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Preliminary studies on using LAB strains isolated from spontaneous sauerkraut fermentation in combination with mineral salt, herbs and spices in sauerkraut and sauerkraut juice fermentations

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The use of mineral salt, herbs and spices in combination with isolated lactic acid bacteria strains in sauerkraut fermentation was studied. Mineral salt differs from ordinary salt because NaCl is partially replaced by KCl. The mineral salt contains 28% KCl and 57% NaCl. The final NaCl content in the sliced white cabbage mixture was 0.5%. In approximately 20 hours the pH dropped to the desired level. All the pressed sauerkraut juices had a good microbiological quality. The sensory quality of all pressed juices was found to be either good or acceptable.

Key-words: sauerkraut, sauerkraut juice, salt, aniseed, fennel seeds, caraway, dill, garlic, mint

Introduction

Even though sauerkraut is mostly produced by spontaneous fermentation starters are also used. The selection criteria for lactic acid bacteria used in vegetable and vegetable juice fermentations has been summarised in 1993 by Buckenhüskes (Buckenhüskes 1993, Daeschel et al. 1987, Lücke et al. 1990, Buckenhüskes 1992). Daeschel and Fleming (1984) have also discussed the selection of lactic acid bacteria to be used in vegetable fermentations and the use of starters has further been studied in several works (Frank 1973, Flem-

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ing et al. 1985, Buckenhüskes et al. 1986, Delclos 1992, Harris et al. 1992, Breidt et al. 1993, Breidt et al. 1995, Halasz et al. 1999, Savard et al. 2000, Gardner et al. 2001, Tolonen et al. 2002, Wiander and Ryhänen 2005, Johanningsmeier et al. 2007, Wiander and Ryhänen 2007, Wiander and Ryhänen 2008, Martinez-Villaluenga et al. 2009, Penas et al. 2010). Commercial starters are available on the market, but they are not widely used in sauerkraut and sauerkraut juice fermentations. The most common reasons have been discussed by Lücke et al. (1990) and Hammes (1991). In spite of the advantages the use of starters also increases fermentation costs. One of the benefits of commercial starters according to Lücke et al. (1990) is that the starters ensure the accurate proceeding of the fermentation process.

There are studies in which lactic acid bacteria strains have been isolated from spontaneous sauerkraut fermentations (Stetter and Stetter 1980, Valdez de et al. 1990, Harris et al. 1992, Wiander and Ryhänen 2008) and further studies in which such isolated lactic acid bacteria strains have been used as starters in the fermentation of sauerkraut (Breidt et al. 1993, Tolonen et al. 2002, Wiander and Ryhänen 2008).

The fermentation of white cabbage into sauerkraut traditionally proceeds in the presence of NaCl. There are reports on using different salt concentrations (Delanoe and Emard 1971, Gangopadhyay and Mukherjee 1971, Mayer et al. 1973, Niven, 1980). Low concentrations of NaCl (0.3% and 0.5%) have been used in sauerkraut fermentations (Viander et al. 2003, Wiander and Ryhänen 2005, Tolonen et al. 2002) and NaCl concentrations of 0.6% have also been used (Pederson 1940, Fleming and McFeeters 1985, Trail et al. 1996). Sauerkraut has been prepared utilising hydrolysed protein and a salt content of 1.0 – 4.5% (Hsu et al. 1984, Wedral et al. 1985). A patent has been worked out for making sauerkraut where part of the normally added salt is replaced by an alcohol/acid mixture (Owades 1991). Delclos (1992) has studied the use of a reduced NaCl concentration in combination with lactic acid bacteria starter cultures, the NaCl concentration being 1%. Kimchi is traditionally produced by using NaCl, but there have been studies on replacing part of the NaCl with KCl (Choi et al. 1994).

In this work we studied the impact of low concentration of mineral salt, different herbs and spices in combination with isolated lactic acid bacteria strains on the fermentation of white cabbage into sauerkraut and sauerkraut juice. The herbs and spices used in this study were aniseed, fennel seeds, caraway, dill, garlic and mint. Mineral salt with low sodium content was used because this is in line with the general trend in industrialized countries of reducing the salt level of foods to prevent cardiovascular diseases.

