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Role Of Lactic Acid Bacteria In Fermentation Of Cabbage Juice

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Abstract: The present research was conducted to determine the suitability of cabbage as a raw material for the production of probiotic cabbage juice by lactic acid bacteria (Lactobacillus plantarum, Lactobacillus delbrueckii). The cabbage juice was inoculated with a 24 h old culture of Lactobacillus plantarum, Lactobacillus delbrueckii and incubated at 30°C for 72 h and observations were made for changes in pH, acidity, sugar content and viable cell counts during fermentation under controlled conditions. The probiotic cabbage juice have an initial pH value of 5.6. The findings of the current research showed that Lactobacillus plantarum actively fermented the cabbage juice and reduced the pH 3.5 and increased the total acidity to about 0.98 per cent. Lactobacillus delbrueckii reduced the pH 3.6 and increased the total acidity 0.94 per cent. After 4 weeks of cold storage at 4°C, the viable cell counts of Lactobacillus plantarum and Lactobacillus delbrueckii were still 3.10x107 and 7.30x106 cfu ml-1 respectively. The outcome of the present research in the form of fermented cabbage juice could serve as a healthy beverage for vegetarians and consumers who are allergic to dairy products.

Keyword: Cabbage juice, Lactobacillus plantarum, Probiotics, Lactobacillus delbrueckii.

INTRODUCTION:

Fermentation of foodstuff is a desirable process of biochemical modification of primary food products with the major role in this respect played by microorganisms and their enzymes. Fermentation improves flavour and taste, extends the shelf life and increases the nutritional value of fermented products.

There are 21 different kinds of vegetable products processed by lactic acid fermentation produced currently in Europe, including vegetables and vegetable juices.

The importance of this method of preserving food in the modern world is underlined by the wide range of uses in both developed and developing countries because of its low price and significant sensory characteristics of thus preserved food.

Development of foods that promote health and well being is one of the key research priorities of food industry (Kalenhammer and Kullen, 1999). This trend has favoured consumption of foods enriched with physiologically active components such as prebiotics, probiotics, vitamins, minerals, dietary fiber, fish oils, plant and starch (Betoret et al., 2003). Probiotics are defined as live microbial feed supplement that beneficially affects the host by improving its intestinal balance (Fuller, 1989). The majority of probiotics recommended are the species of Lactobacillus including L. acidophilus, Lactobacillus plantarum, Lactobacillus casei and Streptococcus lactis etc., The probiotic lactic acid bacteria (Lactobacillus plantarum) was isolated from fish and its efficiency in bacteriocin production against pathogenic microorganisms was studied by Jayachitra and Ramanathan (2012). Probiotication is one of the methods used to produce fermented functional foods. Addition of probiotics to food provides several health benefits including reduction in the level of serum cholesterol, improvement of gastrointestinal function, enhancement of immune system and reduction in risk of colon cancer (Berner and O'Donnell, 1998; Saarela et al., 2002; McNaught and Macfie, 2001; Rafter, 2003). For health benefits probiotic bacteria must be viable and available at a high concentrations typically 106 cfu g-1 of product (Shah, 2001).

Currently probiotic products are usually marketed in the form of fermented milk and yoghurt. However, lactose intolerance and the cholesterol content are two drawbacks related to their consumption. It has been suggested that fruit juice could serve as good medium for cultivating probiotics (Mattila-Sandholam et al., 2002). Fruits and vegetables are healthy foods because they are rich in antioxidants, vitamins, dietary fibres and minerals.Furthermore, fruits and vegetables do not contain any dairy allergens that might prevents usage by certain segments of the population (Luckow and Delahunty, 2004).

Cabbage is a cruciferous, vegetable which is rich in minerals, vitamin C, dietary fibres especially phytochemical (Chu et al., 2002). The objective of this study was to determine the suitability of cabbage as a raw material for production of probiotic cabbage juice by probiotic lactic acid bacteria.

MATERIALS AND METHODS

Preparation of cabbage juice

Cabbage (Brassica oleracea L. var. capitata) was purchased from Chidambaram market and kept at 4°C prior to use. Cabbage juice was obtained with a juice extractor and

sterilized in autoclave for 15 min at 121°C.

