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Influence of *Saccharomyces cerevisiae* strains on fermentation of *Monascus* vinegar from rice pasta by-product

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Abstract

In the present work, rice pasta by-product (RPBP) was used as a raw material for the production of *Monascus* vinegar. Alcoholic fermentation using RPBP and red yeast rice *koji* were carried out, and the fermentative characteristics based on the yeast strains (*Saccharomyces cerevisiae* TISTR 5169, TISTR 5196, and TISTR 5197) were investigated. The compositional changes and functional properties of *Monascus* vinegar were examined. *S. cerevisiae* TISTR 5169 produced a higher yield of alcohol in a shorter time as compared to other strains, in which 10% alcohol was observed after four days of fermentation. Descriptive sensory evaluation showed that *Monascus* wine fermented by *S. cerevisiae* TISTR 5169 (W5169) showed the highest result of intensity in cereal, fruit, and alcohol aroma. Therefore, W5169 was selected for subsequent acetous fermentation. During fermentation for 39 days, the titratable acidity (acetic acid) increased, associated with the decrease in alcohol contents and pH values. Acetification increased total phenolic content in accordance with an increase of antioxidant activity. Moreover, *Monascus* vinegar contained functional ingredients including *Monascus* pigments; yellow (0.17 OD unit/mL), orange (0.08 OD unit/mL), red (0.06 OD unit/mL), monacolin K (0.0141 ppm), and total phenolic content (71.70 µg GAE/mL). *Monascus* vinegar exhibited potential for antioxidant activity (58.8%) and xanthine oxidase inhibitory activity (73.7%), and in particular, was without the mycotoxin citrinin.

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Introduction

Red yeast rice, also called *hong qu*, is obtained from rice fermented with red mould, *Monascus purpureus*. This genus of *Monascus* synthesises bioactive secondary metabolites including monacolin K (anticholesterolemic agent) and pigments. However, *Monascus* spp. also produce mycotoxin citrinin with nephrotoxic effects in animals and humans (Hsieh *et al*., 2013; Jirasatid *et al*., 2019).

Monascus vinegar or *hong qu* vinegar is made from rice with the addition of red yeast rice as the fermentation starter (Hsieh *et al*., 2013). *Monascus* vinegar possesses various physiological functions including antioxidant activity, xanthine oxidase inhibitory (XOI) activity (antihyperuricemia), and protection from cardiovascular diseases due to the presence of abundant amounts of bioactive compounds, particularly acetic acid and monacolin K (Hsieh *et al*., 2013; Jiang *et al*., 2019; Song *et al*., 2020). In recent years, the fermentation of *Monascus* vinegar were investigated by many researchers, for example, Yuan *et al*. (2021) reported that germinated rice was more suitable as raw material for *Monascus* vinegar than that of polished and unhusked rice, because it contained several aromatic compounds and the highest amount of aroma. In addition, Zhang *et al*. (2019) studied the fermentation parameters for high-yield monacolin K and low-yield citrinin. The process parameters during alcoholic fermentation were: temperature, 32°C; inoculum size, 20%; fermentation time, 9 d; and alcohol content, 10%. During acetous fermentation, temperature was 38°C for 12 d, and then reduced to 32°C for 3 d; and it was observed that the content of monacolin K and citrinin was 50 ppm and 0.01 mg/L, respectively.

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Vinegar fermentation from rice generally requires three stages of fermentation which are (1) saccharification, the conversion of starch to sugars by hydrolysis with enzymes or fermentation with mould cultures *e.g. Amylomyces rouxii*, (2) alcoholic fermentation, the conversion of sugars to ethanol by yeast *e.g. Saccharomyces cerevisiae*, and subsequently (3) acetous fermentation, the oxidisation of ethanol to acetic acid by acetic acid bacteria *e.g. Acetobacter pasteurianus* (Ho *et al*., 2017; Phuapaiboon, 2017; Jaikang *et al*., 2019).

Many factors affect vinegar production such as the yeast strains, raw materials, and environmental conditions namely pH and temperature (Ho *et al*., 2017; Roda *et al.,* 2017). Different strains of yeast produce different amounts of ethanol and volatile compounds, thus affecting the quality of the final vinegar, particularly aroma (Ho *et al*., 2017; Jiang *et al*., 2019). Yeast strains producing high yield of ethanol with short fermentation time should be used for vinegar production on an industrial scale since this increases the safety of the product, thereby ensuring quality and reducing production cost (Song *et al*., 2019).