Material and methods

Fermentation trials

In the first trial 0.9% mineral salt was used in combination with isolated *Leuconostoc mesenteroides* (10^5 cfu/g) and isolated *Pediococcus spp.* Nr. 4 (10^3 cfu/g). Fresh mint (2%) was used as a supplement. The treatments were carried out in triplicate and one triplicate treatment without mint was used as a control. Hungarian cabbage was used in the treatments and the amount of sliced cabbage per vessel was 6 kg. The fermentation vessels were made of steel. Mineral salt was mixed with the sliced cabbage, the slices were pressed tightly together and covered with a plastic film, on which water was poured to inhibit air from entering the cabbage mixture and CO_2 from escaping from the mixture. Fermentations were carried out at 20 °C.

In the second trial 0.9% mineral salt was used and the sliced cabbage was inoculated with isolated *Leuconostoc mesenteroides* (1.36 x 10^6 cfu/g) and isolated *Pediococcus dextrinicus* (1.02 x 10^6 cfu/g). Domestic aniseed, fennel seeds, caraway, dill and garlic were used as supplements (1%). Domestic cabbage was used in these treatments and the amount of sliced cabbage per vessel was 1.6 kg. Each treatment was carried out once. The fermentation conditions were otherwise as described above, but the fermentation temperature was 21 °C.

Mineral salt containing 28% KCl and 57% NaCl was used in all treatments. The mineral salt (Pansuola®) contains 57% sodium chloride, 28% potassium chloride, 12% magnesium sulphate, 2% lycine hydrochloride, 1% silicon dioxide and 0,0036% potassium iodide. The mineral salt has been produced for and marketed by Oriola Oy (Espoo, Finland).

Isolation, identification and enrichment of *LAB strains*

The *Leuconostoc mesenteroides* and *Pediococcus dextrinicus* strains were isolated from spontaneous sauerkraut fermentations and identified in a previous study (Wiander and Ryhänen 2008).

Pediococcus spp. Nr. 4 was isolated from spontaneous sauerkraut fermentation and identified by using the API test consisting of API 50 CH strips and API 50 CHL Medium (bioMérieux sa, France).

The isolated lactic acid bacteria strains used in the fermentation trials were enriched in autoclaved juice pressed from sliced raw white cabbage for 2 – 3 days.

Inoculation of cabbage and sampling

The sliced white cabbage mixed with herbs or spices was inoculated either with isolated *Leuconostoc mesenteroides* and isolated *Pediococcus dextrinicus* or isolated *Leuconostoc mesenteroides* and isolated *Pediococcus* spp. Nr. 4.

Samples were taken regularly during the fermentation processes by using sterile pipettes. To get representative samples, equal volumes of cabbage juice were taken from three different places in the fermentation vessels from a depth of approximately 5 cm. The three samples were mixed into one sample.

Microbiological, chemical and sensory analyses

Lactic acid bacteria were enumerated by cultivation on M.R.S nutrition medium (Biokar Diagnostics, France or Difco Labs, USA) containing 0.02% sodium azide and 1.5% agar for 2–3 days at 30 °C. Yeasts and moulds were grown on Yeast extract glucose chloramphenicol agar (Difco Labs, USA) for 7 days at 25 °C. Enterobacteria were cultivated on Violet red bile agar (Biokar Diagnostics or Difco Laboratories) supplemented with 0.1% glucose and grown for 2-3 days at 37 °C. All microbiological analyses were carried out either in duplicate or triplicate.

The pH of the cabbage juice was measured by using a pH-meter (RadiometerPHM93, Radiometer Analytical, Denmark) during the fermentation. Total acidity, given as total lactic acid, was measured by titration using 0.1 N NaOH with phenolphtalein as indicator. All chemical analyses were carried out either in duplicate or triplicate.