Lactic acid bacterial cultures

The isolated culture of Lactobacillus plantarum (LBF1) and Lactobacillus delbrueckii (LBD1) were used for the fermentation of cabbage juice.

Fermentation of probiotic cabbage juice

Fermentation experiments were conducted in 250 ml Erlenmeyer flask, each containing 100 ml of sterile cabbage juice. All the samples were inoculated with 1 ml of 24 h culture (>106 cfu ml^{-1}) of Lactobacillus plantarum and Lactobacillus delbrueckii and incubated at 30°C for 72 h. Samples were taken at 0, 24, 48 and 72 h for chemical and microbiological analysis.

Chemical and microbiological analysis

Samples were taken at 24 h intervals for chemical and microbiological analysis. pH was measured with a pH meter. Total acidity, expressed as percent lactic acid, was determined by titrating with 0.02 N NaOH to pH 8.2. Sugar content was analyzed as glucose by the phenol sulfuric acid method of Dubois et a1. (1956). Viable cell counts (cfu ml^{-1}) were determined by the standard plate method with Lactobacilli MRS medium after 48 h incubation at 30°C.

Effect of cold storage on cell viability in fermented cabbage juice

After 72 h of fermentation at 30°C, the fermented samples were stored at 4°C for four weeks. Samples were taken at weekly intervals, and viability of lactic acid bacterial cultures in fermented cabbage juice was determined and expressed as colony forming unit (cfu ml-1).

Statistical analysis

All experiments were carried out in triplicate, and each sample was analyzed in duplicate. The results are expressed as mean ± S.D (standard deviation). The values that have no common superscript are significantly different ($p<0.05$) according to Duncan's multiple range test. Any two means not marked by the same superscript (for example, a and b or b and c within rows) are significantly different. Any two means marked by the same superscript (for example, a and a or b and b within rows) are not significantly different ($p<0.05$).

RESULTS AND DISCUSSION

In the present investigation, two lactic acid bacteria namely Lactobacillus plantarum and Lactobacillus delbrueckii were used for the fermentation of cabbage juice to produce probiotic cabbage juice. The results showed that both the organisms were found to utilize the cabbage juice for cell synthesis and lactic acid production without nutrient supplementation. The time course of lactic acid fermentation of cabbage juice by Lactobacillus plantarum, Lactobacillus delbrueckii were recorded and the results are presented in Tables 1 and 2.

The fermented cabbage juice have an initial pH value of 5.6. The L. plantarum and L. delbrueckii actively fermented the cabbage juice and lowered pH to 3.5, 3.6 respectively after 72 h fermentation. Individual inoculation of L. plantarum and L. delbrueckii in cabbage juice produced significantly more titrable acidity expressed as per cent lactic acid. L. plantarum and L. delbrueckii produced 0.97 and 0.94 per cent lactic acid.

Similar studies conducted by Kohajdova et al. (2006) reported that pH reduction to 3.6 and titrable acidity of 1.42 per cent lactic acid after 72 h fermentation of cabbage juice by Lactobacillus plantarum. Yoon et al. (2005) also reported that pH reduction to 3.6 and titration acidity expressed in terms of lactic acid of 0.97, 0.95 per cent during 72 h fermentation of cabbage juice by L. plantarum and L. delbrueckii.

Tantipaibulvut et al. (2008) researched roselle juice fermentation with L. plantarum, L. casei. Both lactic acid bacteria reduced the pH to 3.9 and produced titrable acidity of 0.47 and 0.52 per cent after 72 h fermentation of L. plantarum and L. casei respectively. The present results in accordance with the above findings.

In the fermentation of cabbage juice by individual inoculation of Lactobacillus plantarum and Lactobacillus delbrueckii showed initially increased in sugar content between 0 to 24 h whereas a slight decrease in the sugar content was noticed in the fermentation of cabbage juice after 72 h. The sugar content of fermented juice of cabbage in different time intervals by L. plantarum were 34.01±0.01, 37.21±0.40, 33.47±2.10 and 22.43±1.50 mg ml^{-1} respectively for 0, 24, 48 and 72 h of fermentation. The sugar content of fermented juice of cabbage in different time intervals by Lactobacillus delbrueckii were 34.07±0.03, 36.24±2.10, 28.53±1.20, 27.41±0.71 mg ml^{-1} respectively for 0, 24, 48 and 72 h of fermentation. Yoon et al. (2004) found that the lactic acid cultures rapidly fermented tomato juice and reduced the level of sugar. L. plantarum consumed the sugar at a much faster rate than L. acidophilus, L. casei and L. delbrueckii.