Currently, agricultural by-products or wastes containing sugars or starch such as pineapple waste (Roda *et al*., 2017), corncob (Chakraborty *et al.*, 2015), and broken rice noodle (Jaikang *et al*., 2019) have been applied for vinegar production. To the best of our knowledge, no effort has been made to employ rice pasta by-product (RPBP) for *Monascus* vinegar production. RPBP is a by-product obtained from rice pasta processing by extrusion. The utilisation of RPBP as a substrate for *Monascus* vinegar production is an attractive alternative for utilising a by-product from industrial production to produce a high value-added product. Therefore, the present work aimed to produce and assess the quality of *Monascus* vinegar made from RPBP. Firstly, the influence of *Saccharomyces cerevisiae* strains (*S. cerevisiae* TISTR 5169, TISTR 5196, and TISTR 5197) on physicochemical, microbiological, sensorial, and functional properties of *Monascus* wines during alcoholic fermentation was investigated. These strains were suggested as high alcohol producer (Wongpiyachon *et al*., 2007). Further, the compositional changes during acetous fermentation of *Monascus* vinegar were also monitored. Of particular interest was the capability to maintain the functional properties of *Monascus* vinegar such as antioxidant activity and XOI activity after fermentation.

Materials and methods

Materials

Rice pasta by-product (RPBP) from white rice (Chai Nat, non-glutinous rice) was obtained from Family Tree Foods Co. Ltd., Thailand. RPBP were defective pasta in shape after extrusion. RPBP (moisture content < 10%) were ground and used as a raw material for vinegar fermentation. *Sao-Hai*, non-glutinous rice, was acquired from a local market in Chonburi, Thailand, and used as the substrate for red yeast rice fermentation.

Microorganisms

Amylomyces rouxii TISTR 3182, *Monascus purpureus* TISTR 3629, *Acetobacter pasteurianus* TISTR 102, and *Saccharomyces cerevisiae* TISTR 5169, TISTR 5196, and TISTR 5197 were purchased from the Microbiological Resources Centre, Thailand Institute of Scientific and Technological Research (TISTR), Thailand.

Proximate composition analyses of rice pasta by-product (RPBP)

Proximate composition of RPBP was determined following AOAC (2016). Carbohydrate content was calculated by subtracting the total protein, fat, water, and ash content of the whole sample.

Fermentation of red yeast rice koji

Koji of red yeast rice was produced by solid-state fermentation (SSF) following Jirasatid *et al*. (2019). *M. purpureus* TISTR 3629 was cultivated on *Sao-Hai* rice at 30°C for 7 d, ground, and stored at 4°C.

Fermentation of rice pasta by-product koji

A 10 g of RPBP was transferred into a Petri dish. Distilled water (10 mL) was added to adjust the moisture content to 50%. The substrate was steamed at 121°C for 30 min, cooled to room temperature, inoculated with 10% (v/w) of *A. rouxii* spore suspension (1×10^6 spores/mL), and then cultivated at 30°C for 4 d. RPBP *koji* containing approximately 35% reducing sugar (w/w) was used for further alcoholic fermentation.

Alcoholic fermentation

RPBP *koji* (200 g) was mixed with red yeast rice *koji* (10 g) and sterile distilled water (800 mL). Total soluble solid (TSS) was adjusted to 25 °Brix using sucrose. The mixture was transferred to 2,000 mL Erlenmeyer flasks. Submerged fermentation (SmF) was commenced by adding 10% (v/v) of cell suspension (1×10^6 cells/mL) of either *S. cerevisiae* TISTR 5169, TISTR 5196, or TISTR 5197 to the mixture (Lapa *et al*., 2011). The samples were cultivated at room temperature for 14 d, or until the alcohol content reached 12% (v/v) (Hsieh *et al*., 2013; Li *et al*., 2014). When alcoholic fermentation was completed, the samples were filtered through a cotton layer in a Buchner funnel under vacuum (Phuapaiboon, 2017). The supernatant was used for acetous fermentation.

During fermentation, *Monascus* wines were collected. The physicochemical and microbiological properties were evaluated every 2 d, while sensorial and functional properties were determined only after fermentation completed.

Acetous fermentation

Monascus wine was adjusted to 6% alcohol content by adding sterile distilled water, and to 1.5% acetic acid content by adding acetic acid solution (QREC, New Zealand) (Li *et al*., 2014). The wine broth (1,000 mL) was inoculated with the starter suspension of *A. pasteurianus* (3×10^8 CFU/mL, 10% v/v) in a 2,000 mL Erlenmeyer flask, and incubated at ambient temperature without shaking until the acetic acid content reached 4% (w/v), following the United States Food and Drug Administration (FDA) standard (Ho *et al*., 2017). After fermentation, vinegar was filtered through a cotton layer in a Buchner funnel under vacuum, and stored in a refrigerator (4 - 5°C).

The fermented broth was withdrawn at 3-d intervals during fermentation to monitor the physicochemical and microbiological properties, while the functional properties of *Monascus* vinegar were determined after the completion of acetous fermentation.

