Processing characteristics and flavour of full lotus root powder beverage

Junbo Liu, Min Zhang* and Shaojin Wang

Abstract

BACKGROUND: Lotus root beverage is commonly made from raw lotus root (RLR). However, RLR production is strictly limited, because it is prone to decomposition and browning after its short harvest season. In this study an innovative beverage was prepared from full lotus root powder (FLRP) as a substitute for RLR in an attempt to solve this problem.

RESULTS: The components of FLRP basically corresponded to those of RLR, but there was some loss of heat-labile compounds. Using differential scanning calorimetry, a gelatinisation temperature range of 57.08–67.80 °C was determined for FLRP with an average particle size distribution of 70 μm. The optimal conditions for enzymatic treatment of FLRP beverage were determined by response surface methodology as an enzyme concentration of 2.2 g kg⁻¹ at 53 °C for 86 min. Turbidity decreased from 1082 to 280 nephelometric turbidity units following enzymolysis. Properties of FLRP beverage were also studied and a qualitative comparison of flavour compounds between RLR and FLRP beverages was made by electronic nose.

CONCLUSION: Basic flavour compounds were consistent and flavour radar plots had approximately the same shape, area and proportion when all ingredients were identical apart from FLRP and RLR. Therefore, in terms of flavour, FLRP beverage appears to be a feasible substitute for RLR beverage.

Keywords: full lotus root powder; particle size distribution; differential scanning calorimetry; response surface methodology; electronic nose; beverage

INTRODUCTION

Lotus root (Nelumbo nucifera Gaertn.) is a well-known aquatic vegetable in China and contains abundant amounts of protein, amino acids, dietary fibre, starch and vitamins C, B₁ and B₂. It is widely favoured by Asian people because of its hard and crispy texture and distinctive aroma and taste. It is often used to make dishes such as salads, pickled vegetables, stir-fried foods and confections.1 Because lotus root contains a high concentration of polyphenolic compounds,2,3 it possesses good antioxidant activity. Hu and Skibsted2 studied the antioxidant capacity of rhizome extract and rhizome knot extract of edible lotus and found that the total phenol content in the plant extract was correlated with the antioxidant capacity, except for the scavenging of carbon-centred radicals. Lotus rhizome knot, as a waste product from the food industry, could be a potential material for antioxidant extraction. Although lotus root is extremely popular as a functional and edible food because of its favourable properties, its production is strictly limited by its short shelf life and decline in quality after processing.4 Browning is a major problem that is detrimental to storage and processing. Enzymatic browning is the main factor influencing brown pigment accumulation due to polyphenolic compounds. Furthermore, the harvest period is of short duration. Developing products of lotus root may not only solve these problems indirectly but also lead to significant economic benefits.

Vegetable juices are currently receiving much attention because of their high levels of nutritional substances and favourable taste.5,6 Among them, lotus root beverage is widely consumed because of its beneficial effects on heart and lung function.7 In China, freshly harvested raw lotus root (RLR) is often processed into lotus root beverage. However, RLR production is strictly limited by its short harvest season, resulting in a negative impact on the local agricultural economy. Full lotus root powder (FLRP) is a type of flour made from dehydrated RLR. It contains the same basic substances as fresh lotus root, but with some loss of heat-labile compounds. However, FLRP indirectly conserves the raw material, which is prone to postharvest decomposition and browning. If lotus root beverage could be processed with FLRP in place of RLR, its transitory and seasonal production would be significantly extended. FLRP is considered as a semi-material or semi-product of lotus root beverage and has been requested for standardisation in China, which may also reduce the effect of differences in processed material batches on FLRP beverage. Up to now, there have been no reports on FLRP beverage.

