Antioxidant Activity and Optimal Manufacturing Conditions of Purple Sweet Potato Lactic Acid Bacteria Drink

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In the current studies, we produced a purple sweet potato lactic acid bacteria drink (PSPLABD) using a variety of lactic acid bacterial strains. The various PSPLABD were analyzed for color, pH, and flavor. We found that Lactobacillus helveticus B-1 was the most efficient strain for fermentation. In addition, optimal conditions included a purple sweet potato content of 10%, a skim milk content of 7%, a white sugar content of 7.5%, fermentation at 35°C, and a pH adjusted to 3.5 (fermentation time -24 h). PSPLABD had 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity (IC50 = 130 µl) and inhibited lipid peroxidation (equivalent to 103 µM butylated hydroxytoluene). Fermentation had no effect on the antioxidant activity of PSPLABD, but the purple sweet potato and the lactic acid bacteria drink components had a synergistic effect on the inhibition of lipid peroxidation. Thus, the PSPLABD could be used as a health food which has antioxidant activity and an appealing flavor and color.

Keywords: PSP, LABD, PSPLABD, optimum conditions, antioxidant activity

Introduction

Sweet potatoes (Ipomoea batatas L.) are consumed as a fresh vegetable in many parts of the world. They are rich in polyphenols, vitamin B, iron, calcium, zinc, and protein, and are tolerant to many diseases and pests (Odake, Hatanaka et al. 1994; Pace, Sibiya et al. 1985; Yoshimoto, 2001). The Ayamurasaki purple sweet potato (PSP) is also rich in anthocyanins. This PSP has a deep magenta-colored skin and plum-colored flesh.

Anthocyanins are common components of fruits and vegetables that serve as pigments and natural antioxidants (Roy et al. 2002; Timothy and Edwina 1995; Xue et al. 2002). Anthocyanins also have antioxidant and anticarcinogenic activities in vivo (Ghiselli et al. 1998; Hagiwara et al. 2001; Kamei et al. 1995; Satuegracia et al. 1997). In addition to being rich in anthocyanins, the Ayamurasaki PSP provides a higher yield than the commonly used Yamagawamurasaki PSP. Therefore, increased use of the Ayamurasaki PSP as a pigment and as a processed food is now desired.

We have recently considered using PSP to enhance the quality and nutritional content of a lactic acid bacteria drink (LABD). LABD is rich in the nutrients calcium, phosphorus, and protein, and it has been shown to have anti-hypertensive (Nakajima et al. 1995; Nakamura et al. 1995; Yamamoto et al. 1994), antimutagenic (Bakalinsky et al. 1996; Bodana and Rao 1990; Nadathur et al. 1994), and cholesterol-lowering (Beena and Prasad 1997; Nakajima et al. 1992; Rao et al. 1981) activities. Recently in Japan, there has been growing interest in the use of LABD as a health food.

Materials and Methods

PSP and LAB PSP powder was purchased from JA-Miyazaki (Miyazaki, Japan). LAB was purchased from Nippon Nyugyo Gijyutu Kyoukai (Tokyo, Japan). Skim milk and white sugar were purchased from a local grocer. The lactic acid bacteria strains used in this study and their optimal fermentation temperatures are shown in Table I. A sample (10%) of the skim milk solution was pasteurized, inoculated with a starter culture, and fermented at the optimal temperature for 24 h. This fermented milk was used as the starter culture for the PSPLABD.

Preparation of PSPLABD PSP powder (10 g) was dissolved in 100 ml of water and filtered through a No. 2 filter paper (Toyo Roshi, Ltd, Tokyo, Japan). Next, 80 ml of the filtrate was mixed with skim milk (3 to 10 g) and white sugar (5 to 10 g). The mixture was pasteurized at 70°C for 15 min. After cooling, 20 ml of starter (10^7 cells/ml) cultures were added, and the mixture was fermented at 15 to 40°C.

