comparison of carrot and cabbage in itself, since the vegetable cultivar, culture conditions, harvest and storage conditions (time, temperature) can also impact their fermentation (Leff & Fierer, 2013). The impact of the cutting type or size has only been rarely addressed, although this factor can markedly vary, at least in domestic productions (Thierry, Madec, et al., 2023). Regarding the content of NaCl in food, the World Health Organisation has suggested reducing sodium intake by 30% to obtain the WHO guideline of 2 g day<sup>-1</sup> (i.e., 5 g of salt day<sup>-1</sup>) by 2025 (World Health Organization. Regional Office for Europe, 2018). We chose to compare a salt concentration of 1%, which is the concentration of salt generally recommended, with a concentration of 0.8%, i.e. a 20% Na reduction. No significant differences in the microbial and biochemical changes were observed between these two salt concentrations, although mineral diffusion was slightly enhanced at the highest salt concentration (see below). 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. This high number of replicates was of great interest because we observed a large variability from jar-to-jar. Each jar exhibited its own fermentation path and showed some specificity regarding the microbial results, the profile of metabolites, and the acidification rate (Figures 2, 3, 4). Each jar was prepared by taking the required amount of cut vegetables from a large (~10 kg) bin, without previous mixing the bin content. Therefore, we hypothesise that the initial microbiota present in each jar differed from one another, in particular concerning LAB, which are highly sub-dominant or even under detection threshold in raw vegetables. Differences could also result from differences of composition between different carrots and different cabbages. The variations we observed were both quantitative, i.e. differences in the rate of fermentation, and qualitative, e.g. the nature of the dominant taxa that grew over time. For example, yeasts were detected in only 2 out of the 4 jars of leaf cabbage characterised at 40 h fermentation, metataxonomic profiles differed between replicates (Figure 7), and the metabolite profiles also showed quantitative and qualitative differences between replicates (Figure 3). In the industrial sauerkraut production, heterogeneity can occur in the tanks, and the juice is recirculated to avoid this source of potential defects (Pederson & Albury, 1969). Differences between replicates were also observed, but not discussed, in several studies, e.g. the results on 16S-based metataxonomics in fermented radish and carrot (Raghuvanshi et al., 2019), and in paocai (Wang et al., 2020; Wang, Chen, Tang, Ming, Huang, Li, Ye, Fan, Yin, et al., 2022). For example, the

877 abundance of *Enterobacteriaceae* according to the 16S rRNA gene metataxonomics ranged  
878 between ~20% and ~80% in the triplicates of four-days fermented carrot and of one-day radish,  
879 and high pH variations during the first two days of fermentation were also observed  
880 (Raghuvanshi et al., 2019). These results stress the need to include a sufficient number of  
881 replicates in studies on spontaneous vegetable fermentation, in particular for small-scale  
882 fermentation.

### 883 **LAB quickly outcompeted bile-tolerant *Enterobacteriaceae* that dominate plant microbiota** 884 **at the beginning of fermentation**

885 For both studied vegetables, our culturomics as well as metataxonomic results confirm the  
886 sequential establishment of the microbial community, with the presence of a large proportion  
887 of *Pseudomonas* in raw carrot and the early development of bile-tolerant *Enterobacteriaceae*,  
888 referred to as enterobacteria below. The dominance of *Pseudomonas* and enterobacteria during  
889 the first hours of fermentation is consistent with the fact that these two groups constitute a large  
890 part of plant surface microbiota (Lund, 1992; Leff & Fierer, 2013; Jackson et al., 2015).  
891 *Pseudomonas* members are characterised by their great ability to colonise different ecological  
892 niches and were recovered from a wide variety of 77 samples from 11 different vegetables (Ruiz-  
893 Roldán et al., 2021). In the present study, viable enterobacteria were lower in raw carrot than in  
894 raw cabbage, 4.3 and 6.0 log CFU/g, respectively, which may be due to the fact that carrots were  
895 washed before use, in contrast to cabbages. Enterobacteria are largely represented in the  
896 microbiota of fresh vegetables, e.g. at ~5.2 log CFU/g in 41 out of the 45 samples characterised,  
897 which included carrot, cabbage, and five other fresh vegetables (Al-Kharousi et al., 2016). The  
898 first shift conserved among different fermented vegetables was from the initial microbial  
899 population of vegetables to *Enterobacteriales*, according to a recent integrative bioinformatics  
900 approach used to perform a meta-analysis of 10 public amplicon data sets on fermented  
901 vegetables (Junker et al., 2024). For example, members of the *Enterobacteriaceae* family that  
902 included many non-affiliated taxa and *Erwinia* largely dominated on different vegetables,  
903 according to a 16S-based metataxonomic analysis (Raghuvanshi et al., 2019).

904 Our results also confirm the early development of LAB, which manage to outcompete  
905 *Pseudomonas* and enterobacteria notwithstanding their prevalence in raw vegetables. This is in  
906 full agreement with the results of a bioinformatics meta-analysis on different fermented  
907 vegetables, which showed that, after *Enterobacteriales* domination, a second shift led to an  
908 assemblage dominated by *Lactobacillales*, i.e. LAB (Junker et al., 2024). This second shift was  
909 observed for example in carrot juice (Wuyts et al., 2018), cucumber (Stoll et al., 2020), kimchi  
910 (Song et al., 2020; Jung et al., 2022), and paocai (Wang et al., 2020). LAB are able to live as an  
911 endophyte in a large variety of crop plants (Pontonio et al., 2018). In our study, viable LAB were  
912 non-detectable in cabbage and their initial counts were 3 log CFU/g in carrot, in line with the  
913 values of 2 to 4 CFU/g previously reported (Di Cagno et al., 2013). Plant endophytic communities  
914 are dominated by *Gammaproteobacteria*, *Alphaproteobacteria* and *Actinobacteria*. *Firmicutes*  
915 are sub-dominant and mainly represented by *Bacillales*, while *Lactobacillales* are generally  
916 under 0.1% of relative abundance (Hacquard et al., 2015; Kõiv et al., 2019). LAB are often not  
917 detected in the starting ingredients by metataxonomics, as shown in sauerkraut manufacture  
918 (Zabat et al., 2018). The selective pressure of the environmental conditions that result from the  
919 fermentation process applied, in particular the anaerobic conditions, indeed favours the growth

