Food Research 4 (4) : 1038 - 1044 (August 2020)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Effect of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* starter cultures in lower salt concentration fermentation on the sauerkraut quality

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Article history:
Received: 16 January 2020
Received in revised form: 1 February 2020
Accepted: 3 February 2020
Available Online: 10 March 2020

Keywords:
Antioxidant,
Lactobacillus plantarum,
Leuconostoc mesenteroides,
Sauerkraut

DOI:
https://doi.org/10.26656/fr.2017.4(4).029

Abstract

This research was aimed to study the effect of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* starter cultures application in lower salt concentration fermentation on sauerkraut quality. Fresh cut cabbage was fermented with different starter cultures (*L. plantarum*, *L. mesenteroides* or the combination) at different lower salt concentration (0.5% or 1%) at 28°C for 5 days. The obtained sauerkrauts were subjected to evaluation of the quality i.e. total lactic acid bacteria, pH, total acidity, total phenolic content and DPPH scavenging activity analysis. The sulforaphane content analysis was performed by using LC-MS. The starter cultures increased total lactic acid bacteria, total acidity and decreased pH. *L. mesenteroides* resulted in the highest total phenolic content and the lowest IC_{50} value. Sauerkraut with the addition of *L. mesenteroides* contains sulforaphane higher 848.65 ng/g than that of control 776.47 ng/g. The results analysis of LC-MS also detected another compound, namely 2-phenethyl isothiocyanate, an antimicrobial compound. The sauerkraut is potential in functional food development with antiproliferative, anti-inflammatory, antioxidant, and anti-cancer activities.

1. Introduction

Lactic acid fermentation is expected to become an important role in the development of functional fermented vegetables. Sauerkraut, fermented cucumbers and kimchi are the most studied lactic acid fermented vegetables mainly due to their commercial importance (Swain *et al*., 2014). Sauerkraut, means sour cabbage, is a fermentation product of cabbage through spontaneous lactic acid bacteria fermentation. In the fermentation, fresh cabbage is shredded and mixed with 2-2.5% salt to pull out water and nutrients from the cabbage, and the juice will become a substrate for the lactic acid bacteria growth (Johanningsmeier *et al*., 2007). Several lactic acid bacteria play an important role in the fermentation process i.e. *L. mesenteroides, Lactobacillus cucumeris, L. plantarum* and *Lactobacillus pentoacetius* (Lu *et al*., 2003; Plengvidhya *et al*., 2007; Swain *et al*., 2014). Addition of two types of bacteria to determine the performance of bacteria that play an active role when making change to bioactive compounds, and to know the performance of those local bacteria when added to bacteria to cabbage that will be used by the lactic acid bacteria for their growth. Addition of a combination of bacteria to determine the acidity in sauerkraut, if the two types of bacteria added will be equally active and work in the fermentation process.

Concerning on the salt concentration in sauerkraut fermentation, consumers tend to prefer lower sodium foods. Moreover, the brine in the fermentation contains very high in nondegradable chloride ions and BOD. The ability to reduce the salt in sauerkraut fermentation would reduce the concentration of sodium chloride in the waste stream and the volume of brine formed (Johanningsmeier *et al*., 2007). The salt has a function to draw water out of nutrients contained in cabbage that will be used by the substrate for the growth of lactic acid

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bacteria. The addition of 2% salt can accelerate the fermentation process of sauerkraut but is less effective in inhibiting the growth of pathogenic microorganisms. However, the high salt concentration will kill the lactic acid bacteria. Therefore the addition of lactic acid bacteria culture can reduce the salt addition while the fermentation process still go well and increase bioactive compounds (Xiaozhe *et al*., 2019). Several researchers reported that starter cultures favored the low salt sauerkraut fermentation (Tolonen *et al*., 2004; Wiander and Ryhanan, 2005).