Measurement of color and sensory evaluations Color tone was measured using a Color Meter ZE-2000 (Nippon
Table 1. Lactic acid bacteria used in this study and their optimal fermentation temperatures.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Optimal temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus lactis 527</td>
<td>30</td>
</tr>
<tr>
<td>Lactobacillus lactis cremoris H-61</td>
<td>37</td>
</tr>
<tr>
<td>Lactobacillus diacetyl lactis N-7</td>
<td>30</td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
</tr>
<tr>
<td>Calvalaiverse subsp thermophilus 510</td>
<td>37</td>
</tr>
<tr>
<td>Lactobacillus casei subsp casei L-14</td>
<td>37</td>
</tr>
<tr>
<td>Lactococcus</td>
<td></td>
</tr>
<tr>
<td>Lactococcus lactis subsp diacetylactis N-7</td>
<td>37</td>
</tr>
<tr>
<td>Lactococcus subsp bulgaricus B-5b</td>
<td>37</td>
</tr>
<tr>
<td>Lactococcus acidophilus L-54</td>
<td>37</td>
</tr>
<tr>
<td>Lactococcus helveticus B-1</td>
<td>37</td>
</tr>
</tbody>
</table>

1Lactococcus  
2Streptococcus  
3Lactobacillus

Densyoku Kougyou Tokyo). The Hunter a value was measured in duplicate, and determined with a circle cell. The sensory evaluations of PSPLABD were determined by 12 members of the laboratory staff (seven males and five females, age 22 to 35). Panelists evaluated each PSPLABD for flavor based on a five-point hedonic scale ranking from 1 (extreme dislike) to 5 (strong liking). The data were analyzed by ANOVA. Mean separation was conducted using the Duncan Multiple Range test, where alpha = 0.05.

Measurement of lactic acid, reducing sugar, and anthocyanin content PSPLABD was diluted with 20 mM H₃PO₄-NaH₂PO₄ (pH 2.8), and then the solution was centrifuged at 1300 x g for 10 min. The supernatant was filtered through a 20 μm pore PTFE membrane (Toyo Roshi, Ltd. Tokyo). Filtered supernatant fractions (20 μl) were analyzed using a SPD-10AV HPLC (Shimadzu, Ltd. Tokyo) with a YMC-Pack ODS-AQ-304 (300 mm x 4.6 mm) column. Fractions were eluted with 20 mM H₃PO₄-NaH₂PO₄ (pH 2.8) at a flow rate of 1.0 ml/min and at 15°C. Lactic acid was detected and quantified by monitoring the effluent at 220 nm. Quantification of lactic acid was based on comparison with external standards, wherein linear regression was used to obtain the best-fit line for peak height vs. concentration of lactic acid standard. Total reducing sugar content was determined by the method of Somogyi-Nelson (Somogyi 1951; Nelson 1944). Anthocyanins were extracted overnight at 4°C from 5 ml samples with 5 ml of methanol containing 0.1% HCl. After centrifugation at 1000 x g for 5 min, the absorbance of the supernatant was measured at 530 nm. Anthocyanin content was calculated using cyanidin chloride as a standard.

Assay of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity To measure antioxidant activity, the DPPH radical-scavenging assay was carried out as described previously (Yildirim and Mavi 2001) with a slight modification. Briefly, the sample solution was adjusted to pH 3.5, and then the solution was centrifuged at 1300 x g for 10 min. A 0.6 ml sample of the supernatant was mixed with 0.6 ml of 100 μM DPPH in ethanol. The mixture was shaken vigorously and allowed to stand for 2 min at room temperature in the dark. The absorbance of the mixture was determined at 520 nm. The antioxidant activity of test compounds is expressed as IC₅₀, which is defined as the concentration of test compounds required to inhibit DPPH radicals by 50%.

Measurement of the inhibition of linoleic acid auto-oxidation Prevention of the auto-oxidation of emulsified linoleic acid was determined using an assay that measures beta-carotene discoloration by oxidized linoleic acid (Tsushida et al. 1994). Briefly, 0.5 ml of beta-carotene solution (100 mg in 100 ml chloroform), 0.2 ml of linoleic acid solution (10 g in 100 ml chloroform), and 1.0 ml of Tween-40 solution (20 g in 100 ml chloroform) were mixed. After evaporation of the chloroform under a stream of nitrogen gas, the mixture was resuspended with 100 ml of distilled water. A 45-ml portion of the linoleic-beta-carotene solution was mixed with 4 ml of 0.2 M phosphate buffer (pH 7.0). Each sample was diluted 10-fold with 70% ethanol. A 4.9 ml sample of this mixture was combined with 100 μl of sample solution, and the reaction mixture was incubated at 50°C for 30 min. The rate of beta-carotene discoloration was determined as the decrease in absorbance at 470 nm between 0 min and 30 min. Butylated hydroxytoluene (BHT) was used as the control standard, and the rate of prevention of linoleic acid auto-oxidation was expressed as μmol of BHT equivalents per 100 μl of sample.