920 of LAB to the detriment of enterobacteria (Yu et al., 2020). LAB growth leads to a significant  
921 acidification of the environment, thus inhibiting the growth of enterobacteria, which do not  
922 withstand the acidic pH (Ostling & Lindgren, 1993). Concomitantly with acidification, we  
923 effectively observed a drastic reduction in viable enterobacteria, which were no more detected  
924 by plate counting after two weeks in 3 of the 4 conditions studied, i.e. in shredded cabbage and  
925 both thinly and roughly-cut carrot, which were characterised by a pH < 4.0 and a titratable  
926 acidity > 0.45. These results are in line with the safety threshold recommended by the Codex  
927 Alimentarius standard for pickled fruits and vegetables, which stipulates that the product has  
928 to be prepared and packed “to ensure an equilibrium pH of less than 4.6” (FAO, 2007). Our  
929 results further illustrate the ubiquitous nature of LAB in food fermentation, thanks to their  
930 ability to rapidly ferment different carbohydrates into lactic acid (Gänzle, 2015). Besides the  
931 inhibitory activity of the organic acids they produce, LAB could also inhibit enterobacteria  
932 through the synthesis of antimicrobial peptides, since many of the species found in our study  
933 are known to potentially produce bacteriocins (Zacharof & Lovitt, 2012; Hernández-González et  
934 al., 2021). A low proportion of LAB compared to that of *Enterobacteriaceae* was observed using  
935 metataxonomics in the present study, even after one month of fermentation, in particular in  
936 fermented cabbage. In a previous study using the same methods to characterise domestic  
937 samples of fermented vegetables, metataxonomics results showed that LAB had a median  
938 abundance of 90%, mainly represented by members of the *Lactobacillaceae* family, and that  
939 *Enterobacteriales* was the 2<sup>nd</sup> main taxon (Thierry, Madec, et al., 2023). Moreover, a negative  
940 relationship was observed between the abundance of *Enterobacteriales* and the age of the  
941 samples. The lower LAB abundance observed in the present study could thus be explained by  
942 the age of sample, one month maximum in the present study versus a median duration of 6  
943 months (from 2 weeks to 4 years). Similarly, in a study on paocai fermented at 15°C and 25°C  
944 during one year, LAB dominated, representing about 60% of the total abundance, with (former)  
945 *Lactobacillus* as dominant genus (Wang et al., 2020).

946 The first LAB species that grew in both vegetables of the present study were  
947 heterofermentative species, *Leuconostoc* sp. and *L. lactis*, in agreement with previous reports in  
948 other fermented vegetables, such as paocai (Wang, Chen, Tang, Ming, Huang, Li, Ye, Fan, Chi, et  
949 al., 2022). Heterofermentative LAB are capable of degrading a wide variety of carbohydrates  
950 (Gänzle, 2015) and *Leuconostoc* efficiently metabolises sucrose (Cogan & Jordan, 1994).

951 The choice of the metataxonomics markers markedly impact the picture of the bacterial  
952 community. Metataxonomics using both 16S rRNA and *gyrB* genes as markers helped us to  
953 describe the changes in bacterial community during fermentation. The *gyrB* marker, although  
954 rarely used in metataxonomics, provides species-level taxonomic resolution in food ecosystems  
955 (Poirier et al., 2018), as effectively observed in the present study. For the 16S marker, the V3-V4  
956 region is the most commonly used in the field of food microbiology (Parente et al., 2022), but we  
957 chose the V5-V7 region in the present study to compare the results with that of our previous  
958 study on fermented vegetables (Thierry, Madec, et al., 2023). Both markers confirmed the  
959 succession of *Enterobacteriaceae* and LAB, except in some roughly-cut cabbage samples.  
960 However, the *gyrB* marker uniquely detected *Leuconostoc* as the first LAB genus which  
961 appeared, which is also confirmed by the results of the culture-dependent approach. In three  
962 roughly-cut cabbage samples, the 16S marker uniquely detected *Clostridium* taxon. The  
963 detection of this undesirable genus is important since some *Clostridium* can be responsible for

964 food poisoning. We however failed in finding reports on clostridia-related poisoning associated  
965 with the consumption of fermented vegetables.

#### 966 **Metabolites of fermented products as markers of microbial activity**

967 In the present study, the compounds analysed were carbohydrates, organic acids, alcohols  
968 and volatile compounds. Among metabolites, mannitol, lactic and acetic acids were the main  
969 metabolites produced, in agreement with previous reports on fermented carrot juice (Wuyts et  
970 al., 2018) and on sauerkraut (Plengvidhya et al., 2007; Tlais et al., 2022). Mannitol, lactic acid,  
971 and acetic acid respectively accounted for about 45-55%, 22-30%, and 10-12% of total  
972 metabolites in carrot and shredded cabbage after one-month fermentation. These proportions  
973 are similar to those reported in carrot juice (Wuyts et al., 2018), sauerkraut (Plengvidhya et al.,  
974 2007), and other fermented vegetables as kimchi (Jung et al., 2011). These metabolites are  
975 typical markers of LAB metabolism. Heterofermentative LAB, as the *Leuconostoc* members  
976 identified in both cabbage and carrot, convert fructose into mannitol (Wisselink et al., 2002;  
977 Martínez-Miranda et al., 2022). They also convert other carbohydrates into lactic acid, acetic  
978 acid, and ethanol, while homofermentative LAB convert carbohydrates into lactic acid as the  
979 main end-product. In our study, the ratio of lactic and acetic acids after one month of  
980 fermentation varied from one jar to another, from 1.5 to 5 in fermented cabbage and 1.7 to 4.3  
981 in carrot. Butanediol was also detected in fermented samples, at markedly variable  
982 concentrations. Butanediol can be produced from acetoin by *L. mesenteroides* and  
983 *Lactobacillus sakei*. For example, metatranscriptomic analyses in kimchi showed that genes  
984 encoding the pathway from pyruvate to diacetyl/acetoin and butanediol were expressed during  
985 kimchi fermentation (Chun et al., 2017; Kim et al., 2020). Concerning volatiles, most of them  
986 cannot be used as specific markers of microbial activity, because their pathways of formation  
987 are shared by many bacteria groups. For example, isothiocyanates, thiocyanates, and nitriles  
988 were detected by GC-MS in fermented cabbage samples (Table S3). These sulphur-containing  
989 compounds derive from the glucosinolates present in cabbage, which were completely  
990 degraded after one month fermentation (results non shown), as previously reported (Wieczorek  
991 & Drabińska, 2022). Even if some LAB strains can hydrolyse glucosinolates into nitriles, other  
992 bacteria like enterobacteria also do it (Mullaney et al., 2013). Moreover, the activity of plant  
993 myrosinase also results in glucosinolate hydrolysis, and is favoured by both the cutting of  
994 vegetable, which releases glucosinolates and myrosinase from separated cell parts, and by the  
995 pH decrease, favourable to myrosinase activity (Wieczorek & Drabińska, 2022). As for biogenic  
996 amines, which are mainly produced by *Enterobacteriaceae* from amino acid decarboxylation  
997 (Halász et al., 1994), they were not analysed in the present study. Total concentrations of  
998 biogenic amines can reach from ~50 to ~600 mg/kg in different fermented vegetables (Świder et  
999 al., 2020).

#### 1000 **The fermentation rate of cabbage and carrot markedly differed in the present study**

1001 Carrot and cabbage fermentation exhibited a similar succession of the main bacterial  
1002 groups, with *Enterobacteriaceae* quickly replacing the initial microbiota, further replaced within  
1003 a few days by LAB, as detailed above. The main differences between the two vegetables used,  
1004 in the present study, concerned the rate of fermentation. Despite a similar profile of metabolites

1005 in carrot and cabbage, with mannitol, lactic acid, and acetic acid as main products, the  
1006 concentrations produced in carrot were two-fold higher than in cabbage (Figure 3).

