ピタヤ(ドラゴンフルーツ)果汁を用いた麦汁の加工および発泡酒の品質への影響
Wort Processing Using Pitaya (dragon fruit) Juices and its Effects on the Quality of Low-malt Beer

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In this work, we evaluated the use of different pitaya fruits in the preparation of malt wort used in the subsequent production of low-malt beers with characteristic colours. Pitaya extracts and juices were obtained from the fruits of Hylocereus polyrhizus (red pitaya) and H. undatus (white pitaya). Preparation procedures were modified to optimize the influence of natural light and temperature on pitaya betacyanins during pitaya sterilization and storage. Our results showed that a sterilization temperature of 70°C, time of 30 min. and growth in the shade resulted in improved wort sterilization, with the characteristic colour of pitaya juice. Under identical culture conditions, using the wort containing sterilized whole and red flesh pitaya juice with an initial betacyanin concentration of 164 mg/l, the ethanol concentration was greater than 5.9%, with betacyanin retention of over 50% at the end of maturation. Additionally, sensory evaluation of the obtained low-malt beers showed that the use of juice from red flesh pitaya was preferred to that of whole red pitaya by most testers. This is the first report describing the processing of low-malt beer using pitaya juice.

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Key words: pitaya, betacyanin, low-malt beer, sterilization condition, sensory evaluation

Pitaya fruits (dragon fruits) are native to Mexico, Central America, and South America and have been grown in many Asian countries, including Vietnam, Taiwan, the Philippines, Malaysia, and Japan. Pitaya flesh contains high concentrations of sugars (°Brix 12.5-14.8), acids, vitamins, and carotenes. Glucose and fructose are the principal sugars in pitaya flesh, with small amounts of oligosaccharides. Pitaya has a number of varieties, and each can be distinguished based on peel colour and/or flesh colour. In Japan, the widely grown varieties of pitaya are * Hylocereus polyrhizus (red-peeled pitaya with red flesh; red pitaya) and H. undatus (red-peeled pitaya with white flesh; white pitaya). These two species of pitaya have recently attracted attention due to their economic value and potential health benefits. Previous studies have reported the red-purple pigment and betacyanin content in the peel and/or the flesh. The betacyanins present in pitaya are betanin, isobetanin, phyllocactin, and hylocerenin. Recent studies have focused on the antioxidant activities of these compounds, suggesting that these pigments may protect against certain oxidative stress-related disorders. In addition, due to their relative stability in the pH range from 4 to 7, betacyanins have great potential as natural colouring agents that can be added to a variety of foods and beverages.

Around the world, beer is the most widely consumed alcoholic beverage and it continues to be a popular drink. Currently, a large variety of beers is available for consumption. In Japan, low-malt beer is one of the most common alcoholic beverages. In low-malt beer, the wort content is less than two-thirds that of regular beer, and supplementation with sugar syrup and/or other carbohydrate materials, i.e., tomato juice, ginger extract, or orange peel, is used to account for the low wort content. Therefore, the flavour, visual appearance, taste, and food functionality of these beverages are expected to be unique, creating a beer product that is distinct from regular beer. The use of pitaya fruits...
for low-malt beer fermentation also has several advantages, including providing a good carbon source and producing a visually unique beer due to the pigments within pitaya fruits. However, there have been no reports describing the processing of low-malt beer using pitaya fruits. Such an analysis must be studied in greater detail, especially with respect to their influence on pigment stability and the quality of low-malt beer produced from pitaya.

In this study, we prepared pitaya extracts and juices from red and white pitaya fruits and investigated the influence of natural light and temperature conditions during the preparation process on the characteristics of pitaya pigment. We then produced low-malt beer from the selected pitaya juices by yeast fermentation and assessed beer qualities by evaluating the smells and tastes of the beer products. To our knowledge, this is the first report describing the processing of low-malt beer using pitaya juice.

Materials and Methods

1. Sample preparation

   (1) Pitaya extract Two species of pitaya fruit (red pitaya and white pitaya) grown in Okinawa, Japan, were obtained from a local market store. The flesh was separated from the red peel, and the flesh and peel were then homogenized separately with a 2X volume of 80% (v/v) aqueous ethanol solution in a blender at room temperature for 15 min. Each homogenate was then centrifuged at 3,000 rpm for 10 min, and the supernatant was filtered (5 a filter paper, Advantec, Tokyo, Japan). The filtrate was dried under reduced pressure to yield the pitaya extract, which was subsequently used in the beer preparation process.