Results

Effect of LAB on fermentation To determine the effect of LAB on fermentation, PSPLABDs were produced with each strain. Figures 1a-c show the changes in reducing sugar concentration, lactic acid concentrations, and pH in PSPLABDs during fermentation using eight different strains of lactic acid bacteria. The rate of fermentation using the B-1, L-54, and B-5b strains was higher than using other strains, and, overall, the B-1 strain provided the highest fermentation rate. After a 24-h fermentation with the B-1, L-54, and B-5b strains, respectively, the pH was 3.5, 3.6, and 3.6, the lactic acid content was 4.9, 4.5, and 3.1 mg/ml, and the reducing sugar content was 58.2, 59.8, and 62.1 mg/ml. There was no difference in pH, lactic acid content, or reducing sugar content when strains H-61, 527, N-7, or 510 were used. Strain L-14 showed the slowest fermentation rate, with a final pH of 4.6, a lactic acid content of 2.2 mg/ml, and a reducing sugar content of 80.3 mg/ml. The fermentation rates of B-1, L-54, and B-5b strains were faster than those of other strains.

Effect of lactic acid bacteria strain on the color and anthocyanin content of PSPLABD The effect of LAB on the color and anthocyanin content of PSPLABD were observed. Changes in the color of the PSPLABD during fermentation differed significantly for the various strains (Fig. 1-d). The Hunter a value of the PSPLABD during fermentation differed significantly for the various strains (Fig. 1-d). The Hunter a value of the PSPLABD during fermentation differed significantly for the various strains (Fig. 1-d). The Hunter a value of the PSPLABD during fermentation differed significantly for the various strains (Fig. 1-d). The Hunter a value of the PSPLABD during fermentation differed significantly for the various strains (Fig. 1-d).
527, H-61, and N-7 after 4 h of fermentation. After 24 h of fermentation, three different color groups could be discerned: beige with a reddish tinge (strains N-7, H-61, and 527), pink (strains L-14 and 510), and magenta (strains B-5b, L-54, and B-1). Figure 2 shows the anthocyanin contents of the PSPLABD fermented by each strain. The anthocyanin contents after fermentation were different for each strain. PSPLABD fermented by strain B-1 had the highest anthocyanin content in all strains (24.4 μg/ml). The PSPLABD fermented by strains N-7, H-61, and 527, which had low Hunter a values, also had low anthocyanin contents (14.2, 16.4, and 14.7 μg/ml, respectively.). Strain B-1 had the highest anthocyanin content and the fastest fermentation rate of all strains and was used for further experiments.

Effect of component concentration and fermentation temperature on the color and flavor of PSPLABD A panel of 12 individuals found that the PSPLABDs made from a mixture of 5 g skim milk and 7.5 g white sugar at pH 4.0 (fermentation time ~8 h) or from 7 g skim milk, 7.5 g white sugar at pH 4.0 or 3.5 (fermentation time ~ 24 h) had better flavor than the other PSPLABDs (Table 2). In addition, the PSPLABD of the 7 g skim milk/7.5 g white sugar/pH 3.5 mixture showed the highest Hunter a value.

We next examined the effect of fermentation temperature (15, 20, 25, 30, 35, or 40°C) on the color as determined by the Hunter a value. During a 24 h fermentation, the Hunter a value of the PSPLABD increased as the fermentation temperature increased, and the maximum Hunter a values were obtained at fermentation temperatures of 35 and 40°C (Fig. 3-b). Also, we found that the pH of PSPLABD decreased as the fermentation temperature increased and that fermentation temperatures of 35 and 40°C produced the lowest final pH. On the other hand, the initial pH did not affect the color of the PSPLABD after fermentation (data not shown).

DPPH radical scavenging activity of PSPLABD The free radical scavenging activity of the PSPLABD, LABD without PSP, and a 10% PSP solution (which contains the same level of anthocyanin as the PSPLABD prior to fermentation) were determined by examining their abilities to bleach the stable radical DPPH. Figure 4 shows that PSPLABD (IC50 = 130 μl) was more efficient than the LABD without PSP (IC50 = 263 μl) or the 10% PSP solution.
Table 2. Effect of skim milk and white sugar contents on hunter a value and flavor of PSPLABD.