1007 Many factors can be involved in the differences observed between carrot and cabbage,  
1008 among which the specific microbiota of the vegetables used, the microbial changes during the  
1009 processing steps. e.g. the washing of carrot vs only the removal of the external leaves of  
1010 cabbage, and the composition of vegetables in macro- and micronutrients. Carrot generally  
1011 contain 1.4-fold higher amounts of carbohydrates than cabbage (Anses, 2020), which likely  
1012 explains why the concentrations in the main metabolites were higher in the juice of the former  
1013 than in the latter one. Carrot and cabbage juice composition also differ in terms of  
1014 micronutrients, which may also influence the development of micro-organisms. For example,  
1015 our carrot juices contained more P and more K than cabbage, and similar amounts of Mg, while  
1016 cabbage globally contained more vitamins. However, besides the type of vegetable, many other  
1017 factors, such as the vegetable cultivar, its stage of harvest and storage time, and other factors,  
1018 can influence their microbial and biochemical composition. As a result, the differences observed  
1019 in the present study between carrot and cabbage fermentation should not be generalised.

#### 1020 **A thin cutting favours the release of solutes and increases the fermentation rate**

1021 The cutting of vegetables before fermentation varies from thinly grated to large pieces of  
1022 vegetables in domestic productions, as stated above, but the effect of cutting, to our best  
1023 knowledge, has been scarcely studied. The “degree of disintegration” was cited, besides the  
1024 temperature and the type of vegetable, among the factors that influence the fermentation  
1025 (Buckenhueskes, 1993; Buckenhueskes, 2015). The nutrients present inside the vegetable cells  
1026 must be released in the aqueous phase, i.e. juice, to be made available to the microorganisms  
1027 via shredding, slicing, or only piercing, depending on vegetables (Buckenhueskes, 2015). The  
1028 thinner the cutting, the higher the surface of cut plant tissue that can directly release vegetable  
1029 solutes in brine, thus providing LAB with nutrients and increasing the buffering capacity of brine.  
1030 Solutes can also diffuse from entire, free from injury, vegetables, as described in cucumber, but  
1031 at a lower rate (Passos et al., 2005). In a study that aimed at modelling the equilibrium of solutes  
1032 between the brine and entire cucumber, either peeled or not, it was shown that the diffusion  
1033 coefficient of glucose was 9.2 times higher for peeled cucumber than for unpeeled one (Potts et  
1034 al., 1986). The latter study suggests that the surface of cut tissue is an important factor to  
1035 promote the diffusion of solutes into the brine. We therefore calculated the surface of cut  
1036 vegetable, by estimating the mean dimension of the pieces of vegetables used in the present  
1037 study, assimilated either to cylinders (entire, sliced and grated carrot), or to parallelepipeds  
1038 (cabbage leaf pieces and shredded cabbage). Moreover, the experimental mass of vegetable  
1039 and brine weighted per jar differed depending on the vegetable and the cutting, thus resulting  
1040 in differences of the ratio of vegetable vs brine, which varied from 0.73 (leaf cabbage), 0.94  
1041 (shredded cabbage), 1.24 (sliced carrot) to 1.33 (grated carrot). Based on these ratios and the  
1042 estimations of the surface of cut tissue, we calculated the cut surfaces per g of initial brine,  
1043 which were approximately 0.4 cm<sup>2</sup>, 8 cm<sup>2</sup>, 9 cm<sup>2</sup>, and 19 cm<sup>2</sup>, for leaf cabbage, sliced carrot,  
1044 shredded cabbage, and grated carrot, respectively, as detailed in **Supplementary Table S7**. In  
1045 other words, the cut surface of the thinly-cut cabbage was approximately 26-fold higher than  
1046 that of roughly-cut cabbage, while it was only 2-fold for the thinly-cut carrot compared to  
1047 roughly-cut one. We therefore hypothesised that these marked differences of the cut surface

1048 could, at least partly, explain why, in the present study, the two cutting types of cabbage  
1049 markedly differed in the rates of acidification and viable enterobacteria decrease, in contrast to  
1050 the slighter differences observed between sliced and grated carrots (Figure 2).

1051 The hypothesis of a greater diffusion of vegetable solutes into brine for thinly-cut vegetables  
1052 is further supported by our results of the mineral composition of juices. First, juices contained  
1053 18-32% more minerals (K, P, and Mg) coming from the vegetable tissue in shredded than in leaf  
1054 cabbage, and 10-16% more in grated than in sliced carrot. Secondly, carrot juices contained  
1055 more P and K than cabbage juice, although white cabbage is expected to be as rich in K and 1.5  
1056 richer in P compared to carrot, according to Ciqual data (Anses, 2020), thus suggesting a higher  
1057 global diffusion from vegetable into juice in the case of carrot, for which the cut surface was  
1058 greater. As a result, we can hypothesise that the buffering capacity of the juice was higher for  
1059 thinly-cut vegetables compared to roughly-cut ones. This assumption is consistent with the fact  
1060 that, for a given pH, TTA was higher in thinly-cut vegetable. For example, TTA was twice as high  
1061 in grated carrots at T1, compared to sliced carrots, despite a similar pH. The effect of cutting  
1062 was previously studied to compare the survival of pathogenic strains during fermentation of  
1063 cabbage either kept as whole heads or shredded (Niksic et al., 2005). The authors attributed the  
1064 lower survival observed in shredded cabbage to the significantly higher total titratable acidity  
1065 in shredded cabbage juice compared to the one of whole head cabbage, in relation to the higher  
1066 buffering capacity of the juice. The importance of the buffer capacity of vegetable juice has been  
1067 previously highlighted. A pioneer study showed that the composition of cucumbers depended  
1068 on their size, the. Smaller fruit, however, contain lower levels of sugars and a higher natural  
1069 buffering capacity than larger ones and achieved a complete sugar utilisation during  
1070 fermentation (Lu et al., 2002). Buffer models were later developed in cucumber juice of different  
1071 composition, so as to be capable of assessing the relationship between pH and the  
1072 concentration of acids (Breidt & Skinner, 2022).

1073 The addition of salt is known to withdraw nutrients from the vegetable tissue towards the  
1074 juice. We effectively observed, in the present study, a slight but significant effect of the amount  
1075 of added NaCl on the release of P and K in the juice (+ 5-8% in carrot, and + 9-16% in cabbage).

