Evaluation of freeze-concentrated sugar-cane juice
(Penilaian jus tebu konsentrat sejuk beku)

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Key words: freeze-concentration, fresh sugar-cane juice, reconstituted juice

Abstract
Sugar-cane (Saccharum officinarum) juice with initial total soluble solids (TSS) of 15 °Brix was extracted from blanched sugar-cane stalks and was used for the production of a double strength (30 °Brix) sugar-cane juice using a freeze-concentration process. The freeze-concentrated juice was lower in yeast and mould count, pH and colour values, but higher in non-enzymic browning (NEB) index, chlorophyll content, total aerobic count and relative viscosity as compared to the fresh juice. A sensory evaluation performed on the samples indicated that fresh sugar-cane juice had higher hedonic scores in sweetness, flavour, aftertaste and overall acceptability as compared to that of freeze-concentrated juice (30 °Brix).

The sensory scores of concentrated juice, however, improved upon reconstitution with mineral water. Reconstituted juice with TSS of 15 °Brix and 20 °Brix had the highest hedonic scores for the flavour, sweetness, aftertaste and overall acceptability attributes as compared to other reconstituted juice. During storage, the TSS and pH values of freeze-concentrated juice stored at 10 °C and 25 °C decreased considerably with storage times, and the decrease was more pronounced in the juice stored at 25 °C. The TSS and pH values, however, were unchanged at storage temperatures of –18 °C and 4 °C. The colour values and NEB index of all juice were not affected by the storage temperatures used.

Introduction
Fresh sugar-cane juice is a popular thirst-quenching drink in many South East Asia countries due probably to its refreshing sensation of cane’s flavour and sweetness. The high sugar content of 15–18% (Tee et al. 1997; Easa 2000; Yusof et al. 2000) of the juice suggests that sugar-cane juice can potentially be developed into a natural energy drink. Alternatively, the sugar composition of the juice can be modified to obtain the so-called ‘functional sugar-cane juice’; a juice that is high in fructose-oligosaccharides and low in sucrose (Easa 2000). By adding Pectinex Ultra SP-L enzyme followed by an incubation treatment, sugar modification was achieved, producing a potential health enhancing products.

Another feature of the juice is the substantial content of chlorophyll of 1 mg/100 ml (Yusof et al. 2000). This is important since chlorophyll has been suggested as one of the promising anticancer ingredients (Lin 1999), an odour suppressor and wound healer (Humphrey 2004). The similarity between the chemical structures of chlorophyll with blood pigment has also

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been suggested for correcting the effects of anemia (Humphrey 2004). All these will be of significance if the juice can be properly preserved using a technology that is accessible by small operators. This, however, has not been sufficiently developed.

The main problem associated with fresh sugar-cane juice is its short shelf life and heat sensitivity of its flavour and components. Therefore, the juice is typically sold fresh by the roadsides and small eateries throughout South East Asia countries. It is therefore not uncommon for the variation in the total soluble solids (TSS), flavour, colour and other sensory attributes of the juice from eatery to eatery. The risk of contracting food poisoning from drinking spoiled sugar-cane juice is also a concern since most of the sugar-cane juice operators are not trained in the area of food safety. The difficulty in preserving sugar-cane juice stems from the nature of the juice itself. In contrast to fruit juice, the pH of fresh sugar-cane juice is normally around 5.0, that is above 4.6 (Yusof et al. 2000), thus making the juice to be classified as a low acid product. This condition does not favour a long shelf life of pasteurized juice. In addition, the high sugar content of the juice makes it vulnerable to sugar degradation if heated at high temperatures such as during the processes of sterilization, evaporation and drying.

Therefore, most of the attempts to preserve the sugar-cane juice have been focusing on the use of heat treatment with refrigeration, and inclusion of preservatives (Bhupinder et al. 1991; Yusof et al. 2000). These treatments have not been commercially applied since the sensory attributes of the juice were altered. Among these treatments, the preservation using low temperatures has been the most effective method in maintaining the quality of sugar-cane juice (Bhupinder et al. 1991; Yusof et al. 2000) even though this may not be practical for many small scale sugar-cane juice vendors.