Physicochemical analysis

The pH, TSS, and alcohol levels of *Monascus* wines and *Monascus* vinegar were determined. The pH was measured with a pH meter (Lab 850, Schott, Germany) (Hsieh *et al*., 2013). TSS was measured with a handheld refractometer at 25°C (Master, Atago, Japan) in °Brix (Li *et al*., 2014). Alcohol content (%, v/v) was measured using a vinometer (Vin-o-meter, Germany), in which the samples were filtered with filter paper (Whatman No. 4) before analysis. Titratable acidity was measured by the titration of 10 mL of sample with 0.1 N NaOH (Kemaus, Elago Enterprises Pty. Ltd., Australia). Result was expressed as g of acetic acid per 100 mL (or %) (Hsieh *et al*., 2013).

Microbiological analysis

The counts of yeast and mould in *Monascus* wines and *Monascus* vinegar were determined. The sample (10 mL) was homogenised with 90 mL of 0.85% sterile NaCl (Ajax Finechem Pty. Ltd., Australia), and then diluted 10-fold. Colonies of yeast and mould were enumerated separately using compact dry YM (Nissui Pharmaceutical Co. Ltd., Japan) by incubation at 30°C for 4 - 5 d (Yousef and Carlstrom, 2003). In addition, the counts of acetic acid bacteria (AAB) in *Monascus* vinegar were also examined using a spread-plate technique on glucose yeast extract agar (GYEA per litre; 100 g glucose, 10 g yeast extract, 10 g $CaCO_3$, and 20 g agar). Plates were incubated at 30°C for 48 h. Colony counts were expressed as log colony-forming units per mL (log CFU/mL) (Yousef and Carlstrom, 2003; Li *et al*., 2014).

Analysis of monacolin K, pigments, citrinin, total phenolic content, and antioxidant activity

For *Monascus* wines and vinegar, total phenolic content, monacolin K, and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity were determined following Hsieh *et al.* (2013). Total phenolic contents were quantified using the Folin-Ciocalteu reagent. The concentrations of *Monascus* pigments namely yellow, orange, and red were determined using a UV/vis spectrophotometer (Genesys 20, Thermo Scientific, USA) at 400, 470, and 500 nm, respectively (Jirasatid *et al*., 2019). The citrinin content was measured by high performance liquid chromatography (HPLC Model 600E, Waters, USA) following Jirasatid *et al*. (2019).

Analysis of xanthine oxidase inhibitory (XOI) activity

The inhibition of xanthine oxidase activity indicates an antihyperuricemic activity; reduction of uric acid (Kohoude *et al*., 2017). The XOI activity using xanthine as substrate was examined following Hsieh *et al*. (2013). The absorbance was measured at

290 nm using a UV/vis spectrophotometer. The A_{blank} was measured without sample extract. The % XOI activity was expressed using Eq. 1:

$$\% \text{ XOI activity} = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100 \quad \text{(Eq. 1)}$$

Sensory evaluation of Monascus wine

Descriptive sensory analysis of three *Monascus* wines was conducted by 10 trained panellists from the Department of Food Science, Burapha University, Thailand. The panelists were selected from the following criteria: more than 20 years old, experienced consumers of wine, and were not alcohol intolerance. Sensory evaluation was performed in a room with uniform source of lighting, with absence of noise and distracting stimuli. Samples (15 mL) were provided in lidded clear glasses coded with a random order. *Monascus* wine was further fermented to vinegar, thus the eight sensory attributes of appearance (colour and turbidity), aroma (alcohol, fruit, and cereal), and taste (sweet, sour, and bitter) were chosen for evaluation. The reference materials used to define the aroma and taste were included for training of panelists. There were three reference scales to demonstrate the minimum, medium, and maximum intensity of each attributes. The training was completed when the intensities and characteristics of sensory attribute references were accorded by all panelists (Yang *et al*., 2018). The intensities of sensory attributes were scored on a 5-point scale from 0 to 5 (0 = none; 1 = very weak; 2 = ordinary; 3 = moderate; 4 = strong; and 5 = very strong) (Lawless and Heymann, 1998).

Statistical analysis

The experiments were conducted using a completely randomised design (CRD). Data were analysed using analysis of variance (ANOVA). Tukey's multiple comparison test was performed to determine significant differences of the means at $p \leq 0.05$.

Results and discussion

Microbial growth during alcoholic fermentation

The percentages of moisture content, crude ash, crude fat, crude protein, and carbohydrate of RPBP powder were 10.65 ± 0.03, 0.35 ± 0.00, 0.05 ± 0.00, 7.69 ± 0.32, and 81.26 ± 0.34% (dry basis), respectively.

The microbial cultures in alcoholic fermentation of *Monascus* wine were *S. cerevisiae* and *M. purpureus*. The time courses of viable counts of yeast and mould in *Monascus* wines fermented by *S. cerevisiae* TISTR 5169 (W5169), TISTR 5196 (W5196), and TISTR 5197 (W5197) are presented in Table 1.

Table 1. Number of yeast and mould cells (log CFU/mL) in *Monascus* wines fermented by *S. cerevisiae* TISTR 5169 (W5169), TISTR 5196 (W5196), and TISTR 5197 (W5197) during alcoholic fermentation.