After gelatinisation, FLRP requires enzymatic hydrolysis to eliminate carbohydrates, which otherwise can cause instability of the system during storage owing to flocculation of carbohydrate molecules. Gelatinisation, as an indispensable treatment, must be

* Correspondence to: Min Zhang, School of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu, China. E-mail: min@jiangnan.edu.cn

a State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu, China

b Department of Biological Systems Engineering, Washington State University, Pullman, WA 99164-6120, USA
performed before enzymolysis. Differential scanning calorimetry (DSC) is an effective method for determining the gelatinisation temperature of carbohydrates through the transformation of thermometric enthalpy.\textsuperscript{8,9} In addition, enzymolysis significantly increases soluble solid content, an important index of beverages. Temperature, time and enzyme concentration are three important factors influencing the effect of enzymolysis, while the pH of FLRP solution is within the feasible range of amylase (pH 5 – 6). Response surface methodology (RSM) is a statistical procedure frequently used for optimising complex processes and evaluating interactive effects. RSM has been successfully used to optimise process variables.\textsuperscript{10–17} The electronic nose (E-nose) system is a sensor-based technology that creates a unique smell print of total headspace volatiles. E-nose does not resolve the sample’s volatiles into individual components but responds to the whole set of volatiles in a unique digital pattern.\textsuperscript{18} E-nose has been applied to aspects of microbial contamination, sensory quality, discrimination of storage shelf life, non-destructive detection, etc.\textsuperscript{19–23} Flavour differences between FLRP and RLR beverages can also be detected by E-nose.

The objectives of this work were to develop an innovative FLRP beverage by studying its processing properties, especially the optimal conditions for enzymolysis, and to compare the flavours of FLRP and RLR beverages by E-nose.

**MATERIALS AND METHODS**

**Materials**

FLRP was supplied by Tian Tang Food Company (Hangzhou, China). RLR (N. nucifera Gaertn.) was purchased from a local wholesale market (Wuxi, China), transported to the laboratory and stored at 0–4 °C before processing. Amylase of enzymatic activity 20,000 U mg\textsuperscript{−1} was obtained from Jie Nuo Enzyme Co. Ltd (Zaozhuang, China). Sucrose, citric acid, ascorbic acid, Carrageenan, β-cyclodextrin and sodium alginate were purchased from Sinopkarm Chemical Reagent Co. Ltd (Shanghai, China).

**Component comparison between FLRP and RLR**

Official methods of the AOAC\textsuperscript{24} were used to determine moisture (925.10), starch (996.11), protein (2001.11), fat (996.06), soluble and insoluble dietary fibre (996.19) and ash (940.26) contents. Water activity (a\textsubscript{w}) was determined using an Ms-1 precision water activity meter (Novasina Co. Ltd, Zurich, Switzerland).

**Particle size distribution of FLRP**

The particle size distribution (PSD) of FLRP was determined using a Mastersizer 2000 laser diffraction size analyser (Malvern Instruments Co. Ltd, Malvern, UK).

**Gelatinisation temperature of FLRP**

Thermal analysis was performed using a DSC-7 differential scanning calorimeter (Perkin Elmer, Waltham, MA, USA). FLRP (2 mg) and water (4 mg) were sealed in a crucible and held at 4 °C for 24 h. Calibration of the instrument was performed with indium as standard. A heating rate of 5 °C min\textsuperscript{−1} and a scanning range between 40 and 90 °C were used throughout the study.

**Preparation process of FLRP beverage**

The preparation process of FLRP beverage involved the following steps: FLRP → water/gelatinisation → enzymolysis → crude filtration → centrifugation → mixing into beverage → homogenisation → pasteurisation → canning → product. Enzymolysis was the main step studied in this work.

**Pretreatment of FLRP**

FLRP and water (1 : 20 w/w) were stirred and heated with an HJ-3 heating magnetic stirrer (Great Wall Scientific Industrial & Trade Co. Ltd, Zhengzhou, China). Heating was stopped at a temperature of about 70 °C when the FLRP became a completely viscous liquid.

**Experimental design for enzymolysis**

The experimental design for enzymolysis was executed according to RSM. In each experiment, 2 g of FLRP was used.

The variables of the enzymatic process were selected on the basis of preliminary experiments. The final independent variables of the process and their coded (x) and actual (X) levels are shown in Table 1. The variables selected were the temperature (X\textsubscript{1}, 46.6–63.4 °C) and time (X\textsubscript{2}, 39.5–140.5 min) of enzymatic treatment and the enzyme concentration (X\textsubscript{3}, 0.3–3.7 g kg\textsuperscript{−1}) used. The coded and actual values of the independent variables together with the response values for reducing sugar yield are shown in Table 2.