Since consumers are used to drinking fresh juice, the juice’s authenticity becomes an important sensory attribute. Typically ice cubes are added in order to achieve the cool and freshness sensation of the fresh juice. In fact, in many road sides and ‘night-market’ practices throughout Malaysia, freshly extracted juice is chilled by mixing it with a large quantity of crushed ice and left to stand for hours. This practice causes dilution of flavour and sweetness thus affecting the authenticity of the juice. The authors could not find any reference of attempts to standardize the flavour of sugar-cane juice by e.g. controlling the total solid content of the juice or by drying the juice to a powder form.

The objective of this study was to evaluate the use of a freeze-concentration process to produce concentrated sugar-cane juice with a standard soluble solid content. This would be evaluated against fresh juice, and stored at a range of time and temperatures. Freeze-concentration is thought to be of benefit in conditions where heat is damaging to product quality (Despande et al. 1982; Braddock and Marcy 1985). By controlling the freezing process, the total soluble solid (TSS) of ingredients occurring within the fresh juice can be increased without the use of excessive heat such as that applied during the process of evaporation.

Materials and methods

Materials

Sugar-canies (Saccharum officinarum) of ‘yellow variety’, obtained from a plantation in Selangor, Malaysia, were used in this study. The canes were at a maturity stage where they were ready for juice extraction. All canes were stored at 4 °C prior to extraction that was performed within 2–3 days of storage. All chemicals used for the project were of reagent grades.

Extraction of sugar-cane juice

Canes were cut into uniform lengths about 0.4 m long (after removing the nodes and
outer skin from the cane). They were then washed with plain water to remove any dirt or foreign particles from the cane surfaces. Canes intended for freeze-concentration process were then blanched at 80 °C for 15 min using a steaming cabinet (MSM-2001, Malaysia). The stalk blanching method was not performed on the fresh juice. After rinsing, a three-roller power crusher (Mindong Electric, model CH-316, Taiwan) was used to extract the juice. The juice was filtered by passing through a layer of muslin cloth. The extracted juice was collected in a chilled container and chilled immediately before being analysed.

**Production of freeze-concentrated sugar-cane juice**

Extracted juice was filled into polypropylene plastic casings (30.5 cm x 21 cm) that was then sealed and subjected to a rapid chilling treatment to −18 °C using an air blast freezer (Irinox, Italy). Each plastic casing can be filled with 400 ml of juice. The process of rapid chilling from ambient temperature to −18 °C took 30–35 min to complete. At the end of this process the core temperature of the juice-ice mixture was −8 °C.

The juice was then transferred into a domestic freezer (−18 °C; Sharp, Malaysia) and stored for 24 h after which the hardening and completion of ice formation occurred. At this stage, separation of ice and unfrozen phase consisted mainly of concentrated sugar-cane juice was evident. A small opening was made at one end of the plastic casing to allow the flow of the concentrated juice into a volumetric flask. This process of thawing was performed at room temperature and stopped once the total soluble solid of the thawed juice reached 30 °Brix. The thawing process took 50–70 min to complete. The production trials were repeated three times to ensure consistency of the product.

**Colour, pH, microbiological and total soluble solid (TSS) analyses**

The measurements of colour, pH and TSS were performed in triplicates. The colour of sugar-cane juice was determined by using the Minolta colorimeter (model CM-3500d, Japan) with spectra magic software and CIELAB colour system. The values obtained from the colorimeter may be expressed as lightness ‘L*’ (100%, white; 0%, black), redness ‘a*’ (+, red; -, green) and yellowness ‘b*’ (+, yellow; -, blue). The a* value was considered the most relevant for sugar-cane juice as it reflects degree of greenness. The total colour value of the juice was expressed as (Ranganna 1977):

\[
\text{Colour} = (L^*^2 + a^*^2 + b^*^2)^{1/2}
\]

The colour of the juice was also expressed as:

\[
\text{Chroma/Saturation} = (a^*^2 + b^*^2)^{1/2}
\]

The total soluble solids was measured using an Otago refractometer (model HSR-500, Japan; 0–42 °Brix) and pH was measured using a Horiba F series pH meter (model F 21) at 25 °C.

