Effect of herb addition on spontaneous fermentation of radish (Raphanus sativus L.) roots in brine and the fate of L. monocytogenes and E. coli O157:H7

Article *in* Journal of Medicinal Plants Studies · April 2018

CITATION
1

READS
404

6 authors, including:

Spiros Paramithiotis
University of Ioannina
215 PUBLICATIONS **3,533** CITATIONS
SEE PROFILE

Sofia Pappa
Agricultural University of Athens
2 PUBLICATIONS **48** CITATIONS
SEE PROFILE

Marina Papadelli
Technological Educational Institute of Peloponnese
30 PUBLICATIONS **492** CITATIONS
SEE PROFILE

Dimitra Daferera
Agricultural University of Athens
89 PUBLICATIONS **10,884** CITATIONS
SEE PROFILE

Journal of Medicinal Plants Studies

www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
JMPS 2018; 6(2): 32-39

Received: 11-01-2018
Accepted: 13-02-2018

S Pappa
Laboratory of Food Quality Control and Hygiene, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos, Athens, Greece

M Papadelli
Department of Food Technology, Technological Educational Institute of Peloponnese, Kalamata, Greece

S Paramithiotis
Laboratory of Food Quality Control and Hygiene, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos, Athens, Greece

D Daferera
Laboratory of Chemistry, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos, Athens, Greece

MG Polissiou
Laboratory of Chemistry, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos, Athens, Greece

EH Drosinos
Laboratory of Food Quality Control and Hygiene, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos, Athens, Greece

Correspondence
S Paramithiotis
Laboratory of Food Quality Control and Hygiene, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos, Athens, Greece

Effect of herb addition on spontaneous fermentation of radish (*Raphanus sativus* L.) roots in brine and the fate of *L. monocytogenes* and *E. coli* O157:H7

S Pappa, M Papadelli, S Paramithiotis, D Daferera, MG Polissiou and EH Drosinos

Abstract
The aim of the present study was to assess the effect of mint (*Mentha* sp.), lemon thyme (*Thymus citriodorus*) and garlic (*Allium sativum*) on the spontaneous fermentation of *Raphanus sativus* roots in brine and the fate of *Listeria monocytogenes* and *Escherichia coli* O157:H7 inoculated at 4 or 6 log CFU/mL. In all cases, lactic acid bacteria prevailed from the beginning of the fermentation with *Lb. plantarum* being the dominant species at the end of it. Both *L. monocytogenes* and *E. coli* O157:H7 were not detected upon prolongation of the fermentation to 27 and 16 days regarding the inoculum level of 4 and 6 log CFU/mL, respectively. The addition of herbs into the brine, at least in the amount used, had no negative effect on radish root spontaneous fermentation. From a safety perspective, prolongation of the fermentation time may be advised for effective inactivation of *L. monocytogenes* and *E. coli* O157:H7.

Keywords: foodborne pathogens, mint, lemon thyme, garlic

Introduction
The root is the main edible part of the radish plant (*Raphanus sativus* L.), a vegetable of the Brassicaceae family, the consumption of which has been associated with positive effects on health and well-being [1, 2]. Radish roots are considered to be a good source of B-complex vitamins, vitamin C and minerals like potassium, calcium, phosphorus, magnesium etc. [3]. In India they are widely used as a medicinal plant and nowadays there is a growing interest for their use as an ingredient for the production of healthy functional foods due to a wide range of health promoting properties attributed to the presence of glucosinolates and their degradation products, as well as antioxidants like polyphenols, flavonoids and ascorbic acid [2, 4].
Fermentation is frequently employed as a processing method for the extension of shelf-life of highly perishable raw materials [5]. The consumption of fermented radish roots is currently common only in areas of China, Japan and Korea and mostly as an ingredient of Kimchi and Pao Cai [6, 7]. According to the literature, the only product that is exclusively prepared from radish roots is Sinki, a traditional food of Himalaya's area produced by pit fermentation and usually consumed in India, Nepal, and Bhutan [8, 9].
Brine fermentation is the preferred type of lactic acid fermentation in Greece, mostly due to the nature of fruits and vegetables employed. A variety of lactic acid fermented fruits and vegetables including cauliflower, asparagus and green tomatoes has been lately studied [10-12]. Only recently did lactic acid fermentation of radish roots attracted scientific attention [13] due to the nutritional value of the raw materials and the unique organoleptic properties of the final product. Spontaneous radish root fermentation was initiated by *Pediococcus pentosaceus* and then dominated by *Lactobacillus plantarum*. *Lb. brevis* was only detected during the final days of fermentation [13].
A common practice aiming at the flavor enhancement of the final product is the addition of herbs and spices, such as dill, garlic, bay leaves, thyme etc. Such an addition usually takes place during preparation of the raw materials [7, 14-16] and may affect the development of the microecosystem through the release of compounds with antimicrobial properties. It is already well documented that a lot of herbs and spices exert antimicrobial effect against foodborne pathogens and usually employed as natural preservatives during the processing of several

foods [17-21]. However, the effect on the fermentation itself, as this is assessed through the kind of predominant microbiota along with the concomitant acidity development, is often neglected.

In this study, the effect of mint, lemon thyme or garlic addition on spontaneous fermentation of radish roots in brine along with the fate of two major foodborne pathogens, namely *L. monocytogenes* and *E. coli* O157:H7, has been investigated. Especially regarding the latter, apart from the inhibitory effect of lactic acid produced during fermentation, the possibility of secretion of other antimicrobial compounds from the radish roots and the herbs immersed into the brine, which could contribute to the fate of the two pathogens, has been considered. In this frame, the aim of the present study was to investigate the impact of herb addition on spontaneous fermentation of radish roots in brine as well as the effect of the combination of the two factors -fermentation and presence of herbs- on the fate of *L. monocytogenes* and *E. coli* O157:H7.

Materials and Methods

Bacterial Strains

Two pathogens were used for the inoculation of brines used for the radish fermentation: *L. monocytogenes* strain LQC 15257 belonging to serotype 4b, previously isolated from a strawberry sample and *E. coli* O157:H7 strain NCTC 13125. Both strains were stored at -20 ºC in Brain-Heart Infusion (BHI) broth (Biolife, Milano, Italy) containing 20% glycerol (Merck, Darmstadt, Germany). Before use, the bacteria were subcultured twice in BHI broth incubated at 37 ºC for 24 h.

Pickle preparation and sampling

Fermentation of radish (*R. sativus* L.) roots was performed according to a traditional recipe that is used in southern Attica. Seven hundred grams (± 5 g) of radish roots were thoroughly washed with tap water, cut in half and submerged into 1.3 L of brine solution (5% NaCl, w/v). Three kinds of herbs and spices were added separately in the fermentation vessels by immersion of the plant leafs or cloves (in the case of garlic) in the brine, at a concentration of 1.0 % w/v: mint (*Mentha* sp.), lemon thyme (*Thymus citriodorus*) and garlic (*Allium sativum*). A fermentation vessel without any herb or spice was used as a control. Then, the brine solution was inoculated with the appropriate amount of overnight cultures of both *L. monocytogenes* and *E. coli* O157:H7 strains to reach a final level of about 4 (low pathogen inoculum) or 6 (high pathogen inoculum) log CFU/mL. The surface was covered with olive oil and the mixture left to ferment for as long as necessary at 20 ◦C. The fermentation was considered as complete when both pH and TTA values exhibited no statistically significant change between two consecutive samplings. However, fermentation was prolonged until the pathogens were not detectable in two successive samplings. Thus, brine samples were analyzed at days 0, 1, 3, 6, 9, 13, 16, 20 and 27 in the case of low pathogen inoculum and 0, 1, 3, 6, 9, 13 and 16 in the case of high pathogen inoculum. Fermentations were performed in duplicate and the average values are presented.

