Study of Volatile Compounds in Thai Rice Wine (Sato) Produced from Wheat

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Abstract

Metabolism of nitrogen source such as amino acids is an important factor contributing to the production of volatile compounds in alcoholic beverages. In rice wine production, amino acids come from the proteolysis of rice during koji fermentation. This research studied volatile compounds in Sato as affected by raw material containing high nitrogen content such as wheat rice (WR) comparing with glutinous rice (GR, control sample). Total soluble solid (TSS) and total nitrogen (TN) were determined in koji at the end of fermentation (7 days). Free amino acid, ethanol and volatile compounds were measured every 2 days. The TSS values of koji made using WR and GR were 17.13 and 21.53 $^{\circ}$Brix, respectively. The TN values of those samples were 0.240% and 0.085%, respectively. During alcoholic fermentation, higher reduction in TN was observed in the WR sample (0.09% reduction) compared with the control sample (0.04% reduction) during the first 2 days, and its TN content continuously decreased until the end of fermentation. This inversely correlated with an increase in alcohol pattern of the two samples. At the end of fermentation, there were 12.7 and 10.8% (v/v) alcohol in the WR and GR samples, respectively. Higher concentration of 3-methyl-1-butanol, phenylethanol, phenetyl acetate and diethyl succinate was observed in the WR sample compared with the GR sample. However, some of these volatiles were observed at the first day of alcoholic fermentation suggesting that these compounds are already produced during koji fermentation.

Keywords: Sato; Amino acids; volatile compounds

1. Introduction

Flavor is one of many important quality characteristics of alcoholic beverages. Both volatile and non-volatile compounds contributed to flavor characteristics. Many factors have been shown to affect the production of volatile compounds. Garde-Cerdan and Ancín-Azpilicueta (1) observed that wine fermented from nitrogen deficient must supplemented with amino acids resulted in wine with increased total esters, isoamyl acetate and phenylethyl acetate. The study by Hernández-Orte, Ibarz, Cacho and Ferreira (2) showed that wine produced from must which the concentration of amino acids was doubled had fruity and fusel characteristics. Theoretically, amino acids are metabolized to higher alcohols by yeast through Ehrlich pathway (3,4). Hernández-Orte, Ibarz, Cacho and Ferreira (5) found that isoamyl alcohol, isobutanol and b-phenyl ethanol were produced along alcohol fermentation. In addition, they reported that there was lower effect of amino acid on production of isoamyl alcohol compared with b-phenyl ethanol. The later was especially obvious when the concentration of phenylalanine was high. Esters can be produced from either through enzymatic reaction (alcohol acetyltransferases) during primary fermentation as well as chemical esterification between fatty acids and alcohols during wine aging (6).

Due to the fact that higher alcohols are a precursor involed in the synthesis of ester, metabolism of amino acids by yeast would affect concentration of ester in alcoholic beverages and its flavor. In rice wine production, amino acids come from the proteolysis of rice by acid proteases. These acid proteases were produced during koji fermentation (7). However, the amino acids digestion occurred mainly during alcohol fermentation (8). There were several studies about effect of ammonium and amino acid addition on fermentation

kinetic and production of volatile compounds during wine fermentation (2,5,6,9). However, only limited information has been found for the effect of amino acid on production of volatile compounds in rice wine. As in the case of wine, metabolism of amino acids yields positive results of higher alcohol as well as esters formation. Therefore, the use of raw material that is high in protein content other than rice or glutinous rice, in case of Thai rice wine, should yield product with better volatile flavor. This study investigated volatile compounds, particularly higher alcohols and acetate esters, in Sato (Thai rice wine) as affected by raw material containing high protein content such as wheat rice (WR) comparing with glutinous rice (GR) which is used as a control sample.

2. Materials and Methods

2.1 Experimental plan

Thai rice wine (Sato) was made using either glutinous rice or wheat as raw material. *Aspergillus niger* TISTR 3257 (Microbiological Resource Centre, Thailand Instituted of Scientific and Technological Research, Thailand) was used for koji fermentation. *Saccharomyces cerevisiae* SC 90 (kindly provided by Associate Professor Dr. Taworn Vinijsanun, Mahidol University, Kanchanaburi Campus, Thailand) was used during alcoholic fermentation. At the end of koji fermentation, total nitrogen (TN), total soluble solid (TSS) of sugary liquid were measured. During alcoholic fermentation, alcohol level, amino acid content and concentration of volatile compounds were determined. All experiments were carried out in triplicate.