Non-enzymic browning index (NEB index) was estimated following the methods employed by Butchelli and Robinson (1994). Microbiological analysis for total aerobic and yeast and mould count was performed using the standard plate count method (Andrew 1992).

**Analysis of chlorophyll**

Chlorophyll was measured according to the methods of Nagata and Yamashita (1992).

**Estimation of relative viscosity**

Juice was filtered through a 40 μ filter to separate the pulp and the viscosity of the juice measured using a graduated burette (50 ml, 1 mm orifice). Time required for 40 ml freeze-concentrated juice to run out at 3–4 °C was measured and expressed relative to time found for fresh juice.
**Sensory evaluation and reconstitution studies**

To standardize the temperature of samples for sensory evaluation, freshly prepared samples were kept in paper cups and stored at 4 °C for 4 h before serving. Sensory evaluation of the juice was carried out by 10 panellists. Each panellist was instructed to perform the sensory evaluation in one sitting. The panellists were familiarized with sugar-cane juice of various TSS prior to the actual evaluation.

During sensory evaluation, the panellists rated the samples for colour, aroma, sweetness, aftertaste, flavour and overall acceptability using a Hedonic scale of 1–9 (1 = dislike very much, 9 = like very much).

In the reconstitution studies, freeze-concentrated juice was reconstituted with a commercial mineral water to a TSS range of 15–25 °Brix and sensory evaluation performed. One sample of reconstituted juice with TSS of 15, 20, 25 °Brix and the undiluted freeze-concentrated juice were presented to each panellist. Similar procedures and arrangement were performed during the comparison studies of freeze-concentrated juice and fresh juice.

**Storage studies**

Fresh or freeze-concentrated juice was filled into sterilized universal bottles and capped. Three bottles of each sample were stored at –18 °C (Control), 4, 10 and 25 °C for 8 days. Samples were removed at two days interval until eighth day of storage for the analysis of TSS, pH, NEB index and colour.

**Statistical analysis**

Data were analysed by the analysis of variance and Duncan Multiple Range Test using a Statistical Analysis System (SAS) program.

**Results and discussion**

The physico-chemical and microbiological data of the two types of juice are displayed in Table 1. The initial TSS of sugar-cane juice used was 15 °Brix. Typical juice or juice drinks are approximately 84–89% water and 11–16% solids and the soluble solids of these juices are primarily composed of sugars (Clydesdale et al. 1994). It can be seen that the freeze-concentration process employed in this project was able to double the TSS of sugar-cane juice. The increased solid content of the freeze-concentrated juice also increased its relative viscosity. Similar observation in viscosity was noted by Milind and Siddharth (2005) for sugar-cane juice that was freeze-concentrated to beyond 40 °Brix concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Fresh juice</th>
<th>Freeze-concentrated juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (°Brix)*</td>
<td>14.4 ± 1.6</td>
<td>29.9 ± 2.0</td>
</tr>
<tr>
<td>pH+</td>
<td>5.13 ± 0.07</td>
<td>4.84 ± 0.15</td>
</tr>
<tr>
<td>NEB index+</td>
<td>0.121 ± 0.018</td>
<td>0.254 ± 0.019</td>
</tr>
<tr>
<td>Colour*</td>
<td>97.81 ± 0.88</td>
<td>93.73 ± 1.85</td>
</tr>
<tr>
<td>Chroma/saturation</td>
<td>9.0</td>
<td>16.2</td>
</tr>
<tr>
<td>a*</td>
<td>-0.40</td>
<td>-0.70</td>
</tr>
<tr>
<td>Relative viscosity</td>
<td>1.00</td>
<td>1.10</td>
</tr>
<tr>
<td>Chlorophyll (mg/10 ml)</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Microbiology (Total plate count; cfu/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDA</td>
<td>$1.9 \times 10^4$</td>
<td>$3.8 \times 10^3$</td>
</tr>
<tr>
<td>PCA</td>
<td>$7.3 \times 10^2$</td>
<td>$2.9 \times 10^3$</td>
</tr>
</tbody>
</table>