Physico-chemical analyses

Radish fermentation was monitored by measuring the pH value and total titratable acidity (TTA) of the brine. pH value was recorded (WTW, Weilheim, Germany) in brine samples (10 mL) aseptically derived from each fermentation jar. After the addition of 90 mL of distilled water and well stir of the sample, the acidity was titrated using 0.1 N NaOH solution to a final pH of 8.5 and the TTA was expressed in % lactic acid (%LA). All analyses were performed in duplicate and the average values are presented.

Total phenolic content

Total Phenolic Content (TPC) in radish brine samples was determined by the Folin–Ciocalteu colorimetric method based on Tawaha *et al*. [22] procedure. Briefly, 50 μL of the radish brine (after centrifugation) were added to 450 μL of deionized water and 2.5 mL of 0.2 N Folin–Ciocalteu reagent. The mixture was vortexed, and after 5 min, 2 mL of 7.5% (w/v) sodium carbonate solution were added and mixed. The absorbance of the final solution was measured at 765 nm after incubation at room temperature for 1.5 h with intermittent shaking.

A calibration curve was prepared from catechin (Fluka, Germany, (+) catechin hydrate ≥ 96.0%) water solutions at concentrations ranged from 5 to 750 μg/mL. The Total Phenolic Content in all brine samples was expressed as Catechin Equivalents (CE) in μg per mL of brine. The procedure was repeated twice for each sample and the average values are presented.

Microbiological Analyses

Microbiological analyses were performed in the brine samples throughout fermentation. Total aerobic mesophilic count, lactic acid bacteria, yeasts/moulds, pseudomonads, enterococci, and *Enterobacteriaceae* were enumerated according to Pardali *et al*. [13]. The pathogens were detected or enumerated according to ISO 11290-1:1996 and ISO 11290-2:1998 for *L. monocytogenes* and ISO 16654:2001 for *E. coli* O157:H7 [23-25]. All colonies present on MRS agar obtained during the final day of each fermentation were isolated and purified by successive subculturing. DNA extraction from the isolates was performed according to Cocolin *et al*. [26] and identification by species specific PCR according to Aymerich *et al*. [27].

Statistical Analysis

The effect of herb presence during fermentation, for each inoculum level of the pathogens, on the evolution of pH value, TTA and the dynamics of the microbial populations enumerated i.e. total aerobic mesophilic count, lactic acid bacteria, yeasts/moulds, pseudomonads, enterococci, *Enterobacteriaceae*, *L. monocytogenes* and *E. coli* O157:H7 was assessed by one-way analysis of variance (ANOVA).

Results

In Table 1, the evolution of pH value and total titrable acidity (TTA) during spontaneous radish root fermentation at 20 ºC with or without the addition of herbs and inoculated with 4 or 6 log CFU/mL of the pathogens, are shown. The initial pH value ranged from 6.53 to 6.94 and decreased to a final value ranging from 3.42 to 4.18 after 9 to 13 days. Similarly, the initial TTA values ranged from 0.008 to 0.012 % lactic acid (LA) and increased to a final value ranging from 0.26 to 0.36 % LA after 9 to 13 days. Herb addition had no statistically significant effect (P<0.05) on the evolution of pH and TTA values. On the contrary, statistically significant differences were observed in the pH and TTA values between the different inoculum levels (4 or 6 log CFU/mL) of the same treatment (without addition of herbs, addition of *Mentha* sp., *Thymus citriodorus* or *Allium sativum*) during the 1st, 3rd or 6th days of spontaneous radish root fermentation (Table 1). In all

cases, pH value was higher and TTA value was lower when the inoculum level was 4 log CFU/mL.

Total phenolic content (TPC) in brine during spontaneous radish fermentation at 20 ºC, with or without the addition of herbs and inoculated with 4 or 6 log CFU/mL of the pathogens, is presented in Table 2. The initial TPC was determined at 43.72 and 44.56 μg CE/mL at inoculum levels 4 and 6 log CFU/mL, respectively. In the first case, a statistically significant increase took place until the 6th day of fermentation when *T. citriodorus* or *A. sativum* were added and until the 9th day of fermentation when no herb or *Mentha* sp. were added. The TPC values in the final day of fermentation (27th) ranged from 343.17 to 418.17 μg CE/mL. Statistically significant differences were also observed between the different treatments of the same sampling day. This was particularly evident regarding the fermentation in the presence of *T. citriodorus*, in which TPC was higher from the respective in the absence of any herb or in the presence of *Mentha* sp. in most of the sampling days, including the final one (27th). On the contrary, TPC in the presence of *Mentha* sp. or *A. sativum* was statistically different from the respective in the absence of any herb only in the 6th day of fermentation.

Similarly, at 6 log CFU/mL pathogen inoculum level, a statistically significant increase in TPC was observed until the 6th day of fermentation. In that case, the TPC values in the final day of fermentation (16th) ranged from 360.11 to 406.22 μg CE/mL. As in the previous case, statistically significant differences were observed between the different treatments of the same sampling day in many cases without though any particular trend. Interestingly, in the final day of fermentation (16th) TPC was higher when no herb was added that with the addition of garlic.

Statistically significant differences were also detected in the TPC between the different inoculum levels (4 or 6 log CFU/mL) of the same treatment during the 3rd, 6th, 9th or 13th days of spontaneous radish root fermentation. When no herb or *Mentha* sp. were added TPC was lower at 4 log CFU/mL pathogen inoculum level compared to the respective at 6 log CFU/mL during the 3rd, 6th, and 9th days of fermentation in the first case and 3rd and 6th days in the second. Similarly, statistically significant differences were also observed in the case of *T. citriodorus* addition; more accurately TPC was lower during the 3rd day of fermentation but higher during the 6th and 13th days at 4 log CFU/mL pathogen inoculum level compared to the respective at 6 log CFU/mL.

The evolution of total aerobic mesophilic count, lactic acid bacteria, enterococci, pseudomonads, *Enterobacteriaceae*, yeasts/molds, *L. monocytogenes* and *E. coli* O15:H7 during spontaneous radish root fermentation at 20 ºC, with or without the addition of herbs and inoculated with 4 CFU/mL of the pathogens are presented in Table 3. The initial total aerobic mesophilic count ranged from 6.77 to 6.9 log CFU/mL; it was increased to 7.11-7.91 log CFU/mL after 6 days and then reduced to 5.91-6.60 log CFU/mL by the end of fermentation. The initial lactic acid bacteria and enterococci populations were rather low; they ranged between 2.27 and 3.07 log CFU/mL and between 1.77 and 2.81 log CFU/mL, respectively. Both populations increased to 6.95-8.02 log CFU/mL and 6.91 to 7.97 log CFU/mL after 6 to 9 days and then reduced to 5.96-6.61 log CFU/mL and 5.32-6.51 log CFU/mL by the end of fermentation, respectively. Pseudomonads and yeasts/molds were present during the first days of fermentation but their population declined below enumeration limit before the end of it. More accurately, they were initially enumerated at 3.77-3.90 and 3.34-3.44 log CFU/mL, respectively. Then, pseudomonads population declined and was below enumeration limit after 13 days when no herb was added to the fermentation, 3 days when *Mentha* sp. was added and 6 days when *T. citriodorus* or *A. sativum* were added. The population of yeasts/molds increased during the first day of fermentation but then reduced below enumeration limit after 6 days when *Mentha* sp. or *T. citriodorus* were added of 9 days when no herb or *A. sativum* were added. The evolution of the *Enterobacteriaceae* population was quite interesting. It was initially enumerated at 5.00-5.44 log CFU/mL, increased during the first day of fermentation but then decreased below enumeration limit after 13 days of fermentation when no herb was added and 16 days of fermentation when *T. citriodorus* or *A. sativum* were added. Interestingly, when *Mentha* sp. was added, *Enterobacteriaceae* population was enumerated until the end of fermentation. *Listeria monocytogenes* population increased during the first days of fermentation. More accurately, it increased to 4.90-6.13 log CFU/mL during the first three days of fermentation and then decreased to below enumeration limit after 9 days in the presence of *T. citriodorus* or *A. sativum*, 16 days in the absence of herbs and 20 days in the presence of *Mentha* sp. In all cases, absence of the pathogen was verified during the 20th day of fermentation. Similarly, *Escherichia coli* O157:H7 population also increased during the first days of fermentation. More accurately, it reached 5.39 log CFU/mL during the first three days of fermentation in the absence of herbs and to 5.50-6.12 log CFU/mL during the first day in the presence of herbs. Then, the population decreased below enumeration limit after 6 days in the presence of *T. citriodorus* or *Mentha* sp. and 9 days in the presence of *A. sativum* and in the absence of herbs. Absence of the pathogen was verified after 9 days of fermentation in the presence of *A. sativum*, 13 days in the presence of *T. citriodorus* and 16 days in the presence of *Mentha* sp. and in the absence of herbs.