On the other hand, Penas *et al.* (2010) reported that antioxidant activity increase during sauerkraut fermentation, lactic acid bacteria capable to increase the bioactive compounds i.e. phenolic and glucosinolate compounds. Phenolic compounds have the ability to increase antioxidant activity, furthermore can avoid degenerative diseases (Murray, 2009), while sulforaphane is an isothiocyanate derivative that has antiproliferative, anti-inflammatory, antioxidant, and anti -cancer activities. The ability to prevent cancer through DNA protection by modulating enzymes and inhibiting gene mutations (Romeo *et al*., 2018). Antioxidant activity in sauerkraut can inhibit nitric oxide (NO) a factor that causes inflammation that is one of the responses in immune cells (Penas *et al*., 2012).

This research aimed to study the effect of starter cultures application of *L. plantarum* and *L. mesenteroides* at lower salt fermentation on the sauerkraut quality.

2. Materials and methods

2.1 Materials

White cabbage (*Brassica olerace* L. var) used in this experiment was purchased from the local market. Cultures of *L. mesenteroides* FNCC 0023 and *L. plantarum* FNCC 0027 were obtained from Food and Nutrition Culture Collection, Gadjah Mada University. The cultures were maintained routinely on a MRSB medium.

2.2 Starter culture preparation

The starter cultures of *L. mesenteroides* and *L. plantarum* were prepared according to Penas *et al*., (2012). A loopful of the culture was inoculated into 7 mL of MRSB medium and incubated at 37^{o}C for 16 hrs. The culture suspension was harvested by centrifugation at 6000 rpm for 15 mins. The cells were washed with sterile distilled water, then put it into a 100 mL of sterile distilled water with cell density of 10^6 CFU/mL.

2.3 Sauerkraut fermentation

The fresh cabbage was washed, cut, added with salt at different concentration (0.5% or 1%), inoculated with different starter culture (*L. plantarum*, *L. mesenteroides* or the combination) at 20% (v/w), then incubated at room temperature (28^{o}C) for 5 days. Spontaneous fermentation with salt at 2% was used as a control. The obtained sauerkrauts were subjected to evaluation of the quality i.e. total lactic acid bacteria, pH, total acidity, total phenolic content and DPPH scavenging activity analysis. The sulforaphane content analysis was performed on the selected sauerkraut.

2.4 Sauerkraut quality evaluation

2.4.1 Total lactic acid bacteria

Total lactic acid bacteria were determined according to Penas *et al*. (2010). A total of 5 g of the sample was prepared aseptically to make a sample solution, then diluted with buffer peptone water into serial dilution. The diluted sample suspension was poured on MRS Agar and then incubated at 37^{o}C for 48 hrs.

2.4.2 Total acidity and pH

Total acidity was measured according to Ranggana (1977) by using direct titration with NaOH solution of 0.1N and phenol-phtalein indicator. Total acidity was expressed as percentage of lactic acid. pH was measured by using pH meter (Manual pH meter Micro Bench TI 2100).

2.4.3 Total phenolic content

One gram of sample was extracted in 10 mL of methanol, centrifuged at 6000 rpm for 20 mins. 0.5 mL of supernatant was put into the test tube, added with 2.5 mL of 10% Folin Ciocalteau reagent and 2.5 mL of 7.5% Na_2CO_3, and incubated at room temperature for 90 mins. Absorbance was measured at 750 nm with spectrophotometer (Yang *et al*., 2007). Gallic acid was used as a standard. The total phenolic content in sauerkraut was expressed as mg GAE/g.

2.4.4 DPPH scavenging activity

The extract was prepared with the same procedure of the total phenolic content analysis. The supernatant was diluted into 10, 20, 30, 40 and 50 ppm. 4 mL of sample was added into 1 mL of 0.2 mM diphenyl-1-picrylhydrazyl radical (DPPH). The mixture was incubated in the darkroom for 30 mins, then the absorbance was measured at 517 nm with a spectrophotometer (Molyneux, 2004). The DPPH scavenging activity was expressed as IC_{50}.