The sensory quality of the sauerkraut juices was evaluated by a taste panel consisting of 3 trained persons.

Results

Isolated lactic acid bacteria strains in combination with mineral salt and mint

The number of lactic acid bacteria in the raw material was enumerated before the fermentation started and was found to be 10 cfu/g in the cabbage and 15 cfu/g in the mint. The number of yeasts and moulds was 2 250 cfu/g in the cabbage and 13 500 cfu/g in the mint and the number of enterobacteria in the cabbage and mint was 148 cfu/g and 440 cfu/g respectively.

The pH was measured during the fermentation process and was found to decline slightly slower in the mint treatments compared to the control treat-

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ments. However, in approximately 25 hours the pH in all treatments had decreased to 4.0 (Fig. 1).

The number of lactic acid bacteria was enumerated during the fermentation and the number was higher in the pressed sauerkraut juice from the fermentations with mint (0.23 x 10^8 cfu/ml) compared to the juices produced without mint (0.17 x 10^6 cfu/ml).

The number of yeasts and moulds decreased radically as the fermentation process proceeded and was in the pressed sauerkraut juice produced from the fermentation with mint 12 cfu/ml and in the sauerkraut juice produced without mint 22 cfu/ml.

No enterobacteria were detected in any of the pressed sauerkraut juices.

Isolated lactic acid bacteria strains in combination with mineral salt and supplements

In the treatments with aniseed, fennel seeds, caraway, dill and garlic supplements pH was measured and the decrease in pH was quite similar in all treatments, except for the treatment with garlic. The reduction in pH was slower in the treatment with garlic compared to the other treatments. However, in 24 hours the pH in all treatments had decreased to 4.0 (Fig. 2).

The growth of lactic acid bacteria was slowest in the treatment with garlic as supplement (Fig. 3).

The number of yeasts and moulds decreased rapidly during the fermentation and no yeasts and moulds or enterobacteria were detected in any of the pressed sauerkraut juices.

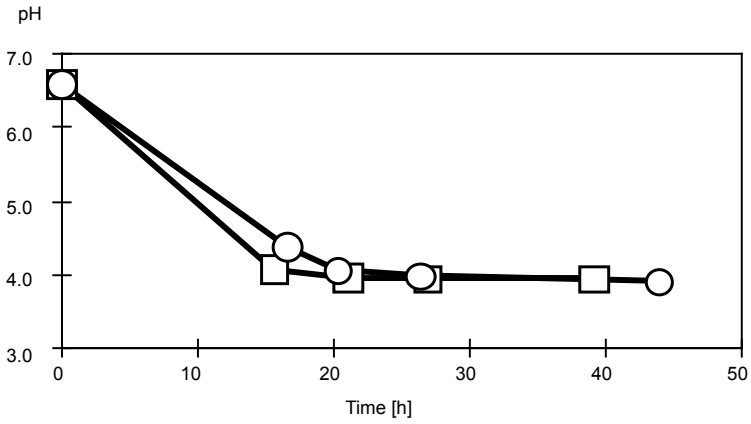

Fig. 1. Change of pH during fermentation of sauerkraut at 20°C by using a mixture of Pediococcus spp. Nr. 4 and Leuconostoc mesenteroides with (○) or without (□) added mint (mean values and standard deviations).

