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Effects of drying methods and storage time on the aroma and milling quality of rice (*Oryza sativa* L.) cv. Khao Dawk Mali 105

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Abstract

The effects of drying methods and storage time on the aroma and milling quality of the aromatic rice, Khao Dawk Mali 105, stored as whole grains, were investigated in order to define the most appropriate post-harvest treatment for the rice. Among the six drying methods that were used, namely: modified air at 30 and 40 °C, hot air at 40, 50, and 70 °C, and sun-drying, the methods that employed lower temperature appeared to provide higher concentrations of the key aroma compound, 2-acetyl-1-pyrroline, and lower amounts of the off-flavour compounds, *n*-hexanal and 2-pentylfuran, regardless of the storage time. The sun-drying method yielded contrasting results. Overall, during 10 months storage, as the time increased, 2-acetyl-1-pyrroline concentrations decreased whereas *n*-hexanal and 2-pentylfuran contents increased. Head rice yield was most clearly affected in the sample dried by hot air at 70 °C, giving a percentage yield slightly less than half of those obtained by the other drying methods. However, no significant variation in the percentages of whiteness was observed among the rice samples obtained from the different drying methods and storage times.

Keywords: KDML 105; 2-Acetyl-1-pyrroline; Solid phase microextraction; SPME; *n*-Hexanal; 2-Pentylfuran; Head rice; Whiteness

1. Introduction

Khao Dawk Mali (KDML) 105, commonly known in food markets as "Jasmine Rice" or "Thai Hom Mali Rice", is the most popular aromatic rice variety grown in Thailand. Because of its famous reputation in appearance, cooking quality and high aroma level, the rice has gained an increasing popularity throughout the world food market. In recent years, great efforts have been made to improve Thai aromatic rice breeding programmes leading to the achievement of high quality and yields that meet the demands of the world's rice consumers. The management of rice grain after harvesting has been reported to play an essential role in posterior maintenance of rice yield and quality. The post-harvest treatments often considered include methods and temperature of drying, storage moisture content, storage conditions and duration, while the properties of rice grains frequently reported are head rice yield, pasting properties, chemical properties and sensory quality (Daniels, Marks, Siebenmorgen, Mcnew, & Meullenet, 1998; Meullenet, Markes, Griffin, & Daniels, 1999; Meullenet, Marks, Hankins, Griffin, & Daniels, 2000; Pearce, Marks, & Meullenet, 2001; Perdon, Marks, Siebenmorgen, & Reid, 1997; Ranalli, Howell, & Siebenmorgen, 2003; Zhou, Robards, Helliwell, & Blanchard, 2002; Zhou, Blanchard, Helliwell, & Robards, 2003).

Aromatic rice possesses a characteristic odour that distinguishes it from ordinary rice. This odour results from the volatile components released from the rice. However, this can be readily affected by pre- and post-harvest treatments, due to the vulnerability to change in

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the composition of the volatile constituents under diverse conditions. From more than 100 rice volatiles identified by many research groups in the past two decades, only some possess an odour threshold value low enough to make their contributions to the characteristic odour of the rice important. 2-Acetyl-1-pyrroline, since it was firstly identified by Buttery, Ling, and Juliano (1982), has been reported and confirmed as the most important aroma compound contributing to the aroma character of rice (Buttery, Ling, Juliano, & Turnbaugh, 1983b; Jezussek, Juliano, & Schieberle, 2002; Laksanalamai & Ilangantileke, 1993; Paule & Powers, 1989). Therefore, concentrations of 2-acetyl-1-pyrroline investigated through a set of post-harvesting processes should reflect changes in aroma quality of the rice affected by the treatments.

In this study, a non-heated method of extraction, utilizing acidic solvent followed by re-extraction with organic solvent, as described in our previous study (Mahatheeranont, Keawsa-ard, & Dumri, 2001; Wongpornchai, Sriseadka, & Choonvisase, 2003), was employed for quantitation of 2-acetyl-1-pyrroline in KDML 105 rice samples which had been subjected to a designed set of drying methods and storage durations. At the same time, overall volatile constituents were examined by the use of a handy but effective solid phase microextraction (SPME) device. Development of some impact volatiles following storage was then investigated. In addition, measurements of milling qualities of the rice samples studied were carried out. Correlation of all data obtained could define an appropriate set of post-harvest treatments for obtaining aromatic rice products with low substantial loss or reduction in quality.