The response function (Y\textsubscript{i}) was the reducing sugar yield, which was related to the coded variables (x\textsubscript{i}; i = 1, 2, 3) by a second-degree polynomial using the method of least squares:\textsuperscript{16}

\[
Y_{i} = b_{0} + b_{1}x_{1} + b_{2}x_{2} + b_{3}x_{3} + b_{11}x_{1}^{2} + b_{22}x_{2}^{2} + b_{33}x_{3}^{2} + b_{12}x_{1}x_{2} + b_{13}x_{1}x_{3} + b_{23}x_{2}x_{3}
\]  

(1)

The polynomial coefficients are represented by b\textsubscript{0} (constant term), b\textsubscript{1}, b\textsubscript{2} and b\textsubscript{3} (linear effects), b\textsubscript{11}, b\textsubscript{22} and b\textsubscript{33} (quadratic effects) and b\textsubscript{12}, b\textsubscript{13} and b\textsubscript{23} (interactive effects).

The pH of the juice was maintained at the natural level of 5–6 in FLRP solutions. The temperature of enzymolysis was adjusted to the desired level using an HHS constant temperature water bath (Great Wall Scientific Industrial & Trade Co. Ltd) with a control precision of ±0.1 °C. At the end of enzymolysis the enzyme in the sample was inactivated by heating the suspension at 95 °C for 5 min in the water bath,\textsuperscript{14} then the treated juice was centrifuged at 3000 × g prior to the determination of reducing sugar yield.

**Reducing sugar yield**

Reducing sugar yield was the response to the degree of amyloglucosidase of FLRP and was determined by UV–visible spectrophotometry (Precision Science Instrument Co. Ltd, Shanghai, China) using the HCl hydrolysis/3,5-dinitrosalicylic acid (DNS) method.\textsuperscript{25} Results are reported in g kg\textsuperscript{−1}.

| Table 1. Independent variables of process and their corresponding levels |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Independent variable | Symbols | Levels | Levels | Levels |
| x1 | 46.6 | 50 | 55 | 60 | 63.4 |
| x2 | 39.5 | 60 | 90 | 120 | 140.5 |
| x3 | 0.3 | 1 | 2 | 3 | 3.7 |

The turbidity and SSC of FLRP beverage were determined by the methods described above.

The pH of FLRP beverage was determined using a pHS-2C acidimeter (Analyzer Instrument Co. Ltd, Shanghai, China).

Flavour comparison between RLR and FLRP beverages

Before flavour comparison, RLR beverage was prepared. RLR was cleaned, peeled and cut into 3–5 mm slices, which were blanched at 100 °C for 90 s to inactivate polyphenol oxisdase and gelatinise the starch contained in RLR. The blanched slices were then pulped at an RLR/water ratio of 1:4 (w/w). After amylohydrolysis the juice was blended into RLR beverage using the same ingredients as for FLRP beverage.

The flavour of RLR and FLRP beverages was determined using a PEN3 E-nose (Airsense Co. Ltd, Schwerin, Germany), which is a portable model with ten metal oxide sensors that each detect a different type of flavour. FLRP and RLR beverages (10 mL) were injected into 25 mL vials and sealed by membrane for 1 h to strengthen their flavour. A probe was then inserted into each vial of beverage to extract the flavour for E-nose measurement. The flow rate of air towards the detection system was maintained at 7.747 mL min⁻¹ and lasted for 50 s per measurement. This was sufficient to obtain stable values of odour with a counting speed of 1 s⁻¹. Each experiment was replicated three times. The response signal was expressed by the ratio of measured conductivity (G) and initial conductivity (G0). Because the detectable baseline is unity, the response signal will deviate depending on whether the flavour concentration is increasing or decreasing. The G/G₀ ratio (away from the baseline) therefore indicates the property of flavour. In each case the average value of counts from 41 to 50 s was analysed for comparison.

Statistical analysis

The RSM data were subjected to analysis of variance (ANOVA) using SAS Version 8.00 (SAS Institute, Cary, NC, USA). Mean values were considered significantly different at P < 0.05.

RESULTS AND DISCUSSION

Properties of FLRP

The component comparison between FLRP and RLR is shown in Table 3. Although FLRP contained the same basic components as RLR, including starch, protein and soluble and insoluble dietary fibre, some heat-labile compounds were obviously lost during its processing into powder. However, because of their processing properties, there was little loss of starch and insoluble dietary fibre between RLR and FLRP. The αw of FLRP was 0.362 at 15 °C, which will significantly prolong its shelf life, because micro-organisms can hardly survive at αw < 0.85. This advantage may overcome the limitations of RLR if FLRP beverage is acceptable in other respects.