1076 In the case of leaf cabbage, which had by far the smallest cut surface, we also observed faulty  
1077 fermentation. For example, on the four jars of leaf cabbage characterised after one month  
1078 fermentation, two still contained alive enterobacteria (321-a and 331-b), the third (321-b)  
1079 contained lower viable LAB counts (7.6 log CFU/mL) and a high content in ethanol (5.2 g/mL vs  
1080 1.6 g/mL in all other samples at that stage) and for the fourth one (331-a), the whole lactic  
1081 population consisted of enterococci, associated with an atypical metabolite profile without  
1082 mannitol (Figures 2, 3, and 4) and a distinct volatile profile, with for example the highest  
1083 amounts of esters of butanoic acid. These four samples also contained *Clostridium* (321b, 331a,  
1084 331b) and/or *Lachnoclostridium* (321a, 321b, 331a) taxa. The mean pH of these four samples was  
1085 4.0, versus 3.6 at the same stage in all other samples. It is noteworthy to mention that many leaf  
1086 cabbage samples had a very unpleasant and atypical smell. We therefore hypothesise that the  
1087 cutting of cabbage into large leaf pieces was insufficient to provide LAB enough nutrients and  
1088 that there could exist a threshold cut surface under which a rapid lactic fermentation is hardly  
1089 achieved.

1090 In brief, a fine cutting, besides salting, favours the release of solutes towards the juice, thus  
1091 providing microorganisms the nutrients they need to grow, and increasing the buffer capacity



1092 of brine. Consequently, a quicker acidification, a higher titratable acidity, and quicker decrease  
1093 of the number of viable enterobacteria can be expected, as effectively observed in the present  
1094 study in particular for cabbage, for which the surface generated by the cutting step was much  
1095 greater (> 20-fold) in shredded cabbage than in leaf cabbage. The difference of salt  
1096 concentration also intensified the release of solutes, as observed in the present study for some  
1097 minerals, but the possible consequences on microbial growth were probably masked by the  
1098 variability observed from jar-to-jar. In line with the importance of the buffer capacity, the  
1099 targeted values of both the pH (< 4) and titratable acidity (1%) are given in the specifications for  
1100 the protected geographical indication of “Sauerkraut of Alsace”, to satisfy safety and sensory  
1101 property requirements (EU Commission implementing regulation, 2018).

## 1102 **Some health benefits and risks associated with fermented vegetables consumption**

1103 Besides the preservation of vitamins, discussed below, fermented foods have been  
1104 associated with potential health benefits, which result from two main factors. The first is the  
1105 microbial production of metabolites of interest for human nutrition, e.g. vitamins, bioactive  
1106 peptides, or that can positively affect human health, e.g. mannitol,  $\gamma$ -aminobutyric acid (Lenhart  
1107 & Chey, 2017). The second is the presence of live microorganisms that can interact with the  
1108 intestinal microbiota (Rezac et al., 2018). Initially much explored for dairy-based fermented  
1109 foods (Kok & Hutkins, 2018; Companys et al., 2020) and the Korean kimchi (Cha et al., 2023), this  
1110 is now also well demonstrated for other non-dairy foods (Wuyts et al., 2020; Valero-Cases et al.,  
1111 2020).

1112 On the other hand, in the case of poorly controlled production, the consumption of altered  
1113 fermented products can lead to health risks. Concerning fermented vegetables, the high  
1114 number of enterobacteria present at the beginning of fermentation is associated with the risk  
1115 of toxin and biogenic amine production. The presence of spore-forming bacteria, among which  
1116 toxin producers such as some *Clostridium* sp., has also been reported. In a recent South Korean  
1117 quantitative risk assessment study for *Clostridium perfringens* foodborne illness via kimchi  
1118 consumption, the authors conclude that the risk was “very low” (Choi et al., 2020).  
1119 Enterobacteria disappear as soon as the pH is low enough for a period of time, and not all of  
1120 them present hazards. For example, in the present study, we mainly identified *Hafnia alvei*  
1121 among the clones isolated on the VRBG medium from the leaf cabbage samples in which  
1122 enterobacteria were alive after one-month fermentation. *H. alvei* is commonly isolated from and  
1123 even used as an adjunct culture in raw milk Camembert cheese. One of the leaf cabbage samples  
1124 also contained clostridia. Fortunately, all these samples would not have been consumed  
1125 because they had a very unpleasant odour.

1126 We focused in this study on the vitamin content in raw vegetables and after fermentation.  
1127 According to literature, the final concentration in fermented vegetables of the vitamins first  
1128 depends on their initial concentration in raw vegetables, even if fermentation can result in  
1129 variations in their vitamin content. The vitamin concentration has been shown to decrease  
1130 during fermentation in most of the reported cases, as stated in a recent literature review  
1131 (Thierry, Baty, et al., 2023). The contents in vitamins C and K1 and beta-carotene in raw carrot  
1132 and/or cabbage observed in the present study are in accordance with the expected values from  
1133 nutritional food tables, while the vitamin B9 content was in the lower range of the reported  
1134 values (Anses, 2020). We only observed an increase for the vitamin C content during

1135 fermentation, which may be due to microbial activity of ascorbigen degradation (Berger et al.,  
1136 2020). In contrast, vitamin B9 content was not improved, in agreement with previous report  
1137 (Jägerstad et al., 2004). Vitamins K2 and B12 are absent from raw vegetables and can result from  
1138 microbial synthesis. Vitamin K2 is mainly present in fermented foods (Walther & Chollet, 2017)  
1139 and can be synthesised by some LAB strains (Capozzi et al., 2012). Vitamin B12 is present in foods  
1140 of animal origin (National Institutes of Health) and can also be synthesised by some bacteria as  
1141 propionibacteria (Falentin et al, 2010). We did not observe the production of vitamins K2 and  
1142 B12 in the present study. In the context of human nutrition, vitamin contents were expressed in  
1143 the present study per quantity of fresh matter of fermented vegetables (without brine), i.e. as  
1144 they are generally consumed. In our study, cabbage and carrot remained a good source of  
1145 vitamin C and beta-carotene, respectively, after fermentation, since they bring 24 to 31% of  
1146 nutritional value of reference in vitamin C for 100 g of fermented cabbage and 167 to 204 % of  
1147 pro-vitamin A (in beta-carotene form) for 100 g fermented carrot.

1148

### **Acknowledgments**

1149 We thank Gilles Drege from CTCPA (agri-food technical centre), Nantes, France for their help  
1150 in the organisation and preparation of jars of sliced and grated carrot, at the Oniris platform,  
1151 Nantes, and Patrice de Martrin and Geoffroy Branjonneau, PROTIAL, Beaucouzé, France, for  
1152 their help in the organisation and preparation of jars of shredded and leaf cabbage.

1153 We are grateful to the INRAE MIGALE bioinformatics facility (MIGALE, INRAE, 2020. Migale  
1154 bioinformatics Facility, doi: 10.15454/1.5572390655343293E12) for providing help, computing  
1155 and storage resources.

1156 We also thank Cécile Grondin, UMR SPO, INRAE, Université de Montpellier, Institut Agro,  
1157 Montpellier, France, for yeast isolates identification, Véronique Broussole and Stephanie Oriol,  
1158 UMR408 SQPOV, INRAE, Montpellier, France for *Bacillus* spp. identification, and Arlette Leduc,  
1159 UMR STLO, INRAE, Institut Agro, Rennes, France for her help in mineral analysis.