2. Materials and methods

2.1. Rice sample

Khao Dawk Mali 105 rice (*Oryza sativa* L.) was grown in a paddy of a local farm in Amphur Thatum, Surin Province, located in the northeastern part of Thailand, during August and November 2001. It was harvested by a combined harvester when containing approximately 28% moisture.

2.2. Chemicals

2-Acetyl-1-pyrroline was synthesized as outlined by Buttery, Juliano, and Ling (1983a), starting by hydrogenating 2-acetylpyrrole in methanol solution using 5% rhodium on an activated alumina catalyst (Aldrich Chemical Co., Milwaukee, WI) at room temperature under 10 psi of H_2 pressure. 2-(1-Hydroxyethyl) pyrrolidine was obtained as the major product and was isolated. It was then weighed and subjected to oxidation by refluxing with a stirred suspension of silver carbonate (Aldrich Chemical Co., Milwaukee, WI) on Celite in benzene solution under a nitrogen atmosphere. As the resulting mixture was found to contain many other products, purification of the 2-acetyl-1-pyrroline was accomplished by utilizing a gas chromatographic column packed with 3% dimethylpolysiloxane coated on 80/100 mesh solid supports. The principal peak for 2-acetyl-1-pyrroline emerging from the GC detector outlet was collected in 3-mm-o.d. Pyrex tubes. The collecting tube was then sealed under vacuum and stored at −20 °C for further use. Structural examination of this purified 2-acetyl-1-pyrroline by capillary gas chromatography–mass spectrometry (GC–MS) and infrared spectroscopy (IR) showed mass and infrared spectra consistent with the structure of this compound (Buttery, Ling, & Juliano, 1985).

Stock acid solution containing 0.25 mg/l concentration of an internal standard was prepared by accurate weighing of 2,4,6-trimethylpyridine, 99% purity (Aldrich Chemical Co., Milwaukee, WI), and dissolution in a precisely measured volume of 0.1 M HCl. Standard solutions of 2-acetyl-1-pyrroline were made by dissolving a known weight of the purified product in a precisely measured volume of the stock acid solution.

2.3. Drying methods and storage condition

Fresh paddy, weighing approximately 3000 kg and containing approximately 28% moisture, was subjected to six different methods of drying: in modified air at 30 and 40 °C, in hot air at 40, 50, and 70 °C, and by sun-drying until the moisture contents were reduced to 13–15%. Moisture contents were measured by a moisture tester (Dole, model 400, Seedburo Equipment Company, USA) and the results expressed on a wet matter basis, as summarized in Table 1. The experiments were conducted at the Seed Processing Plant Agriculture Unit, Faculty of Agriculture, Khon Kaen University, located in the north-eastern part of Thailand, during November 2001. After the drying treatments, each rice sample was kept as whole grains in gunnysacks and stored under ambient conditions (20–35 °C and 70–85% relative humidity) for 4 weeks. Rice samples were then taken from the middle of the gunnysacks for further

Table 1
Drying methods used for KDML 105 rice and the drying time required for each method to obtain the resulting moisture contents

Drying methods	Drying time (h)	Moisture content (%)
Sun-drying	54	14.12
Modified air, 30 °C	11	13.87
Modified air, 40 °C	9	13.60
Hot air, 40 °C	9	13.96
Hot air, 50 °C	9	13.56
Hot air, 70 °C	8	13.03

experiments. These experiments were conducted at monthly intervals for a storage time of 10 months.