In Table 4, the evolution of total aerobic mesophilic count, lactic acid bacteria, enterococci, pseudomonads, *Enterobacteriaceae*, yeasts/molds, *L. monocytogenes* and *E. coli* O15:H7 during spontaneous radish root fermentation at 20 ºC, with or without the addition of herbs and inoculated with 6 log CFU/mL of the pathogens, are presented. The initial total aerobic mesophilic count ranged from 6.18 to 6.77 log CFU/mL; it increased to 7.54-7.99 log CFU/mL after 6 to 9 days and then reduced to 6.16-7.46 log CFU/mL by the end of fermentation. The initial lactic acid bacteria and enterococci populations were enumerated between 2.54 and 4.09 log CFU/mL and between 1.80 and 2.63 log CFU/mL, respectively. Both populations increased to 7.46-8.03 log CFU/mL and 7.02 to 7.77 log CFU/mL after 6 to 9 days and then reduced to 6.17-6.90 log CFU/mL and 4.47-6.23 log CFU/mL by the end of fermentation, respectively. Pseudomonads, *Enterobacteriaceae* and yeasts/molds were present during the first days of fermentation. More accurately, they were initially enumerated at 3.05-3.30, 5.21-5.75 and 3.12-3.60 log CFU/mL, respectively. Then, in all case yeasts/molds population decreased below enumeration limit after 3 days of fermentation, while *Enterobacteriaceae* population increased during the first day of fermentation but reduced below enumeration limit after 6 days of fermentation. In the case of pseudomonads population, it declined below enumeration limit after 3 days of fermentation when no herb or *Mentha* sp. were added and after 6 days of fermentation when *T. citriodorus* or *A. sativum* were added. An increase in the *L. monocytogenes* population to 6.56-7.10 log CFU/mL

was noticed during the first day of fermentation; then it decreased to below enumeration limit after 13 days in the absence of any herb and in the presence of *Mentha* sp. and *T. citriodorus*, and after 6 days in the presence of *A. sativum*. In both cases, absence of the pathogen was also verified during the 13th and the 9th days of fermentation, respectively. As in the previous case, *E. coli* O157:H7 population increased to 5.95-7.13 log CFU/mL during the first day of fermentation and then decreased below enumeration limit after three days in the absence of herbs and in the presence of *Mentha* sp., and after 6 days in the presence of *T. citriodorus* or *A. sativum*. Absence of the pathogen was verified after 9 days of fermentation in all cases.

In many cases, statistically significant differences were observed between the populations enumerated during spontaneous radish root fermentation under the same treatment but different pathogen inoculum level or under different treatments but the same pathogen inoculum level. Despite these differences, no particular trend different from the ones already described was identified.

A total of 120 colonies grown on MRS agar during the final day of each fermentation were isolated and subjected to species-specific PCR. The required amplicon of 219 bp was detected in all cases and thus all isolates were classified as *Lb. plantarum*.

Table 1: Evolution of pH and total titrable acidity (TTA) during spontaneous radish root fermentation in brine at 20 ºC, with or without the addition of herbs and inoculated with 4 or 6 log CFU/mL *L. monocytogenes* and *E. coli* O157:H7

Time (days)	**inoculated with 4 log CFU/mL of *L. monocytogenes* and *E. coli* O157:H7**							
	pH values				TTA values			
	Without any herb	*Mentha* sp.	*T. citriodorus*	*A. sativum*	Without any herb	*Mentha* sp.	*T. citriodorus*	*A. sativum*
0	6.87 (0.12)$^{a, n}$	6.53 (0.11)$^{a, n}$	6.94 (0.12)$^{a, n}$	6.71 (0.11)$^{a, n}$	0.010 (0.002)$^{a, n}$	0.012 (0.003)$^{a, n}$	0.008 (0.001)$^{a, n}$	0.009 (0.002)$^{a, n}$
1	5.82 (0.14)$^{a, y}$	5.92 (0.12)$^{a, y}$	5.89 (0.13)$^{a, y}$	6.01 (0.24)$^{a, y}$	0.025 (0.008)$^{a, y}$	0.029 (0.002)$^{a, y}$	0.028 (0.003)$^{a, y}$	0.018 (0.005)$^{a, y}$
3	5.36 (0.17)$^{a, y}$	5.43 (0.23)$^{a, n}$	5.37 (0.18)$^{a, n}$	5.40 (0.17)$^{a, n}$	0.117 (0.012)$^{a, y}$	0.072 (0.005)$^{a, y}$	0.099 (0.004)$^{a, y}$	0.072 (0.006)$^{a, y}$
6	3.65 (0.42)$^{a, n}$	4.03 (0.28)$^{a, n}$	4.53 (0.25)$^{a, n}$	3.90 (0.44)$^{a, n}$	0.198 (0.051)$^{a, n}$	0.153 (0.062)$^{a, y}$	0.198 (0.024)$^{a, n}$	0.211 (0.021)$^{a, n}$
9	3.44 (0.23)$^{a, n}$	3.92 (0.27)$^{a, n}$	4.02 (0.27)$^{a, n}$	4.18 (0.23)$^{a, n}$	0.324 (0.032)$^{a, n}$	0.261 (0.052)$^{a, n}$	0.288 (0.032)$^{a, n}$	0.283 (0.035)$^{a, n}$
13	4.07 (0.38)$^{a, n}$	3.90 (0.16)$^{a, n}$	4.15 (0.22)$^{a, n}$	4.17 (0.34)$^{a, n}$	0.261 (0.018)$^{a, n}$	0.306 (0.028)$^{a, n}$	0.283 (0.042)$^{a, n}$	0.297 (0.036)$^{a, n}$
16	3.44 (0.12)$^{a, n}$	4.18 (0.40)$^{a, n}$	3.99 (0.20)$^{a, n}$	4.10 (0.18)$^{a, n}$	0.288 (0.008)$^{a, n}$	0.306 (0.030)$^{a, n}$	0.279 (0.050)$^{a, n}$	0.297 (0.050)$^{a, n}$
20	3.54 (0.22)$^{a, NA}$	3.81 (0.36)$^{a, NA}$	3.93 (0.30)$^{a, NA}$	4.17 (0.32)$^{a, NA}$	0.288 (0.021)$^{a, NA}$	0.315 (0.009)$^{a, NA}$	0.297 (0.030)$^{a, NA}$	0.301 (0.040)$^{a, NA}$
27	3.75 (0.20)$^{a, NA}$	3.90 (0.25)$^{a, NA}$	3.95 (0.11)$^{a, NA}$	3.92 (0.25)$^{a, NA}$	0.270 (0.009)$^{a, NA}$	0.297 (0.024)$^{a, NA}$	0.297 (0.020)$^{a, NA}$	0.315 (0.020)$^{a, NA}$
	inoculated with 6 log CFU/mL of *L. monocytogenes* and *E. coli* O157:H7							
	pH values				TTA values			
	Without any herb	*Mentha* sp.	*T. citriodorus*	*A. sativum*	Without any herb	*Mentha* sp.	*T. citriodorus*	*A. sativum*
0	6.70 (0.12)^{a}	6.80 (0.12)^{a}	6.80 (0.12)^{a}	6.70 (0.13)^{a}	0.010 (0.003)^{a}	0.011 (0.002)^{a}	0.010 (0.003)^{a}	0.009 (0.004)^{a}
1	5.35 (0.18)^{a}	5.50 (0.13)^{a}	5.47 (0.14)^{a}	5.48 (0.18)^{a}	0.042 (0.001)^{a}	0.045 (0.002)^{a}	0.045 (0.002)^{a}	0.035 (0.009)^{a}
3	4.95 (0.11)^{a}	5.04 (0.15)^{a}	5.04 (0.23)^{a}	5.12 (0.11)^{a}	0.143 (0.004)^{a}	0.134 (0.015)^{a}	0.153 (0.015)^{a}	0.125 (0.011)^{a}
6	3.44 (0.46)^{a}	4.66 (0.32)^{a}	4.66 (0.41)^{a}	4.36 (0.50)^{a}	0.251 (0.001)^{a}	0.278 (0.020)^{a}	0.176 (0.056)^{a}	0.248 (0.030)^{a}
9	3.78 (0.24)^{a}	4.12 (0.34)^{a}	4.12 (0.36)^{a}	4.00 (0.36)^{a}	0.360 (0.019)^{a}	0.327 (0.034)^{a}	0.285 (0.041)^{a}	0.291 (0.040)^{a}
13	3.82 (0.26)^{a}	3.68 (0.42)^{a}	3.68 (0.31)^{a}	3.42 (0.24)^{a}	0.280 (0.021)^{a}	0.290 (0.036)^{a}	0.327 (0.023)^{a}	0.318 (0.018)^{a}
16	4.13 (0.24)^{a}	4.12 (0.25)^{a}	4.12 (0.25)^{a}	3.95 (0.18)^{a}	0.285 (0.040)^{a}	0.314 (0.024)^{a}	0.340 (0.041)^{a}	0.352 (0.030)^{a}