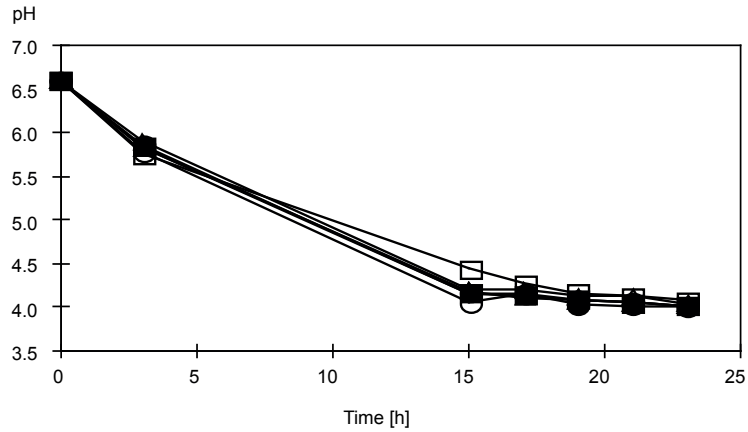

Fig. 2. Change of pH during fermentation of sauerkraut at 21°C by using a mixture of Leuconostoc mesenteroides and Pediococcus dextrinicus with added herbs or spices. (●) aniseed, (■) fennel seeds, (▲) caraway, (○) dill, (□) garlic and (△) control without added herbs or spices.

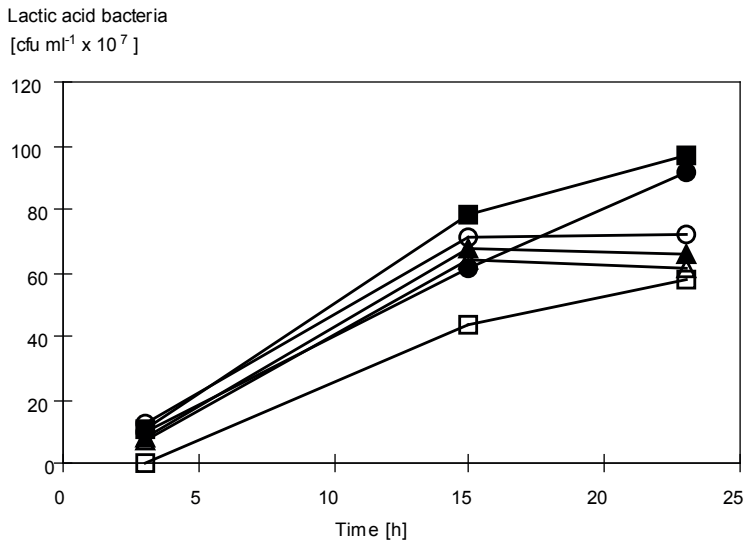

Fig. 3. Number of lactic acid bacteria during fermentation of sauerkraut at 21°C by using a mixture of Leuconostoc mesenteroides and Pediococcus dextrinicus. (●) aniseed, (■) fennel seeds, (▲) caraway, (○) dill, (□) garlic and (△) control without added herbs or spices.

Sensory evaluation

The preliminary results of the sensory evaluation suggest that the kind of sauerkraut juices produced in this study could be accepted by the consumers. The taste of the pressed sauerkraut juices produced from the treatments with mint, aniseed, fennel seeds, and dill was considered the best (5 scores of 5), garlic was considered good (4 scores of 5), whereas the taste of the juice from the treatment with caraway was not considered very appealing although it was acceptable (2 scores of 5). The taste of the sauerkraut juices produced from the control fermentations was considered good (4 scores of 5).

Discussion

The results of this preliminary study show that it is possible to produce sauerkraut and sauerkraut juice of good quality and with a pH of approximately 3.8 by using mineral salt with a final concentration of 0.5% NaCl. The sauerkraut juices were considered to have a smoother taste compared to sauerkraut juices produced by using ordinary NaCl which is in line with earlier studies (Tolonen et al. 2002, Viander et al. 2003, Wiander and Ryhänen 2005, Wiander and Ryhänen 2008). By using herbs and spices in combination with isolated lactic acid bacteria strains as starters and mineral salt with a low NaCl content it was possible to produce sauerkraut and sauerkraut juices with a good microbiological and sensory quality. The pH dropped in all treatments below 4 in 20 - 25 hours which is a considerably shorter fermentation time compared to what is needed in natural sauerkraut fermentations. This study also showed that isolated *Pediococcus* spp. Nr. 4 can be used in sauerkraut fermentations with good results.

These products with low sodium content are in accordance with the general trend in trying to lower the salt content in foods and promote the consumption of healthier foods. The products are alternatives to consumers who want to eat fermented products with low sodium content.

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