Relationship Between Yeast Strains and Nutritive Supplements and Its Influence on Enological Parameters of Santol (*Sandoricum koetjape* Merr.) Wine Production

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ABSTRACT

To improve the enological characteristics of santol wine production, two yeast strains (G74 and SIHA3) under five nutritive supplements (0.3 and 1.0 g L^{-1} DAP, 0.6 mg L^{-1} thiamine, 0.4 g L^{-1} Fermaid E, 0.4 g L^{-1} Fermaid K) and a control were investigated. Santol juice-based wine contained 18 g L^{-1} glucose, 22 g L^{-1} fructose, 2.8 g L^{-1} citric acid and 0.4 g L^{-1} succinic acid. The concentrations of most organic acids were within the limit range of good wines, except for the large amount of 3 g L^{-1} citric acid. The addition of 1.0 g L^{-1} DAP for both strains produced more than 40 g L^{-1} d^{-1} CO_2 during fermentation and showed a lower sulfur binding capacity of 18-19 mg L^{-1}.

Key words: santol wine, diammonium hydrogenphosphate, thiamine, organic acids, sulfur binding capacity

INTRODUCTION

Santol (*Sandoricum koetjape* Merr.), which belongs to the *Meliaceae* family, is a large ornamental as well as a fruit tree from the Asiatic tropics and is grown in the Philippines, Malaysia, Cambodia, Laos, Vietnam, Indonesia, Sarawak, Brunei, India, Guam and Thailand. Fruits are round, yellow-orange, sour-sweet, of apple size and are consumed in fresh or picked form. It is one of the indigenous Thai fruits used for wine production in Thailand. Since the liberalization of alcoholic beverage production in 2000, the wine industry has developed from a monopoly concession to a new market-driven scheme, which allows small and medium-sized industries to enter into this business countrywide. Scientists have seriously attempted to exploit better techniques for the fruit-wine industry to produce good-quality wines and obtain more market share (Jitjaroen, 2007).

Selection of yeasts has been based on the ability to ferment under the adverse conditions found in grape musts, such as a high level of sulfur dioxide, low pH, low vitamin concentration and low nitrogen. Microbiological control during vinification is essential in the production of high-quality wines (Reynolds *et al.*, 2001). Boulton (1980) studied the influence of nitrogen on fermentation kinetics; low initial nitrogen concentration caused nitrogen exhaustion and the growth rate of yeasts declined rapidly. Higher

initial concentrations of nitrogen delayed or prevented the decline in growth rate. The concentration of alcohol was related to the initial nitrogen concentration. It was suggested that ethanol tolerance of yeasts was, therefore, a function of nitrogen concentration. The maximal carbon dioxide evolution rate was directly proportional to the initial nitrogen concentration in the medium. A high initial nitrogen concentration stimulated and sustained the maximum carbon dioxide production rate. Intermediate concentrations had a neutral effect, while a low initial concentration reduced the carbon dioxide production rate and shortened the phase at the maximum rate (Henschke and Jiranek, 1992).

Santol wine fermentation was studied in detail by Jitjaroen (2007). The starchy odour resulting from the remaining starch in the juice-based wine was reduced in santol wine using two commercial amylase enzymes (Distizym BA-T, and Fructamyl FCT). The starch degradation by 0.05% Distizym BA-T enzyme after 1 h was beneficial; it contributed to the production of the largest concentration of 24 g L^{-1} glucose and 27.9 g L^{-1} fructose. The sugar yield formed after starch degradation was rather low and added about 25% to the natural santol juice. This step seemed to be superfluous for santol juice-based wine preparation. Therefore, in this study the relationship between yeast strains and nutritive supplements was investigated in terms of the kinetic parameters. The nutrients were used in the form of single and complex nutrients in combination with different yeast strains. The objective of this study was to identify methods and techniques that would enable santol wine makers to improve wine quality.

MATERIALS AND METHODS

Analytical methods

Santol juice was analyzed for pH value,

titratable acidity, D-glucose, D-fructose and organic acids. Santol wines were analyzed for carbon dioxide production, organic acids, pH value, titratable acidity, total and free sulfur dioxide (free SO_2).