The average values are presented; standard deviation is given in parenthesis

In each sampling day, different superscript letters (a, b, c and d) denote significant differences (P<0.05) between the different treatments (without addition of herbs, addition of *Mentha* sp., *T. citriodorus* or *A. sativum*) of each parameter (pH or TTA) and inoculum level (4 or 6 log CFU/mL) during spontaneous radish root fermentation in brine at 20 °C.

In each sampling day, different superscript letters (y or n) denote significant differences (P<0.05; y: detection of statistically significant differences; n: no detection of statistically significant differences) between the different inoculum level (4 or 6 log CFU/mL) of the same treatment (without addition of herbs, addition of *Mentha* sp., *T. citriodorus* or *A. sativum*) of each parameter (pH or TTA) during spontaneous radish root fermentation in brine at 20 °C.

NA: Not Applicable

Table 2: Evolution of total phenolic content in brine (in µg CE/mL) during spontaneous radish root fermentation in brine at 20 ºC, with or without the addition of herbs and inoculated with 4 or 6 log CFU/mL *L. monocytogenes* and *E. coli* O157:H7

Time (days)	**inoculated with 4 log CFU/mL of *L. monocytogenes* and *E. coli* O157:H7**			
	Without any herb	*Mentha* sp.	*T. citriodorus*	*A. sativum*
0	43.72 (24.41)			
1	54.83(10.74)$^{a, n}$	52.33 (19.92)$^{a, n}$	54.83 (18.86)^{a,n}	60.67 (23.35)$^{a, n}$
3	82.61 (33.04)$^{a, y}$	110.11 (30.97)$^{ab, y}$	117.89 (29.82)$^{ab, y}$	134.56 (25.57)$^{b, n}$
6	243.17 (24.70)$^{a, y}$	186.59 (13.21)$^{b, y}$	354.28 (24.71)$^{c, y}$	333.17 (32.33)$^{c, n}$
9	308.17 (28.07)$^{a, y}$	292.61 (22.19)$^{a, n}$	378.44 (34.51)$^{b, n}$	330.94 (52.78)$^{ab, n}$
13	354.83 (32.22)$^{a, n}$	335.39 (36.05)$^{a, n}$	422.06 (41.63)$^{b, y}$	386.78 (54.78)$^{ab, n}$
16	376.78 (31.14)$^{a, n}$	374.00 (30.48)$^{a, n}$	409.83 (57.17)$^{a, n}$	401.78 (46.32)$^{a, n}$
20	338.72 (8.81)$^{a, NA}$	347.06 (29.29)$^{a, NA}$	446.78 (36.27)$^{b, NA}$	382.06 (55.08)$^{ab, NA}$
27	343.17 (27.99)$^{a, NA}$	346.50 (37.36)$^{a, NA}$	418.17 (29.91)$^{b, NA}$	378.17 (37.84)$^{ab, NA}$
	inoculated with 6 log CFU/mL of *L. monocytogenes* and *E. coli* O157:H7			
	Without any herb	*Mentha* sp.	*T. citriodorus*	*A. sativum*
0	44.56 (3.33)			
1	65.67 (29.90)^{a}	76.78 (22.68)^{a}	80.11 (25.53)^{a}	78.31 (31.23)^{a}
3	222.06 (44.25)^{a}	170.39 (17.60)^{a}	166.50 (22.13)^{a}	164.28 (23.57)^{a}
6	384.83 (47.43)^{a}	327.06 (63.35)^{ab}	299.56 (33.56)^{b}	321.22 (30.69)^{ab}

9	363.72 (24.34)[a]	329.56 (26.71)[a]	339.56 (71.42)[a]	329.00 (17.43)[a]
13	359.28 (16.26)[a]	325.11 (22.12)[b]	326.22 (52.63)[ab]	314.56 (40.08)[ab]
16	406.22 (11.82)[a]	380.11 (33.68)[ab]	393.44 (27.01)[ab]	360.11 (35.11)[b]

The average values are presented; standard deviation is given in parenthesis

In each sampling day, different superscript letters (a, b, c and d) denote significant differences (P<0.05) between the different treatments (without addition of herbs, addition of *Mentha* sp., *T. citriodorus* or *A. sativum*) of each inoculum level (4 or 6 log CFU/mL) during spontaneous radish root fermentation in brine at 20 °C.

In each sampling day, different superscript letters (y or n) denote significant differences (P<0.05; y: detection of statistically significant differences; n: no detection of statistically significant differences) between the different inoculum level (4 or 6 log CFU/mL) of the same treatment (without addition of herbs, addition of *Mentha* sp., *T. citriodorus* or *A. sativum*) of each parameter (pH or TTA) during spontaneous radish root fermentation in brine at 20 °C.

NA: Not Applicable

Table 3: Evolution of total aerobic mesophilic count, lactic acid bacteria, enterococci, pseudomonads, *Enterobacteriaceae*, yeasts/molds, *L. monocytogenes* and *E. coli* O15:H7 during spontaneous radish root fermentation at 20 °C, with or without the addition of herbs and inoculated with 4 log CFU/mL of the pathogens.