2.2 Materials

Glutinous rice was purchased from a local market in Kanchanaburi, Thailand. Wheat rice was kindly provided from Piyasattree, Ltd., Nakhonpathom, Thailand. These raw materials were kept in a dessicator

at room temperature throughout experiment.

2.3 Production of Sato

Glutinous rice (*Oryza satava var glutinosa*) and wheat rice (*Triticum aestivum* L.) were mixed with water at 1:2 and 1:3 for glutinous rice and wheat rice, respectively. These ratios were used to ensure optimum koji fermentation (data not shown). They were soaked overnight prior to subject to heat at 121°C for 15 min. Ten milliliters of *A. niger* TISTR 3257 spore suspension (10^7 spores/mL) were mixed with the cooled rice (600 g). The koji fermentation was carried out at 30°C for 7 days. After that, the TSS of koji was adjusted to 22°Brix. Free sulfur dioxide was adjusted to 200 ppm using sodium metabisulphite. Ammonium sulphate was added into the sample to 1 g/L concentration. Then, *S. cerevisiae*, previously grown overnight (10^7 CFU/mL) in pineapple juice supplemented with 1 g/L of ammonium sulphate, was inoculated at 4% (v/v). The fermentation was carried out in 1 L Erlenmeyer flask under static condition at 30°C for 8 days.

2.4 Analysis during koji and alcoholic fermentation

Glutinous rice and wheat rice were analyzed for their compositions including moisture, protein, total nitrogen, fat and ash content according to AOAC (10). Total soluble solid was measured using a hand refractometer. Alcohol content of samples was examined using an Ebuliometer. Free amino acid contents including leucine, isoleucine, phenylalanine and valine were determined by using high performance liquid chromatography (HPLC 200 series, Perkin elmer, U.S.A.) connected with diode array detector (338 nm) according to method described by Soufleros and others (11). The column used was a UPS C18, 5 m column, 4.6 x 250 mm, Vertical Chromatography, Thailand). Volatile compounds including 3-methyl-1-butanol, 2-methyl-1-propanol, phenyl ethanol, isobutyl acetate, isopentyl acetate, phenetyl acetate and diethyl succinate were determined by using gas chromatography-mass spectrophotometry (GC-MS 7890A, Agilent Technologies, U.S.A.) coupling with a 007-CW capillary column (30 m x 0.25 mm diameter, 0.25 mm film thickness (Quadrex, U.S.A.). Liquid-liquid extraction and running condition were carried out according to method described by Ortega, López, Cacho and Ferreira (12).

2.5 Statistical analysis

The experimental results were analyzed using Student's t test at 95% confidence.

3. Results and Discussion

3.1 Composition of glutinous and wheat rice

Compositions of Glutinous Rice (GR) and Wheat Rice (WR) are shown in Table 1. The WR sample contains significantly higher protein content (~6.6), total nitrogen (~1.2), fat content (~0.9) and ash content (~1.4) than the GR sample ($p < 0.05$). In contrast, the GR sample has significantly higher moisture content (~3% higher) and total carbohydrate (~9% higher) compared with the WR sample.

Table 1. Compositions of Glutinous rice and Wheat rice (%, dry basis)

Composition (%)	Glutinous rice	Wheat rice
Moisture content	13.17 ± 0.05^a	10.60 ± 0.07^b
Protein content	6.98 ± 0.07^b	13.53 ± 0.06^a
Total nitrogen	1.17 ± 0.01^b	2.37 ± 0.01^a
Fat content	1.65 ± 0.07^b	2.59 ± 0.08^a
Ash content	0.31 ± 0.02^b	1.70 ± 0.01^a
Total carbohydrate*	91.06 ± 0.08^a	82.18 ± 0.13^b

[a,b] Mean values showing different superscript letter differ significantly (p < 0.05)

* Total carbohydrate = 100 – (Protein – Total nitrogen – Fat – Ash content)

