Influence of the brewing process and degree of milling on the taste characteristics of pigmented rice wine

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Abstract

The taste characteristics of pigmented rice wine were investigated with respect to brewing conditions, and the extent to which the rice had been milled to remove the bran. Both the saccharification and the subsequent alcoholic fermentation processes were monitored over time at 25 ºC and 30 ºC. The following conditions were selected based on maximising ethanol content and minimizing acetic acid: saccharification for two days at 30 ºC and alcoholic fermentation for nine days at 30 ºC. This brewing process was applied to pigmented rice which had been milled to various degrees (0%, 30%, 50% and 65%) and the wine was analysed for taste compounds (sugars, organic acids, amino acids and cyclic dipeptides (2,5-diketopiperazines)). The results showed that the higher degree of milling significantly increased the glucose in the wine, however there was a concomitant loss of glutamic acid ($p<0.05$). Cyclo(Pro-Val), cyclo(Pro-Ile), cyclo(Pro-Leu) and cyclo(Pro-Pro) were detected in pigmented rice wine for the first time. They can impart bitter and metallic tastes, but they were present at concentrations below their reported taste thresholds. Their formation increased as the degree of milling increased and the pH decreased. Based on reported taste thresholds, the compounds most likely to contribute to the taste of pigmented rice wine are acetic acid and glutamic acid.

Introduction

Rice wine or *Sake* is a traditional fermented alcoholic beverage which is becoming increasingly popular in some Asian countries [1]. The production of sake is well-documented. Chinese or Japanese rice wine is prepared using high quality polished glutinous rice, wheat and *koji*, a starter culture which contains both the fungi for the saccharification step, and the yeast for the subsequent fermentation step. During these processes, the brewing temperature is important as it affects the cell growth, cell density, starch hydrolysis and the production of ethanol and organic acids [1]. The taste of rice wine has been described as having sweet, sour, harmonious, mellow, and fresh characteristics [2], which are mainly generated during fermentation when proteins present in the rice are converted to small peptides and amino acids by proteases from the microorganisms [3]. Other non-volatile metabolites, including organic acids and sugars can contribute to the taste of rice wine.

Polished glutinous rice is not the only rice used for wine manufacture. Unpolished pigmented rice, where the bran is retained, is also used to produce pigmented rice wine, especially in countries such as Thailand and the Philippines. However, contrary to *Sake*, it has a unique savoury flavour, and much less is known about the compounds contributing to the characteristic taste and aroma of the pigmented rice wine. Therefore, this study investigated the influence of (i) the brewing process and (ii) degree of milling on the generation of the taste characteristics of pigmented rice wine.

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Experimental

Materials

Glutinous pigmented rice (Double Elephant, Thailand) was purchased from a local supplier at Reading, UK. Brewing microorganisms were *Aspergillus oryzae* ATCC 22787 and *Saccharomyces cerevisiae* NCYC 478 obtained from LGC Standards (UK) and The National Collection of Yeast Cultures (UK) respectively, and cyclic dipeptide standards (2,5-diketopiperazines) were purchased from Bachem AG (Switzerland).

Selection of brewing process

Saccharification

Pigmented rice was steamed for 60 min at 100 ºC and inoculated with the fungi *A. oryzae*, followed by incubation at 25 ºC or 30 ºC for 8 days. Sugars and organic acids were analysed every 24 h. The optimum saccharification process was determined by the conditions (time and temperature) that produced the highest concentration of glucose.

Alcoholic fermentation

The optimum saccharification process was applied to the steamed pigmented rice, which was subsequently inoculated with *S. cerevisiae* and left to ferment for 10 days at either 25 ºC or 30 ºC. Samples were collected every day to determine the levels of sugars, organic acids and the ethanol content. The optimum fermentation conditions were selected on the basics of high ethanol content and reduced levels of acetic acid. Samples were pasteurized at 70 ºC for 10 min.

The brewing of rice wine from pigmented rice with different degree of milling

Pigmented rice was milled in a Twinbird rice polishing machine (Japan) to (partially) remove bran and produce rice of various degrees of milling (DM0% (whole grain), DM30%, DM50% and DM65% (fully polished grain)). The grains were used for brewing under the selected brewing conditions.