Time (days)	TAMC	LAB	pseudomonads	*Enterobacteriaceae*	Yeasts/molds	enterococci	*L. monocytogenes*	*E. coli* O157:H7
Without any herb								
0	6.90 (0.35)[a]	2.36 (0.25)[a]	3.77 (0.42)[a]	5.44 (0.36)[a]	3.44 (0.41)[a]	2.39 (0.33)[a]	4.25 (0.17)[a]	4.12 (0.41)[a]
1	7.46 (0.63)[a]	4.02 (0.42)[a]	3.32 (0.52)[a]	6.60 (0.45)[a]	4.30 (0.53)[a]	4.98 (0.42)[a]	4.80 (0.36)[a]	4.45 (0.25)[a]
3	7.43 (0.54)[a]	5.37 (0.52)[a]	2.79 (0.38)[a]	6.69 (0.28)[a]	2.83 (0.29)[a]	6.17 (0.25)[a]	5.13 (0.25)[a]	5.39 (0.33)[a]
6	7.47 (0.48)[a]	7.43 (0.42)[ab]	3.12 (0.42)	4.95 (0.52)[ab]	2.07 (0.54)[a]	6.91 (0.51)[a]	3.07 (0.23)[a]	3.14 (0.24)[a]
9	6.38 (0.20)[a]	6.52 (0.33)[a]	3.43 (0.33)	4.24 (0.38)[ab]	<2	5.98 (0.44)[ab]	2.45 (0.41)[a]	presence
13	6.26 (0.40)[a]	6.25 (0.59)[a]	<2	<1	<2	5.78 (0.21)[a]	1.45 (0.22)[a]	presence
16	6.03 (0.25)[a]	6.11 (0.31)[a]	<2	<1	<2	5.57 (0.25)[a]	presence	absence
20	6.01 (0.60)[a]	6.09 (0.33)[a]	<2	<1	<2	5.20 (0.20)[a]	absence	absence
27	5.98 (0.25)[a]	5.96 (0.47)[a]	<2	<1	<2	5.32 (0.32)[a]	absence	absence
Addition of *Mentha* sp.								
0	6.77 (0.41)[a]	2.60 (0.42)[ab]	3.90 (0.52)[a]	5.41 (0.42)[a]	3.34 (0.33)[a]	1.84 (0.20)[b]	4.03 (0.34)[a]	4.24 (0.14)[a]
1	7.03 (0.22)[a]	4.90 (0.38)[b]	3.84 (0.36)[a]	6.39 (0.52)[a]	4.73 (0.42)[ab]	3.60 (0.23)[b]	4.80 (0.41)[a]	5.50 (0.18)[b]
3	6.95 (0.30)[a]	5.56 (0.18)[a]	<2	6.36 (0.33)[ab]	3.57 (0.44)[b]	5.95 (0.33)[a]	6.13 (0.25)[b]	4.67 (0.32)[b]
6	7.11 (0.52)[a]	6.69 (0.33)[a]	<2	5.33 (0.47)[a]	<2	6.91 (0.34)[a]	3.07 (0.33)[a]	presence
9	6.78 (0.49)[ab]	6.95 (0.54)[a]	<2	4.97 (0.59)[b]	<2	5.30 (0.58)[a]	2.05 (0.24)[a]	presence
13	6.76 (0.35)[a]	6.51 (0.42)[a]	<2	4.04 (0.31)[a]	<2	6.01 (0.32)[a]	2.38 (0.15)[b]	presence
16	6.38 (0.38)[ab]	6.36 (0.33)[a]	<2	2.01 (0.25)	<2	5.78 (0.42)[a]	1.30 (0.20)	absence
20	6.12 (0.46)[a]	6.19 (0.20)[a]	<2	2.56 (0.31)	<2	5.79 (0.47)[ab]	absence	absence
27	6.10 (0.44)[ab]	6.09 (0.45)[a]	<2	2.98 (0.17)	<2	5.77 (0.44)[a]	absence	absence
Addition of *T. citriodorus*								
0	6.84 (0.44)[a]	2.27 (0.45)[a]	3.77 (0.42)[a]	5.34 (0.42)[a]	3.39 (0.25)[a]	2.81 (0.42)[a]	4.22 (0.20)[a]	4.05 (0.25)[a]
1	7.46 (0.20)[a]	5.17 (0.25)[bc]	3.90 (0.25)[a]	6.92 (0.37)[a]	5.25 (0.42)[b]	3.84 (0.25)[b]	5.15 (0.31)[a]	6.12 (0.42)[c]
3	7.34 (0.18)[a]	6.86 (0.33)[b]	3.03 (0.37)[a]	6.21 (0.25)[b]	3.00 (0.24)[ab]	7.21 (0.51)[b]	6.13 (0.42)[b]	5.65 (0.26)[a]
6	7.68 (0.33)[a]	7.77 (0.45)[b]	<2	4.36 (0.42)[b]	<2	7.36 (0.44)[ab]	4.39 (0.18)[b]	presence
9	6.90 (0.28)[b]	6.90 (0.25)[a]	<2	3.34 (0.64)[a]	<2	6.39 (0.49)[b]	presence	presence
13	6.45 (0.5)[a]	6.46 (0.36)[a]	<2	3.77 (0.3)[a]	<2	6.13 (0.36)[a]	presence	absence
16	6.49 (0.22)[b]	6.46 (0.12)[a]	<2	<1	<2	6.13 (0.45)[a]	presence	absence
20	6.17 (0.41)[a]	6.19 (0.42)[a]	<2	<1	<2	6.01 (0.45)[b]	absence	absence
27	5.91 (0.25)[a]	6.00 (0.52)[a]	<2	<1	<2	5.77 (0.20)[a]	absence	absence
Addition of *A. sativum*								
0	6.90 (0.42)[a]	3.07 (0.32)[b]	3.84 (0.52)[a]	5.00 (0.28)[a]	3.39 (0.25)[a]	1.77 (0.25)[b]	4.20 (0.22)[a]	4.19 (0.22)[a]
1	7.36 (0.50)[a]	5.79 (0.22)[c]	3.90 (0.38)[a]	6.77 (0.42)[a]	4.54 (0.36)[ab]	3.77 (0.36)[b]	4.57 (0.32)[a]	5.96 (0.21)[c]
3	7.11 (0.36)[a]	6.71 (0.52)[b]	1.77 (0.45)[b]	6.19 (0.41)[ab]	3.77 (0.42)[b]	6.47 (0.52)[ab]	4.90 (0.27)[a]	4.67 (0.32)[b]
6	7.91 (0.38)[a]	8.02 (0.44)[b]	<2	4.60 (0.63)[ab]	2.85 (0.52)[a]	7.97 (0.44)[b]	3.00 (0.24)[a]	3.07 (0.41)[a]
9	7.36 (0.52)[b]	7.04 (0.23)[a]	<2	4.43 (0.52)[b]	<2	7.26 (0.60)[b]	presence	absence
13	6.26 (0.24)[a]	6.14 (0.50)[a]	<2	4.21 (0.47)[a]	<2	6.16 (0.20)[a]	presence	absence
16	5.98 (0.60)[ab]	6.00 (0.42)[a]	<2	<1	<2	5.89 (0.28)[a]	presence	absence
20	6.21 (0.24)[a]	6.19 (0.33)[a]	<2	<1	<2	5.95 (0.36)[b]	absence	absence
27	6.60 (0.20)[b]	6.61 (0.22)[a]	<2	<1	<2	6.51 (0.24)[b]	absence	absence

TAMC: Total Aerobic Mesophilic Count; LAB: Lactic Acid Bacteria

microbial population are presented in log CFU/mL, standard deviation is given in parenthesis

in each column, different superscript letters (a, b, c and d) denote significant differences (P<0.05) between the different treatments (without addition of herbs, addition of *Mentha* sp., *T. citriodorus* or *A. sativum*) during spontaneous radish root fermentation in brine at 20 °C.