PDA = Potato Dextrose Agar: PCA = Plate Count Agar

*Mean ± Standard Deviation of three determinations
The yield of fresh and concentrated juice can be estimated from the weight of canes used and the amount of juice obtained after extraction and freeze-concentration process. Sugar-canes of 4 kg yielded approximately 2 litres of fresh juice and this was later processed to produce approximately 500 ml of freeze-concentrated juice. The exact yield of the freeze-concentrated juice could not be established since the TSS of freeze-concentrated juice was controlled at 30 °Brix. After the freeze-concentrated juice was obtained, a quantity of unthawed ice remaining was discarded. The TSS of this unthawed ice was 2 °Brix. Despite differing in TSS value, the freeze-concentrated juice was visually similar to the fresh juice.

Changes in pH, NEB index and colour values were apparent after the freeze-concentration process; freeze-concentrated juice was lower in pH and colour values, but higher in chroma value and NEB index. Thus the freeze-concentrated juice was higher in organic acid content, darker and more saturated in colour and higher in the content of water soluble materials than the fresh juice. Despite the lower pH attained, the freeze-concentrated juice was still considered as a low acid product as its pH value was higher than 4.6. The slight darkening of the concentrated juice could have been attributed to some level of non-enzymic browning occurring during stalk blanching (Butchelli and Robinson 1994).

The level of chlorophyll in the sugar-cane juice was 0.1 mg/10 ml and this value increased to 0.2 mg/10 ml following freeze-concentration. The ratio of chlorophyll a to chlorophyll b was 3:2, which was close to the typical ratio of most green plants, which is at a ratio of about 3:1 (Humphrey 2004). Since chlorophyll is the main pigment responsible for the colour of sugar-cane juice (Yusof et al. 2000) it may have had accumulated in the concentrated juice and contributed to the colour change. The shift of the a* values towards a more negative value supports this explanation.

The freeze-concentrated juice was slightly lower in yeast and mould count than that of the fresh juice even though the freeze-concentration process took more than 24 h to complete. This could be contributed by the blanching treatment of cane stalks prior to extraction and rapid freezing rate employed during the early stage of the freeze-concentration juice production that was enhanced by a freezing storage of 24 h.

Even though the total aerobic count of the freeze-concentrated juice was slightly higher than the fresh juice, this level was not considered harmful for normal consumption. Thus the treatment employed for the production of freeze-concentrated juice resulted in an acceptable microbial quality even though enzymic browning reactions could have proceeded at a higher rate due to the higher concentration of compounds.

One feature of the freeze-concentrated juice is its low acceptability of its sensory attributes (Figure 1). Fresh juice scored higher in the attributes of flavour, sweetness, aftertaste and overall acceptability as compared to that of freeze-concentrated juice (p <0.05). However, no difference in the score of colour and aroma was detected.

![Figure 1. Comparison of sensory attributes of fresh sugar-cane juice (15 °Brix) with freeze-concentrated sugar-cane juice (30 °Brix). A bar with different letter as another bar is significantly different from the other (p <0.05)](image-url)
Freeze-concentrated sugar-cane juice

between the two juices. This suggests that the freeze-concentration process had changed some of the sensory attributes of the sugar-cane juice and this could well be due to the concentrating, rather than freezing effect.

A sugar-cane juice with a higher TSS (Table 1) may be too ‘grassy’ in flavour and aftertaste, and too sweet in sweetness perception thus reducing overall acceptability. Another reason for the differing sensory attributes were due probably to the blanching process that was not employed on the fresh sugar-cane juice.