3.2 Total soluble solid (^{o}Brix) and total nitrogen (%, dry basis) of koji

At the end of koji fermentation, both samples (GR and WR sample) were analyzed for total soluble solid (Figure 1a) and total nitrogen (Figure 1b). The total soluble solid in the GR sample is higher than in the sample made from wheat, 21.53 vs. 17.13 ^{o}Brix, respectively. On the other hand, the WR sample contains higher total nitrogen content compared with the control sample, 0.24 vs. 0.085% (w/w), respectively. It is well known that *Aspergillus* spp. produces both amylases and acid proteases (5,13,14). These enzymes hydrolyze substrates such as starch and protein into sugars and amino acids. The results of total soluble solid and total nitrogen show similar trends as that found in compositional analysis of raw material (Table 1, total carbohydrate and total nitrogen). This might suggest that the difference in concentration of sugars and nitrogen of koji is due to composition of raw materials used. Nevertheless, the finding may be partly due to influence of raw materials on activity of hydrolyzing enzymes produced by the fungus.
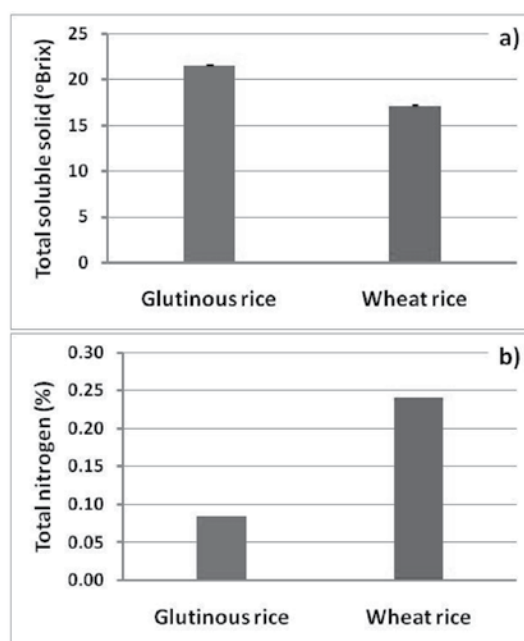

Figure 1. Total soluble solid (^{o}Brix) and total nitrogen (%, dry basis) of koji at the end of fermentation (7 days)

3.3 Total soluble solid, total nitrogen and alcohol content during alcohol fermentation

The results of total soluble solid, total nitrogen and alcohol content during alcoholic fermentation are presented in Figure 2. At the beginning of fermentation, the sugar level of both samples was adjusted to 22 ^{o}Brix (Figure 2a). The sugar level decreased rapidly in the

sample made using wheat rice during the first 2 days from 22 to 10 ^{o}Brix. On the other hand, in the case of the control sample, the sugar level slowly declined from 22 to 17 ^{o}Brix. Thereafter, the utilization of sugar reached a plateau at day 6 for the GR sample and day 4 for the WR sample, respectively. Pattern of nitrogen reduction is similar to that of sugar utilization where there was higher reduction of total nitrogen of the WR sample compared with the control sample during the first 2 days, and it continued to decline until the end of fermentation (Figure 2b). However, in the case of total nitrogen, the initial concentration in the WR sample (2.8%) was higher than that of the control sample (1.4%) (Figure 2b). By taking the value of total nitrogen into consideration, the increase in sugar utilization in the WR sample is likely to be due to its nitrogen content. Similar justification should also be suitable for explaining the result of alcohol content (Figure 2c). The alcohol content of both samples rose steeply during the first 2 days with the WR sample showing higher rate of alcohol production compared with the control sample. Then, the alcohol content of both samples slowly increased with comparable rate until the end of fermentation. At the end of fermentation, the GR and WR samples contain 10.8 and 12.7% (v/v), respectively. Similar results about the effect of total nitrogen (both free amino acids and ammonium) on sugar utilization and alcohol production have been reported by Hernández-Orte, Ibarz, Cacho and Ferreira (5), Arias-Gil, Garde-Cerdán and Ancín-Apilicueta (15) and Taillandier, Portugal, Fuster and Strehaiano (16). It has been shown that yeast consumed amino acids mainly in the first quarter of alcohol fermentation (5). In addition, the rate of amino acid consumption is positively correlated with its initial concentration (16). However, their experiment did not found a correlation between rate of sugar assimilation and concentration of nitrogen.
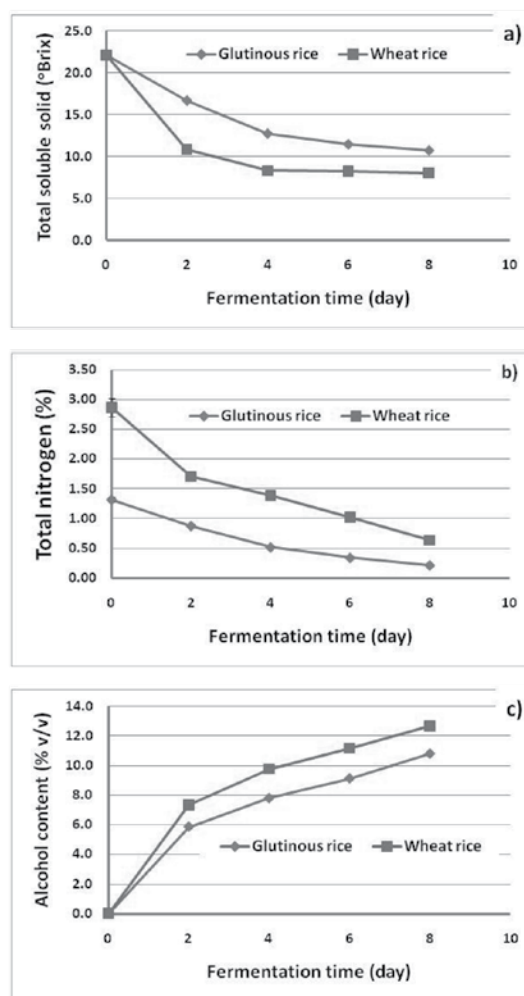

