

Research Article

Impact of commercial yeast strains on wine fermentation and formation of metabolites of yellow passion fruit (*Passiflora edulis* Sims *f. flavicarpa* Degner)

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Abstract

Juice of yellow passion fruit (YPF) was fermented with different commercial yeast strains of *Saccharomyces*. This research aimed to investigate the impact of one commercial strain of *Saccharomyces bayanus* (Strain A) and two strains of *Saccharomyces cerevisiae* (Strain B and C) on the fermentation kinetics and formation of metabolites produced by wine yeast in YPF wines. The results showed that most parameters were significantly different depending on the yeast strain used. The YPF wine fermented with the A and B strain showed the most diverse fermentation parameters and produced significantly higher concentrations of fatty acid ethyl esters, which contribute to very pleasant odours of various fruity notes in YPF wine. However, the B strain also produced high concentrations of acetaldehyde, acetic acid and acetic acid esters in YPF wines. The C strain developed the highest amounts of lactic acid. This strain also produced larger concentrations of keto acids, principally pyruvate and α -ketoglutarate, which have implications for wine stability and quality due to their abilities to bind sulphur dioxide.

Keywords: yeast strains, metabolites, yellow passion fruit, *Passiflora edulis* Sims *f. flavicarpa* Degner, *Saccharomyces*, Thailand

Introduction

During alcoholic fermentation, *Saccharomyces* yeasts do not only convert sugars to ethanol and carbon dioxide [41], they also produce a wide range of metabolites, for example, glycerol, acetic acid, acetaldehyde, pyruvate and lactic acid [3]. The keto acids, principally pyruvate and α -ketoglutarate, have implications for wine stability and quality due to their abilities to bind sulphur dioxide and to react with phenols [2, 15, 17, 18, 19]. Acetic acid is of particular importance, as it imparts a vinegar-like character and becomes objectionable at concentrations of 0.7-1.1 g/L. Depending on the style of wine and yeast strain, the acceptable concentration is 0.2-0.7 g/L [6, 22, 33, 36]. Glycerol is a major product of alcoholic fermentation [24, 36], which imparts tastes of slightly sweet, as well as having an oily and heavy mouth-feel [41]. Its metabolism by yeasts plays several important roles during anaerobic fermentation of sugar, for example, to provide precursors for the synthesis of phospholipids, which are components of cell membranes [14, 36, 41]. Rankine [16] and Reynolds *et al.* [22] reported a range of 5-6.4 g/L in wines fermented by various yeasts. Acetaldehyde is the major carbonyl compound found in wine, which contributes to flavour with aroma descriptors such as “bruised apple” and “nutty” but can also be a sign of wine oxidation, especially in white wine [41].

A vast number of volatile compounds are also formed and modulated by yeast during alcoholic fermentation that have a significant impact on the flavour and overall quality of wines [11]. The choice of the yeast strain used by the winemaker is increasingly motivated by the potential impact of that strain on the wine characteristics [23]. However, not all *Saccharomyces* strains have the same capacity to reveal these compounds [23, 28]. The use of different *Saccharomyces* strains for wine fermentation has been shown to result in wines with differing secondary metabolites, through varied relative concentrations of acetic acid esters, fatty acid ethyl esters and higher alcohols [3, 9, 13, 29], which are sensorially important volatile metabolites giving wines its vinous character [20, 38, 40]. The volatile esters represent the largest and most important group of flavour compounds produced during fermentation [26]. The characteristic fruity odours of wine and other grape-derived alcoholic beverages are primarily due to a mixture of acetic acid hexyl ester (hexyl acetate), hexanoic acid ethyl ester (ethyl hexanoate) and octanoic acid ethyl ester (ethyl octanoate) (apple-like aroma), acetic acid 3-methylbutyl ester (banana-like aroma) and acetic acid 2-phenylethyl ester (fruity, flowery flavour with a honey note) [12, 28, 41].