The reconstitution study of concentrated juice was performed with mineral water to a range of concentration 15–25 °Brix before subjecting the diluted juice to a sensory evaluation (Table 2). The scores for colour and aroma were not significantly different ($p < 0.05$) between the freeze-concentrated and reconstituted samples. On the other hand, the scores for sweetness, flavour, aftertaste and overall acceptability improved with dilution. This trend is similar to that observed in Figure 1. A similar sensory response of overall acceptance of reconstituted ‘Ya Pak King’ (a type of fruit) juice with TSS has been shown by Suntornsuk et al. (2004).

Reconstituted juice with excessive level of sugars and flavour were least accepted, while those with similar TSS to the original juice were most preferred. The most acceptable TSS of the reconstituted freeze-concentrated juice was juices with the TSS of 15 °Brix and 20 °Brix which was similar to the freshly extracted juice at 15 °Brix. This indicates the possibility of reconstitution of the freeze-concentrated sugar-cane juice with water to achieve a similar sensory perception of the fresh juice. As many consumers would prefer drinking sugar-cane juice with ice cubes, the dilution of the freeze-concentrated juice with ice may yield sensory sensation similar to that of fresh juice.

The sensory attributes of fresh juice and reconstituted (15 °Brix) juice were not significantly different for all sensory attributes tested ($p < 0.05$; results not shown). Therefore water can be added into freeze-concentrated juice to bring it to 15 °Brix juice concentration without affecting sensory appeal. This result suggests that most of the sensory attributes and overall acceptability of sugar-cane juice were governed by the TSS of the juice.

The TSS of sugar-cane juice comprised mainly of sugars, natural flavourings, pigments and other nutrients. At this stage, the main contributing components of the juice responsible for the sensory scores and acceptance cannot be ruled out. It is thought, however, that a threshold level of TSS could have existed for the sugar-cane juice that

<table>
<thead>
<tr>
<th>Final Total Soluble Solid (TSS) after reconstitution with mineral water</th>
<th>15 °brix</th>
<th>20 °brix</th>
<th>25 °brix</th>
<th>30 °brix (original)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>5.7a ± 1.50</td>
<td>6.5a ± 1.36</td>
<td>6.3a ± 1.99</td>
<td>6.0a ± 2.17</td>
</tr>
<tr>
<td>Aroma</td>
<td>5.3a ± 1.68</td>
<td>6.3a ± 1.16</td>
<td>5.6a ± 1.92</td>
<td>5.7a ± 2.06</td>
</tr>
<tr>
<td>Sweetness</td>
<td>6.4a ± 1.55</td>
<td>6.2a ± 1.70</td>
<td>4.1b ± 2.45</td>
<td>3.8b ± 2.21</td>
</tr>
<tr>
<td>Flavor</td>
<td>6.5a ± 1.06</td>
<td>5.9b± ± 1.39</td>
<td>4.9cb ± 2.9</td>
<td>3.9c ± 2.15</td>
</tr>
<tr>
<td>Aftetaste</td>
<td>6.7a ± 1.39</td>
<td>6.1a ± 1.19</td>
<td>3.9b ± 1.94</td>
<td>3.7b ± 1.95</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>6.9a ± 1.51</td>
<td>5.8a ± 1.47</td>
<td>4.3b ± 2.23</td>
<td>3.9b ± 2.43</td>
</tr>
</tbody>
</table>

Mean ± Standard Deviation of ten determinations
Means with different letters are significantly different
determines the optimum sensory perception and acceptability. This threshold is yet to be determined.