Time (d)	Number of yeast cells (log CFU/mL)			Number of mould cells (log CFU/mL)		
	W5169	W5196	W5197	W5169	W5196	W5197
0	$6.89 \pm 0.41^{A,ns}$	6.76 ± 0.49^{AB}	6.85 ± 0.54^{A}	$4.97 \pm 0.21^{NS,ns}$	5.61 ± 0.27^{NS}	4.69 ± 0.24^{NS}
2	$7.19 \pm 0.67^{A,ns}$	7.24 ± 0.73^{A}	7.21 ± 0.74^{A}	5.69 ± 1.23^{ns}	6.02 ± 0.71	5.37 ± 0.21
4	$7.64 \pm 0.56^{A,ns}$	5.97 ± 1.05^{AB}	7.02 ± 0.39^{A}	5.17 ± 0.49^{ns}	5.42 ± 0.28	5.18 ± 0.93
6	$5.16 \pm 1.06^{B,ns}$	6.02 ± 0.06^{AB}	6.39 ± 0.32^{A}	4.18 ± 1.49^{ns}	4.44 ± 1.30	4.90 ± 1.27
8	$4.67 \pm 0.03^{BC,ns}$	5.14 ± 0.09^{B}	6.01 ± 1.39^{AB}	3.80 ± 1.14^{ns}	4.13 ± 1.84	5.02 ± 0.71
10	$4.17 \pm 0.08^{BC,ns}$	5.29 ± 0.08^{B}	5.80 ± 1.31^{AB}	3.59 ± 1.64^{ns}	4.31 ± 1.12	4.10 ± 1.80
12	$< 3^{C,ns}$	$< 3^{C}$	$< 3^{C}$	4.36 ± 0.31^{ns}	4.80 ± 0.30	5.23 ± 0.27
14	$< 3^{C,ns}$	$< 3^{C}$	$< 3^{C}$	4.29 ± 0.27^{ns}	4.53 ± 0.50	4.82 ± 0.12

Values are mean ± SD. Means followed by different uppercase superscripts in the same column indicate significant difference ($p \leq 0.05$). ^{NS}not significantly different in the same column ($p > 0.05$). ^{ns}not significantly different in the same row for each microbial ($p > 0.05$).

The numbers of yeast cells in all samples increased during the early stage of fermentation (0 - 4 d), and then decreased significantly throughout fermentation ($P \leq 0.05$) due to ethanol stress, thus impacting the cellular processes of yeast cells such as inhibition of transport systems of glucose and amino acids, and damage of their cell membrane (Silva *et al*., 2013; Singkong, 2015; Udom *et al*., 2019). Maximum counts of yeast (7.64 log CFU/mL) were obtained in sample W5169, followed by W5196 (7.24 log CFU/mL) and W5197 (7.21 log CFU/mL). However, there was insignificant difference among all samples studied at the same fermentation time ($p > 0.05$) (Table 1). Similar pattern of change in the number of yeast cells in the present work agreed with Palaniveloo and Vairappan (2013) and Shin *et al.* (2017), in which the yeast cells increased in primary stage of fermentation, and then decreased in the later stage of alcoholic fermentation.

The mould colonies found on compact dry YM showed only the red colonies, thus indicating *M. purpureus*. The population of mould in each sample was stable during fermentation for 14 d ($p > 0.05$) (Table 1). The counts of mould in sample W5169, W5196, and W5197 varied within the range of 3.59 - 5.69, 4.13 - 6.02, and 4.10 - 5.37 log CFU/mL, respectively. In addition, there was no significant difference in viable counts of mould between the assessed samples at the same cultivation period ($p > 0.05$) (Table 1). This suggested that *M. purpureus* was able to survive in a high alcohol environment (10 - 12%), and also had more tolerance to ethanol than *S. cerevisiae*. This was in agreement with Boonprab and Matsui (2018) who demonstrated that *Monascus* sp. was more resistance to ethanol as compared to yeast. This in turn indicated that RPBP was suitable substrate for the growth of yeast and mould; RPBP substrate was rich in rapidly utilised sugar such as glucose, which was obtained from *koji* fermentation (saccharification), thus resulting in growth support for *S. cerevisiae* and *M. purpureus*.

Physicochemical characteristics of Monascus wine during alcoholic fermentation

The values of the initial and final pH of samples prepared using different *S. cerevisiae* strains were within the range 3.34 - 3.62 and 3.43 - 3.51, respectively (Figure 1a). The pH values of each sample decreased slightly throughout the 14 d of fermentation ($p > 0.05$), thus suggesting that organic acids such as lactic acid and acetic acid were produced by *S. cerevisiae* and *M. purpureus* (Shin *et al*., 2017). TSS, mainly sugars, decreased from 25 °Brix to values of 8.7 - 13.0 °Brix (Figure 1b), and alcohol contents increased from 0 to 12% in all samples (Figure 1c).