Analysis of compounds responsible for taste

Sugars, organic acids and ethanol content

The analysis of sugars, organic acids and ethanol was performed as described by Zeppa et al. [4]. Separation was carried out on an Aminex HPX-87H column (300 x 7.8 mm, 9µm) from Bio-Rad (UK) with 5 mM sulfuric acid as the mobile phase for the separation of the compounds of interest. The selected wavelength for the organic acids was 210 nm, whereas an RI detector was used for the analysis of sugars and ethanol.

Free amino acids

Free amino acids were analysed using the EZfaast™ amino acid derivatization technique (Phenomenex, Torrance, CA), followed by GC-MS (Agilent, Germany) as described by Elmore et al. [5].

Cyclic dipeptides

Analysis of cyclic dipeptides was carried out as described by Oruna-Concha et al. [6]. Briefly, pigmented rice wine (15 mL) was mixed with 50 µL of 3-chlorophenol (100 mg/L) as internal standard, and then passed through the SPE cartridge (Strata-X 33 µm polymeric reversed phase giga tube, Phenomenex). HPLC water and methyl acetate were used for washing and elution, respectively. The eluent was concentrated by flushing with N_2, and then injected into the GC-MS equipped with a ZB-Wax column.

Results and discussion

Selection of brewing conditions for the production of pigmented rice wine

Saccharification is one of the important steps during brewing, as the starch present in cooked rice is converted to simple sugars, thus acting as nutrients for the subsequent fermentation stage and contributing to the taste and flavour of the rice wine [1]. Sugars including maltotriose, maltose and glucose were monitored throughout the saccharification process (Figure 1). Sugar levels were low on day 1 regardless of the temperature, however the concentration of maltotriose and maltose significantly increased by day 2 as the rice starch was degraded to maltotriose and maltose by the fungi. From day 2, an increase in glucose levels was observed as both maltotriose and maltose were converted to glucose. The highest levels of glucose were observed at day 6. After that, the sugars levels decreased as their rate of formation was less than their rate of consumption by *A. oryzae*. Slightly higher levels of sugars were observed at 30 ºC and therefore the optimum saccharification process for this study was set at 30 ºC for 2 days.

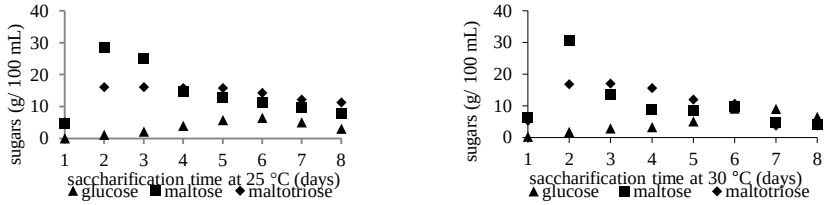

Figure 1: Glucose, maltose and maltotriose generated from pigmented rice during saccharification by *A. oryzae*; n=3 brews. The standard deviation was generally <2%, and <5% in all cases.

Following saccharification, the rice was inoculated with *S. cerevisiae* for alcoholic fermentation. During this step, and regardless of temperature, the levels of maltose and glucose decreased, whereas an increase in ethanol was observed, particularly on day 9 (Figure 2). Malic, lactic, succinic and acetic acid were also formed by yeast metabolism which used glucose as a substrate [1]. Moreover, fermentation at 30 ºC produced more ethanol and lower levels of acetic acid ($p<0.05$). Although acetic acid is the most abundant volatile acid in wine, its excessive concentration (>0.9 g/L) affects negatively the quality of wine because it can contribute a bitter or sour aftertaste [7]. Therefore, the optimum fermentation conditions for this study were set at 30°C for 9 days.