Figure 2. Total soluble solid (^{o}Brix), total nitrogen (%, wet basis) (b) and alcohol content (c) during alcoholic fermentation

3.4 Concentration of valine, phenylalanine, leucine and isoleucine during alcohol fermentation

Concentration of free amino acids including valine, phenylalanine, leucine and isoleucine measured during alcohol fermentation of the GR and WR samples is shown in Figure 3. The concentration of all amino acids studied in the GR sample (10-80 mg/L) was lower than that of the WR sample (15-130 mg/L). This might be due to the difference of their initial nitrogen concentration (Figure 1b). Moreover, in the GR sample, there was an increasing trend in concentration of all four amino acids

from the beginning to the end of fermentation (Figure 3a). On the contrary, in the WR sample (Figure 3b), only concentration of valine and isoleucine increased to their maximum at day 4 and 6, respectively, and declined afterward. This is not the case for phenylalanine and leucine. Their concentration firstly declined during the first 2 days followed by a steeply increased to its maximum at day 4 (phenylalanine) and day 6 (leucine). Then, their concentration decreased again from day 6 to day 8.
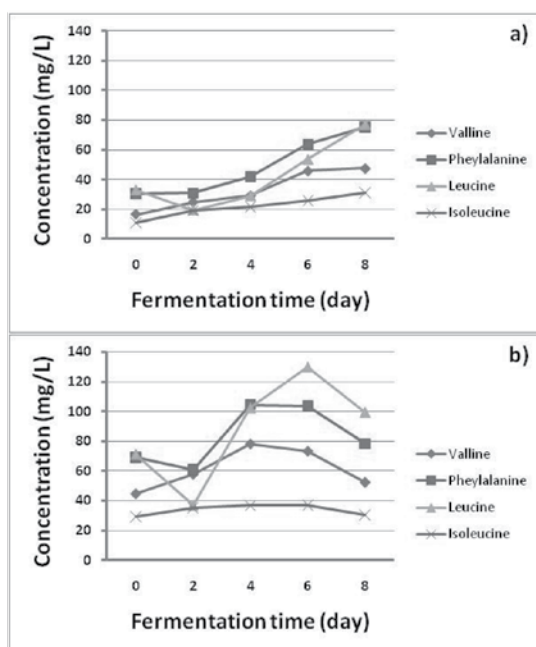

Figure 3. Concentration of free amino acids (valine, phenylalanine, leucine and isoleucine) in the sample made with glutinous rice (a) and wheat rice (b) measured 2 days interval during alcohol fermentation

As can be seen, the results of free amino acid content (Figure 3) contradicts to the change of total nitrogen (Figure 2b). Generally, the amino acids and peptides are used by yeast (17). However, some amino acids, such as isoleucine and leucine, can be produced by yeast during wine fermentation (15). Iemura, Yamada,

Takahashi, Furukawa and Hara (18) observed that when the initial amino acids in medium are high, yeast prefers to assimilate amino acids from the medium over peptides. This decreases final amino acid concentration. Barrajón-Simancas, Giese, Arévalo-Villena and Briones (17) reported similar result that the concentration of most free amino acids in wine decreases from the beginning to the end of fermentation. These findings oppose to our result that the concentration of some amino acids increased continuously during alcohol fermentation. This discrepancy might be due to the fact that the proteolysis of rice protein by enzyme produced from koji occurred during fermentation as observed by Negi and Banerjee (13,14) and Hashizume *et al.* (8), while the proteolysis of grape protein is limited during wine fermentation. Although there is no enough evidence from our experiment about the cause of such results, it is possible that there is higher rate of free amino acid production (proteolysis by acid proteases or production by yeast) than rate of amino acid utilization (yeast metabolism). High concentration of free amino acids in the WR sample could be due to effects of high nitrogen content of wheat rice (Table 1.). However, the difference in protease activity between the GR and WR koji should also be taken into consideration.