2.4. Quantitative analysis of 2-acetyl-1-pyrroline

2.4.1. Solvent extraction of 2AP from brown rice

The rice grain samples were husked by hand to yield brown rice seeds on the day of experiment. The brown rice seeds were ground and then screened through a 35-mesh sieve where crude particles were filtered out. Five grams of the ground rice powder was weighed and three replications of each sample were carried out. The samples were then placed to 125-ml flasks containing 50 ml of 0.25 mg/l 2,4,6-trimethylpyridine internal standard solutions. Each mixture was stirred for 30 min at room temperature. After filtration of the rice seed particles, 40 ml of the filtrate was transferred to a 125-ml pear-shaped separatory funnel, followed by the addition of approximately 1.2 ml of 5.0 M NaOH to make the solution slightly basic. Then, 50 ml of dichloromethane was immediately added as an organic solvent. The extraction was carried out twice and 90.0 ml of the combined dichloromethane portions collected. After being dried with anhydrous sodium sulfate, this extract was concentrated to 1 ml by removal of dichloromethane using a rotary evaporator under reduced pressure at a temperature of 28 °C. The resulting concentrated extract was transferred to a V-shaped vial and left open to the air at room temperature until its volume decreased to 0.1 ml before it was subjected to quantitative analysis by capillary GC with a flame ionization detector (FID). The whole experimental process was repeated for the extraction and analysis of standard 2-acetyl-1-pyrroline of known amounts in a dilution series in order to obtain a standard calibration curve.

2.4.2. GC conditions

An Agilent 6890 GC equipped with an Agilent 7683 injector and a FID (Agilent Technologies, Palo Alto, CA) was utilized for analysis of 2-acetyl-1-pyrroline in the rice seed extracts. A fused silica capillary column HP-5MS, biphenyldimethylpolysiloxane, with dimensions of 30 m × 0.25 mm i.d. and 0.25 μm film thickness (Agilent Technologies) was programmed, starting at 45 °C. The temperature was ramped to 120 °C at a rate of 5 °C/min, resulting in an overall separation time of 15 min. The injector temperature was set at 250 °C and was operated in a split mode with a split ratio of 10:1. Purified helium was used as the GC carrier gas at a flow rate of 1 ml/min.

2.5. Analysis of rice volatiles

2.5.1. SPME

The rice grain samples were husked by hand to obtain brown rice seeds which were then chilled at 4 °C for 24 h before they were ground and screened through a 35-mesh sieve. The rice powder, weighing exactly 8.00 g, was added to 100 μl of an internal standard solution containing 10.0 mg/l of 2,4,6-trimethylpyridine and then sealed in a 27-ml bottle fitted with a PTFE/silicone septum (Restek Corp., Bellefonte, PA) and an aluminium cap. The sample bottle was left at room temperature (27 °C) for 15 min before it was incubated in an oil bath at 80 °C for 30 min. Extraction of the rice sample headspace volatiles was performed using an SPME fibre (Supelco, Bellefonte, PA) of 1 cm in length, coated with poly(dimethylsiloxane) (PDMS) of 100-μm thickness which had been preconditioned in a GC injection port at 200 °C for 1 h. The fibre, mounted in the manual SPME holder (Supelco), was up in its protective sheath when it was inserted through the septum of the rice sample bottle being incubated in an oil bath at 80 °C. Inside the bottle, the fibre was lowered to expose it to the rice sample headspace at a position 1 cm above the sample surface. It was left there for 30 min prior to desorption of the volatiles into the splitless injection port of the GC–MS instrument with 1-min desorption time.

2.5.2. GC–MS

Analyses of the rice headspace volatiles extracted by SPME were performed on a gas chromatograph–mass spectrometer (Agilent 6890 and HP 5973 mass-selective detector, Agilent Technologies) equipped with a fused silica capillary column, HP-1MS, with dimethylpolysiloxane as the non-polar stationary phase (30 m × 0.25 mm i.d. × 0.25 μm) (Agilent Technologies). The injection port temperature was set at 250 °C. The column temperature programme started at 35 °C upon injection and was increased at a rate of 2 °C/min to 50 °C, then at a rate of 3 °C/min to 100 °C, and finally at a rate of 5 °C/min to 180 °C. Purified helium gas, at a flow rate of 1 ml/min, was used as the GC carrier gas. The GC–MS transfer line was set to 280 °C. The mass spectrometer was operated in the electron impact (EI) mode with an electron energy of 70 eV, ion source temperature, 230 °C, quadrupole temperature, 150 °C, mass range m/z 29–350, scan mass rate, 6.35 scan/s, and electron multiplier voltage, 1341 V.