Passion fruit is known for its natural attractive colouring, unique flavour properties and medicinal purposes. It not only has high amounts of vitamin A and C, potassium, dietary fibre, carotenoids and polyphenolics [43], but also is the best tropical fruit having a floral, estery aroma with an exotic tropical sulphury note [32]. However, its high acidity limits its use as an ingredient in the formulation of various preparations such as beverages, ice cream, marmalade, cocktails, etc. The compounds involved in passion fruit aroma described until now were terpenes and norisoprenoids, present in both free and glycosylated form, glycosides of benzyl alcohol, 3-methyl-2-buten-1-ol, and 2-hydroxy-2-phenylacetonitrile (mandelonitrile) [4, 5, 7, 32]. Jordán *et al.* [10] reported that the most abundant compounds in yellow passion fruit aqueous essence are linalool, octanol, hexanoic acid ethyl ester and butanoic acid ethyl ester. Sulphur-containing volatiles also play important roles in the flavour of yellow passion fruit, like 2-methyl-4-propyl-1,3-oxathiane, 3-mercaptohexanol (3MH, which contributes to aromas of passion fruit and grapefruit), 3-mercaptohexyl acetic acid ester (3MHA, which contributes to aromas of boxwood, grapefruit zest and passion fruit). 3-methylthiohexanol and the acetic acid esters, butanoic acid esters (butanoates) and hexanoic acid esters (hexanoates) of the two

alcohols have been identified and described also as key odorants of yellow passion fruit [37, 7, 8, 27, 30, 31, 32]. All these volatiles are among the most potent components responsible for the typical tropical-fruity notes of passion fruit [10, 32].

However, only little information is available on the improvement of passion fruit wine quality by optimal choice of yeasts and nearly no information is available to optimise the release and preservation of volatile flavours in passion fruits wine during the alcoholic fermentation. Therefore, this study was aimed at examining the impact of commercial yeast strains on fermentation kinetic and formation of metabolites, for example organic acids, acetaldehyde, keto acids (pyruvate and α -ketoglutarate), glycerol and higher alcohols of passion fruit wine.

Research Methodology

The fermentation for this study was conducted at the department of Food Science and Technology, Lampang Agricultural Research and Training Centre, Lampang, Thailand and YPF wine samples were then transported to the Section of Microbiology and Biochemistry, Geisenheim Research Centre, Germany for further investigation.

Passion fruit juice

The frozen YPF puree employed for this study is the hybrid variety of *Passiflora edulis* Sims f. *flavicarpa* Degner. It was purchased from Thai Nutri-Juice Co., Ltd., Thailand and kept at -25°C until use. The properties of YPF puree were TSS (total soluble solid) 14.4°Brix (sugar content 152 g/L), pH 2.81, titratable acidity (TA) 51.1 g/L (data not shown). The frozen YPF puree was thawed at ambient temperature and deacidified by addition of hot water until its pH was 3.15-3.2. The sugar content of deacidified YPF juice was TSS 1°Brix (sugar content 10 g/L), then sugar content was adjusted to provide sugar quantities of 200 g/kg juice by addition of cane sugar (sucrose) and 500 mg/kg diammonium hydrogen phosphate $[(\text{NH}_4)_2\text{HPO}_4]$ was supplemented. The juice was filled into two brown glass bottles of 0.75 L with YPF juice volume of 0.65 L in order to provide two fermentation replicates, then 50 mg/L of sulphur dioxide was adjusted by addition of potassium metabisulphite ($\text{K}_2\text{S}_2\text{O}_5$). Juice bottles were left for approximately 12 hours to suppress undesirable microorganism growth as well as to function as an antioxidant. The properties of the prepared YPF juice were TSS 16.4°Brix (sugar content 175 g/L), pH 3.5, TA 6.3 g/L (data not shown).

Yeast strains

The three commercial wine yeast strains used were characterized as follows: *Saccharomyces bayanus*, Strain A (Lallemand, Danstar Ferment AG, Zug, Switzerland), which produces low amounts of undesirable fermentation by-products such as SO_2 , hydrogen sulphide (H_2S), acetaldehyde and volatile acid, *Saccharomyces cerevisiae*, Strain B (DSM Food Specialities, Delft, The Netherlands), which produces low to average amounts of SO_2 , glycerol, volatile acidity and acetaldehyde and *Saccharomyces cerevisiae*, Strain C (Laffort Oenologie, Bordeaux, France), which produces low amounts of undesirable fermentation by-products but releases fruity aroma.