3.5 Monitor of volatile compounds

Production of some volatile compounds commonly detected in alcoholic beverages including 3-methyl-1-butanol, 2-methyl-1-propanol, phenyl ethanol, isobutyl acetate, isopentyl acetate, phenetyl acetate and diethyl succinate were monitored during fermentation (Figure 4). These compounds represent pleasant and unpleasant odor description as described in Table 2. The concentration of 3-methyl-1-butanol, phenyl ethanol, phenetyl acetate and diethyl succinate was higher in the WR sample compared with the GR sample. The first two compounds increased steeply during the first 2 days from 20 to 110 mg/L for

3-methyl-1-butanol and from 36 to 69 mg/L for phenyl ethanol in the sample, respectively. Their concentration rose to the maximum (122 mg/L for 3-methyl-1-butanol and 89 mg/L for phenyl ethanol) at day 6. Then, the concentration of phenyl ethanol declined dramatically. The concentration of the other two compounds (phenetyl acetate and diethyl succinate) just slightly increased during fermentation. Their concentration were between 6.0 to 7.0 mg/L (GR sample), 7.0 to 8.0 mg/L (WR sample) for phenetyl acetate and 0.2 to 0.4 mg/L (GR sample), 0.4 to 0.5 mg/L (WR sample) for diethyl succinate. For isopentyl acetate and isobutyl acetate, their concentrations were relatively constant in the case of WR sample. However, there was higher fluctuation in concentration for the GR sample. The concentrations of both compounds were between 2.0 to 3.5 mg/L (isopentyl acetate) and 1.0 to 3.0 mg/L (isobutyl acetate) in both samples. The GR sample contained higher concentration of 2-methyl-1-propanol compared with the WR sample throughout alcohol fermentation. Their concentrations were between 18 to 50 mg/L for the GR sample and 15 to 35 mg/L for the WR sample.

In general, these compounds belong to two groups, which are higher alcohol (3-methyl-1-butanol, 2-methyl-1-propanol, phenyl ethanol) and ester (isobutyl acetate, isopentyl acetate, phenetyl acetate, diethyl succinate). Esters give floral and fruity aroma to alcoholic beverages such as wine, beer and sake (4). Higher alcohols at a suitable concentration (less than 300 mg/L) have been reported to give complexity to wine (19,20). When the concentrations of them are higher than 400mg/L, they give an unpleasant aroma. Valine, phenylalanine, leucine and isoleucine are precursors of 2-methyl-1-propanol, phenyl ethanol, 3-methyl-1-butanol, and 2-methyl-1-butanol, respectively (3). Higher alcohols, one of the substrate involved in esters formation, are formed from amino acids available in fermenting medium by