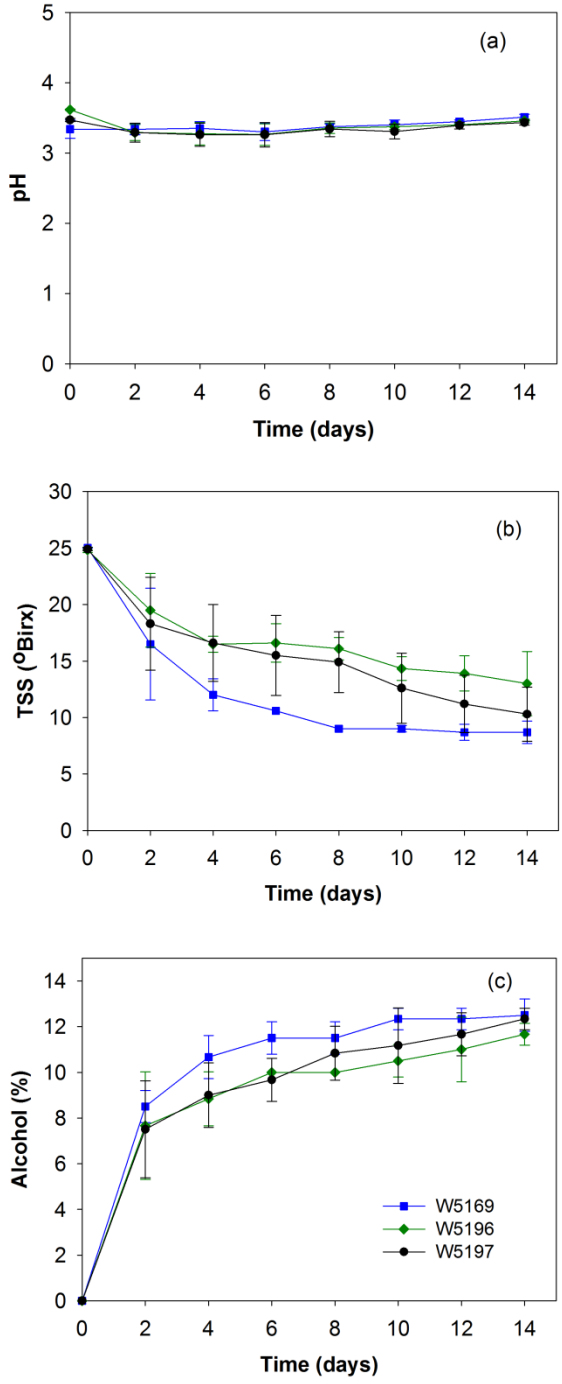

Figure 1. (a) Changes in pH, **(b)** total soluble solid (TSS), and **(c)** alcohol levels of *Monascus* wines fermented by *S. cerevisiae* TISTR 5169 (W5169), TISTR 5196 (W5196), and TISTR 5197 (W5197) during alcoholic fermentation.

TSS decreased significantly during fermentation ($p \leq 0.05$), thus showing that microorganisms (*S. cerevisiae* and *M. purpureus*) utilised carbon sources for growth and driving their metabolic processes. This was associated with the progressive increase of alcohol content over the course of the fermentation time ($p \leq 0.05$). Generally, yeasts used 95% of sugar to produce alcohol and CO_2, while the other 5% was taken up for growth and other metabolic activities (Singkong, 2015). Additionally, *Monascus* was able to produce ethanol in the exponential phase under aerobic condition (Rosenblitt *et al.*, 2000; Boonprab and Matsui, 2018). High alcohol contents (10%) in all samples were achieved after about 4 - 6, thus resulting in the decrease in the number of yeast cells during fermentation. Interestingly, *S. cerevisiae* TISTR 5169 showed great potential for ethanol production, with more than 10% on the fourth day. At the end of fermentation, sample W5169, W5196, and W5197 had alcohol contents of 12.5, 11.7, and 12.3%, respectively ($p > 0.05$). The presence of high alcohol content indicated successful fermentation by utilisation of RPBP as a substrate. The alcohol content achieved in the present work was similar to the study by Takeshita *et al*. (2015) in which *Monascus* wine contained 7.9 - 8.6% ethanol after fermentation by industrial *sake* yeast *S. cerevisiae* K7 for 7 d. The yield of alcohol depended on the number of yeast cells during fermentation, the type of fermentable sugars, the capacity of sugar consumption and alcohol production, and the alcohol tolerance of the yeast strain (Palaniveloo and Vairappan, 2013; Shin *et al*., 2017).

Contents of functional ingredients and antioxidant activity of Monascus wine

Monacolin K or lovastatin is an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which inhibits the conversion of HMG-CoA to mevalonate, a rate-limiting step of the cholesterol biosynthesis route in the liver (Manzoni and Rollini, 2002). In the present work, monacolin K was very low, thus could be ignored in *Monascus* wines. A previous study reported that monacolin K contents of red *koji* wine were 2 ppm (Hsieh *et al*., 2013).