Carbon dioxide production was examined by daily weighing, total and free SO_2 by the Ripper titrametric method, residual sugar and alcohol contents by the Rebelein titrametric method (Zoecklein et al., 1995), D-glucose and D-fructose by an enzymatic method (Mannheim, 1998) and organic acids using a Hewlett Packard (HP series 1100) high performance liquid chromatograph (HPLC) (Schneider et al., 1987). Yeast cultures

The yeast strains *Saccharomyces cerevisiae* SIHA3 and *S. cerevisiae* G74 were used for alcoholic fermentation. The former is a commercial, dry yeast obtained from Begerow GmbH, Germany, which was rehydrated for 25-30 min at 35°C and was added to the must at 0.02% (v/v). G74 was a liquid yeast received from the Department of Microbiology and Biochemistry, Research Institute of Geisenheim, Germany and was inoculated at 3% (v/v).

Experimental fermentations

Two sets of santol wine production were undertaken. Firstly, santol juice was analyzed for its physico-chemical composition. Secondly, santol must was fermented with two yeast strains (G74 and SIHA3) with one of five nutritive supplements (0.3 and 1.0 g L^{-1} diammonium hydrogenphosphate (DAP), 0.6 mg L^{-1} thiamine, 0.4 g L^{-1} Fermaid E (Fer.E), 0.4 g L^{-1} Fermaid K (Fer.K)), or a control, thus giving twelve different fermentation treatments. Experiments were conducted at the Department of Microbiology and Biochemistry, Research Institute of Geisenheim, Germany. All treatments were replicated twice and evaluated parameters presented in the form of mean values.

Santol wine fermentation with different yeast strains and nutritive supplements

The fermentation base was adjusted to a sugar content of 200 g L^{-1} by the addition of sucrose and added sulfur dioxide to a level of 50 mg L^{-1}. Samples of 650 mL of must were placed in one-litre white bottles and mixed well with the different nutritive supplements and yeast strains, fitted with a fermentation lock and incubated at 25°C until attenuation. Subsequently, sulfur dioxide was added and calculated on the basis of residual 50 mg L^{-1} free SO_2 in the finished wine.

RESULTS AND DISCUSSION

Santol juice components

Santol is a highly acidic fruit with a yellow-orange juice initially containing 18.2 g L^{-1} glucose, 22.3 g L^{-1} fructose, with 7.4 g L^{-1} total acidity (as citric acid) and a pH of 3.3. The main acids were 2.8 g L^{-1} citric acid, 0.37 g L^{-1} succinic acid, 0.13 g L^{-1} malic acid and 0.0004 g L^{-1} shikimic acid (results not shown). Therefore, the fermentation base was adjusted to a sugar content of 200 g L^{-1} initially by sucrose, while pH and acidity were appropriate for yeast fermenting (Jackson, 1994).

Carbon dioxide production

Yeast growth is usually described in terms of the kinetic parameters associated with the different stages of growth. The maximum fermentation rate was about 21 g L^{-1} d^{-1} CO_2, which occurred when assimilable nitrogen was maximal at 300 mg L^{-1} (Henschke and Jiranek, 1992). In the present study, the development of santol wine was determined by the fermentation rate based on the maximum rate of carbon dioxide production. The supplements were investigated based on the addition of a single nutrient, such as DAP or complex nutrients such as Fermaid E and Fermaid K, which are commercially blended yeast nutrients of ammonia salts (DAP) α-amino nitrogen, sterols, unsaturated fatty acids, magnesium sulfate, thiamine, folic acid, niacin, biotin, calcium pantothenate and inactive yeast.

The selected yeast strains and nutritive supplements clearly affected CO_2 production during fermentation (Figure 1). The addition of nutritive sources like DAP and Fermaid E enhanced CO_2 evolution up to 40-45 g $L^{-1}d^{-1}$ and exceeded that of the thiamine and control groups. The minimum amounts of CO_2 were released by strain SIHA3 in the control and
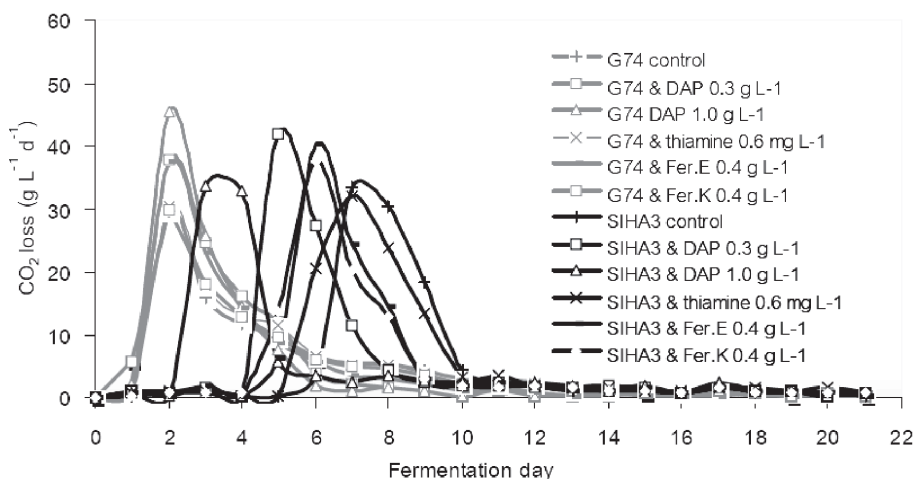