2.6. Measurement of percentages of head rice and whiteness

The percentage of head rice and the whiteness of the milled rice are significant indices of the rice milling quality. Two hundred grams rough rice samples from each drying treatment, after storage for 8 weeks, were dehulled in a McGill sample sheller (Seedburo Equipment Company, USA). Brown rice samples were subsequently weighed and milled in a McGill No. 2 miller (Seedburo Equipment Company, USA). Milled rice samples were weighed and separated into head rice and

broken rice by different sieve sizes in an automated sizing device. After the weights of head rice samples had been obtained, the percentages of head rice were calculated. Milled rice samples were taken to measure percentages of whiteness using an automated colour meter (Minolta-C300, Japan).

3. Results and discussion

A number of research groups have reported concentrations of 2-acetyl-1-pyrroline in KDML 105 rice in the range 0.05–0.20 ppm, which were found to be as high as those contained in other well-accepted types of Asian aromatic rice, such as Basmati 370 (India and Pakistan) and Milagrosa (Philippines) (Buttery et al., 1983b; Paule & Powers, 1989; Tanchotikul & Hsieh, 1991). In these previous studies, a typical extraction technique for volatile components, employing steam distillation and solvent extraction (SDS), was utilized in conjunction with analysis of the extracts by gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS). Regardless of the thermal effect of the SDS technique in causing degradation of the compounds of interest, this conventional method has been employed by most research groups, partly due to the assumption that 2-acetyl-1-pyrroline may form during cooking of rice or other foods. Recently, our reports on the quantification of 2-acetyl-1-pyrroline in KDML 105 rice (Mahatheeranont et al., 2001) and other plant materials (Wongpornchai et al., 2003) have, however, revealed its presence in plants as a naturally occurring compound because the non-heated solvent extraction method was utilized for extraction of 2-acetyl-1-pyrroline in raw rice and raw plant materials. This was confirmed by the report of Yoshihashi, Huong, and Inatomi (2002) in their studies of the biological formation of 2-acetyl-1-pyrroline in KDML 105 rice, which showed that the compound appeared to occur in the aerial part of the rice at temperatures below that in the bread-baking process. Concentrations of 2-acetyl-1-pyrroline in fresh uncooked brown rice of KDML 105 reported later by our group were found to be as high as 3.00 ppm.

Extraction of 2-acetyl-1-pyrroline from rice seeds by acidic aqueous solution has the advantage over the SDS technique that no heat is involved, so that, oxidations or Maillard reactions of some molecules are prevented. Other reactions of organic components which may result in formation or degradation of compounds, especially the target 2-acetyl-1-pyrroline, are also minimized. Furthermore, the relative concentration of 2-acetyl-1-pyrroline, a slightly basic compound, as well as other basic components, can be enhanced in acidic solution relative to those of non-basic components contained in rice. Thus, the rice seed extracts yield a less complex mixture of components than that obtained from the conventional simple steam distillation or SDS technique. Consequently, quantification of the aroma compound 2-acetyl-1-pyrroline by the use of an internal standard method is more efficient.

For quantitative analysis of 2-acetyl-1-pyrroline in KDML 105 rice samples, a known amount of 2,4,6-trimethylpyridine, as internal standard, was added to the 0.1 M HCl used as the first extracting solvent. When ground rice seed was immersed in an appropriate quantity of 0.1 M HCl, 2-acetyl-1-pyrroline and 2,4,6-trimethylpyridine were readily partitioned in the acidic solution due to their basic properties. Repeating this process one more time allowed recovery of the maximum amount of both 2-acetyl-1-pyrroline and 2,4,6-trimethylpyridine from 5.00 g of ground rice sample. This was confirmed by an undetectable signal of the compounds by GC analysis of the extract of the same rice sample that had been extracted twice. After the acidic solution was made slightly basic by adding 1.0 M NaOH, almost the entire amount of 2-acetyl-1-pyrroline and 2,4,6-trimethylpyridine was removed from the aqueous phase to the organic dichloromethane phase in the secondary step of the extraction. In order to recover all amounts of 2-acetyl-1-pyrroline and 2,4,6-trimethylpyridine from the aqueous layer, extraction by dichloromethane was performed twice. The complete extraction of both compounds into the organic phase was confirmed by their undetectable signals in the GC chromatogram of the second dichloromethane extract. As a result, the rice extracts were presumed to contain virtually all of the 2-acetyl-1-pyrroline contained in the corresponding rice samples.