2.4.5 Sulforaphane content analysis

Sulforaphane was analyzed by using Liquid

Chromatography-Mass Spectrometry (LC-MS) according to Kim *et al.* (2017). Sample preparation was conducted according to Liang *et al*. (2006) with the following procedure: 5 g of the sample was extracted with methylene chloride, dissolved in acetonitrile and filtered through a 0.22 µm membrane. The extract was injected to an HPLC system (Agilent Seris 1200) connected to electrospray ionization (ESI) with API 400 Q TRAP Mass Spectrometry system. The LC operating conditions were reversed-phase C18 column in an oven set at 30°C, mobile phase of 20% acetonitrile in water then changed linearly to 60% acetonitrile with flow rate was 1 mL/min. The MS operating conditions were as follow: ESI positive ion ([M + H] +), ion spray voltage (5.5 kV), gas (20 psi), nebulisation gas (50 psi), heater gas (50 psi), high purity nitrogen (N2), heater gas temperature (550°C), declustering potential (100 V), entrance potential (10 V), and spectrum range (m / z 100 -1000). Then the sulforaphane content calculation was done using a standard sulforaphane curve and expressed as µg/g.

2.5 Statistical analysis

The data were analyzed by analysis of variance (ANOVA), followed by the LSD test at $p<0.05$.

3. Results and discussion

3.1 Lactic acid bacteria growth and activity

In sauerkraut fermentation, the lactic acid bacteria grow and transforms substrates into lactic acid and others. The lactic acid bacteria growth and activity are indicated from the differences data of total lactic acid bacteria, total acidity and pH before and after the fermentation presented in Table 1 and Table 2. The addition of 6 log CFU load starter cultures at initial fermentation increased 2 log cycles during the 5 days fermentation. This is related to the increase in total acidity and decreasing pH values during the sauerkraut fermentation. The pH decreased due to the lactic acid bacteria produce organic acids and release H^+ ion cause an acidic atmosphere in the fermentation (Goh *et al*., 2012).

There were significant ($p<0.05$) starter culture differences in the total LAB at the final fermentation, with the combination starter cultures having the highest total LAB (Table 1). Combination of the cultures yielded higher growth than the single culture. The LAB growth demonstrated is thought to be due to the different contribution of both cultures, as reported by Plengvidhya *et al.* (2007) that *L. mesenteroides* dominate at the beginning of fermentation, then *L. plantarum* and *Lactobacillus brevis* continuing the fermentation. Addition of *L. mesenteroides* starter culture yielded higher growth than that of *L. plantarum*. This may indicate the different growth ability of the cultures to

Table 1. Total Lactic Acid Bacteria (LAB) total acidity before and after in sauerkraut fermentation at different starter cultures and salt concentrations

Culture	Salt (%)	Total of LAB	
		Day 0	Day 5
		10^6 (CFU/mL)	10^8 (CFU/mL)
L. plantarum	0.5	2.4±0.11	3.4±0.06^{d}
L. mesenteroides		2.7±0.10	2.4±0.10^{e}
L. plantarum + L. mesenteroides		2.3±0.14	5.5±0.12^{b}
L. plantarum	1	2.5±0.01	4.6±0.11^{c}
L. mesenteroides		2.3±0.14	3.1±0.09^{d}
L. plantarum + L. mesenteroides		2.6±0.23	6.6±0.10^{a}
Control (Spontaneous fermentation)	2	2.6±0.23	2.6 x 10^7±0.23

The values are expressed as a mean ± SD (n = 3), and values in a column with the same letters are not significantly (P > 0.05) different.