The lactic acid bacterial count of cabbage juice fermented by L. plantarum initially having population of 7.7±2.4x106 cfu ml-1 after 24 h slightly increased population of L. plantarum was noticed (8.3±3.0x108 cfu ml-1) and the highest populations of L. plantarum 16.54±0.00x10^{8} cfu ml^{-1} and 18.34±1.5x10^{8} cfu ml^{-1} were noticed at 48 and 72 h respectively. The lactic acid bacterial count of cabbage juice fermented by L. delbrueckii initially having population of 5.7±3.2x10^{6} cfu ml^{-1}. After 24 h, slightly increased population of L. delbrueckii was noticed (8.4±2.5x10^{8} cfu ml^{-1}) and the highest population of L. delbrueckii was noticed (13.75±0.40x10^{8} cfu ml^{-1}) at 48 h. The decrease in the population of L. delbrueckii was noticed (11.35x1.20 x10^{8} cfu ml^{-1}) after 72 h of fermentation.

Yoon et al. (2004) found that the four lactic acid bacterial cultures grew rapidly in tomato juice and reached a viable cell population of greater than 1.0x10^{8} cfu ml^{-1} after 48 h fermentation at 30°C. Extending the fermentation time from 48 to 72 h did not result in a significant increase in viable cell counts. This could be due to low pH and high acidity in fermented tomato juice. The results are supported the present findings.

Yoon et al. (2005) found that L. plantarum and L.

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delbrueckii grew rapidly on cabbage juice and reached nearly $10x10^8$ cfu ml-1 after 48 h fermentation at 30°C. Extending the fermentation, beyond 48 h did not result in a significant increase in the viable cell counts of lactic acid bacteria.

The results of effect of cold storage on the viability of two species of lactic acid bacteria in fermented cabbage juice are presented in Table 3.

Tantipaibulvut et al. (2008) studied that the effect of cold storage on the viability of L. plantarum and L. casei in fermented roselle juice after four weeks. The viable cell counts of Lactobacillus plantarum and L. casei $1.8x10^6$ cfu ml^{-1} and $1.6x10^6$ cfu ml^{-1} respectively for 48 and 72 h of fermentation.

In the present study, Lactobacillus plantarum and Lactobacillus delbrueckii were capable of surviving in the fermented cabbage juice at 4°C for several weeks. The viable cell counts of Lactobacillus plantarum and Lactobacillus delbrueckii $3.1\pm0.14x10^7$ and $7.3\pm3.20x10^6$ cfu ml^{-1} respectively after four weeks of storages at 4°C. These results are in accordance with the findings of Yoon et al. (2005). Also they found the viable cell counts of four lactic acid bacteria (L. acidophilus, L. plantarum, L. casei, L. delbrueckii) in the fermented tomato juice ranged from 10^6 to 10^8 cfu ml^{-1} after four weeks of cold storage at 4°C in a different study.

For the maximum health benefits, the minimum number of probiotic microorganisms in a food product should be 10^6 cfu g^{-1} (Shah, 2001). Several factors could affect the cell viability of lactic acid cultures in probiotic food products. Probiotic cultures are commonly used in the dairy industry and some products produced during lactic acid fermentation such as lactic acid, diacetyl and acetaldehyde could be associated with loss of viability of probiotic bacteria (Post, 1996). Lactic acid starters are reported to produce bacteriocin against probiotic bacteria and vice versa (Dave and Shah, 1997). Therefore, the viability of lactic cultures is most important factor during refrigerated or frozen storage. The viability of probiotic organisms is dependent on the level of oxygen in products, oxygen permeation of the package, fermentation time and storage temperature (Shah, 2000). The viability of probiotic bacteria is also affected by inhibitory substances such as lactic acid produced during production and cold storage. Other factor for loss of viability of probiotic organisms have been attributed to the decrease in pH of the medium and accumulation of organic acid as a result of growth and fermentation (Hood and Zottola, 1988; Shah and Jelen, 1990). In the present research, we found both L. plantarum and L. delbrueckii could survive in high acidity condition and low pH in fermented cabbage juice.