Fermentation

The yeast cultures were rehydrated following the recommendations of the manufacturer prior to inoculation in order to achieve 10^5 – 10^6 cells/mL of each strain and were randomly assigned to each of two bottle replicates. The bottles were fitted with airlocks and the fermentations were carried out at 20°C in a controlled environment. The progress of fermentation was

followed by monitoring CO₂ production, which was determined by weight loss during fermentation. After the weight losses of the samples were constant, the YPF wines were cold stabilized at 4-5°C for 7 days and racked into previously sterilized bottles. Then potassium metabisulphite was added corresponding to 80 mg/L YPF wine and the bottles were sealed with sterilized crown caps. Bottled YPF wines were stored at below 15°C until the transportation to Germany for further investigation.

Analyses

TSS was analysed by hand refractometer (range 0 - 32°Brix, Atago, N-1 E, Japan) and the pH was analysed by pH meter (Hanna 8520 instrument: HANNA instruments® Inc. 584 Park East Drive Woonsocket, RI 02895, USA). Residual sugar (as glucose) and titratable acidity (TA, as citric acid) were analysed according to Iland *et al.* [35]. Organic acids were determined by Hewlett Packard (HP) Series 1100 high performance liquid chromatograph (HPLC) equipped with a multiwavelength detector (UV/Visible) according to a modified procedure from Schneider *et al.* [25]. Organic acids were resolved on an Allure® Organic Acids column (259 mm x 4.6 mm i.d., 5 µm particle sizes) using diluted sulphuric acid as eluent and UV detection at 210 nm. Keto acids, glycerol and acetaldehyde were determined enzymatically by an UV/VS spectrometer Lambda 2 (Perkin Elmer GmbH, Überlingen, Germany) and wavelength at 340 nm equipped with a refrigerated/heating circulator, Model F25-ME (JULABO Labortechnik GmbH, Seelbach, Germany) and controlled at 25°C isothermic condition according to instructions of Boehringer Mannheim Company [34]. Esters and higher alcohols were detected by HP 5890 Series II gas chromatograph equipped with a cooled injection system CIS-3 (Gerstel GmbH, Mühlheim an der Ruhr, Germany) and HP 5972 mass selective detector (MSD) according to a modified procedure from Rapp *et al.* [21].

Statistical analysis

The one-way analysis of variance (ANOVA) and least significant difference (LSD) test were performed using MSTATC statistical program [46] to interpret mean differences in mean values.

Results and Discussion

YPF is a highly acidic fruit having TA (as citric acid) 51.1 g/L and pH 2.81 (data not shown). The main acids were citric acid 46.3 g/L, followed by malic acid 6.5 g/L and acetic acid 0.3 g/L (Fig. 1a). Thus, it is necessary to perform deacidification by dilution with water in order to adjust to the optimal acidity for sensory preferences as well as yeast growth [36, 41, 45].

Fermentation kinetics

The YPF wines were fermented with A, B and C yeast strain until the weight losses were constant. Residual sugar concentrations were below 2 g/L after 35-38 days of fermentation (Table 1). The A strain had similarly fast fermentation kinetics to that of the B strain, while the fermentation of the C strain was slightly slower (Fig. 1b). The reason for this phenomenon could probably be that this strain usually has slow-acting fermentation kinetics.

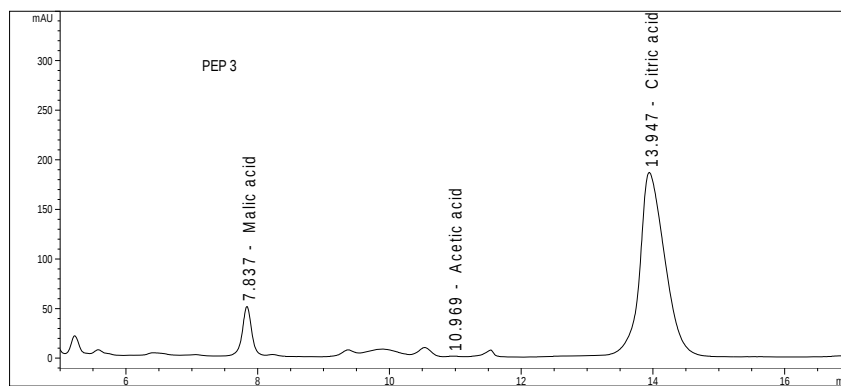


Figure 1a. Chromatogram of organic acids from YPF puree using HPLC.