1160

### **Funding**

1161 This work was financially supported by the Conseil Régional de Bretagne, France, the Conseil  
1162 Régional des Pays de la Loire, France, and Fonds de dotation EKIP, Paris, France, in the  
1163 framework of the FLEGME inter-regional project, under the scientific coordination of INRAE and  
1164 managed by the VEGEPOLYS VALLEY competitive cluster.

1165

### **Conflict of interest disclosure**

1166 The authors declare that they comply with the PCI rule of having no financial conflicts of  
1167 interest in relation to the content of the article.

1168

### **Data, scripts, code, and supplementary information availability**

1169 The sequence data for this study have been deposited in the European Nucleotide Archive  
1170 (ENA) at EMBL-EBI and are available online: PRJEB79032 on the webpage hosting the data  
1171 <https://www.ebi.ac.uk/ena/browser/view/PRJEB79032>.  
1172

1173 Supplementary information including data, and scripts is available online: DOI of the webpage  
1174 hosting the supplementary information <https://doi.org/10.57745/MJWSJQ>; Thierry et al., 2024.

1175

## References

1176 Al-Kharousi ZS, Guizani N, Al-Sadi AM, Al-Bulushi IM, Shaharoon B (2016) Hiding in Fresh Fruits and  
1177 Vegetables: Opportunistic Pathogens May Cross Geographical Barriers. *International Journal*  
1178 *of Microbiology*, **2016**, 4292417. <https://doi.org/10.1155/2016/4292417>

1179 Anses (2020) Ciqual French food composition table. <https://doi.org/10.5281/zenodo.4770202>

1180 Ashaolu TJ, Reale A (2020) A Holistic Review on Euro-Asian Lactic Acid Bacteria Fermented Cereals  
1181 and Vegetables. *Microorganisms*, **8**, 1176. <https://doi.org/10/ghggp2>

1182 Beckers B, Op De Beeck M, Thijs S, Truyens S, Weyens N, Boerjan W, Vangronsveld J (2016)  
1183 Performance of 16s rDNA Primer Pairs in the Study of Rhizosphere and Endosphere Bacterial  
1184 Microbiomes in Metabarcoding Studies. *Frontiers in Microbiology*, **7**.  
1185 <https://doi.org/10/ghwscw>

1186 Berger MD, Vakula A, Horecki AT, Rakić D, Pavlić B, Malbaša R, Vitas J, Jerković J, Šumić Z (2020)  
1187 Cabbage (*Brassica oleracea* L. var. capitata) fermentation: Variation of bioactive compounds,  
1188 sum of ranking differences and cluster analysis. *LWT*, 110083.  
1189 <https://doi.org/10.1016/j.lwt.2020.110083>

1190 Breidt F, Skinner C (2022) Buffer Models for pH and Acid Changes Occurring in Cucumber Juice  
1191 Fermented with *Lactiplantibacillus pentosus* and *Leuconostoc mesenteroides*. *Journal of Food*  
1192 *Protection*, **85**, 1273–1281. <https://doi.org/10.4315/JFP-22-068>

1193 Buckenhueskes HJ (2015) 22 - Quality improvement and fermentation control in vegetables. In:  
1194 *Advances in Fermented Foods and Beverages* Woodhead Publishing Series in Food Science,  
1195 Technology and Nutrition. (ed Holzapfel W), pp. 515–539. Woodhead Publishing.  
1196 <https://doi.org/10.1016/B978-1-78242-015-6.00022-0>

1197 Buckenhuskes HJ (1993) Selection criteria for lactic acid bacteria to be used as starter cultures for  
1198 various food commodities. *FEMS Microbiology Reviews*, **12**, 253–272.

- 1199 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High-  
1200 resolution sample inference from Illumina amplicon data. *Nature Methods*, **13**, 581–583.  
1201 <https://doi.org/10.1038/nmeth.3869>
- 1202 Canon F, Mariadassou M, Maillard M-B, Falentin H, Parayre S, Madec M-N, Valence F, Henry G,  
1203 Laroute V, Daveran-Mingot M-L, Cocaign-Bousquet M, Thierry A, Gagnaire V (2020) Function-  
1204 Driven Design of Lactic Acid Bacteria Co-cultures to Produce New Fermented Food  
1205 Associating Milk and Lupin. *Frontiers in Microbiology*, **11**, 584163. <https://doi.org/10/ghmt26>
- 1206 Capozzi V, Russo P, Teresa Duenas M, Lopez P, Spano G (2012) Lactic acid bacteria producing B-group  
1207 vitamins: a great potential for functional cereals products. *Applied Microbiology and*  
1208 *Biotechnology*, **96**, 1383–1394. <https://doi.org/10.1007/s00253-012-4440-2>
- 1209 Cha J, Kim YB, Park S-E, Lee SH, Roh SW, Son H-S, Whon TW (2023) Does kimchi deserve the status of  
1210 a probiotic food? *Critical Reviews in Food Science and Nutrition*, **0**, 1–14.  
1211 <https://doi.org/10.1080/10408398.2023.2170319>
- 1212 Choi Y, Kang J, Lee Y, Seo Y, Lee H, Kim S, Lee J, Ha J, Oh H, Kim Y, Byun K-H, Ha S-D, Yoon Y (2020)  
1213 Quantitative microbial risk assessment for *Clostridium perfringens* foodborne illness  
1214 following consumption of kimchi in South Korea. *Food Science and Biotechnology*, **29**, 1131–  
1215 1139. <https://doi.org/10.1007/s10068-020-00754-2>
- 1216 Chun BH, Kim KH, Jeon HH, Lee SH, Jeon CO (2017) Pan-genomic and transcriptomic analyses of  
1217 *Leuconostoc mesenteroides* provide insights into its genomic and metabolic features and  
1218 roles in kimchi fermentation. *Scientific Reports*, **7**, 11504. <https://doi.org/10/gbx7hq>
- 1219 Cogan TM, Jordan KN (1994) Metabolism of *Leuconostoc* Bacteria. *Journal of Dairy Science*, **77**, 2704–  
1220 2717. [https://doi.org/10.3168/jds.S0022-0302\(94\)77213-1](https://doi.org/10.3168/jds.S0022-0302(94)77213-1)
- 1221 Companys J, Pedret A, Valls RM, Solà R, Pascual V (2020) Fermented dairy foods rich in probiotics and  
1222 cardiometabolic risk factors: a narrative review from prospective cohort studies. *Critical*  
1223 *Reviews in Food Science and Nutrition*, **0**, 1–10.  
1224 <https://doi.org/10.1080/10408398.2020.1768045>