Table 4: Evolution of total aerobic mesophilic count, lactic acid bacteria, enterococci, pseudomonads, *Enterobacteriaceae*, yeasts/molds, *L. monocytogenes* and *E. coli* O15:H7 during spontaneous radish root fermentation at 20 °C, with or without the addition of herbs and inoculated with 6 log CFU/mL of the pathogens.

Time (days)	TAMC	LAB	pseudomonads	*Enterobacteriaceae*	Yeasts/molds	enterococci	*L. monocytogenes*	*E. coli* O157:H7
Without any herb								
0	6.50 (0.33)[a, n]	3.51 (0.42)[a, y]	3.25 (0.35)[a, n]	5.75 (0.35)[a, n]	3.39 (0.47)[a, n]	1.80 (0.21)[a, n]	5.77 (0.24)[a]	6.14 (0.21)[a]
1	6.54 (0.42)[a, n]	5.60 (0.36)[a, y]	3.00 (0.42)[a, n]	6.47 (0.28)[a, n]	3.30 (0.36)[a, y]	4.12 (0.41)[a, n]	6.56 (0.31)[a]	6.59 (0.35)[a]
3	7.07 (0.35)[a, n]	7.07 (0.18)[a, y]	<2	4.47 (0.36)[a, y]	<2	6.04 (0.25)[a, n]	5.69 (0.22)[a]	presence
6	7.90 (0.52)[ab, n]	8.03 (0.25)[a, n]	<2	<1	<2	6.86 (0.33)[ab, n]	3.30 (0.27)[a]	presence

9	6.77 (0.20)$^{a, n}$	7.14 (0.29)$^{a, n}$	<2	<1	<2	7.02 (0.34)$^{a, y}$	2.79 (0.10)^{a}	absence
13	6.78 (0.58)$^{ab, n}$	7.00 (0.36)$^{ab, n}$	<2	<1	<2	6.07 (0.52)$^{a, n}$	absence	absence
16	6.68 (0.42)$^{ab, n}$	6.62 (0.42)$^{a, n}$	<2	<1	<2	6.23 (0.42)$^{a, n}$	absence	absence
Addition of *Mentha* sp.								
0	6.23 (0.60)$^{a, n}$	2.68 (0.34)$^{b, n}$	3.30 (0.41)$^{a, n}$	5.56 (0.32)$^{a, n}$	3.60 (0.40)$^{a, n}$	2.07 (0.20)$^{ab, n}$	5.84 (0.31)^{a}	6.14 (0.31)^{a}
1	6.46 (0.52)$^{a, n}$	4.87 (0.25)$^{b, n}$	3.29 (0.36)$^{a, n}$	6.32 (0.38)$^{a, n}$	3.60 (0.33)$^{ab, y}$	4.56 (0.35)$^{ab, y}$	6.89 (0.25)^{a}	7.13 (0.28)^{a}
3	6.25 (0.42)$^{b, n}$	6.00 (0.53)$^{b, n}$	<2	3.44 (0.42)$^{b, y}$	<2	5.80 (0.25)$^{a, n}$	5.47 (0.35)^{a}	presence
6	7.30 (0.36)$^{a, n}$	7.46 (0.49)$^{a, n}$	<2	<1	<2	6.60 (0.42)$^{a, n}$	4.32 (0.44)^{b}	presence
9	7.54 (0.35)$^{b, n}$	7.62 (0.44)$^{a, n}$	<2	<1	<2	7.47 (0.60)$^{a, y}$	2.55 (0.32)^{a}	absence
13	7.43 (0.52)$^{a, n}$	7.53 (0.21)$^{a, y}$	<2	<1	<2	5.00 (0.24)$^{b, y}$	absence	absence
16	7.36 (0.40)$^{a, y}$	6.90 (0.37)$^{a, n}$	<2	<1	<2	4.47 (0.35)$^{b, y}$	absence	absence
Addition of *T. citriodorus*								
0	6.18 (0.34)$^{a, n}$	2.54 (0.33)$^{b, n}$	3.28 (0.54)$^{a, n}$	5.21 (0.24)$^{a, n}$	3.12 (0.54)$^{a, n}$	2.34 (0.35)$^{ab, n}$	5.84 (0.18)^{a}	6.16 (0.14)^{a}
1	6.28 (0.52)$^{a, y}$	5.97 (0.42)$^{a, y}$	3.33 (0.47)$^{a, n}$	6.42 (0.44)$^{a, n}$	3.90 (0.23)$^{b, y}$	4.69 (0.45)$^{ab, y}$	6.65 (0.32)^{a}	6.39 (0.22)^{ab}
3	6.65 (0.32)$^{ab, y}$	6.77 (0.33)$^{ab, n}$	2.69 (0.32)$^{a, n}$	3.89 (0.38)$^{ab, y}$	<2	5.65 (0.52)$^{a, y}$	5.84 (0.32)^{a}	3.30 (0.08)^{a}
6	7.88 (0.54)$^{ab, n}$	7.83 (0.52)$^{a, n}$	<2	<1	<2	7.55 (0.45)$^{b, n}$	5.18 (0.31)^{c}	presence
9	7.56 (0.25)$^{b, y}$	7.60 (0.41)$^{a, n}$	<2	<1	<2	7.38 (0.60)$^{a, n}$	3.47 (0.30)^{b}	absence
13	6.68 (0.62)$^{ab, n}$	6.49 (0.42)$^{b, n}$	<2	<1	<2	5.69 (0.24)$^{a, n}$	absence	absence
16	7.46 (0.50)$^{a, y}$	6.41 (0.28)$^{a, n}$	<2	<1	<2	5.14 (0.35)$^{b, y}$	absence	absence
Addition of *A. sativum*								
0	6.77 (0.32)$^{a, n}$	4.09 (0.25)$^{a, y}$	3.05 (0.36)$^{a, n}$	5.36 (0.52)$^{a, n}$	3.60 (0.47)$^{a, n}$	2.63 (0.42)$^{b, y}$	5.84 (0.24)^{a}	5.80 (0.34)^{a}
1	6.32 (0.24)$^{a, y}$	5.26 (0.25)$^{ab, n}$	3.84 (0.47)$^{a, n}$	6.35 (0.37)$^{a, n}$	3.60 (0.45)$^{ab, y}$	5.12 (0.51)$^{b, y}$	7.10 (0.42)^{a}	5.95 (0.12)^{b}
3	6.62 (0.52)$^{ab, n}$	6.47 (0.14)$^{ab, n}$	2.25 (0.42)$^{a, n}$	4.48 (0.42)$^{a, y}$	<2	6.12 (0.15)$^{a, n}$	4.79 (0.25)^{b}	3.30 (0.28)^{a}
6	7.99 (0.25)$^{b, n}$	7.94 (0.33)$^{a, n}$	<2	<1	<2	7.77 (0.60)$^{b, n}$	presence	presence
9	6.53 (0.48)$^{a, n}$	6.63 (0.21)$^{b, n}$	<2	<1	<2	6.78 (0.25)$^{a, n}$	absence	absence
13	6.44 (0.36)$^{b, n}$	6.30 (0.44)$^{b, n}$	<2	<1	<2	5.30 (0.25)$^{ab, y}$	absence	absence
16	6.16 (0.21)$^{b, n}$	6.17 (0.41)$^{a, n}$	<2	<1	<2	5.95 (0.58)$^{ab, n}$	absence	absence

TAMC: Total Aerobic Mesophilic Count; LAB: Lactic Acid Bacteria

microbial population are presented in log CFU/mL, standard deviation is given in parenthesis

in each column, different superscript letters (a, b, c and d) denote significant differences (P<0.05) between the different treatments (without addition of herbs, addition of *Mentha* sp., *T. citriodorus* or *A. sativum*) during spontaneous radish root fermentation in brine at 20 °C.