The average PSD of FLRP was 70 µm (Fig. 1). The gelatinisation temperature of FLRP at this PSD is shown in Fig. 2. The DSC curve became concave when the temperature reached 57.08 °C. The value of thermometric enthalpy (3.92 J g⁻¹) was significantly lower than that (5.7 J g⁻¹) reported by Min et al., mainly because the PSD of FLRP in the present study (70 µm) was much smaller than that of general lotus root powder (>150 µm). The temperature of gelatinisation was subsequently fixed at 70 °C to ensure complete gelatinisation.

Table 2. Central composite design together with response values for reducing sugar yield

<table>
<thead>
<tr>
<th>No.</th>
<th>x1: Temperature (°C)</th>
<th>x2: Time (min)</th>
<th>x3: Enzyme concentration (g kg⁻¹)</th>
<th>x4: Reducing sugar yield (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−1.682 (60.5)</td>
<td>0 (90)</td>
<td>1 (2)</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>1.682 (65.4)</td>
<td>0 (90)</td>
<td>1 (2)</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>0 (55)</td>
<td>1.682 (39.5)</td>
<td>0 (2)</td>
<td>721</td>
</tr>
<tr>
<td>4</td>
<td>1.682 (64.5)</td>
<td>0 (90)</td>
<td>1.682 (3.7)</td>
<td>803</td>
</tr>
<tr>
<td>5</td>
<td>0 (55)</td>
<td>0 (90)</td>
<td>1.682 (3.7)</td>
<td>803</td>
</tr>
<tr>
<td>6</td>
<td>1.682 (65.4)</td>
<td>0 (90)</td>
<td>0 (2)</td>
<td>769</td>
</tr>
<tr>
<td>7</td>
<td>0 (55)</td>
<td>0 (90)</td>
<td>0 (2)</td>
<td>769</td>
</tr>
<tr>
<td>8</td>
<td>1.682 (65.4)</td>
<td>0 (90)</td>
<td>1.682 (3.7)</td>
<td>803</td>
</tr>
<tr>
<td>9</td>
<td>−1.682 (46.6)</td>
<td>0 (90)</td>
<td>1 (2)</td>
<td>721</td>
</tr>
<tr>
<td>10</td>
<td>1.682 (63.4)</td>
<td>0 (90)</td>
<td>1 (2)</td>
<td>769</td>
</tr>
<tr>
<td>11</td>
<td>0 (55)</td>
<td>−1.682 (39.5)</td>
<td>0 (2)</td>
<td>721</td>
</tr>
<tr>
<td>12</td>
<td>1.682 (65.4)</td>
<td>0 (90)</td>
<td>1 (2)</td>
<td>769</td>
</tr>
<tr>
<td>13</td>
<td>0 (55)</td>
<td>0 (90)</td>
<td>−1.682 (0.3)</td>
<td>455</td>
</tr>
<tr>
<td>14</td>
<td>0 (55)</td>
<td>0 (90)</td>
<td>1.682 (3.7)</td>
<td>803</td>
</tr>
<tr>
<td>15</td>
<td>0 (55)</td>
<td>0 (90)</td>
<td>0 (2)</td>
<td>769</td>
</tr>
<tr>
<td>16</td>
<td>0 (55)</td>
<td>0 (90)</td>
<td>0 (2)</td>
<td>769</td>
</tr>
<tr>
<td>17</td>
<td>0 (55)</td>
<td>0 (90)</td>
<td>0 (2)</td>
<td>769</td>
</tr>
<tr>
<td>18</td>
<td>0 (55)</td>
<td>0 (90)</td>
<td>0 (2)</td>
<td>769</td>
</tr>
<tr>
<td>19</td>
<td>0 (55)</td>
<td>0 (90)</td>
<td>0 (2)</td>
<td>769</td>
</tr>
<tr>
<td>20</td>
<td>0 (55)</td>
<td>0 (90)</td>
<td>0 (2)</td>
<td>769</td>
</tr>
</tbody>
</table>

Turbidity

The turbidity of the solution before and after enzymolysis was determined using an STZA24 turbidimeter (Model Bright Turbidimeter Co. Ltd, Wuxi, China) under optimised enzymolysis conditions. Results are reported in nephelometric turbidity units (NTU).