Figure 1 The daily carbon dioxide loss during santol wine fermentation using two yeast strains and five nutritive supplements or a control.

thiamine treatment groups ranging from 29 to 32 g $L^{-1}d^{-1}$. The fermentation activity was initiated sooner by strain G74, on the second day, as compared to strain SIHA3, which started on the third to the seventh day. All treatments produced an alcohol content of 12-13% (v/v), concomitant with residual sugar contents of 2-5 g L^{-1}. The yeasts could not perfectly complete their fermentation period within 21 days. This conclusion was derived from the remaining slight carbon dioxide evolution at attenuation and the residual sugar content in the finished wine. This may have been due to depletion of nutrients, which were essential in the fermentation pathway, as well as to the capability of each yeast strain to utilize sugar during wine fermentation.

Organic acids

Some differences were noted between the treatments in terms of production and/or utilization of organic acids. These are reflected by the low concentrations of tartaric, malic and citric acids in the wine. In the current study, small changes were obtained in pH ranging from 3.3 to 3.5-3.6 corresponding with a decrease of acidity from 7.4 to 6.2-7.1 g L^{-1} and depending on the extent of changes in the organic acids content (Table 1). These organic acids apparently increased slightly in all wine samples and differed among the treatments. The concentrations of most organic acids were within the range known from grape wines (up to 1 g L^{-1} malic acid, 0.1-1 g L^{-1} lactic acid and 0.6-0.9 g L^{-1} acetic acid), except for a large amount of 3-3.5 g L^{-1} citric acid, which was present in concentrations significantly beyond the upper limit reported for good wine quality, for example in the range from 0.5 to 1 g L^{-1} (Amerine *et al*., 1972; Amerine and Ough, 1980; Ribereau-Gayon *et al*., 2000; Radler, 1982). Therefore, the expected level of ethanol, the formation of appropriate organic acid concentrations and the carbonyl compounds that are present regularly in finished wine may be related to the capability of

the yeast to metabolize glucose via glycolysis and to the fermentation pathway forming pyruvic acid, which is oxidized to acetaldehyde and subsequently reduced to ethanol (Zeeman *et al*., 1982; Radler, 1982; 1986). The occurrence of a large amount of citric acid originated from the natural juice itself.

Sulfur binders and sulfur binding capacity

The contents of the three metabolic carbonyl compounds differed among the treatments (Table 1). In most samples, there was no pyruvate detected. Less acetaldehyde was produced (16 mg L^{-1} for strain G74 and 22 mg L^{-1} for strain SIHA3), when DAP was added at 1.0 g L^{-1}. Yeast strain G74 as well as SIHA3, when supplemented with 1.0 g L^{-1} DAP, formed the smallest amount of total binding capacity (18-19 mg L^{-1}) concomitant with less acetaldehyde, puruvate and ·-ketoglutarate. These carbonyl compounds depended upon yeast strains and increasing nitrogen source in the present study. The accumulation of carbonyl compounds in most samples appears to be a result of thiamine deficiency (Ribereau-Gayon *et al*., 2000). A sufficient concentration of thiamine reduces the synthesis of carbonyl compounds that bind to sulfur dioxide, thereby diminishing the amount of sulfur dioxide needed to control spoilage organisms adequately (Jackson, 1994). Lafon-Lafourcade *et al*. (1967) stated that thiamine was not only a growth factor but could also limit the rate of sulfur dioxide evolution during wine storage by promoting the decarboxylation of ketonic acids (pyruvic and ketoglutanic acids).