in each column, different superscript letters (y or n) denote significant differences (P<0.05; y: detection of statistically significant differences; n: no detection of statistically significant differences) between the different inoculum level (4 or 6 log CFU/mL) of the same treatments (without addition of herbs, addition of *Mentha* sp., *T. citriodorus* or *A. sativum*) during spontaneous radish root fermentation in brine at 20 °C

Discussion

The outcome of spontaneous fermentations is determined by the environmental conditions such as salt concentration and temperature as well as the indigenous microbial populations of the raw materials. Lactic acid bacteria usually constitute only a minor portion of the initial microecosystem but tend to prevail due to their metabolic capacity [5]. During this study, pH and TTA were continuously monitored as quality parameters depicting the fermentation process. Moreover, the composition of the lactic acid bacteria microbiota during the final day of fermentation was also assessed. Spontaneous fermentation of *R. sativus* roots at 20 ºC is usually completed after 11 to 17 days [13]. This was also the case in the present study suggesting that the amount of the aromatic plants added and the population of the pathogens inoculated had no effect on the outcome of the fermentation. Indeed, similar conclusion was drawn by Paramithiotis *et al*. [28] when *L. monocytogenes* and *Salmonella* Typhimurium were added from the beginning of spontaneous cauliflower fermentation. Furthermore, dominance of *Lb. plantarum* ensured that the fermentation was not diverted. *Lb. plantarum* is generally characterized by the ability to rapidly adapt to dynamic environmental conditions [29] and therefore it is very often reported to dominate such fermentations [9, 30, 31]. As far as acidity development was concerned, it was slower when the pathogens were inoculated at 4 log CFU/g compared to the respective at 6 log CFU/g pathogen inoculum level. This may be either due to differences in the composition of the LAB microecosystem before *Lb. plantarum* dominance or to the higher pathogen inoculum itself. Indeed, both pathogens are capable of catabolizing carbohydrates to organic acids affecting thus acidity development [32, 33], especially in the early stages of fermentation. Concomitantly, the delayed inactivation particularly of *Enterobacteriaceae* and secondarily of pseudomonads may be due to this slower acidity development and a transient induction of acid tolerance response [34]. The inoculum size affected the survival of the pathogen. Indeed, when the pathogens were inoculated at 4 log CFU/mL, a longer fermentation time was required until they were undetectable by selective enrichment. Similar effect was also observed by Cho *et al*. [35] during radish kimchi fermentation inoculated with *E. coli* O157:H7 and cabbage kimchi fermentation inoculated with *L. monocytogenes*. This effect has been extensively studied, especially when the minimum inhibitory concentration of inhibitory compounds or when growth/no growth interface under specified conditions were assessed [36-38]. Several explanations have been offered in order to clarify the so-called inoculum effect; some of them may be generally applied [39] while others may refer only to specific levels of inocula [40, 41]. However, this effect was not further assessed in the present study, and thus it is not clear whether it is due to the slower acidity development or to the inoculum effect mentioned above.

Radish roots are a natural source of various polyphenols, mainly belonging to phenolic acids and flavonoids [2, 4]. Both groups of compounds are usually found in plant sources in the form of glycosides [42]. Water extracts of radish roots have been reported to be rich in catechin and sinapic acid [2]; therefore, release of such water-soluble compounds in the brine during radish root fermentation is possible. Indeed, Jing *et al*. [43] reported the detection of 4-hydroxybenzoic, gentisic, vanillic, syringic, *p*-coumaric, ferulic, sinapic and salicylic acids, as well as flavonoids, mainly kaempherol derivatives and anthocyanins in red radish brines during lactic acid fermentation.

Total phenolic content in radish brine is associated to the polyphenolic content and composition of the initial source.

Jing *et al*. [43] reported that TPC in radish brine remained between 206–208 μg/mL throughout the 14 days of fermentation, indicating that changes in total phenolics were not significant. On the contrary, a gradual and significant increase in TPC was observed in the present study; TPC at the end of fermentation was 5 to 6 times higher than the respective original. This gradual increase of TPC in brine could be the result either of their controlled extraction from the initial plant material or of their glycosides' hydrolysis, due to the decrease of the pH value, which leads to the releasing of the hydroxyl groups of aromatic rings, which can enhance quantitatively the phenolic compounds based to the complexation method used for their determination.

Mint and lemon thyme are also well known natural sources of phenolic compounds. Methanolic extracts of six *Mentha* species have been reported to contain phenolics ranged from 14.66 to 43.21 mg Gallic Acid Equivalent per g of dry material [44]. Among the studied mint species, *M. aquatica*, *M. arvensis* and *M. piperita* have been reported as the richest. TPC in water extract of *M. piperita* was estimated at 40.7 mg GAE/g, with ferulic, hesperidin, ellagic acid, and sinapic acid being the major compounds [45]. Regarding lemon thyme, the phenolic composition of its ethanolic extract has been found to be rich in rosmarinic acid and in luteolin-7-O-α-glucuronide. Its phenolic profile also contained derivatives of the flavones luteolin, chrysoeriol and apigenin, the flavanones eriodictyol and naringenin and the flavonol quercetagetin, which consisted about the 7.5 mg/g of dried plant [46].

Garlic, by means of bulbs of mature plants, is well known for its volatile organosulfur compounds, rather than its phenolic composition. TPC in methanolic extracts of mature garlic bulbs has been quantified at 0.18 mg GAE/g, while total flavonoids at 5.78 μg QE/g [47].

Taking into account the above mentioned phenolic composition of mint, lemon thyme, and garlic, it could be expected that their phenolic bulk could contribute to the total phenolic content of the radish brine. This was particularly evident in the case of *T. citriodorus* addition but only during fermentation when the pathogen inoculum level was 4 log CFU/g. However, no particular trend could be identified, thus the statistically significant differences that were observed could be assigned to the artisanal nature of the fermentation and more precisely to the uncontrolled quality of the raw materials used.

Regarding the effect of herbs on the fermentation or the survival of the pathogens, no definite conclusions could be drawn since the statistically significant differences that were observed could be characterized as occasional and assigned to the spontaneous nature of the fermentation.

Conclusions

The addition of herbs into the brine, at least in the amount used, had no negative effect on radish root spontaneous fermentation. From a safety perspective, prolongation of the fermentation time may be advised for effective inactivation of *L. monocytogenes* and *E. coli* O157:H7.