   (2) Pitaya juice For red pitaya, whole fruits (flesh and peel) and flesh were homogenized in a blender at room temperature for 15 min. Each homogenate was then centrifuged at 3,000 rpm for 10 min, and the supernatant was obtained. The supernatant was used as the pitaya juice during the study.

2. Influence of natural light on pitaya colour quality

   The pitaya extract was mixed with a commercial beer (Kirin, Tokyo, Japan), and the final betacyanin concentration was adjusted to 56.5 mg/ℓ. The mixture was added to colourless storage bottles and stored in a room with natural light (non-shaded conditions) or a darkroom (shaded conditions) at 25°C for 32 days. The colours of the bottled samples were measured with a Chroma Meter (Minolta CR-200, Tokyo, Japan) using an illuminant D65 and a 10° observer angle. Data were expressed as L*, a*, and b* [9]. Colour differences (∆E*) were assessed by applying the equation

   $$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

3. Total betacyanin content

   The betacyanin content was assessed using the procedure described by STINTZING et al. [10] with slight modifications. Data were expressed as betanin equivalents using the following formula:

   $$\text{Betacyanin concentration (mg/ℓ)} = A_{\text{ub}} \times DF \times MW \times (1,000/\varepsilon \times L)$$

   where $A_{\text{ub}}$ is the absorbance at 530 nm, DF is the dilution factor, MW is the molecular weight of betanin (MWbetanin : 550 g/ℓ), $\varepsilon$ is the extinction molar coefficient ($\varepsilon_{\text{betanin}}$ : 60,000 mol/ℓ·cm), and L is the cell path (1cm).

4. Sterilization conditions

   Juices prepared from whole fruits and flesh of red pitaya were mixed with water to adjust the final betacyanin concentration to 246.4 mg/ℓ. Juices were then processed in an autoclave (MLS–2420; Sanyo, Osaka, Japan) under sterilization conditions at several temperatures (70–121°C) and for several durations (5–30 min). To quantify the number of aerobic heterotrophic bacteria (total viable bacteria count [TVC]), the autoclaved juice was plated onto plates containing YM medium (10g/ℓ glucose, 3g/ℓ yeast extract, 3g/ℓ malt extract, 5g/ℓ peptone [pH 6.5]) and incubated at 36°C. Colonies were counted after 48 h. Plate counts of aerobic heterotrophic bacteria were expressed as colony-forming units per ml (CFU/ml).

5. Low-malt beer processing

   The brewing process included several steps, i.e., mashing, wort processing, fermentation, and maturation (Fig. 1).

   (1) Mashing and wort processing Sixty-six grams of spray-dried malt (Muntons Plc., Suffolk, UK) was dissolved in 800ml water together with sufficient glucose to achieve 6.9% w/v glucose, and the solution was boiled for 5 min. After boiling, 2 g of dried hops was added, and the solution was
Figs. 1 Schematic showing the process for preparing bottled low-malt beer using the pitaya extract

boiled for another 15 min. The solution obtained was filtered through both 500-μm and 75-μm stainless steel meshes, and the filtrate was mixed with the autoclaved pitaya juice to adjust the final betacyanin concentration to 82, 104, or 164 mg/100 g. These wort products were used as the base worts in the following experiments.

(2) Fermentation and maturation The dried ale yeast Safale 04, *Saccharomyces cerevisiae* (Fermentis, Marcqen Barouel, France), was used in this study. The dried yeast (0.5 g) was added to 10 mL of aqueous glucose solution (10%) in 50-mL Erlenmeyer flasks and incubated at 25°C for 20 min. The base wort solution (800 mL) was inoculated with the rehydrated yeast solution and then cultivated under light-shielded conditions at 20°C for 6 days (primary fermentation). After fermentation, an aliquot (320 mL) of the yeast culture supplemented with 1.8 g glucose was added to a 330-mL coloured pressure bottle. The bottle was capped, and the mixture was further fermented at 20°C for 2 days (secondary fermentation) before final maturation at 4°C for 21 days.