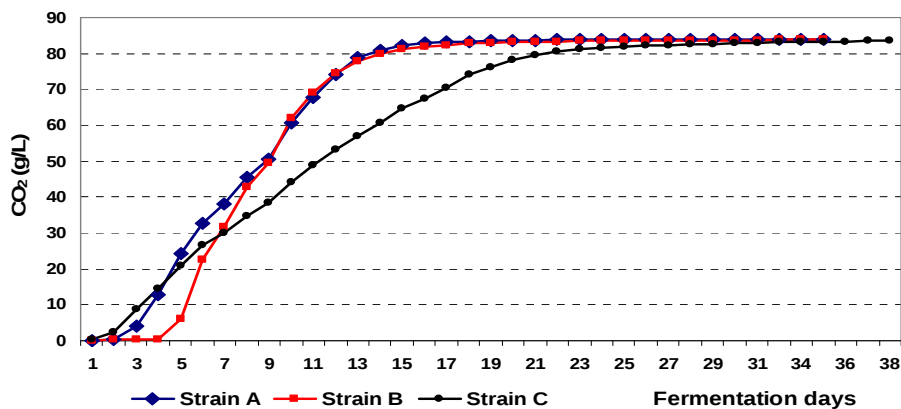


Figure 1b. Effect of different yeast strains on fermentation kinetics during fermentation of YPF juices

Composition of passion fruit wines

The results (Table 1) showed that there were significant differences among the various YPF wines in respect to lactic acid and acetic acid ($p < 0.05$), whereas reducing sugar and other organic acid concentrations were not significantly different among YPF wines. The B strain wine had the highest acetic acid concentration, while the C strain had the highest lactic acid concentration. The concentration of these acids in YPF wines were within the range known from grape wines (malic acid up to 1 g/L, tartaric acid 2-8 g/L, acetic acid 0.2-0.7 g/L and lactic acid 0.1-1 g/L) as well as from some fruit wines [42]. It was exceptional for citric acid, which was present in concentrations beyond the upper limit for good wine quality (0.5-1 g/L) [6, 22, 33, 36]. However, the occurrence of large amounts of citric acid is assumed to originate from the natural juice itself as shown in Fig.1 and in accordance with the literature [42].

Table 1. Some chemical compositions of prepared YPF juice and YPF wines as affected by three different yeast strains.

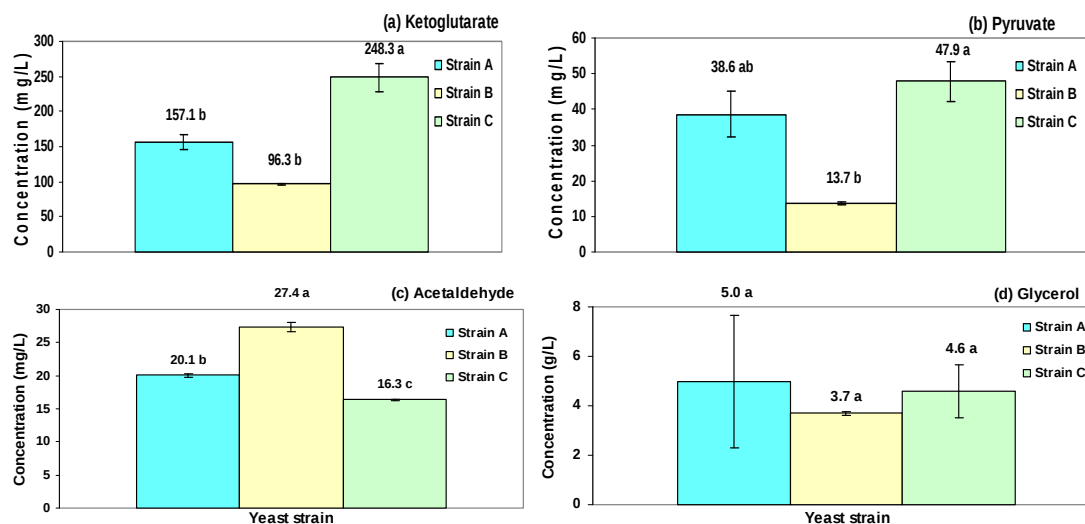
Compounds	YPF wines from different yeast strains		
	Strain A	Strain B	Strain C
Residual sugar (g/L)	0.42 a	0.47 a	0.27 a
Citric acid (g/L)	3.78 a	3.78 a	3.81 a
Lactic acid (g/L)	0.22 b	0.11 c	0.28 a
Tartaric acid (g/L)	0.02 a	0.03 a	0.01 a
Acetic acid (g/L)	0.25 b	0.40 a	0.27 b
Malic acid (g/L)	0.79 a	0.84 a	1.02 a