TSS and pH values have been used as chemical indicator of microbial growth of sugar-cane juice stored at low temperatures. During storage of freeze-concentrated juice, TSS and pH of juice stored at 10 °C and 25 °C decreased with storage time, and the decrease was the fastest in the juice stored at 25 °C. This is not unexpected since a high storage temperature is known to promote microbial infestation of juice (Bhupinder et al. 1991) and caused a subsequent drop in pH. The decreased pH of the juice can be related to the development of acidity of sugar-cane juice during storage (Bhupinder et al. 1991; Yusof et al. 2000) that was thought to be due to acetic acid and lactic acid production.

The slight decrease in TSS with storage might be due to the loss of sucrose that may have been consumed by microbes (Yusof et al. 2000). The sucrose loss could have been reduced if sugar cane juice is prepared with preservatives (Bhupinder et al. 1991). The TSS and pH values of juice stored at −18 and 4 °C remained almost unchanged throughout storage indicating the suitability of these storage temperatures for the juice preservation for a period of 8 days.

The NEB index and colour values for all juice remained almost unchanged throughout storage for all temperatures used (results not shown). Similar patterns of response of TSS, pH, NEB index and colour of fresh sugar-cane juice with storage time was observed (results not shown) and these were similar to the results shown by Yusof et al. (2000). Therefore the pattern of change in most of the physical parameters of freeze-concentrated juice was similar to those of the fresh juice.

It is possible to suggest the incorporation of preservatives such as potassium metabisulphite (Bhupinder et al. 1991) to improve the keeping quality of the freeze-concentrated juice. Depectinization studies, i.e. the addition of pectinesterases on yield of sugar-cane juice are also suggested. Once concentrated, the concentrate can be prepared for retail or consumers by combining with other blends or water for juice standardization.

Realizing this potential, a faster process for water removal of sugar-cane juice through the use of high temperature short-time evaporators, or microwave drying (Suntornsuk et al. 2004) which operate under vacuum should be initiated. Another innovative approach is to blend the juice with a fruit juice or product that will cause a decrease in pH without greatly affecting the sensory attributes. This new blend can be expected to have better shelf life following pasteurization and chilling.

Fruit juice concentrates with high soluble solids are normally produced through a high temperature short-time evaporative process under vacuum to minimize thermal damage to the juice. However, it is also possible to use a heat pump based Freeze Concentration System to concentrate sugar cane juice from 20–40 °Brix in a jaggery making process (Milind and Siddharth 2005). The high technology involving the use of sophisticated equipment is often too expensive for the small and medium industries to venture. The freeze-concentration process employed in this paper may be useful in standardizing the soluble solids in the sugar-cane juice as these will vary with cane maturity, variety and growing conditions.

Conclusion
A freeze-concentration process was successful in producing concentrated sugar-cane juice with a TSS of 30 °Brix. The freeze-concentrated juice differed in most of the sensory attributes as compared to the fresh juice or reconstituted juice concentrate. However the storability of the freeze-concentrated juice was broadly similar to that of fresh sugar cane-juice.
Freeze-concentrated sugar-cane juice

Acknowledgement
Short term grant (Grant no. 304/PTEKIND/634148) from Universiti Sains Malaysia, Pulau Pinang, Malaysia is gratefully acknowledged.

References
Abstrak
Jus tebu (Saccharum officinarum) dengan total pepejal terlarut (TSS) 15 °Brix diperah dari batang tebu tercelur dan digunakan untuk penghasilan jus tebu konsentrat sejuk beku (30 °Brix) menggunakan proses pemekatan sejuk beku. Jus tebu konsentrat sejuk beku menunjukkan kiraan yis dan kulapuk, nilai pH dan warna yang lebih rendah, manakala indeks pemerangan bukan-enzimatik (indeks NEB), kandungan klorofil, kiraan plat aerob dan kelikatan relatif yang lebih tinggi berbanding dengan jus tebu segar. Penilaian sensori menunjukkan jus segar mempunyai skor Hedonik lebih tinggi bagi atribut kemanisan, perisa, ‘aftertaste’ dan penerimaan keseluruhan berbanding dengan jus yang dikonsentrat sejuk beku.


Accepted for publication on 7 August 2006