6. Quality parameters

Quality parameters, namely the sugar content (Brix), pH, betacyanin retention, and ethanol content, were measured throughout the primary fermentation and after the completion of maturation. Brix was measured using a digital refractometer (ATAGO, Tokyo, Japan) and was expressed in Brix units. The pH was measured using a pH meter (S 20; Mettler Toledo, Tokyo, Japan). The betacyanin retention (%) was calculated by comparing betacyanin concentrations in final matured beer samples to that prior to fermentation. The ethanol content was determined by gas chromatography on a polyethylene glycol 1000 column (SUS 5 m × 0.3 mm i.d.; GL Science, Tokyo, Japan) coupled with a flame ionization detector. All experiments were carried out in triplicate, and all data were expressed as means.

7. Sensory evaluation

The sensory profiles of low-malt beers prepared using the whole pitaya or the pitaya flesh juices from red pitaya were analysed using quantitative descriptive analysis (QDA) by a trained taste panel (seven tasters). Sample temperature was regulated to 8 ± 2°C prior to service, and approximately 50 mL was poured into each glass. Tasters rated six attributes for flavour (hoppy and off-flavour), visual appearance (form stability), taste (gustatory feel and tingling), and overall perception, scoring the intensity of each attribute on a scale of 0~5 (0 = no aroma or no taste or extremely bad; 5 = strong aroma or strong taste or excellent). The colour (body colour and form colour) was also judged on a scale of 0~5 (0 = no red colour; 5 = extremely intense red colour). One beer was duplicated in order to determine the consistency of the scoring.

Results and Discussion

1. Influence of natural light on the colour quality of pitaya extracts

First, we evaluated the stability of the betacyanin pigment derived from pitaya extracts subjected to natural light during storage. Two plant parts (the flesh and peel) from two species (red pitaya and white pitaya) were used in this study. Table 1 shows the betacyanin concentrations of the individual pitaya fruits. In the case of red pitaya, the betacyanin concentrations in both the flesh and peel were 16.2 and 14.3 mg/100 g fresh weight (FW), respectively. Conversely, white pitaya contained betacyanin in only the peel, and this pigment was not detected in the flesh. Thus, white pitaya flesh
may not be considered a good source of betacyanins. The effects of natural light on the colour quality of the red pitaya flesh and peel extracts and the white pitaya peel extract were then examined. Initially, the extracts were mixed with a commercially available beer for examination under conditions that were as close as possible to those of the finished product. Table 2 lists changes in the colours of pitaya extracts after storage for 32 days with or without exposure to natural light. When exposed to natural light, the colour differences were significantly more pronounced in extracts derived from the red or white pitaya peels (2.21 and 2.94 respectively), as compared to that obtained from the flesh of the red pitaya (1.18). In contrast, colour differences of the extracts from red and white pitaya peels decreased to 0.68 and 0.77, respectively, when samples were stored in the shade. Interestingly, there was little difference in the colour of the red pitaya flesh extract, regardless of storage conditions. These data indicated that natural light had a greater effect on the pigments in red and/or white pitaya peels than on that in red pitaya flesh. Woo et al. reported that the colour change in pitaya juice could likely be due to changes in betacyanin structure. In addition, betanin/iso betanin and betanin/phyllocactin ratios have been shown to change with light exposure, indicating that alterations in pigment patterns may be induced upon illumination, especially using UV light. Therefore, pitaya extracts stored in the shade should provide the available pigment because colour value differences of less than 1.5 cannot be easily detected by human eyes. Additionally, the red pitaya flesh and peel are likely to be the most practical choice for low-malt beer processing compared with white pitaya due to the total betacyanin concentration.

### Table 1 The betacyanin concentration in the extracts prepared from the 2 varieties of pitaya

<table>
<thead>
<tr>
<th>Variety</th>
<th>Parts</th>
<th>Total betacyanin (mg/100g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red pitaya</td>
<td>Flesh</td>
<td>16.2</td>
</tr>
<tr>
<td></td>
<td>Peel</td>
<td>14.3</td>
</tr>
<tr>
<td>White pitaya</td>
<td>Flesh</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>Peel</td>
<td>10.3</td>
</tr>
</tbody>
</table>

### Table 2 Chromatic parameters of low-malt beer prepared using pitaya extract under non-shading or shading conditions

<table>
<thead>
<tr>
<th>Variety</th>
<th>Parts</th>
<th>Colour difference (ΔE°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-shading</td>
</tr>
<tr>
<td>Red pitaya</td>
<td>Flesh</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>Peel</td>
<td>2.21</td>
</tr>
<tr>
<td>White pitaya</td>
<td>Peel</td>
<td>2.94</td>
</tr>
</tbody>
</table>