- 1225 Di Cagno R, Coda R, De Angelis M, Gobbetti M (2013) Exploitation of vegetables and fruits through  
1226 lactic acid fermentation. *Food Microbiology*, **33**, 1–10. <https://doi.org/10/f4gjhh>
- 1227 EU Commission implementing regulation (2018) *Commission Implementing Regulation (EU) 2018/938*  
1228 *of 20 June 2018 entering a name in the register of protected designations of origin and*  
1229 *protected geographical indications ('Choucroute d'Alsace' (PGI)).*
- 1230 FAO (2007) Standard for pickled fruits and vegetables, CODEX ALIMENTARIUS FAO-WHO.
- 1231 Gänzle MG (2015) Lactic metabolism revisited: metabolism of lactic acid bacteria in food  
1232 fermentations and food spoilage. *Current Opinion in Food Science*, **2**, 106–117.  
1233 <https://doi.org/10.1016/j.cofs.2015.03.001>
- 1234 Gänzle M (2022) The periodic table of fermented foods: limitations and opportunities. *Applied*  
1235 *Microbiology and Biotechnology*, **106**, 2815–2826. [https://doi.org/10.1007/s00253-022-](https://doi.org/10.1007/s00253-022-11909-y)  
1236 [11909-y](https://doi.org/10.1007/s00253-022-11909-y)
- 1237 Guo M, Yuan C, Tao L, Cai Y, Zhang W (2022) Life barcoded by DNA barcodes. *Conservation Genetics*  
1238 *Resources*, **14**, 351–365. <https://doi.org/10.1007/s12686-022-01291-2>
- 1239 Hacquard S, Garrido-Oter R, González A, Spaepen S, Ackermann G, Lebeis S, McHardy AC, Dangl JL,  
1240 Knight R, Ley R, Schulze-Lefert P (2015) Microbiota and Host Nutrition across Plant and  
1241 Animal Kingdoms. *Cell Host & Microbe*, **17**, 603–616. <https://doi.org/10/f7fv5t>
- 1242 Halász A, Baráth Á, Simon-Sarkadi L, Holzapfel W (1994) Biogenic amines and their production by  
1243 microorganisms in food. *Trends in Food Science & Technology*, **5**, 42–49.  
1244 [https://doi.org/10.1016/0924-2244\(94\)90070-1](https://doi.org/10.1016/0924-2244(94)90070-1)
- 1245 Hernández-González JC, Martínez-Tapia A, Lazcano-Hernández G, García-Pérez BE, Castrejón-Jiménez  
1246 NS (2021) Bacteriocins from Lactic Acid Bacteria. A Powerful Alternative as Antimicrobials,  
1247 Probiotics, and Immunomodulators in Veterinary Medicine. *Animals*, **11**, 979.  
1248 <https://doi.org/10.3390/ani11040979>
- 1249 Jackson CR, Stone BWG, Tyler HL (2015) Emerging Perspectives on the Natural Microbiome of Fresh  
1250 Produce Vegetables. *Agriculture*, **5**, 170–187. <https://doi.org/10.3390/agriculture5020170>

- 1251 Jägerstad M, Jastrebova J, Svensson U (2004) Folates in fermented vegetables—a pilot study. *LWT -*  
1252 *Food Science and Technology*, **37**, 603–611. <https://doi.org/10.1016/j.lwt.2003.11.008>
- 1253 Jung M-J, Kim J, Lee SH, Whon TW, Sung H, Bae J-W, Choi Y-E, Roh SW (2022) Role of combined  
1254 lactic acid bacteria in bacterial, viral, and metabolite dynamics during fermentation of  
1255 vegetable food, kimchi. *Food Research International*, **157**, 111261.  
1256 <https://doi.org/10.1016/j.foodres.2022.111261>
- 1257 Jung JY, Lee SH, Kim JM, Park MS, Bae JW, Hahn Y, Madsen EL, Jeon CO (2011) Metagenomic analysis  
1258 of kimchi, a traditional Korean fermented food. *Applied and Environmental Microbiology*, **77**,  
1259 2264–2274.
- 1260 Junker R, Valence F, Mistou M-Y, Chaillou S, Chiapello H (2024) Integration of metataxonomic data  
1261 sets into microbial association networks highlights shared bacterial community dynamics in  
1262 fermented vegetables. *Microbiology Spectrum*, **0**, e00312-24.  
1263 <https://doi.org/10.1128/spectrum.00312-24>
- 1264 Kim KH, Chun BH, Baek JH, Roh SW, Lee SH, Jeon CO (2020) Genomic and metabolic features of  
1265 *Lactobacillus sakei* as revealed by its pan-genome and the metatranscriptome of kimchi  
1266 fermentation. *Food Microbiology*, **86**, 103341. <https://doi.org/10.1016/j.fm.2019.103341>
- 1267 Kõiv V, Arbo K, Maiväli Ü, Kisand V, Roosaare M, Remm M, Tenson T (2019) Endophytic bacterial  
1268 communities in peels and pulp of five root vegetables. *PLOS ONE*, **14**, e0210542.  
1269 <https://doi.org/10/ghwsdb>
- 1270 Kok CR, Hutkins R (2018) Yogurt and other fermented foods as sources of health-promoting bacteria.  
1271 *Nutrition Reviews*, **76**, 4–15. <https://doi.org/10.1093/nutrit/nuy056>
- 1272 Leff JW, Fierer N (2013) Bacterial Communities Associated with the Surfaces of Fresh Fruits and  
1273 Vegetables. *PLOS ONE*, **8**, e59310. <https://doi.org/10.1371/journal.pone.0059310>
- 1274 Lenhart A, Chey WD (2017) A Systematic Review of the Effects of Polyols on Gastrointestinal Health  
1275 and Irritable Bowel Syndrome. *Advances in Nutrition*, **8**, 587–596.  
1276 <https://doi.org/10.3945/an.117.015560>

- 1277 Li M, Lao F, Pan X, Yuan L, Zhang D, Wu J (2024) Insights into the mechanisms driving microbial  
1278 community succession during pepper fermentation: Roles of microbial interactions and  
1279 endogenous environmental changes. *Food Research International*, **179**, 114033.  
1280 <https://doi.org/10.1016/j.foodres.2024.114033>
- 1281 Liu Z, Li J, Zhou X, Wei B, Xie S, Du T, Zhao X, Jiang L, Xiong T (2021) The lactic acid bacteria and yeast  
1282 community of home-made sauerkraut from three provinces in Southwest China. *Archives of*  
1283 *Microbiology*. <https://doi.org/10/gj94ng>
- 1284 Lu Z, Fleming H p., McFeeters R f. (2002) Effects of Fruit Size on Fresh Cucumber Composition and the  
1285 Chemical and Physical Consequences of Fermentation. *Journal of Food Science*, **67**, 2934–  
1286 2939. <https://doi.org/10.1111/j.1365-2621.2002.tb08841.x>
- 1287 Lund BM (1992) Ecosystems in vegetable foods. *Society for Applied Bacteriology Symposium Series*,  
1288 **21**, 115S–26S. <https://doi.org/10.1111/j.1365-2672.1992.tb03631.x>
- 1289 Martin F, Lee J, Azevedo-Scudeller L, Paul A, Delaplace G, Burgain J, Rousseau F, Tanguy G, Famelart  
1290 M-H, Jeantet R, Le Floch-Fouéré C (2022) Heat treatment of milk protein concentrates affects  
1291 enzymatic coagulation properties. *Food Research International*, **162**, 112030.  
1292 <https://doi.org/10.1016/j.foodres.2022.112030>
- 1293 Martínez-Miranda JG, Chairez I, Durán-Páramo E (2022) Mannitol Production by Heterofermentative  
1294 Lactic Acid Bacteria: a Review. *Applied Biochemistry and Biotechnology*, **194**, 2762–2795.  
1295 <https://doi.org/10.1007/s12010-022-03836-5>
- 1296 McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and  
1297 graphics of microbiome census data. *PloS One*, **8**, e61217.  
1298 <https://doi.org/10.1371/journal.pone.0061217>
- 1299 Medina-Pradas E, Perez-Diaz IM, Garrido-Fernandez A, Noe Arroyo-Lopez F (2017) Review of  
1300 Vegetable Fermentations With Particular Emphasis on Processing Modifications, Microbial  
1301 Ecology, and Spoilage. In: *Microbiological Quality of Food: Foodborne Spoilers* (eds