Monascus pigments contain three colour ranges: yellow (monascin and ankaflavin), orange (rubrapunctatin and monascorubrine), and red (rubropunctamine and monascorubramine). These pigments show anti-inflammation behaviour. In particular, the yellow pigment exerts antihyperlipidemic, antiatherosclerotic, and antioxidant activities (Lee *et al*., 2010; Lee and Pan, 2012; Hsu *et al*., 2013). Yellow, orange, and red pigments were observed in *Monascus* wines in ranges of 0.33 - 0.37, 0.15 - 0.16, and 0.16 - 0.17 OD unit/mL, respectively (Table 2).

On the toxicological perspective, low levels of citrinin in food supplements containing red yeast rice is an important parameter in terms of food safety. As seen in Table 2, citrinin content (0.0088 - 0.0094 ppm) was very low in the wines due to the instability of citrinin in acidic solutions (Hsieh *et al.*, 2013).

Table 2. Total phenolic content (TPC), monacolin K, citrinin, *Monascus* pigments, antioxidant activity (DPPH), and xanthine oxidase inhibitory activity (XOI) of *Monascus* wines and/or *Monascus* vinegar.

Functional ingredient	*Monascus* wine			*Monascus* vinegar
	W5169	W5196	W5197	(V5169)
TPC (μg GAE/mL)	40.79 ± 3.03^{ns}	41.70 ± 7.95	41.16 ± 6.42	71.70 ± 13.30
Monacolin K (ppm)	ND	ND	ND	0.0141 ± 0.0023
Citrinin (ppm)	0.0094 ± 0.0027^{ns}	0.0089 ± 0.0007	0.0088 ± 0.0027	ND
***Monascus* pigment included:**				
Yellow pigments (OD unit/mL)	0.37 ± 0.14^{ns}	0.33 ± 0.06	0.36 ± 0.09	0.17 ± 0.07
Orange pigments (OD unit/mL)	0.16 ± 0.07^{ns}	0.15 ± 0.05	0.15 ± 0.06	0.08 ± 0.01
Red pigments (OD unit/mL)	0.17 ± 0.01^{ns}	0.16 ± 0.00	0.16 ± 0.04	0.06 ± 0.00
DPPH (%)	15.4 ± 1.52^{ns}	15.0 ± 2.56	15.8 ± 2.91	58.8 ± 2.48
XOI (%)	-	-	-	73.7 ± 10.04

Values are mean ± SD. ^{ns}not significantly different in the same row for wine samples ($p > 0.05$). ND: not detected.

Considering the contents of pigments and citrinin, yellow pigment of all *Monascus* wines did not differ significantly as well as orange pigment, red pigment, and citrinin ($p > 0.05$), because red yeast rice *koji* was added in samples with the same mass (1 g red yeast rice *koji* per 100 mL wine broth). The low quantity of monacolin K, pigments, and citrinin in *Monascus* wines was possibly due to low amounts of red yeast rice *koji* as a fermentation starter. Moreover, Tan *et al*. (2014) reported that in medium containing 4% ethanol, although cell dry weight of *M. purpureus* increased, the secondary metabolites pathway was inhibited. Therefore, under alcoholic fermentation of *Monascus* wine, even though *Monascus* was able to survive, secondary metabolites may not be produced.

Total phenolic contents and DPPH radical scavenging activity of *Monascus* wines after fermentation fluctuated within the range 40.79 - 41.70 µg GAE/mL and 15.0 - 15.8%, respectively (Table 2). There was no significant difference in total phenolic contents ($p > 0.05$) as well as antioxidant activity ($p > 0.05$) of various wines, thus indicating the insignificant role of *S. cerevisiae* strains on these effects. Cai *et al.* (2019) demonstrated that the polyphenols in rice wine were significantly affected by using various rice materials, but they could be less influenced by using different *Jiuqu* starters.

Sensory evaluation of Monascus wine

The mean intensity rating of *Monascus* wines is illustrated in Figure 2.

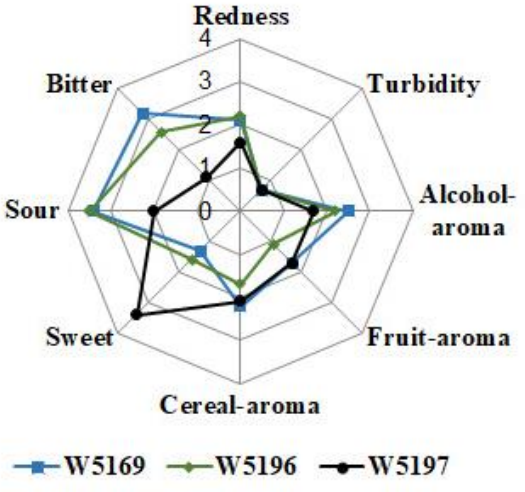

Figure 2. Descriptive sensory evolution of *Monascus* wines fermented by *S. cerevisiae* TISTR 5169 (W5169), TISTR 5196 (W5196), and TISTR 5197 (W5197).