### Table 3 The influence of sterilization on total viable bacterial count and betacyanin concentration in red pitaya juice

<table>
<thead>
<tr>
<th>Sterile conditions</th>
<th>TVC (CFU/mL)</th>
<th>Betacyanin (mg/L)</th>
<th>Betacyanin retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole</td>
<td>Flesh</td>
<td>Whole</td>
</tr>
<tr>
<td>70°C, 5 min</td>
<td>15</td>
<td>1</td>
<td>231.4</td>
</tr>
<tr>
<td>70°C, 15 min</td>
<td>17</td>
<td>1</td>
<td>205.6</td>
</tr>
<tr>
<td>70°C, 30 min</td>
<td>0</td>
<td>0</td>
<td>181.4</td>
</tr>
<tr>
<td>80°C, 15 min</td>
<td>0</td>
<td>0</td>
<td>169.8</td>
</tr>
<tr>
<td>100°C, 15 min</td>
<td>0</td>
<td>0</td>
<td>153.7</td>
</tr>
<tr>
<td>121°C, 15 min</td>
<td>0</td>
<td>0</td>
<td>21.6</td>
</tr>
</tbody>
</table>

Not heated >100 >100 246.4 246.4 100 100

The influence of temperature on colour quality in pitaya is also an important factor when the pitaya juice is sterilized, as the betacyanin content can easily be decreased by heat treatment. To evaluate the optimum conditions for aerobic heterotrophic bacteria sterilization, with the lowest possible pigment degradation, the juices from whole fruits and the flesh of red pitaya were heated to various temperatures for 5-30 min and the TVC and betacyanin concentrations were measured (Table 3). As expected, in samples heated for 15 min, the betacyanin concentrations in juices prepared from whole fruits and flesh decreased with increasing temperatures, especially at temperatures higher than 121°C. Interestingly, heated whole pitaya juice had a slightly higher betacyanin concentration compared to that in the pitaya juice prepared from the heated flesh, indicating greater retention of betacyanin pigments in the former. Wu et al. reported that the concentration of phyllocactin, a major component of betacyanins, in the red pitaya peel was higher than that in the flesh. In addition, after heat treatment, most of the red betanin was converted into a bright yellow betacyanin degradation product, whereas red phyllocactin was transformed into red betanin with a nearly identical
colour. Hence, the total retention of betacyanin pigments and red compounds appears to be lower in whole pitaya juice as compared to that in the flesh, under the same temperatures. However, the TVC values of juice prepared from whole fruits and those of juice prepared from the flesh were under the detection limit following heat treatment of the juice at temperatures greater than 80°C for 15 min. Interestingly, increasing the heating time at 70°C to 30 min was found to sterilize aerobic heterotrophic bacteria completely, with minimal betacyanin degradation. Taken together, it was inferred that the optimum condition for red pitaya juice sterilization was heating the juice at 70°C for 30 min, and subsequent experiments were carried out under these conditions.

3. Quality characteristics of low-malt beer using various pitaya juices

During fermentation and maturation, a number of parameters (Brix, pH, betacyanin retention, and ethanol content) were monitored. Fig. 2 shows the Brix, pH, and betacyanin retention profiles of wort samples containing different concentrations of whole and red flesh pitaya juices (final betacyanin concentrations of 82, 104, and 164 mg/l) during the 6-day fermentation period (primary fermentation) and at the end of maturation (after 29 days). Brix values reflect the concentration of sugar (the major component), followed by the concentrations of other soluble solid contents, which constitute the minority. The Brix values of each wort for which the red pitaya juice was added before fermentation ranged from 13.5 to 14.9, and the values were reduced similarly from 6.7 to 7.5 (Fig. 2a). With a decrease in Brix, ethanol was successfully produced, regardless of whether whole fruit or red flesh pitaya juices were used in the wort preparation (Fig. 3). The low-malt beer obtained at the end of maturation was characterized by an acceptable ethanol content of approximately 6.4% (range, 5.9–6.9%). However, the betacyanin retention decreased with reduction in pH in all samples during primary fermentation (Fig. 2b and Fig. 2c). In general, betacyanins were stable in a pH range of 4 to 7.