1302 Bevilacqua A, Corbo MR, Sinigaglia M), pp. 211–236. Woodhead Publ Ltd, Cambridge.  
1303 <https://doi.org/10.1016/B978-0-08-100502-6.00012-1>

1304 Mullaney JA, Kelly WJ, McGhie TK, Ansell J, Heyes JA (2013) Lactic Acid Bacteria Convert  
1305 Glucosinolates to Nitriles Efficiently Yet Differently from Enterobacteriaceae. *Journal of*  
1306 *Agricultural and Food Chemistry*, **61**, 3039–3046. <https://doi.org/10.1021/jf305442j>

1307 Müller A, Rösch N, Cho G-S, Meinhardt A-K, Kabisch J, Habermann D, Böhnlein C, Brinks E, Greiner R,  
1308 Franz CMAP (2018) Influence of iodized table salt on fermentation characteristics and  
1309 bacterial diversity during sauerkraut fermentation. *Food Microbiology*, **76**, 473–480.  
1310 <https://doi.org/10/ghrq6k>

1311 National Institutes of Health Office of Dietary Supplements - Vitamin B12.

1312 Niksic M, Niebuhr SE, Dickson JS, Mendonca AF, Kozickowski JJ, Ellingson JLE (2005) Survival of  
1313 *Listeria monocytogenes* and *Escherichia coli* O157 : H7 during Sauerkraut fermentation.  
1314 *Journal of Food Protection*, **68**, 1367–1374. <https://doi.org/10/ghzr86>

1315 Ostling CE, Lindgren SE (1993) Inhibition of enterobacteria and *Listeria* growth by lactic, acetic and  
1316 formic acids. *The Journal of Applied Bacteriology*, **75**, 18–24. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2672.1993.tb03402.x)  
1317 [2672.1993.tb03402.x](https://doi.org/10.1111/j.1365-2672.1993.tb03402.x)

1318 Parente E, Zotta T, Ricciardi A (2022) A review of methods for the inference and experimental  
1319 confirmation of microbial association networks in cheese. *International Journal of Food*  
1320 *Microbiology*, **368**, 109618. <https://doi.org/10.1016/j.ijfoodmicro.2022.109618>

1321 Passos FV, Felder RM, Fleming HP, McFeeters RF, Ollis DF (2005) Dynamic model for mass transfer of  
1322 solutes in cucumber fermentation. *Journal of Food Engineering*, **68**, 297–302.  
1323 <https://doi.org/10.1016/j.jfoodeng.2004.06.002>

1324 Pederson CS, Albury MN (1969) The Sauerkraut Fermentation. *Bulletin, New York State Agricultural*  
1325 *Experiment*, 84 pages.



- 1326 Plengvidhya V, Breidt F, Lu Z, Fleming HP (2007) DNA Fingerprinting of Lactic Acid Bacteria in  
1327 Sauerkraut Fermentations. *Applied and Environmental Microbiology*, **73**, 7697–7702.  
1328 <https://doi.org/10.1128/AEM.01342-07>
- 1329 Pogačić T (2015) A methodological approach to screen diverse cheese-related bacteria for their  
1330 ability to produce aroma compounds. *Food Microbiology*, **9**. <https://doi.org/10/ghmt3p>
- 1331 Poirier S (2018) Detection of an amplification bias associated to Leuconostocaceae family with a  
1332 universal primer routinely used for monitoring microbial community structures within food  
1333 products. , **5**.
- 1334 Poirier S, Rué O, Peguilhan R, Coeuret G, Zagorec M, Champomier-Vergès M-C, Loux V, Chaillou S  
1335 (2018) Deciphering intra-species bacterial diversity of meat and seafood spoilage microbiota  
1336 using gyrB amplicon sequencing: A comparative analysis with 16S rDNA V3-V4 amplicon  
1337 sequencing. *PLOS ONE*, **13**, e0204629. <https://doi.org/10.1371/journal.pone.0204629>
- 1338 Pontonio E, Di Cagno R, Tarraf W, Filannino P, De Mastro G, Gobbetti M (2018) Dynamic and  
1339 Assembly of Epiphyte and Endophyte Lactic Acid Bacteria During the Life Cycle of *Origanum*  
1340 *vulgare* L. *Frontiers in Microbiology*, **9**. <https://doi.org/10.3389/fmicb.2018.01372>
- 1341 Potts EA, Fleming HP, McFEETERS RF, Guinnup DE (1986) Equilibration of Solutes in Nonfermenting,  
1342 Brined Pickling Cucumbers. *Journal of Food Science*, **51**, 434–439.  
1343 <https://doi.org/10.1111/j.1365-2621.1986.tb11149.x>
- 1344 Raghuvanshi R, Grayson AG, Schena I, Amanze O, Suwintono K, Quinn RA (2019) Microbial  
1345 Transformations of Organically Fermented Foods. *Metabolites*, **9**, 165.  
1346 <https://doi.org/10/ghvkgr>
- 1347 Rezac S, Kok CR, Heermann M, Hutkins R (2018) Fermented Foods as a Dietary Source of Live  
1348 Organisms. *Frontiers in Microbiology*, **9**, 1785. <https://doi.org/10.3389/fmicb.2018.01785>
- 1349 Ruiz-Roldán L, Rojo-Bezares B, Lozano C, López M, Chichón G, Torres C, Sáenz Y (2021) Occurrence of  
1350 *Pseudomonas* spp. in Raw Vegetables: Molecular and Phenotypical Analysis of Their

1351 Antimicrobial Resistance and Virulence-Related Traits. *International Journal of Molecular*  
1352 *Sciences*, **22**, 12626. <https://doi.org/10.3390/ijms222312626>

1353 Song HS, Whon TW, Kim J, Lee SH, Kim JY, Kim YB, Choi H-J, Rhee J-K, Roh SW (2020) Microbial niches  
1354 in raw ingredients determine microbial community assembly during kimchi fermentation.  
1355 *Food Chemistry*, **318**, 126481. <https://doi.org/10.1016/j.foodchem.2020.126481>