Since there is regulatory pressure to minimize the total quantity of sulfite in foods, it is important to minimize the sulphite-binding capacity of finished wines as far as possible in order to obtain an adequate amount of free SO_2 in the packaged product (Beech and Jarvis, 1989). It may be that 30 mg L^{-1} of free SO_2 was too high in one wine at lower pH (Zoecklein *et al*., 1995).

Table 1 Mean values of the chemical composition of santol must and wines as affected by two yeast strains and six nutritive supplements.

Nutritive supplements	pH	Acidity [a]	Alcohol	Total sugar	Malic acid	Shikimic acid	Lactic acid	Acetic acid	Citric acid	Succinic acid	Pyruvate	Acetal-dehyde	α-Keto glutarate	Bound SO$_2$ [b]	Free SO$_2$	Total SO$_2$
		g L^{-1}	% v/v	g L^{-1}	g L^{-1}	g L^{-1}	g L^{-1}	g L^{-1}	g L^{-1}	g L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
Strain G74																
Control	3.5	6.5	12.7	2.6	0.43	0.004	0.14	0.30	3.33	0.22	nd [c]	35	159	38	136	174
DAP 0.3 g L^{-1}	3.5	6.4	13.2	2.3	0.44	0.01	0.09	0.24	3.10	0.23	1	31	nd	45	60	105
DAP 1.0 g L^{-1}	3.5	6.4	12.8	1.6	0.32	0.02	0.14	0.30	2.77	0.17	nd	16	72	18	72	90
Thiamine0.6mg L^{-1}	3.5	6.4	13.0	2.3	0.36	0.004	0.18	0.32	3.32	0.23	nd	33	18	33	68	101
Fer.E 0.4 mg L^{-1}	3.5	6.3	13.2	2.2	0.40	0.01	0.10	0.25	3.16	0.22	nd	31	39	45	72	118
Fer.K 0.4 mg L^{-1}	3.5	6.2	13.0	2.2	0.39	0.004	0.18	0.28	3.31	0.20	nd	33	19	44	69	113
Strain SIHA3																
Control	3.6	6.6	12.4	4.4	0.34	0.002	0.32	0.20	3.65	0.35	nd	27	nd	51	37	88
DAP 0.3 g L^{-1}	3.5	6.9	12.4	4.6	0.33	0.01	0.18	0.21	3.50	0.34	nd	29	nd	37	48	84
DAP 0.1 g L^{-1}	3.4	7.0	12.0	2.7	0.20	0.005	0.18	0.22	3.07	0.36	nd	22	nd	19	52	72
Thiamine0.6mg L^{-1}	3.5	6.9	12.2	3.7	0.40	0.002	0.34	0.18	3.54	0.39	nd	30	nd	49	37	86
Fer.E 0.4 g L^{-1}	3.5	6.9	11.9	4.7	0.34	0.002	0.25	0.15	3.64	0.39	nd	35	nd	50	52	103
Fer.K 0.4 g L^{-1}	3.5	7.1	12.3	3.3	0.45	0.018	0.20	0.24	3.52	0.31	nd	40	nd	77	44	121

a   compared with citric acid
b   calculated on the basis of residual 50 mg L^{-1} free SO$_2$ in the finished wine
c   not detected

In most of the samples in the current study, free SO_2 levels were higher than the value of 50 mg L^{-1}, which is regarded as the appropriate amount in finished wine. The sulfiting resulted from predicting 50 mg L^{-1} free SO_2 based on the sulphur-binding capacity remaining in the finished samples. Addition of DAP at 1.0 g L^{-1} gave the lowest level of total sulfur dioxide related to the lowest binding capacity.

CONCLUSIONS

Selected yeast strains and nutritive sources were evaluated in terms of their impact on the chemical composition of fermenting santol must. Fermentation activity stayed on a low level in the attenuation stage of santol juice-based wine because it contained insufficient essential nutrients required for maximum growth and survival during fermentation. The sulfur-binding capacity remained high consequently, resulting in excessive sulfiting and an unpleasant, volatile sulfur aroma in the finished wine. More research is needed to minimize the sulfite-binding capacity of wine as much as possible in order to limit the sulfur dioxide level in the packaged product. Besides, finished wines were not entirely consumer-acceptable yet, due to an unpleasant, cloudy appearance. Hence, the elimination of the remaining starch is another very important point which should be considered in santol wine production, for instance by filtration and addition of pectinase enzyme.

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