Table 2. Total acidity and pH before and after in sauerkraut fermentation at different starter cultures and salt concentrations

Culture	Salt (%)	Total acidity (%)		pH	
		Day 0	Day 5	Day 0	Day 5
L. plantarum		0.35±0.02	1.33±0.04^{c}	5.67±0.03	3.69^{a}±0.19
L. mesenteroides	0.5	0.37±0.03	1.31±0.01^{c}	5.75±0.12	3.79^{a}±0.20
L. plantarum + L. mesenteroides		0.34±0.01	2.05±0.05^{a}	5.72±0.05	2.60^{b}±0.13
L. plantarum		0.37±0.01	1.60±0.03^{b}	5.68±0.02	3.39^{a}±0.06
L. mesenteroides	1	0.39±0.01	1.51±0.03^{bc}	5.68±0.02	3.46^{a}±0.10
L. plantarum + L. mesenteroides		0.38±0.01	2.11±0.18^{a}	5.72±0.05	2.54^{b}±0.19

The values are expressed as a mean ± SD (n = 3), and values in a column with the same letters are not significantly (P > 0.05) different.

eISSN: 2550-2166

maintain a pH gradient at high organic acid concentration. McDonald *et al*. (2009) revealed that the growth of *L. mesenteroides* stopped when a pH of 5.4 to 5.7 was reached, while the growth of *L. plantarum* stopped when pH of 4.6 to 4.8 was reached. In the sauerkraut fermentation, the initial pH in a range of 5.67 and 5.75. Consequently, the pH shift for *L. mesenteroides* growth was lower than that for *L. plantarum*. The results of the analysis of variance showed that the addition of culture and salt treatments gave a significant effect (P<0.05) on the increasing of total lactic acid bacteria. Fermentation at 1% salt resulted higher total LAB than that of 0.5%. Salt can pull out the juice containing nutrients in the cabbage. Higher salt concentration, more juice pulled out, consequently more nutrients are available for the LAB growth. However, total LAB in sauerkraut control was lower although the fermentation was carried out at higher salt concentration Table 1. It may be due to the lower LAB loaded at initial fermentation in the control without starter culture. Other researchers also reported that the addition of starter culture can expedite the fermentation process and increase total lactic acid bacteria (Beganovic *et al*., 2011; Yang *et al*., 2019).

Addition of starter culture affected significantly on the total acidity and pH after fermentation at salt 0.5% and 1.0%. It reflects the LAB produce organic acid during the fermentation. Those are supported by the data of pH values, whereby the values at final fermentation were lower than those at the initial. These chemical changes are related to the LAB growth during the fermentation. Total acidity and pH of the fermentation product at the combination cultures treatment respectively higher and lower than those at single culture treatment. This agrees with several researchers reports that *L. mesenteroides* produce lactic acid and acetic acid, which caused pH decreasing. *L. plantarum* and *L. brevis* continuing the fermentation until the pH around 3 (Lu *et al*., 2003; Plengvidhya *et al*., 2007; Swain *et al*., 2014). The bacteria oxidize ethanol to acetaldehyde to acetic acid (Chu and Chen, 2006).

3.2 Antioxidant activity and total phenolic contents

The *in vitro* DPPH scavenging ability increased after fermentation of the sauerkraut at all the treatments, reflected from the lower values of IC_{50} at after fermentation than those at before fermentation as presented in Table 3. Lactic acid bacteria are able to activate enzymes that having the function of breaking down the phenolic complex into simple compounds (Tolonen *et al*., 2004). Invertase, cellulase and amylase are able to break complex bonds between phenolic and other compounds so that increase in total phenolic content during fermentation (Essawet *et al*., 2015). The antioxidant activities correlate to the total phenolic contents, which naturally present in the cabbage and increased during the fermentation. Other researchers also reported that the increase of total phenol in sauerkraut goes hand in hand with an increase of antioxidant activity (Ciska *et al*., 2005; Martinez-Villaluenga *et al*., 2012; Penas *et al*., 2012). The bioactive compounds are able to convert free radical compounds into more stable compounds by donating hydrogen atoms and their aromatic hydroxyl (OH) groups (Dipti *et al*., 2003).

Both *L. plantarum* and *L. mesenteroides* are able to metabolize phenolic compounds in foods. Surprisingly, the lowest IC_{50} values, both at salt 0.5% and 1%, were found at the addition of *L. mesenteroides* culture. The highest increment of total phenolic content occurred in the addition of *L. mesenteroides* culture, consistent with the antioxidant activity.