1356 Stoll DA, Müller A, Meinhardt A-K, Dötsch A, Greiner R, Kulling SE, Huch M (2020) Influence of salt  
1357 concentration and iodized table salt on the microbiota of fermented cucumbers. *Food*  
1358 *Microbiology*, **92**, 103552. <https://doi.org/10/ghrq5x>

1359 Świder O, Roszko MŁ, Wójcicki M, Szymczyk K (2020) Biogenic Amines and Free Amino Acids in  
1360 Traditional Fermented Vegetables—Dietary Risk Evaluation. *Journal of Agricultural and Food*  
1361 *Chemistry*, **68**, 856–868. <https://doi.org/10.1021/acs.jafc.9b05625>

1362 Tamang JP, Cotter PD, Endo A, Han NS, Kort R, Liu SQ, Mayo B, Westerik N, Hutkins R (2020)  
1363 Fermented foods in a global age: East meets West. *Comprehensive Reviews in Food Science*  
1364 *and Food Safety*, **19**, 184–217. <https://doi.org/10/ghmf4m>

1365 Tamang JP, Watanabe K, Holzapfel WH (2016) Review: Diversity of Microorganisms in Global  
1366 Fermented Foods and Beverages. *Frontiers in Microbiology*, **7**.  
1367 <https://doi.org/10.3389/fmicb.2016.00377>

1368 Thierry A, Baty C, Marché L, Chuat V, Picard O, Lortal S, Valence F (2023) Lactofermentation of  
1369 vegetables: An ancient method of preservation matching new trends. *Trends in Food Science*  
1370 *& Technology*, **139**, 104112. <https://doi.org/10.1016/j.tifs.2023.07.009>

1371 Thierry A, Madec M-N, Chuat V, Bage A-S, Picard O, Grondin C, Rué O, Mariadassou M, Marché L,  
1372 Valence F (2023) Microbial communities of a variety of 75 homemade fermented vegetables.  
1373 *Frontiers in Microbiology*, **14**. <https://doi.org/10.3389/fmicb.2023.1323424>

1374 Tlais AZA, Lemos Junior WJF, Filannino P, Campanaro S, Gobbetti M, Di Cagno R (2022) How  
1375 Microbiome Composition Correlates with Biochemical Changes during Sauerkraut

1376 Fermentation: a Focus on Neglected Bacterial Players and Functionalities. *Microbiology*  
1377 *Spectrum*, **10**, e00168-22. <https://doi.org/10.1128/spectrum.00168-22>

1378 Valero-Cases E, Cerda-Bernad D, Pastor J-J, Frutos M-J (2020) Non-Dairy Fermented Beverages as  
1379 Potential Carriers to Ensure Probiotics, Prebiotics, and Bioactive Compounds Arrival to the  
1380 Gut and Their Health Benefits. *Nutrients*, **12**, 1666. <https://doi.org/10.3390/nu12061666>

1381 Walther B, Chollet M (2017) Menaquinones, Bacteria, and Foods: Vitamin K2 in the Diet. In: *Vitamin*  
1382 *K2 - Vital for Health and Wellbeing*, p. . IntechOpen. <https://doi.org/10.5772/63712>

1383 Wang D, Chen G, Tang Y, Li H, Shen W, Wang M, Liu S, Qin W, Zhang Q (2020) Effects of temperature  
1384 on paocai bacterial succession revealed by culture-dependent and culture-independent  
1385 methods. *International Journal of Food Microbiology*, **317**, 108463.  
1386 <https://doi.org/10.1016/j.ijfoodmicro.2019.108463>

1387 Wang D, Chen G, Tang Y, Ming J, Huang R, Li J, Ye M, Fan Z, Chi Y, Zhang Q, Zhang W (2022) Study of  
1388 bacterial community succession and reconstruction of the core lactic acid bacteria to  
1389 enhance the flavor of paocai. *International Journal of Food Microbiology*, **375**, 109702.  
1390 <https://doi.org/10.1016/j.ijfoodmicro.2022.109702>

1391 Wang D, Chen G, Tang Y, Ming J, Huang R, Li J, Ye M, Fan Z, Yin L, Zhang Q, Zhang W (2022) Effect of  
1392 non-core microbes on the key odorants of paocai. *LWT*, **172**, 114211.  
1393 <https://doi.org/10.1016/j.lwt.2022.114211>

1394 Wieczorek MN, Drabińska N (2022) Flavour Generation during Lactic Acid Fermentation of Brassica  
1395 Vegetables—Literature Review. *Applied Sciences*, **12**, 5598.  
1396 <https://doi.org/10.3390/app12115598>

1397 Wisselink HW, Weusthuis RA, Eggink G, Hugenholtz J, Grobben GJ (2002) Mannitol production by  
1398 lactic acid bacteria: a review. *International Dairy Journal*, **12**, 151–161.  
1399 [https://doi.org/10.1016/S0958-6946\(01\)00153-4](https://doi.org/10.1016/S0958-6946(01)00153-4)

1400 World Health Organization. Regional Office for Europe (2018) *Using dietary intake modelling to*  
1401 *achieve population salt reduction: a guide to developing a country-specific salt reduction*  
1402 *model*. World Health Organization. Regional Office for Europe.

1403 Wuyts S, Van Beeck W, Allonsius CN, van den Broek MF, Lebeer S (2020) Applications of plant-based  
1404 fermented foods and their microbes. *Current Opinion in Biotechnology*, **61**, 45–52.  
1405 <https://doi.org/10.1016/j.copbio.2019.09.023>

1406 Wuyts S, Van Beeck W, Oerlemans EFM, Wittouck S, Claes IJJ, De Boeck I, Weckx S, Lievens B, De  
1407 Vuyst L, Lebeer S (2018) Carrot Juice Fermentations as Man-Made Microbial Ecosystems  
1408 Dominated by Lactic Acid Bacteria. *Applied and Environmental Microbiology*, **84**, UNSP  
1409 e00134. <https://doi.org/10.1128/AEM.00134-18>

1410 Yu AO, Leveau JHJ, Marco ML (2020) Abundance, diversity and plant-specific adaptations of plant-  
1411 associated lactic acid bacteria. *Environmental Microbiology Reports*, **12**, 16–29.  
1412 <https://doi.org/10/gk4wpq>

1413 Zabat M, Sano W, Wurster J, Cabral D, Belenky P (2018) Microbial Community Analysis of Sauerkraut  
1414 Fermentation Reveals a Stable and Rapidly Established Community. *Foods*, **7**, 77.  
1415 <https://doi.org/10.3390/foods7050077>

1416 Zacharof MP, Lovitt RW (2012) Bacteriocins Produced by Lactic Acid Bacteria a Review Article.  
1417 *APCBEE Procedia*, **2**, 50–56. <https://doi.org/10.1016/j.apcbee.2012.06.010>

1418