The three samples indicated similar intensity rating in turbidity. W5169 showed strong intensity in cereal, fruit, and alcohol aroma. The high level of alcohol aroma in W5169 was in agreement with high alcohol content (Figure 1c). W5169 and W5196 were intense in sour taste. In contrast, W5197 showed high intense in sweet taste. The results revealed that each *Monascus* wine had unique aroma and taste/flavour characteristics. This was in agreement with Chen and Xu (2010) who reported that the different yeast strains contributed significantly to the different volatile flavour compounds in Chinese rice wine. Based on our results, W5169 presented a mellower taste than W5196 and W5197. Therefore, W5169 was chosen for further acetous fermentation.

Microbial growth during acetous fermentation

During acetous fermentation of *Monascus* vinegar (V5169) for 39 d, the growth and/or survivability of AAB, *A. pasteurianus*, and *M. purpureus* were observed, while the yeast did not survive (Figure 3).

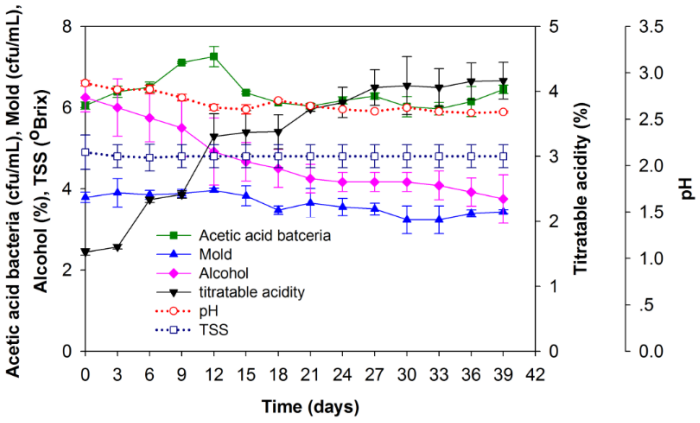

Figure 3. Changes in the viable counts of acetic acid bacteria and mould, alcohol content, total soluble solid (TSS), titratable acidity, and pH of *Monascus* vinegar during acetous fermentation.

The viable counts of AAB increased slightly from 6.05 to 7.26 log CFU/mL over 12 d. Subsequently, the growth was maintained between 5.97 - 6.45 log CFU/mL during the next 12 d of fermentation, in which the decrease in population could have been due to acid stress (titratable acidity > 3%) (Wu *et al.*, 2018). In addition, the viable counts of mould were constant throughout fermentation, and ranged between 3.24 - 3.96 log CFU/mL. This indicated that AAB grew, and mould (*M. purpureus*) could also survive under high acidic/low pH (pH of

2.57 - 2.89) conditions. This was similar to results reported by Li *et al*. (2014) who studied the growth of *Acetobacter aceti* during fermentation of *Hericium erinaceus* vinegar. The number of *A. aceti* cells increased over 3 d, and then decreased gradually to 0 log CFU/mL after 9 d of fermentation.

Physicochemical characteristics of Monascus vinegar during acetous fermentation

As shown in Figure 3, the TSS of *Monascus* vinegar broth was almost constant (4.80 - 4.90 °Brix) over the course of the fermentation. The alcohol content decreased progressively from 6.25 to 3.75% (v/v) after fermentation for 39 d. There was a corresponding significant increase in titratable acidity from 1.53 to 4.16% (w/v). Alcohol (ethanol) is a major substrate used for acetic acid production by AAB. Ethanol is oxidised to acetic acid by the catalyst's alcohol dehydrogenase and acetaldehyde dehydrogenase under aerobic conditions (Ho *et al*., 2017). The results showed that pH decreased slightly from 2.89 to 2.58 over 39 d of fermentation. The decrease in pH values was due to the accumulation of acetic acid and other volatile short chain organic acids such as tartaric, citric, and succinic acid, which are important for the development of flavour and aroma in vinegar (Hsieh *et al*., 2013; Kongkiattikajor, 2015).

The vinegar produced in the present work was within the standard of the USFDA of no less than 4 g of acetic acid per 100 mL (Ho *et al*., 2017). The final acidity (acetic acid) was higher than that of vinegar obtained from germinated pigmented rice (2.82 - 3.18%) (Phuapaiboon, 2017), and organic broken rice noodles (3.52%) (Jaikang *et al*., 2019), but similar to *Monascus* vinegar (4.5%) (Hsieh *et al*., 2013). The different amounts of acidity in vinegars could have resulted from the various raw materials, the number cells of AAB added, the initial alcohol content, and the fermentation period (Li *et al*., 2014; Jaikang *et al*., 2019).