Table 1. Time course of lactic acid fermentation of cabbage juice by Lactobacillus plantarum

Time (h)	pH	Acidity (% lactic acid)	Glucose (mg ml^{-1})	Lactic acid bacterial count (cfu ml^{-1})
0	5.6 ± 0.0^a	0.12 ± 0.1^c	34.01 ± 0.01^b	$7.7\pm2.4x10^{6\ b}$
24	4.5 ± 0.2^a	0.25 ± 0.04^b	37.21 ± 0.40^a	$8.3\pm3.0x10^{8\ b}$
48	3.6 ± 0.2^b	0.75 ± 0.01^a	33.47 ± 2.10^b	$16.54\pm0.0x10^{8\ a}$
72	3.5 ± 0.1^b	0.98 ± 0.02^a	22.43 ± 1.50^c	$18.34\pm1.5x10^{8\ a}$

Means and standard deviations for n = 3. The experimental values within rows that have no common superscript are significantly different ($p<0.05$) according to Duncan's multiple test range.

Table 2. Time course of lactic acid fermentation of cabbage juice by Lactobacillus delbrueckii

Time (h)	pH	Acidity (% lactic acid)	Glucose (mg ml^{-1})	Lactic acid bacterial count (cfu ml^{-1})
0	5.6 ± 0.0^a	0.11 ± 0.0^b	34.07 ± 0.03^a	$5.7\pm3.2x10^{6\ c}$
24	4.3 ± 0.3^a	0.28 ± 0.01^b	36.4 ± 2.10^a	$8.4\pm2.5x10^{8\ b}$
48	3.8 ± 0.1^b	0.71 ± 0.03^a	28.53 ± 1.20^b	$13.75\pm0.4x10^{8\ a}$
72	3.6 ± 0.0^b	0.94 ± 0.01^a	27.41 ± 0.71^b	$11.35\pm1.20x10^{8\ a}$

Means and standard deviations for n = 3. The experimental values within rows that have no common superscript are significantly different ($p<0.05$) according to Duncan's multiple test range.

Table 3. Effect of cold storage on the viability of lactic acid bacteria in fermented cabbage juice

Time (weeks)	Colony forming units ml^{-1}	
	Lactobacillus plantarum	*Lactobacillus delbrueckii*
72 h	$18.13\pm8.06x10^{8\ a}$	$11.35\pm41.80x10^{8\ a}$
1	$11.71\pm2.15x10^{8\ b}$	$8.70\pm1.65x10^{8\ b}$
2	$9.50\pm1.72x10^{7\ b}$	$14.38\pm2.10x10^{7\ b}$
3	$6.40\pm0.75x10^{7\ c}$	$20.12\pm6.54x10^{6\ c}$
4	$3.10\pm0.14x10^{7\ d}$	$7.30\pm3.20x10^{6\ d}$

Means and standard deviations for n = 3. The experimental values within rows that have no common superscript are significantly different ($p<0.05$) according to Duncan's multiple test range.

CONCLUSION

Lactic acid bacteria (Lactobacillus plantarum, Lactobacillus delbrueckii) were found capable of utilizing cabbage juice for cell synthesis and lactic acid production without nutrient supplement. The inoculation of Lactobacillus plantarum and Lactobacillus delbrueckii actively fermented the cabbage juice and resulting in reduction of sugar content and pH. During cabbage juice fermentation acidity and viable counts of lactic acid bacteria

increased. The viable cell count of Lactobacillus plantarum and Lactobacillus delbrueckii in fermented cabbage juice were found viable even after storing for a period of four weeks at 4°C. Hence the lactic acid bacteria fermented cabbage juice serve as probiotic juice for better health of vegetarians and other consumers who are allergic to dairy products.

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