When the concentrated extracts of the rice samples were subjected to quantitative analysis by GC–FID, a similar pattern of component separation was achieved for all of the rice samples analysed. Fig. 1 shows a GC–FID chromatogram of an extract of one of the rice samples which was dried by hot air at 40 °C until the water content was reduced to 13.96% and then stored at room temperature as whole grains for 4 months. Chromatographic peaks, identified as 2-acetyl-1-pyrroline and 2,4,6-trimethylpyridine, appeared at retention times of 9.385 and 11.296 min, respectively, without any interfering peaks. Determination of 2-acetyl-1-pyrroline in the rice extracts was performed by measurements of peak areas of the compound and its internal standard with the aid of the instrument's digital integrator. Correlating ratios of peak areas of 2-acetyl-1-pyrroline and 2,4,6-trimethylpyridine with concentrations of 2-acetyl-1-pyrroline in rice extracts was performed by means of a standard calibration curve obtained by measuring a mixture of varying concentrations of five different 2-acetyl-1-pyrroline standards, together with a fixed concentration of the internal standard, 2,4,6-trimethylpyridine. A linear calibration curve resulted with a regression coefficient of 0.9991 over the concentration

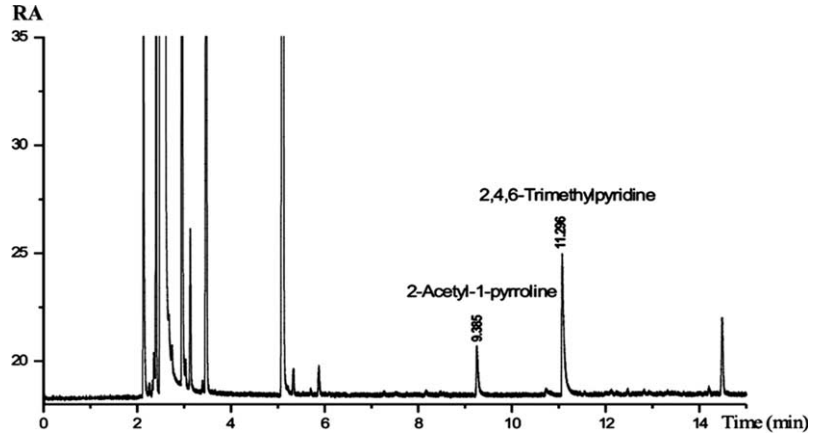

Fig. 1. GC–FID chromatogram of an extract of KDML 105 rice sample dried by hot air at 40 °C and stored at room temperature as whole grains for 4 months.

range of 0.10–5.00 mg/l for 2-acetyl-1-pyrroline. The relative standard deviation (RSD) calculated for each data point of concentration was less than 10%, based on 4 independent runs.

After being subjected to six different drying methods, concentrations of 2-acetyl-1-pyrroline were determined for each set of rice samples at monthly intervals up to 10 months. The results are represented by the bar graph shown in Fig. 2. The RSD for each 2-acetyl-1-pyrroline concentration analysed in triplicate was calculated as being less than 10%. The effect of the drying methods on the concentration of 2-acetyl-1-pyrroline in brown rice stored as whole grains was most significant during the first 2 months of storage. Although the variations in concentration of 2-acetyl-1-pyrroline between the different drying methods at each monthly storage interval did not follow a set pattern, in general, higher concentrations were found from the drying methods that employed lower temperature, for example, hot and modified air at 40 °C and, especially, modified air at 30 °C. The changes in concentration of 2-acetyl-1-pyrroline, after 10 months storage for each drying method, were found to follow the same trend. The 2-acetyl-1-pyrroline concentrations showed the highest rates of decrease at the beginning of storage. Subsequently, the rate decreased with increasing storage time. The average 2-acetyl-1-pyrroline concentration of all of the rice samples subjected to the six different drying methods after 1 month of storage (4.02 ± 0.60 ppm) was slightly more than double that after 4 months (1.88 ± 0.27 ppm) and more than four times that after 10 months of storage (0.89 ± 0.12 ppm).