Soluble solid content

Soluble solid content (SSC) was determined using a WYA Abbe refractometer (Precision Science Instrument Co. Ltd) under optimised enzymolysis conditions. Results are reported in °Brix.

Treatmente amplyrolysis

Following amylohydrolysis, the juice was filtered through a 100 µm screen and centrifuged at 2500 × g for 20 min. The supernatant was blended into the beverage as the initial juice. FLRP beverage comprised 900 g kg⁻¹ initial juice, 60 g kg⁻¹ sucrose, 1 g kg⁻¹ citric acid and composite pectins of 0.2 g kg⁻¹ carageenan, 0.8 g kg⁻¹ β-cyclodextrin and 0.5 g kg⁻¹ sodium alginate. The homogenate was pressurised at 10 MPa twice before pasteurisation at 98 °C for 5 min. The beverage was finally canned.

Viscosity, clarity, turbidity, SSC and pH of FLRP beverage

The viscosity of FLRP beverage was determined using the third measuring unit of an NDJ-79 rotary viscosimeter (Tongji University M & E Equipment Co. Ltd, Shanghai, China). Results are reported in mPa s.

The clarity of FLRP beverage was determined by measuring the absorbance at a wavelength of 660 nm with a UV–visible spectrophotometer (Precision Science Instrument Co. Ltd). Distilled water was used as reference.

The pH of FLRP beverage was determined using a pHS-2C acidimeter (Analyzer Instrument Co. Ltd, Shanghai, China).

Flavour comparison between RLR and FLRP beverages

Before flavour comparison, RLR beverage was prepared. RLR was cleaned, peeled and cut into 3–5 mm slices, which were blanched at 100 °C for 90 s to inactivate polyphenol oxisdase and gelatinise the starch contained in RLR. The blanched slices were then pulped at an RLR/water ratio of 1:4 (w/w). After amylohydrolysis the juice was blended into RLR beverage using the same ingredients as for FLRP beverage.

The flavour of RLR and FLRP beverages was determined using a PEN3 E-nose (Airsense Co. Ltd, Schwerin, Germany), which is a portable model with ten metal oxide sensors that each detect a different type of flavour. FLRP and RLR beverages (10 mL) were injected into 25 mL vials and sealed by membrane for 1 h to strengthen their flavour. A probe was then inserted into each vial of beverage to extract the flavour for E-nose measurement. The flow rate of air towards the detection system was maintained at 7.747 mL min⁻¹ and lasted for 50 s per measurement. This was sufficient to obtain stable values of odour with a counting speed of 1 s⁻¹. Each experiment was replicated three times. The response signal was expressed by the ratio of measured conductivity (G) and initial conductivity (G₀). Because the detectable baseline is unity, the response signal will deviate depending on whether the flavour concentration is increasing or decreasing. The G/G₀ ratio (away from the baseline) therefore indicates the property of flavour. In each case the average value of counts from 41 to 50 s was analysed for comparison.

Statistical analysis

The RSM data were subjected to analysis of variance (ANOVA) using SAS Version 8.00 (SAS Institute, Cary, NC, USA). Mean values were considered significantly different at P < 0.05.
Table 3. Component comparison between RLR and FLRP