The same letter (only 'a') adjacent to means in the same horizontal line indicates significant difference ($p > 0.05$), whereas the different letters ('a', 'b' or 'c') adjacent to means in the same horizontal line indicate significant difference ($p < 0.05$) according to the LSD test.

Formation of metabolites

Keto acids, acetaldehyde and glycerol

The results (Fig. 2) clearly show that the formation of keto acids and acetaldehyde in YPF wines depends on the yeast strain employed ($p < 0.05$), and that the data correspond to those reported in the literature [15, 17, 18, 19, 42]. The C strain produced the highest concentration of keto acids in YPF wine (Fig. 2a, ketoglutarate; Fig. 2b, pyruvate) but the lowest concentration of acetaldehyde (Fig. 2c). However, the amounts of keto acids were within the range found in grape wine [6, 18, 19, 26] and some fruit wine [42]. Rankine [15, 17, 19] reported that the amounts of keto acids normally present in wine are unlikely to have any direct effect on the aroma and flavour of wine, but could significantly effect the binding of sulphur dioxide, which reduces its effectiveness. The amounts of acetaldehyde were also within those usually found in grape wines [26, 36] and in some fruit wines [42]. The amounts of glycerol in YPF wines did not differ between the yeast strains (**Fig. 2d**), and mean values for the three yeast strains ranged from 3.7 to 5 g/L in YPF wines, which are within those usually found in grape wines [36] and in some fruit wines [42].

**Figure 2.** Concentration of metabolite by-products in YPF wines produced by 3 different yeast strains.

(a) α -Ketoglutarate, (b) Pyruvate, (c) Acetaldehyde, and (d) Glycerol

Vertical bars represent standard deviations from two fermentations. Means followed by different letters on the top of the bar are significantly different ($p < 0.05$), whereas means followed by same letters on the top of the bar are not significantly different ($p > 0.05$) according to the LSD test.

Esters

The results (Fig. 3) showed that the B strain produced the highest concentration of acetic acid esters (Fig. 3a-c) in YPF wine ($p < 0.05$). Ribéreau-Gayon *et al.* and Swiegers *et al.* [1, 36] reported that the acetate esters contribute positively to wine aroma at very low concentrations such as acetic acid ethyl ester 50-80 mg/L, acetic acid 3-methylbutyl ester 0.1-3.4 mg/L and acetic acid phenylethyl ester 0-18.5 mg/L. However, the concentrations of these acetic acid esters were generally low in comparison with the data known in grape wines [36, 41]. In addition, the higher amounts of acetic acid developed by the B strain (Table 1), were probably responsible for its acetate ester accumulation in YPF wine as well.

The concentrations of ethyl esters in YPF wines were different depending on yeast strains ($p < 0.05$), except for decanoic acid ethyl ester (Fig. 3e). The A and B strain produced a significantly higher concentration of hexanoic acid ethyl ester and butanoic acid ethyl ester in YPF wines (Fig. 3d, f). Several authors reported that these ethyl esters have very pleasant odours of fruity notes, which contribute to the aromatic fineness of white wines [12, 28, 36, 41]. Our results showed that the concentration of ethyl esters in YPF wines were generally within the ranges known from grape wine [36, 41].