Ehrlich pathway (20). Amino acids are converted to α-keto acid followed by conversion to higher alcohols. Acetate esters (phenethyl acetate, isopentyl acetate) are formed from the reaction of acetyl- CoA and higher alcohols. As can be seen from the results of free amino acids (Figure 3), higher concentration of higher alcohol (3-methyl-1-butanol and phenyl ethanol) in the WR sample could be due to the high concentration of leucine and phenylalanine. The increase of phenyl ethanol and phenyl acetate in this study is similar to the results of Hernández-Orte, Ibarz, Cacho and Ferreira (5) which the increased volatile compounds was associated with an increase in concentration of phenylalanine, aspartic acid, threonine and alanine. However, high concentration of valine in the WR sample did not cause any increase in 2-methyl-1-propanol. In addition, the production of acetate esters does not correlate with the concentration of their amino acid precursors. These results could be due to yeast strains as observed by Patel and Shibamoto (21) and Torrea, Fraile, Carde and Ancín (22). They observed that different strains of *S. cerevisiae* showed different ability on production of volatile compounds. For acetate esters, the concentrations of the two samples were relatively constant. This suggests that yeast does not produce these compounds during alcohol fermentation regardless of the effect of amino acid concentration. Our results contradict to those of other studies (2,5,9,15,16). Verstrepen et al. (4) has reviewed that oxygen is one contributing factor for repression of alcohol acetyltransferases, the enzymes responsible for esters formation. In our study, the fermentation was carried out in a Erlenmeyer flask covered with a cotton plug. It is possible that oxygen can penetrate into the flask. This would repress alcohol acetyltransferases of yeast and prevent esters fermentation in our study.
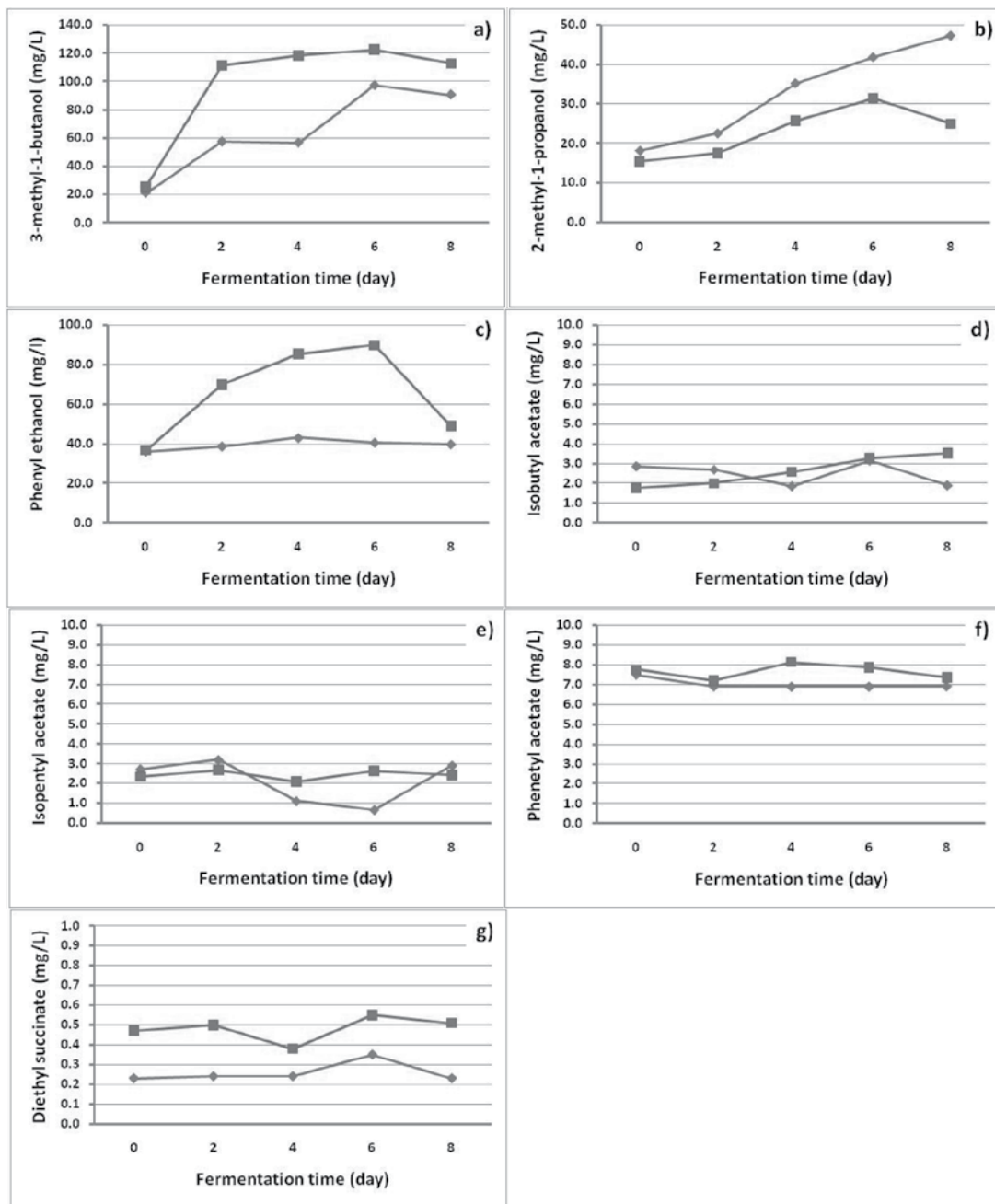
Figure 4. Volatile compounds including 3‑methyl‑1‑butanol (a), 2‑methyl‑1‑propanol (b), phenyl ethanol (c), isobutyl acetate (d), isopentyl acetate (e), phenetyl acetate (f) and diethyl succinate (g) of rice wine made using glutinous rice (◆) and wheat rice (■) during alcohol fermentation.