<table>
<thead>
<tr>
<th>Component</th>
<th>RLR</th>
<th>FLRP</th>
<th>0.01</th>
<th>0.05</th>
<th>0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g kg⁻¹)</td>
<td>81.5 ± 0.2</td>
<td>65.0 ± 0.4</td>
<td>629 ± 7</td>
<td>618 ± 4</td>
<td>63.4 ± 0.2</td>
</tr>
<tr>
<td>Starch (g kg⁻¹)</td>
<td>196 ± 4</td>
<td>173 ± 5</td>
<td>9.3 ± 0.1</td>
<td>7.1 ± 0.3</td>
<td>20.8 ± 0.3</td>
</tr>
<tr>
<td>Soluble dietary</td>
<td>36.4 ± 0.2</td>
<td>24.5 ± 0.3</td>
<td>196 ± 4</td>
<td>173 ± 5</td>
<td>9.3 ± 0.1</td>
</tr>
<tr>
<td>Soluble dietary</td>
<td>16.3 ± 0.3</td>
<td>10.8 ± 0.5</td>
<td>16.3 ± 0.3</td>
<td>10.8 ± 0.5</td>
<td>16.3 ± 0.3</td>
</tr>
<tr>
<td>Fat (g kg⁻¹)</td>
<td>9.3 ± 0.1</td>
<td>7.1 ± 0.3</td>
<td>20.8 ± 0.3</td>
<td>9.5 ± 0.2</td>
<td>153 ± 6</td>
</tr>
<tr>
<td>Ash (g kg⁻¹)</td>
<td>783 ± 5</td>
<td>153 ± 6</td>
<td>153 ± 6</td>
<td>153 ± 6</td>
<td>153 ± 6</td>
</tr>
<tr>
<td>Moisture (g kg⁻¹)</td>
<td>0.939 ± 0.001</td>
<td>0.362 ± 0.001</td>
<td>0.939 ± 0.001</td>
<td>0.362 ± 0.001</td>
<td>0.939 ± 0.001</td>
</tr>
</tbody>
</table>

*All components determined on RLR dry basis except moisture and αm.

Figure 1. Particle size distribution of FLRP.

Enzymolysis analysis

Model analysis

It has been demonstrated that RSM is useful to evaluate effects of multiple parameters on response variables in lipid or enzyme processes. The experimental values for reducing sugar yield under different conditions in Table 2 reflect the effects of the independent variables, i.e. temperature, time and enzyme concentration, on the response function. The experimental data were used to calculate the coefficients of the quadratic polynomial Eqn (1), which were then used to predict the values of amylolysis. The predicted model can be described by the following equation in terms of coded values:

\[ Y_1 = 766.7737 + 10.9503x_1 + 62.3593x_2 + 71.3389x_3 
- 15.1687x_1^2 + 6.1250x_1x_2 - 13.8750x_1x_3 
- 43.80658x_2^2 - 26.1250x_2x_3 - 46.2814x_3^2 \] (2)

ANOVA was used to evaluate the significance of the coefficients of the quadratic polynomial model (Table 4). For any of the terms in the model a large F value and a small P value (P < 0.05) indicate a more significant effect on the corresponding response variable. Table 4 shows that the variables with the largest effect were the linear terms of time (x₂) and enzyme concentration (x₃) and the quadratic terms of time (x₂²) and enzyme concentration (x₃²), all with P < 0.05. P value of the interactive effect of time and enzyme concentration (x₂x₃) was provided with lower significance at P < 0.25 level, although the P value was higher than 0.05.

The P value of the model was about 0.0002, suggesting that the model could be used to accurately estimate the actual values. The coefficient of determination (R²) is the proportion of the variability in the response variable accounted for the enzyme treatment analysis. The closer the value of R² is to unity, the better the empirical model fits the actual data. If the R² value for the response variable was higher than 0.8, it would indicate that the model explained the reaction well. The R² value for the reducing sugar yield was 0.916 (Table 4), suggesting that the proposed model was adequate for further predictions.

Response surface analysis

Response surfaces can be illustrated with three-dimensional plots by presenting the response as a function of two factors while keeping the third factor constant. The response surface plots for reducing sugar yield (RSY) in relation to temperature (TEM), time (TIME) and enzyme concentration (EC) are shown in Fig. 3.

It is evident from Fig. 3(a) that at a fixed enzyme concentration of 2 g kg⁻¹ the reducing sugar yield initially increased rapidly with increasing time, then the rate of increase started to decline after about 90 min. This could be because little undecomposed carbohydrate remained. As seen in Fig. 3(b), with increasing enzyme concentration the reducing sugar yield clearly increased at a fixed time and temperature. However, the effect of temperature was insignificant (P > 0.05) compared with that of enzyme concentration. This could be due to the small range of temperature variation (50–60 °C) for the enzyme. Because both time and enzyme concentration significantly (P < 0.05) influenced the reducing sugar yield (Table 4), their interactive effect on the yield at a fixed temperature of 55 °C is illustrated in Fig. 3(c).