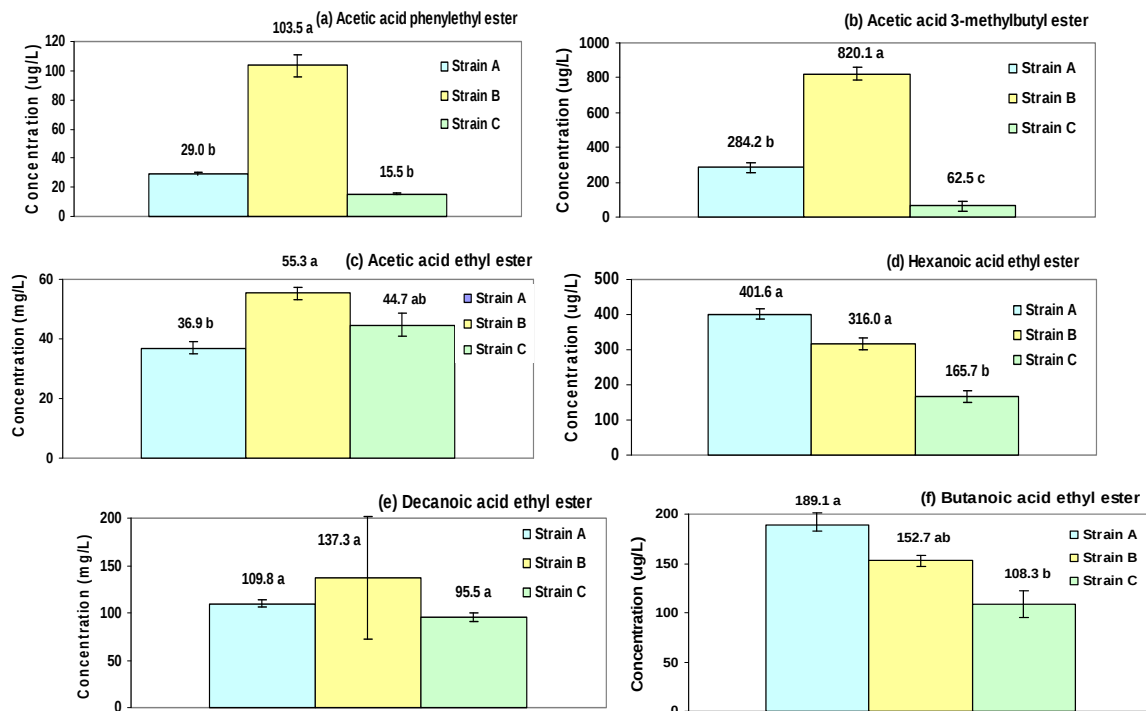


Figure 3. Concentration of acetate and ethyl esters in YPF wines produced by 3 different yeast strains.

(a) Acetic acid phenylethyl ester (fruity, flowery flavour with a honey note), (b) Acetic acid 3-methylbutyl ester/isoamyl acetate (banana-like aroma), (c) Acetic acid ethyl ester (pineapple-like aroma), (d) Hexanoic acid ethyl ester (apple-like aroma), (e) Decanoic acid ethyl ester (pineapple-like aroma) and (f) Butanoic acid ethyl ester (apple, pineapple-like aroma) [39, 44].

Vertical bars represent standard deviations from two fermentations. Means followed by different letters on the top of the bar are significantly different ($p < 0.05$), whereas means followed by same letters on the top of the bar are not significantly different ($p > 0.05$) according to the LSD test.

Conclusion

The trial with three different commercial yeast strains showed a varying influence on the compositions and volatile aroma compounds of YPF wines. All three yeast strains can produce YPF wine of good quality and contribute to typical fruity wine aroma. Strain A and B showed the most diverse fermentation parameters and produced high concentrations of fatty acid ethyl esters, which contribute to very pleasant odours of several fruity notes in YPF wine. However, the B strain also produced high concentrations of acetaldehyde, acetic acid and acetate esters in YPF wines. The C strain developed the highest amounts of lactic acid and keto acids, principally pyruvate and α -ketoglutarate, which have implications for wine stability and quality.

Future work will be focused on the effect of yeast strains and nutrient supplements on the fermentation kinetic, volatile thiol compounds and varietal aroma compounds.

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