3.3 Sulforaphane content

The sauerkraut with the highest antioxidant activity was subjected to sulforaphane content analysis by using LC-MS. The chromatogram of the sample and standard are shown in Figure 1. Sulforaphane is a glucosinolate derivative compound widely found as biologically active compound in cabbage. Sulforaphane content in the sauerkraut with- and without *L. mesenteroides* culture is presented in Table 4. Sulforaphane content in the sauerkraut with *L. mesenteroides* starter culture was higher than that of the control. This indicates that the addition of *L. mesenteroides* culture can increase myrosinase activity to break down the glucose bonds on glucoraphanin so that sulforaphane compound is active as antioxidants. Moreover, it has antiproliferative, anti-inflammatory and anti-cancer activities (Xu *et al*., 2005; Sayed *et al*., 2014). Sulforaphane has a role in apoptosis of cell proliferation, cancer evolution and stimulation of tumor necrosis factor (TNF-α), IL-1, Lipopolysaccharide (LPS) and in oxidative stress (Suganuma *et al*., 2011; Thakur *et al*., 2014; Nallasamy *et al*., 2014; Greaney *et al*., 2016). Another compound was also detected by the

Table 4. Sulforaphane content in sauerkraut

Product	Sulforaphane (µg/g)
Sauerkraut control (Without culture)	776.47±3.21[a]
Sauerkraut (*L. mesenteroides)*	848.65±2.14[b]

The values are expressed as a mean ± SD (n = 3). The different letter in the same column indicate a significant difference between (p<0.05).

LC-MS with m/z value of 105 and Rt 1.58 namely 2-phenethyl isothiocyanate antimicrobial compound (Abbaoui *et al*., 2015).

Table 3. Total phenolic content and antioxidant activities before and after in sauerkraut fermentation at different starter cultures and salt concentrations

Culture	Salt (%)	Total Phenolic Content (mg GAE/g)		Antioxidant Activities, IC_{50} (ppm)	
		Day 0	Day 5	Day 0	Day 5
L. plantarum	0.5	29.99±0.01	58.33±1.46^{c}	155.55±0.12	120.07±3.64^{a}
L. mesenteroides		29.89±0.03	71.50±0.20^{a}	152.45±0.31	95.55±2.37^{c}
L. plantarum + *L. mesenteroides*		29.99±0.18	64.10±0.96^{b}	150.44±1.21	99.62±5.68^{bc}
L. plantarum	1	29.00±1.17	59.80±0.20^{c}	150.73±2.00	110.13±0.74^{ab}
L. mesenteroides		29.50±0.18	72.99±0.88^{a}	154.01±1.67	94.85±1.66^{c}
L. plantarum + *L. mesenteroides*		29.68±0.13	65.48±0.69^{b}	151.52±1.60	98.84±1.36^{c}

The values are expressed as a mean ± SD (n = 3). The different letter in the same column indicate a significant difference between data on the day 0 and day 5 ($p<0.05$). GAE = Gallic acid equivalent, IC_{50} (inhibition concentration at 50% scavenging).