Optimisation of enzymolysis conditions

From the response surfaces the optimal values of coded variables x₁, x₂ and x₃ were calculated as −0.37, −0.14 and 0.19 respectively, corresponding to actual levels of enzyme concentration, temperature and time of 2.2 g kg⁻¹, 53 °C and 86 min respectively. The predicted value of reducing sugar yield was 774 g kg⁻¹ under these optimal conditions. The turbidity of the hydrolysis system under optimal conditions is shown in Fig. 4, where the change in turbidity reflects the effect of hydrolysis indirectly. FLRP formed a turbid solution in water owing to the FLRP granules. Following enzymatic treatment, however, the insoluble FLRP granules decomposed into soluble polysaccharide molecules, as a result of which the turbidity decreased rapidly by about 75%. Correspondingly, the SSC was 3.75 °Brix after enzymolysis, while the initial value before enzymolysis was just above 0 °Brix owing to a small amount of soluble protein and other substances (Fig. 4). Turbidity and SSC only reflect the effect...
of enzymolysis on FLRP indirectly and are indices of qualitative comparison. However, reducing sugar yield can reflect details of the process of enzymolysis directly. As a result, turbidity and SSC were only determined under optimal conditions to evaluate the effect of enzymolysis, while reducing sugar yield was mainly determined as the response in the RSM model used in this study.

Properties of FLRP beverage

Some properties of FLRP beverage are shown in Table 5. The colour of FLRP beverage was light yellow, the same as that of RLR beverage obtained from a local supermarket (Yi Wei Lian Food Co. Ltd, Baoying, China). The pH of FLRP beverage was 3.7, indicating that it belonged to the category of acidic foods (pH < 4.6). Pasteurisation can improve shelf life by protecting against micro-organisms. SSC is an important index in fruit and vegetable beverages. The SSC of FLRP beverage was 10.8 °Brix, with soluble polysaccharides and added sucrose being the main components, which conforms to the Chinese standard (>8 °Brix) for beverages. The viscosity of FLRP beverage was influenced mainly by stabilising agents such as carrageenan and sodium alginate. Turbidity and clarity reflect the visual appearance of a beverage. The turbidity and clarity of FLRP beverage were 92.3 NTU and 81.3% respectively, indicating the presence of some microaggregates even though the juice had been subjected to enzymolysis and centrifugation.

Flavour analysis

Representative response signals recorded by E-nose are shown in Fig. 5. The E-nose model used had ten metal oxide sensors that each detected a different type of flavour (aromatic, broadrange, Ammonia, hydrogen, aro-aliph, broad-methane, sulphur-organic, broad-alcohol, sulph-chlor and methane-aliph). The two flavour radar plots in Fig. 5 show that the main flavours were the compounds detected by sensors 8, 2, 6 and 4 in both FLRP (Fig. 5(a)) and RLR (Fig. 5(b)) beverages. These four flavours respectively represent broad-alcohol, broadrange, broad-methane and hydrogen flavour compounds as the main and characteristic flavour compounds of lotus root when other ingredients have no effect on flavour. The value of sensor 2 for FLRP beverage was 2.51, higher than the value of 1.98 for RLR beverage, while the value of sensor 8 for FLRP beverage was 2.68, lower than the value.

Table 4. Variance analysis of second-order model on reducing sugar yield

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$x_1$</td>
<td>1</td>
<td>1637.57</td>
<td>1637.57</td>
<td>0.9681</td>
<td>0.3484</td>
</tr>
<tr>
<td>$x_2$</td>
<td>1</td>
<td>53107.13</td>
<td>53107.13</td>
<td>31.3948</td>
<td>0.0002**</td>
</tr>
<tr>
<td>$x_3$</td>
<td>1</td>
<td>69502.90</td>
<td>69502.90</td>
<td>41.0873</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Quadratic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$x_1^2$</td>
<td>1</td>
<td>3315.88</td>
<td>3315.88</td>
<td>1.9602</td>
<td>0.1917*</td>
</tr>
<tr>
<td>$x_2^2$</td>
<td>1</td>
<td>27655.44</td>
<td>27655.44</td>
<td>16.3488</td>
<td>0.0023**</td>
</tr>
<tr>
<td>$x_3^2$</td>
<td>1</td>
<td>30868.50</td>
<td>30868.50</td>
<td>18.2482</td>
<td>0.0016**</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$x_1x_2$</td>
<td>1</td>
<td>300.13</td>
<td>300.13</td>
<td>0.1774</td>
<td>0.6825</td>
</tr>
<tr>
<td>$x_1x_3$</td>
<td>1</td>
<td>1540.13</td>
<td>1540.13</td>
<td>0.9105</td>
<td>0.3625</td>
</tr>
<tr>
<td>$x_2x_3$</td>
<td>1</td>
<td>5460.13</td>
<td>5460.13</td>
<td>3.2278</td>
<td>0.1026*</td>
</tr>
<tr>
<td>Model</td>
<td>9</td>
<td>185519.90</td>
<td>20613.32</td>
<td>12.1858</td>
<td>0.0002**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>16915.90</td>
<td>1691.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>202435.80</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DF, degrees of freedom; SS, sum of squares; MS, mean square.