<table>
<thead>
<tr>
<th>Skim milk (g)</th>
<th>White sugar (g)</th>
<th>pH 4.0(a) Hunter a</th>
<th>Flavor(c)</th>
<th>pH 3.5(b) Hunter a</th>
<th>Flavor(c)</th>
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<tr>
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<td>17.7</td>
<td>3.19</td>
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<td>10</td>
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<td>16.0</td>
<td>2.77</td>
<td>18.9</td>
<td>1.96</td>
</tr>
</tbody>
</table>

a pH after 8 h of fermentation
b pH after 24 h of fermentation
c Flavor based on a five-point hedonic scale ranking from 1 (extreme dislike) to 5 (strong liking)

Fig. 3. Effect of fermentation temperature on pH (a) and Hunter a value (b) in PSPLABD. 15°C; ○, 20°C; ▲, 25°C; △, 30°C; ■, 35°C; □, 40°C.

Fig. 4. DPPH radical scavenging activity of PSPLABD, LABD without PSP, and a 10% PSP solution. ○, PSPLABD; ▲, 10% PSP solution; ■, LABD without PSP.

Fig. 5. Prevention of autoxidation in emulsified linoleic acid by PSPLABD, LABD without PSP, and 10% PSP solution. μM BHT equivalents of activity. PSPLABD was more potent at preventing the auto-oxidation of linoleic acid than the sum of the PSP and LABD activities, indicating that there is a synergistic effect of PSP and LABD in the PSPLABD.
Discussion

We focused on the impact of the starter strain on the fermentation rate, retention of the PSP color, and production of antioxidant activity. The fermentation rate in each strain was evaluated by measuring the pH and contents of lactic acid and reducing sugars during fermentation.

We found that all of the strains metabolized the reducing sugars (lactose, glucose, and galactose) in skim milk to produce lactic acid. However, the production of lactic acid and the decrease of pH did not agree because each of the strains produced varying amounts of other organic acids from the reducing sugars (data not shown). These studies further showed that fermentation was fastest with strains B-1, L-54, and B-5b strains and that the color and anthocyanin contents of the PSPLABD produced by each strain were very different. These color differences are not surprising because the appearance of the anthocyanin pigment is known to change with the pH value, becoming blue in alkaline conditions, purple at neutral pH, and red under acidic conditions (Francis 1989). Despite this color change, changes in the Hunter a value did not coincide with changes in the pH. Therefore, we suspect that the lactic acid bacteria metabolized the anthocyanins in the PSPLABDs with low Hunter a values, but further studies must be performed to validate this hypothesis. Finally, our results showed that strain L. helveticus B-1 provided the highest fermentation rate and best retention of PSP red color.

The importance of the antioxidant constituents in the maintenance of health is raising interest among scientists. Thus, we next investigated the antioxidant activity of PSPLABD and its related constituents. We first evaluated the free radical scavenging activity of PSPLABD, LABD without PSP, and a 10% PSP solution by measuring their ability to quench the synthetic DPPH radical. The free radical scavenging activity in the 10% PSP solution is attributed to the anthocyanin pigment from PSP. Free radical scavenging activity in LABD without PSP, in contrast, is probably due to sulphydryls in the milk casein (Taylor and Richardson 1980) or a metabolic or degradation product produced by the lactic acid bacteria. In fact, Kudoh et al. (2001) have reported that an antioxidative peptide is produced from casein by Lactobacillus delbrueckii subsp. bulgaricus IFO 13953 (Kudoh et al. 2001). However, in our strain, Lactobacillus helveticus B-1, the DPPH radical scavenging activity in PSPLABD was the same before and after fermentation (data not shown). This suggests that each LAB has a different protease for degrading casein and that Lactobacillus helveticus B-1 does not have an antioxidative peptide-producing protease. Thus, the free radical scavenging activity in LABD without PSP is due to sulphydryls in the milk casein, and the free radical scavenging activity in PSPLABD is due to anthocyanin in the PSP and sulphydryls in the milk casein.

In addition, we found that the PSPLABD had more antioxidative activity than the sum of the activities of PSP and LABD alone. However, this synergistic activity was not found in the DPPH radical scavenging activity assay. By contrast, a synergistic effect of antioxidative activity of PSP and LABD was shown by measuring the rate of discoloration of β-carotene coupled with the oxidation of linoleic acid. This discrepancy is probably due to different principles (water-based system and oil-based system) used in two methods. Similar to our results, in an oil-based system, a mixture of α-tocopherol and sulphydryls in milk casein act synergistically to inhibit lipid peroxidation (Tong et al. 2000). This suggests that the synergistic antioxidative activity of PSP and LABD could be due to some kind of oil-soluble antioxidant in the PSP. The mechanism of the synergistic effect requires additional investigation.

In conclusion, we produced a new type of LABD that has an appealing color and flavor. Also, our study showed that PSPLABD has antioxidative activity and could, therefore, be useful as a health food.

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References