References

1. Talalay P, Fahey JW. Phytochemicals from cruciferous plant protect against cancer by modulating carcinogen metabolism. Journal of Nutrition 2001; 131:3027S-3033S.
2. Beevi SS, Mangamoori LN, Gowda BB. Polyphenolics profile and antioxidant properties of *Raphanus sativus* L. Natural Product Research. 2012; 26:557-563.
3. Pushkala R, Raghuram PK, Srividya N. Chitosan based powder coating technique to enhance phytochemicals and shelf life quality of radish shreds. Postharvest Biology and Technology, 2013; 86:402-408.
4. Goyeneche R, Roura S, Ponce A, Vega-Galvez A, Quispe-Fuentes I, Uribe E *et al*. Chemical characterization and antioxidant capacity of red radish (*Raphanus sativus* L.) leaves and roots. Journal of Functional Foods. 2015; 16:256-264.
5. Paramithiotis S, Papoutsis G, Drosinos EH. Lactic acid fermentation of fruits and vegetables; an overview In: Paramithiotis S (Ed.), Lactic acid fermentation of fruits and vegetables. Boca Raton: CRC Science. 2017, 1-18.
6. Yan PM, Xue WT, Tan SS, Zhang H, Chang XH. Effect of inoculating lactic acid bacteria starter cultures on the nitrite concentration of fermenting Chinese paocai. Food Control. 2008; 19:50-55.
7. Patra JK, Das G, Paramithiotis S, Shin HS. Kimchi and other widely consumed traditional fermented foods of Korea: a review. Frontiers in Microbiology. 2016; 7:1493.
8. Tamang JP, Sarkar PK. Sinki: a traditional lactic acid fermented radish tap root product. Journal of General and Applied Microbiology. 1993; 39:395-408.
9. Tamang JP, Tamang B, Schillinger U, Franz CMAP, Gores M, Holzapfel WH. *et al*. Identification of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. International Journal of Food Microbiology. 2005; 105:347-356.
10. Paramithiotis S, Hondrodimou OL, Drosinos EH. Development of the microbial community during spontaneous cauliflower fermentation. Food Research International 2010; 43:1098-1103.
11. Paramithiotis S, Doulgeraki AI, Karahasani A, Drosinos. Microbial population dynamics during spontaneous fermentation of *Asparagus officinalis* L. young sprouts. European Food Research and Technology. 2014; 239:297-304.
12. Paramithiotis S, Kouretas K, Drosinos EH. Effect of ripening stage on the development of the microbial community during spontaneous fermentation of green tomatoes. Journal of the Science of Food and Agriculture 2014; 94:1600-1606.
13. Pardali E, Paramithiotis S, Papadelli M, Mataragas M, Drosinos EH. Lactic acid bacteria population dynamics during spontaneous fermentation of radish (*Raphanus sativus* L.) roots in brine. World Journal of Microbiology and Biotechnology. 2017; 33:110.
14. Boesi A. Traditional knowledge of wild food plants in a few Tibetan communities. Journal of Ethnobiology and Ethnomedicine. 2014; 10:75.
15. Kim J, Bang J, Beuchat LR, Kim H, Ryu JH. Controlled fermentation of kimchi using naturally occurring antimicrobial agents. Food Microbiology 2012; 32:20-31.
16. Das G, Patra JK, Singdevsachan SK, Gouda S, Shin HS. Diversity of traditional and fermented foods of the Seven Sister states of India and their nutritional and neutraceutical potential: a review. Frontiers in Life Science. 2016; 9(4):292-312.
17. Burt S. Essential oils: Their antibacterial properties and potential applications in foods – A review. International Journal of Food Microbiology. 2004; 94: 223-253.
18. Holley RA, Patel D. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke

antimicrobials. Food Microbiology 2005; 22:273-292.
19. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. Food Control 2010; 21:1199-1218.
20. Negi PS. Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food application. International Journal of Food Microbiology 2012; 156:7-17.
21. Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. Food Control. 2014; 46:412-429.
22. Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chemistry 2007; 104:1372-1378.
23. International Organization for Standardization (ISO). Microbiology of food and animal feeding stuff—horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1. Detection method. International Organization for Standardization, Geneva. 1996.
24. International Organization for Standardization (ISO). Microbiology of food and animal feeding stuff—horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 2. Enumeration method. 1998. International Organization for Standardization, Geneva.
25. International Organization for Standardization (ISO). Microbiology of food and animal feeding stuff—horizontal method for the detection of *Escherichia coli* O157. International Organization for Standardization, Geneva. 2001.
26. Cocolin L, Rantsiou K, Iacumin L, Urso R, Cantoni C, Comi G. Study of the ecology of fresh sausages and characterization of populations of lactic acid bacteria by molecular methods. Applied and Environmental Microbiology 2004; 70:1883-1894.
27. Aymerich T, Martin B, Garriga M, Hugas M. Microbial quality and direct PCR identification of lactic acid bacteria and nonpathogenic staphylococci from artisanal low-acid sausages. Applied and Environmental Microbiology. 2003; 69:4583-4594.
28. Paramithiotis S, Doulgeraki AI, Tsilikidis I, Nychas GJE, Drosinos EH. Fate of *Listeria monocytogenes* and *Salmonella* sp. during spontaneous cauliflower fermentation. Food Control. 2012; 27:178-183.
29. Klaenhammer TR, Barrangou R, Buck BL, Azcarate-Peril MA, Altermann E. Genomic features of lactic acid bacteria effecting bioprocessing and health. *FEMS* Microbiology Reviews. 2005; 29:393-409.
30. Dahal NR, Karki TB, Swamylingappa B, Li Q, Gu G. Traditional foods and beverages of Nepal - a review. Food Reviews International. 2005; 21:1-25.
31. Maifreni M, Marino M, Conte L. Lactic acid fermentation of *Brassica rapa*: chemical and microbial evaluation of a typical Italian product (brovada). European Food Research and Technology. 2004; 218:469-473.
32. Cozzone AJ. Regulation of acetate metabolism by protein phosphorylation in enteric bacteria. Annual Review of Microbiology. 1998; 52:127-164.
33. Romick TL, Fleming HP, McFeeters RF. Aerobic and anaerobic metabolism of *Listeria monocytogenes* in defined glucose medium. Applied and Environmental Microbiology. 1996; 62:304-307.
34. Booth IR, Cash P, O'Byrne C. Sensing and adapting to acid stress. Antonie van Leeuwenhoek. 2002; 81:33-42.
35. Cho GY, Lee MH, Choi C. Survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during kimchi fermentation supplemented with raw pork meat. Food Control. 2011; 22:1253-1260.
36. Steels H, James SA, Roberts IN, Stratford M. Sorbic acid resistance: the inoculum effect. Yeast 2000; 16:1173-1183.
37. Masana MO, Baranyi J. Growth/no growth interface of *Brocothrix thermosphacta* as a function of pH and water activity. Food Microbiology. 2000; 17:485-493.
38. Koutsoumanis KP, Sofos JN. Effect of inoculum size on the combined temperature, pH and a_w limits for growth of *Listeria monocytogenes*. International Journal of Food Microbiology. 2005; 104:83-91.
39. Bidlas E, Du T, Lambert RJW. An explanation for the effect of inoculum size on MIC and the growth/no growth interface. International Journal of Food Microbiology. 2008; 126:140-152.
40. Augustin JC, Brouillaud-Delattre A, Rosso L, Carlier V. Significance of inoculum size in the lag time of *Listeria monocytogenes*. Applied and Environmental Microbiology. 2000; 66:1706-1710.
41. Robinson TP, Aboaba OO, Kaloti A, Ocio MJ, Baranyi J, Mackey BM *et al.* The effect of inoculum size on the lag phase of *Listeria monocytogenes*. International Journal of Food Microbiology 2001; 70:163-173.
42. Polissiou M, Daferera D. Major dietary antioxidants and their food sources In: Zampelas, A., Micha, R., (Eds), Antioxidants in health and disease, 2015, pp 23-43. Boca Raton: CRS Press.
43. Jing P, Song H, Shen SO, Zhao SJ, Pang J, Qian BJ *et al.* Characterization of phytochemicals and antioxidant activities of red radish brines during lactic acid fermentation. Molecules. 2004; 19:9675-9688.
44. Benabdallah A, Rahmoune C, Boumendjel M, Aissi O, Messaoud C. Total phenolic content and antioxidant activity of six wild Mentha species (Lamiaceae) from northeast of Algeria. Asian Pacific Journal of Tropical Biomedicine. 2016; 6:760-766.
45. Mahdavikia F, Saharkhiz MJ. Secondary metabolites of peppermint change the morphophysiological and biochemical characteristics of tomato. Biocatalysis and Agricultural Biotechnology. 2016; 7:127-133.
46. Pereira O, Peres A, Silva A, Domingues M, Cardoso S. Simultaneous characterization and quantification of phenolic compounds in *Thymus citriodorus* using a validated HPLC–UV and ESI–MS combined method. Food Research International. 2013; 54:1773-1780.
47. Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). Food Chemistry. 2008; 111:925-929.