The coefficient of determination ($R^2$) of the predicted model was 0.916.

Significance:

* $P < 0.25$;

** $P < 0.05$.

Figure 2. DSC curve of FLRP between 40 and 90 °C at a heating rate of 5 °C min$^{-1}$.  

Area = 7.841 mJ
$\Delta H = 3.9203$ J g$^{-1}$
Figure 3. Response surfaces for reducing sugar yield (RSY) of enzymolysis on FLRP beverage: (a) effects of temperature (TEM, °C) and time (TIME, min) at constant enzyme concentration of 2 g kg\(^{-1}\); (b) effects of temperature and enzyme concentration (EC, g kg\(^{-1}\)) at constant time of 90 min; (c) effects of time and enzyme concentration at constant temperature of 55 °C.

Figure 4. Changes in turbidity and SSC due to enzymolysis.

As a result, FLRP beverage maintains the characteristic lotus root flavour that is consistent with RLR beverage. In terms of flavour, FLRP appears to be a feasible substitute for RLR in lotus root beverage.

CONCLUSIONS

The components of FLRP basically corresponded to those of RLR, but there was some loss of heat-stable compounds. The range of gelatinisation temperature was 57.08–67.80 °C for FLRP with an average PSD of 70 μm. Statistical analysis using RSM proved to be a valuable tool for optimising the effects of temperature, time and enzyme concentration on enzymolysis in processing FLRP.

of 3.01 for RLR beverage. Thus slight differences were observed between the two beverages, probably because heating of FLRP led to the loss of some heat-labile flavour compounds. However, the flavour compounds detected by sensors 6 and 4 were similar between FLRP and RRP beverages, because the two flavour radar plots had approximately the same shape, area and proportion when the ingredients were identical apart from FLRP and RLR. As a result, FLRP beverage maintains the characteristic lotus root flavour that is consistent with RLR beverage.
beverage. The optimal conditions were an enzyme concentration of 2.2 g kg\(^{-1}\) at 53 °C for 86 min.

Some properties of FLRP beverage were analysed. In particular, its colour was similar to that of RLR beverage available in the marketplace. In addition, other properties of FLRP beverage such as turbidity, pH and SSC may meet the market standard of RLR beverage.

Flavour compounds were similar between RLR and FLRP beverages, because the two flavour radar plots had approximately the same shape, area and proportion. Therefore FLRP could potentially be used as a substitute for RLR in lotus root beverage.

### Table 5. Properties of FLRP beverage\(^a\)

<table>
<thead>
<tr>
<th>Colour</th>
<th>System</th>
<th>pH</th>
<th>Viscosity (mPa s)</th>
<th>Turbidity (NTU)</th>
<th>Clarity (%)</th>
<th>SSC (°Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light yellow</td>
<td>Stability and unity</td>
<td>3.7 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>92.3 ± 3.1</td>
<td>81.3 ± 1.5</td>
<td>10.8 ± 0.3</td>
</tr>
</tbody>
</table>

\(^a\) Data expressed as mean of three replications ± standard deviation.

**ACKNOWLEDGEMENTS**

This research was supported by a project grant from the National Natural Science Foundation of China (20776062). The authors would also like to acknowledge the State Laboratory of Food Science and Technology as well as Hangzhou Tian Tang Food Co. Ltd for making this work possible.

### REFERENCES