Interestingly, some of the volatiles studied were observed at the first day of alcoholic fermentation suggesting that these compounds are already produced during koji fermentation. According to the study of Mo, Xu and Fan (23), they observed volatile compounds produced in Qu, a molded cereal used for Chinese rice wine production. This would confirm our speculation that the volatile compounds are already produced during koji fermentation. However, there was no difference in the initial concentration of volatile compounds among two samples. This might suggest that there was no effect of raw material, which is high in nitrogen content, on production of the volatile compounds studied during koji fermentation.

With regard to odor activity, the odor active value (OAV), which represents activity of volatile in sample, can be calculated by dividing the concentration of volatile compounds with the odor threshold value (24). The OAV of 3-methyl-1-butanol, 2-methyl-1-propanol, phenyl ethanol, Isobutyl acetate, Isopentyl acetate, phenetyl acetate and diethyl succinate at the end of fermentation are 1.51, 0.63, 0.20, 1.18, 18.25, 3.84 and 0.19 for the GR sample and 1.88, 0.33, 0.24, 2.19, 15.19, 4.09 and 0.43 for the WR sample, respectively (Table 2.). This result shows that esters are potent volatile compound in rice wine (value more than 1). Based on the OAV values of 3-methyl-1-butanol, isobutyl acetate, isopentyl acetate and phenetyl acetate, the GR and WR samples would have different flavor characteristics (fruity, sweet, banana and flowery). In addition, although there are volatile compounds that have the OAV value lower than 1 in both samples, Ryan, Prenzler, Saliba and Scollary (25) have pointed out that volatile compounds which have low impact odorants (OAV less than 1.0) influence global sensory perception of wine. Therefore, the WR sample containing higher OAV values of phenyl ethanol and diethyl succinate (giving positive flavor characteristics) than in the GR sample may give better flavor characteristic over the GR sample. However, other volatiles that have not been determined in this study also contribute to flavor characteristic of rice wine. Thorough analysis of volatile compounds as well as sensory evaluation is needed to be carried out to confirm our presumption.

Table 2. Odor active value (OAV) of volatile compounds

Volatile compounds	Odor threshold (mg/L)*	Glutinous rice	Wheat rice	Odor description
3-methyl-1-butanol	60.00	1.51±0.03	1.88±0.15	Solvent
2-methyl-1-propanol	75.00	0.63±0.05	0.33±0.01	Alcohol, nail polish
phenyl ethanol	200.00	0.20±0.07	0.24±0.10	Rose, honey
Isobutyl acetate	1.60	1.18±0.54	2.19±0.79	Fruity, apple, banana
Isopentyl acetate	0.16	18.25±2.13	15.19±1.01	Banana, fruity, sweet
phenetyl acetate	1.80	3.84±0.31	4.09±0.09	Flowery
diethyl succinate	1.20	0.19±0.01	0.43±0.01	Fruity, melon

*Values of odor threshold (mg/L) come from Peinado, Moreno, Bueno, Moreno and Mauricio (26)

4. Conclusion

In conclusion, it can be seen that the use of raw material that is high in nitrogen content such as wheat rice used in this study affects koji and alcohol fermentation as well as some volatile compounds. The effect of free amino acids as a result of proteolysis during alcohol fermentation on wine volatile compounds mainly influences higher alcohols (3-methyl-1-butanol and phenyl ethanol). As certain volatile compounds can be detected at the first day of fermentation, this suggests that they are already produced by mold during koji fermentation.

5. References

(1)   Garde-Cardán T, Ancín-Azpilicueta C. Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. LWT-Food Sci Technol. 2008;41: 501-10.

(2)   Hernández-Orte P, Ibarz MJ, Cacho J, Ferreira V. Effect of the addition of ammonium and amino acids to musts of Airen variety on aromatic composition and sensory properties of the obtained wine. Food Chem. 2005;89: 163-74.

(3)   Ribéreau-Gayon P, Dubourdieu D, Doneche B, Lonvaud A. Handbook of Enology, The Microbiology of Wine and Vinifications. 2nd ed. USA, John Wiley & Sons; 2006.

(4)   Verstrepen KJ, Derdelinckx G, Dufour JP, Winderickx J, Thevelein JM, Pretorius IS, et al. Flavor-active esters: adding fruitiness to beer-review. J Biosci Bioeng. 2003;96: 110-18.

(5)   Hernández-Orte P, Ibarz MJ, Cacho J, Ferreira V. Addition of amino acids to grape juice of the Merlot variety: Effect on amino acid uptake and aroma generation during alcoholic fermentation. Food Chem. 2006; 98: 300-10.