Contents of functional ingredients and bioactivity of Monascus vinegar

Even though monacolin K was not detected in *Monascus* wines, low values of monacolin K (0.0141 ppm) were obtained in *Monascus* vinegar (Table 2). Natural monacolin K synthesised by fungus occurs in two structures: the β-hydroxyl acid form and lactone form (Manzoni and Rollini, 2002). In acidic solutions like vinegar, the proportion of monacolin K in the lactone form increase with decreasing pH. Hence, monacolin K in its lactone form is the dominant form existing in vinegar (Hsieh *et al*., 2013). Therefore, total monacolin K was analysed in the lactone form. This may have resulted in low values of monacolin K in vinegar. However, a higher content of monacolin K in red *koji* vinegar (1.14 ppm) was reported by Hsieh *et al*. (2013).

Lower levels of yellow (0.17 OD unit/mL), orange (0.08 OD unit/mL), and red pigments (0.06 OD unit/mL) were observed in *Monascus* vinegar (0.35 - 0.50 times) as compared to W5169 (Table 2). This suggested that the pigments were unstable in acidic solutions, thus inducing their degradation in the vinegar production. However, the presence of bioactive pigment compounds in *Monascus* vinegar may improve its bioactivity.

According to the European Union (EU), the maximum citrinin content authorised in food supplements based on rice fermented with red yeast *M. purpureus* is 2 mg/kg (2 ppm) (EU, 2014). Japan allows a maximum citrinin level of 0.2 mg/kg in *Monascus* pigment (Shin *et al*., 2017). In the present work, citrinin was non-detectable in *Monascus* vinegar, as citrinin was degraded in organic acid rich environment (Hsieh *et al*., 2013). Similar to our results, Hsieh *et al*. (2013) observed a decrease in citrinin in red *koji* wine to vinegar from 3.6 to 0.06 ppm. Therefore, the *Monascus* vinegar obtained in the present work was safe for consumption.

In addition, it was found that the total phenolic content and antioxidant activity increased following acetous fermentation. Total phenolic content (71.70 µg GAE/mL) and antioxidant activity (58.8%) of *Monascus* vinegar were about 1.8 and 3.8 times higher than that of W5169 (Table 2). The phenolic compounds in rice wine and rice vinegar mainly originate from the rice material. The phenolic compounds such as dihydroferulic acid and ferulic acid in rice vinegar act as antioxidants (Ho *et al*., 2017). Antioxidant activity in *Monascus* wine and *Monascus* vinegar could also come from *Monascus* pigments (Hsieh *et al*., 2013; Takeshita *et al*., 2015). The fermentation employing AAB also improved the antioxidant activity of the product. This is related to an increase in the free form of phenolic compounds and the other antioxidants like acetic acid obtained through fermentation (Kongkiattikajor, 2015). This agreed with Hsieh *et al*. (2013) who found that total phenolic compounds increased with the time of vinegar fermentation, and acetic acid exhibited significant antioxidant activity. Besides,

Kongkiattikajor (2015) reported that the total phenolic content and antioxidant capacity of roselle vinegar were higher as compared to roselle wine.

The conversion of hypoxanthine to xanthine is catalysed by xanthine oxidase. Subsequently, xanthine is oxidised to uric acid and excreted in urine. High levels of uric acid in blood (hyperuricemia) lead to the accumulation of uric acid crystals in tissues and joints, thus causing arthritis known as gout (Lin *et al*., 2012; Hsieh *et al*., 2013). As reported in a previous study, two bioactive compounds with XOI activity including 5-hydroxymethyl-2-furfural (5-HMF) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) have been identified in red *koji* vinegar (Lin *et al*., 2012). A high level of XOI activity was found in *Monascus* vinegar (73.7%), thus indicating its capability for antihyperuricemia (Table 2). Allopurinol, which is a drug commonly used for the alleviation of gout pain, at 15 µg/mL (ppm) showed XOI activity of 84.1%. Therefore, *Monascus* vinegar was equivalent to 13 µg/mL of allopurinol. This result was higher than a previous study that reported XOI activity of red *koji* vinegar of 55.9% (Hsieh *et al*., 2013).

Conclusion

The present work successfully utilised RPBP as a substrate for *Monascus* vinegar production. *S. cerevisiae* TISTR 5169 produced a higher yield of alcohol with a shorter fermentation time as compared to other yeast strains (*S. cerevisiae* TISTR 5196 and 5197). Descriptive sensory evaluation showed that W5169 presented a mellower taste than those of other samples. Moreover, *Monascus* vinegar (V5169) contained 4% acetic acid after 27 days of fermentation, and contained various functional ingredients such as monacolin K, *Monascus* pigments (yellow, orange, and red), and phenolic compounds. These compounds contributed to the pharmacological effects including antioxidant activity and XOI activity. Importantly, *Monascus* vinegar was without the mycotoxin citrinin. The loss of citrinin by vinegar fermentation is of importance for food safety. The present work proposed low-cost production of *Monascus* vinegar for industrial scale with high antioxidant activity (58.8%) and XOI activity (73.7%), which can be of health benefit and safety to consumers.

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