Further investigation of the rice volatiles by the SPME–GC–MS technique in the same rice samples confirmed consistent degradation patterns of 2-acetyl-1-pyrroline (Fig. 3(a)), except for the 9 and 10 months storage samples. In this technique, the relative amounts of the aroma compound in rice seeds were mainly derived from the ratios of the peak areas of 2-acetyl-1-pyrroline and 2,4,6-trimethylpyridine added into the rice

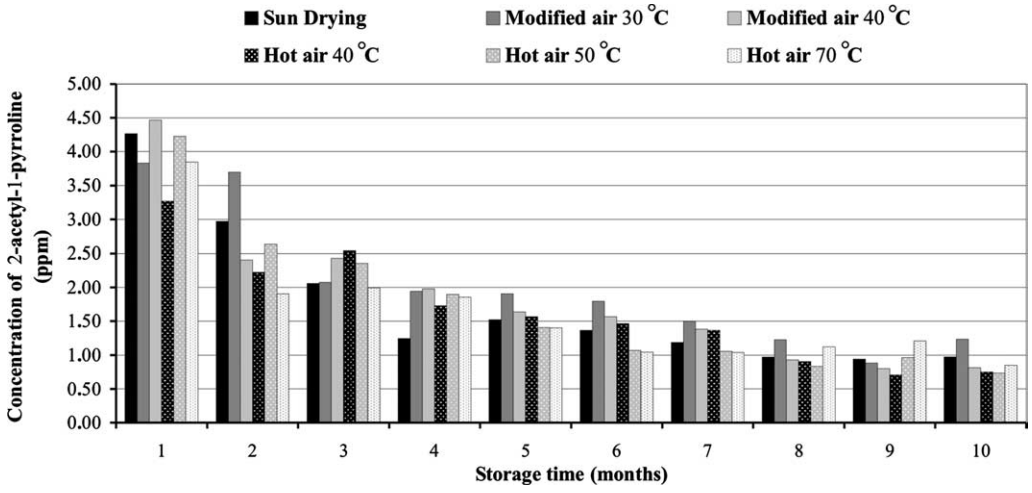

Fig. 2. Effects of drying methods and storage duration on concentration of the aroma compound 2-acetyl-1-pyrroline determined in KDML 105 rice samples stored as whole grains.