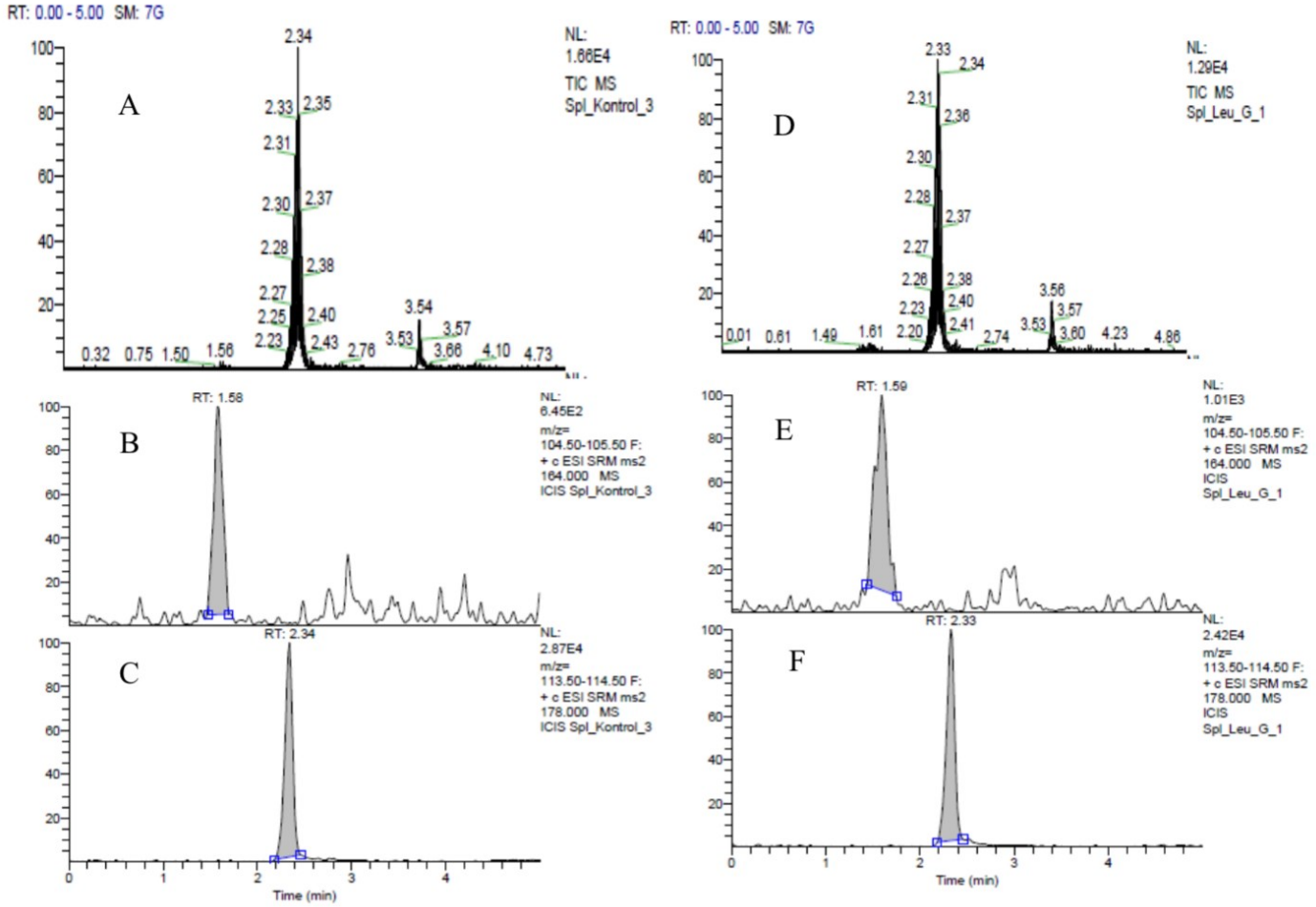

Figure 1. Total ion chromatogram (TIC) of LC-MS analysis of: (A) 2-phenethyl isothyocianate (control); (B) Sulforaphane (control); (C), (D) TIC (sauerkraut + *L. mesenteroides*); (E) 2-phenethyl isothyocianate (sauerkraut + *L. mesenteroides*), and (F); Sulforaphane (sauerkraut + *L. mesenteroides*)

4. Conclusion

The starter cultures application of *L. plantarum* and/ or *L. mesenteroides* at lower salt fermentation increased total lactic acid bacteria, total acidity and decreased pH. *L. mesenteroides* resulted in the highest total phenolic content and the lowest IC_{50} value. Sauerkraut with the addition of *L. mesenteroides* contains sulforaphane higher than that of control.

Conflict of interest

The authors declare no conflict of interest

Acknowledgments

This work was financially supported through Professor Research Grant Brawijaya University with grant number 2571/UN10.F10/PN/2019.

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eISSN: 2550-2166