The pH values of whole fruit and flesh samples prepared with different concentrations of betacyanins for the first 3 days of primary fermentation were estimated to be approximately 3.9, 4.0, and 4.2 for samples prepared with 82, 104, and 163 mg/l betacyanins, respectively, which may explain the different betacyanin retention rates among samples with various betacyanin concentrations. Interestingly, betacyanin retention in all samples further decreased during the secondary fermentation and maturation, despite minimal changes in pH values, indicating that betacyanin decomposition may be
attributable not only to pH but also to other mechanisms. A more detailed comparison showed that the betacyanin retention in beer samples prepared with whole red pitaya juice was slightly higher than that in samples prepared from pitaya flesh and with the same concentration of betacyanins. This observation may be attributable to the same process of betacyanin decomposition described in section 3.2. Among the worts tested, only the worts for juices prepared from whole fruits or flesh, with an initial betacyanin concentration of 164 mg/ℓ, retained over 50% of the betacyanin content at the end of maturation. Whole and red flesh pitaya juice samples with an initial betacyanin concentration of 164 mg/ℓ could thus be used to produce low-malt beer having a good red colour, and the beer produced from such extracts should perform very well in the sensory evaluation.

4. Sensory evaluation

Two types of low-malt beers using red pitaya juice prepared from whole fruits (BPW) and flesh (BPF) were used in this study and were considered top fermented ale beers with 5.9% and 6.5% ethanol and 92.5 and 87.6 mg/ℓ betacyanin, respectively. Tasters evaluated six attributes for the flavour (hoppy and off-flavours), appearance (whole colour, form colour, and form stability), taste (mouth feel and tingling), and overall perception. Fig. 4 shows the preference scores from this evaluation. In terms of flavour, the sensory scores of both low-malt beers were similar with regard to hoppy and off-flavours and the scores were relatively low, indicating that the peel did not affect the hoppy aroma or off-flavour of the beer during fermentation and maturation as compared with the effects of the flesh.

The wort containing red pitaya juice had a strong red colour, which reflected the appropriate colour attributes of BPW and BPF. The body colours of both BPW and BPF were a strong red and received the same score of 5.0 from the tasters. Interestingly, BPF had a significantly stronger red foam colour than BPW. BPF showed acceptable foam stability (3.8), whereas the foam stability of BPW was relatively poor (3.3). Isohumulone derived from hops and malt proteins, contributes to the stabilization of beer foam, and promotes foam cling. Additionally, various polyphenols easily interact with malt proteins and form precipitates, which lead to a decrease in beer foam. Generally, the total polyphenol content in red pitaya peel is higher than that in the flesh, and this may therefore explain the poor foam stability of BPW compared to that of BPF. Many physical and chemical properties of beer such as form head and the carbon dioxide, protein, and polyphenol content have been described in the literature as contributors to mouth feel. Although both BPW and BPF had acceptable tastes, BPF had a slightly superior taste than BPW, which was likely due to the higher foam stability of BPF. The tingling sensation produced by beer is mainly due to the beer alcohol concentration. The difference in the ethanol levels of BPW (5.9%) and BPF (6.5%) was clearly perceived by testers, which was reflected in a higher tingling score for BPF compared with that of BPW. The overall perception score of BPF (3.9) was also higher than that of BPW (3.3), although the general impressions for both BPW and BPF were similar with regard to flavour, body colour, and mouth feel. Therefore, low-malt beer prepared using red flesh pitaya juice was preferred to that prepared using whole red pitaya juice by most testers.

Conclusions

In the present work, we evaluated the basic colour qualities of pitaya fruits that were used to modify the characteristics of malt wort. Our aim was to apply the wort to the brewing and the production of low-malt beer having characteristic pitaya colour. In this study, we used two species of
pitaya (red pitaya and white pitaya) and found that betacyanin was present in most pitaya products, with the concentration being the highest in red pitaya flesh (16.2 mg/100 g), followed by red pitaya peel and white pitaya peel. The exception was the white pitaya flesh, which contained no detectable betacyanin. Natural light had a greater influence on both red and white pitaya peel extracts than on red pitaya flesh. Therefore, we concluded that protection from natural light was required to prevent colour degradation of the pitaya juice during brewing and storage. Whole fruit and red flesh pitaya juices were completely sterilized with minimal betacyanin degradation at 70°C for 30 min. Under identical conditions using wort containing pitaya (red pitaya and white pitaya) and found that minimal betacyanin degradation at 70°C for 30 min.