(6)   Sumby KM, Garbin PR, Jiranek V. Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. Food Chem. 2010;121: 1-16.

(7)   Kitano H, Kataoka K, Furukawa K, Hara S. Specific expression and temperature-dependent expression of the acid protease-encoding gene (pepA) in *Aspergillus oryzae* in solid-state culture (rice-koji). J Biosci Bioeng. 2002; 93: 563-67.

(8)   Hashizume K, Okuda M, Sakurao S, Numata M, Koseki T, Aramaki I, et al. Rice protein digestion by sake koji enzymes: comparison between steamed rice grains and isolated protein bodies from rice endosperm. J Biosci Bioeng. 2006;102: 340-45.

(9)   Torrea D, Varela C, Ugliano M, Ancín-Apilicueta C, Francis IL, Henschke PA. Comparison of inorganic and organic nitrogen supplementation of grape juice-Effect on volatile composition and aroma profile of a Chardonnay wine fermented with *Saccharomyces cerevisiae* yeast. Food Chem. 2011;127:1072-83.

(10)   AOAC International 2000 Official Method of Analysis 17th ed. AOAC International. Gaithersburg: MD.

(11)   Soufleros EH, Bouloumpasi E, Tsarchopoulos C, Biliaderis, CG. Primary amino acid profiles of Greek white wines and their use in classification according to variety, origin and vintage. Food Chem. 2003;80: 261-73.

(12)   Ortega C, López R, Cacho J, Ferreira V. Fast analysis of important wine volatile compounds: development and validation of a new method based on gas chromatographic-flame ionisation

detection analysis of dichloromethane microextracts. J Chromtogr A. 2001;923: 205–14.

(13) Negi S, Benerjee, R. Optimization of amylase and protease production from *Aspergillus awamori* in single bioreactor through EVOP factorial design technique. Food Technol Biotech. 2006;44: 257–61.

(14) Negi S, Benerjee R. Characterization of amylase and protease produced by *Aspergillus awamori* in a single bioreactor. Food Res Int. 2009;42: 443-8.

(15) Arias-Gil M, Garde-Cerdán T, Ancín-Apilicueta C. Influence of addition of ammonium and different amino acid concentrations on nitrogen metabolism in spontaneous must fermentation. Food Chem. 2007;103: 1312-8.

(16) Taillandier P, Portugal FR, Fuster A, Strehaiano P. Effect of ammonium concentration on alcoholic fermentation kinetics by wine yeasts for high sugar content. Food Microbiol. 2007; 24: 95-100.

(17) Barrajón-Simancas N, Giese E, Arévalo-Villena M, Briones UA. Amino acid uptake by wild and commercial yeasts in single fermentations and co-fermentations. Food Chem. 2011;127: 441-45.

(18) Iemura Y, Yamada T, Takahashi T, Furukawa K, Hara S. Influence of amino acid content in seed mash on peptide uptake by yeast cells in main mash in sake brewing process. J Biosci Bioeng. 1999;88: 679-81.

(19) Rapp A, Mandery H. Wine aroma. Experientia. 1986;42: 873-84.

(20) Hazelwood LA, Darn JM, van Maris AJA, Pronk JT, Dickinson JR. The Ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. Appl Environ Microb. 2008;74: 2259-66.

(21) Patel S, Shibamoto T. Effect of 20 different yeast strains on the production of volatile components in symphony wine. J Food Compos Anal. 2003;16: 469-76.

(22) Torrea D, Fraile P, Carde T, Ancín, C. Production of volatile compounds in the fermentation of chardonnay musts inoculated with two strains of *Saccharomyces cerevisiae* with different nitrogen demands. Food Control. 2003;14: 565-71.

(23) Mo X, Xu Y, Fan W. Characterization of aroma compounds in chinese rice wine Qu by solvent-assisted flavor evaporation and headspace solid-phase microextraction. J Agr Food Chem. 2010:58: 2462-69.

(24) Jackson RS. Wine Science: Principles and applicagtions. 3rd ed. Canada: Elsevier Inc.; 2008.

(25) Ryan D, Prenzler PD, Saliba AJ, Scollary GR. The significance of low impact odorants in global odour perception. Trends Food Sci Tech. 2008:19: 383-9.

(26) Peinado RA, Moreno J, Bueno JE, Moreno JA, Mauricio JC. Comparative study of aromatic compounds in two young white wine subjected to pre-fermentative cryomaceration. Food Chem. 2004;84: 585-90.