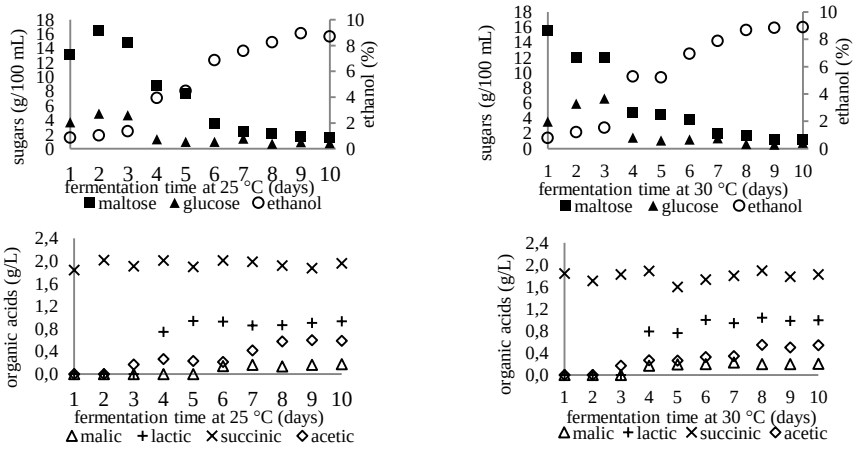

Figure 2: Sugars, ethanol and organic acids produced during fermentation from pigmented rice; n=3 brews. The standard deviation was generally <2%, and <5% in all cases.

Effect of degree of milling on the characteristic taste compounds of pigmented rice wine

Four proline-based cyclic dipeptides, namely cyclo(Pro-Val), cyclo(Pro-Ile), cyclo(Pro-Leu) and cyclo(Pro-Pro) were identified in pigmented rice wine (Table 1) however their concentrations were lower than the reported threshold [8] and they are therefore unlikely to contribute to the taste of rice wine. It is likely that these cyclic dipeptides are generated by the action of the yeast [6] during the brewing process. A Pearson correlation ($p=0.01$) showed a correlation between pH and the formation of cyclic dipeptides, the lower pH being more favourable for formation of cyclic dipeptides [8].

Table 1 shows that glucose significantly increased (0.79-1.78 g/L, $p<0.05$) as the DM increased, however no significant differences were observed in the ethanol content. The predominant acid was acetic acid (0.36-0.65 g/L) and glutamic acid was the predominant amino acid (0.62-1.17 g/L), all present at concentrations higher than their reported thresholds [9], thus contributing respectively to the unique sour and umami taste characteristics of pigmented rice wine. Moreover, this study has shown that retaining the bran increases the glutamic acid in pigmented rice wine ($p<0.05$).

Table 1: pH, ethanol and taste compounds found in pigmented rice wines

taste compounds		*degree of milling*				*threshold*
		0%	*30%*	*50%*	*65%*	
pH		4.76^{d}	4.24^{c}	3.94^{b}	3.69^{a}	-
ethanol (%)		11.9^{a}	12.2^{a}	11.6^{a}	11.8^{a}	-
glucose (g/L)		0.79^{a}	1.20^{b}	1.19^{b}	1.78^{c}	3.24 [9]
glutamic acid (g/L)		1.17^{c}	0.81^{b}	0.62^{a}	0.62^{a}	0.18 [9]
organic acids (g/L)	*lactic acid*	0.73^{a}	0.90^{a}	1.04^{a}	0.76^{a}	1.39 [9]
	acetic acid	0.65^{c}	0.39^{a}	0.36^{a}	0.48^{b}	0.12 [9]
cyclic peptides (mg/L)	*cyclo(Pro-Val)*	1.07^{a}	1.88^{b}	1.79^{b}	2.06^{b}	251 [8]
	cyclo(Pro-Ile)	5.04^{a}	14.2^{b}	16.3^{b}	17.2^{b}	101 [8]
	cyclo(Pro-Leu)	8.96^{a}	9.74^{a}	9.34^{a}	9.81^{a}	250 [8]
	cyclo(Pro-Pro)	2.98^{a}	4.14^{b}	4.27^{b}	4.94^{c}	501 [8]

Values are the mean of three replicates. Means with different letters are significantly different at $p=0.05$.

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