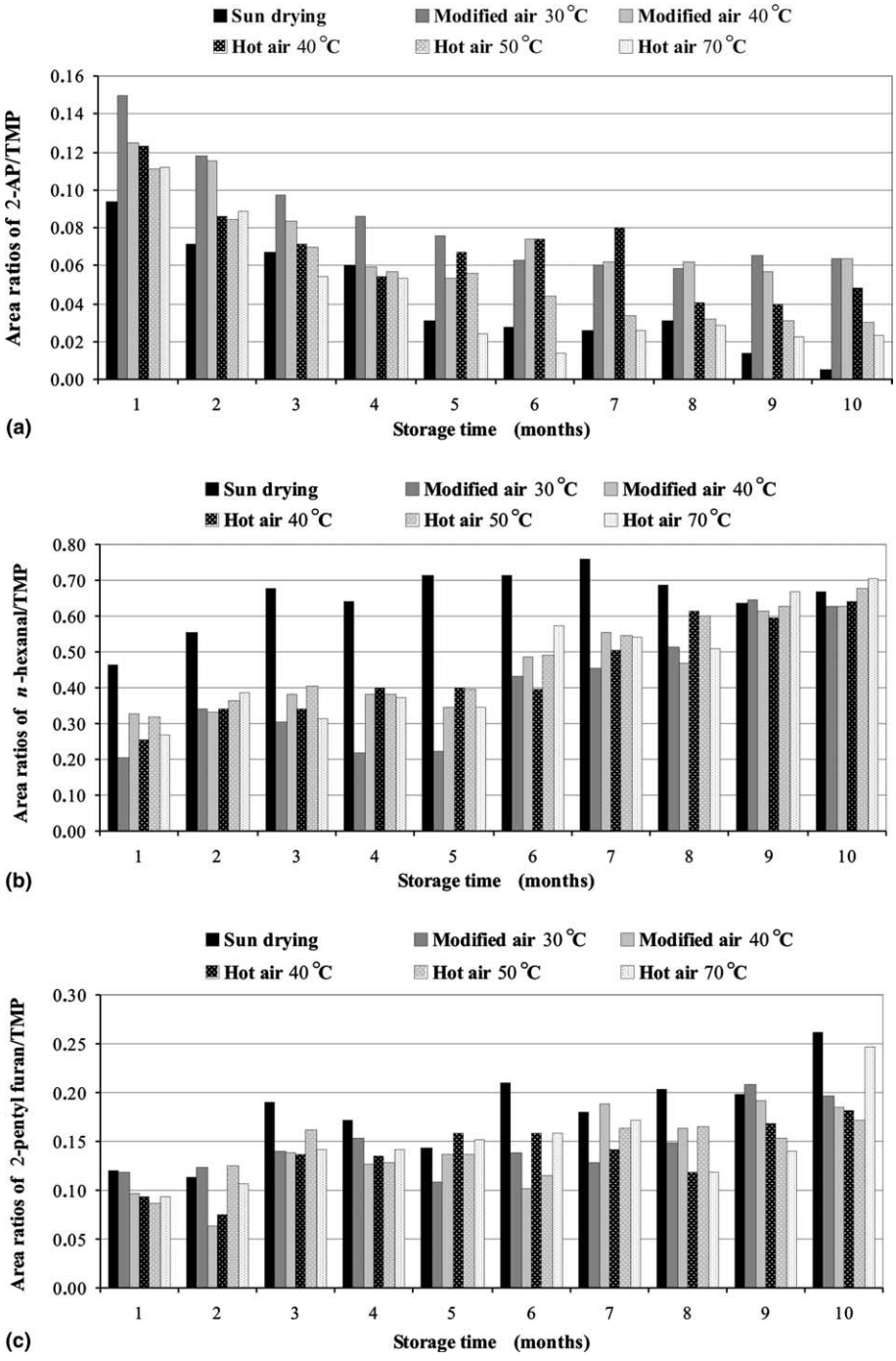

Fig. 3. Effects of drying methods and storage duration on relative amounts of (a) 2-acetyl-1-pyrroline, (b) *n*-hexanal, and (c) 2-pentylfuran obtained by SPME–GC–MS of KDML 105 rice samples stored as whole grains. 2-AP, 2-acetyl-1-pyrroline; TMP, 2,4,6-trimethylpyridine.

samples at constant concentration as standard compounds. Apart from 2-acetyl-1-pyrroline, 53 other volatile components were detected and 30 volatiles were tentatively identified as being mainly from a group of single carbonyl compounds, aldehydes, ketones, alcohols and esters, with a few aliphatic hydrocarbons and terpenoids. Among these identified rice headspace volatiles, two impact off-flavour compounds, *n*-hexanal and 2-pentylfuran, were determined in terms of their relative amounts in rice headspace. Figs. 3(b) and (c) show how the relative amounts of *n*-hexanal and 2-pentylfuran in the rice grain samples subjected to the six different drying methods developed increasingly as a function of time. These developments were relevant to those reported elsewhere for stored brown and white rice (Shin, Yoon, Rhee, & Kwon, 1986; Widjaja, Craske, & Wootton, 1996). It was observed in this work that the method of drying was an important factor affecting the

rice volatile contents. For the aroma compound, 2-acetyl-1-pyrroline, drying methods using low temperature (30 and 40 °C) yielded higher amounts of the aroma compound than those methods using high temperature. Moreover, drying methods at high temperature supported the increases in the off-flavours, *n*-hexanal and 2-pentyl furan. These effects of the drying method continued throughout the experimental period of 10 months. There was also a remarkable variation in the *n*-hexanal contents for rice samples subjected to sun-drying in comparison with the other drying methods, with substantially higher contents being observed during 8 months of storage.

The effect of the drying method on the milling quality of the rice samples stored as whole grains was evaluated, based on the percentage of head rice and whiteness of the milled rice, as shown in Figs. 4 and 5. It was obvious that drying paddy with hot air at high temperature (70 °C) resulted in the lowest head rice yield. The average percentage of head rice obtained was 25.84%. The percentages of head rice obtained from paddy dried with modified air at 30 and 40 °C and hot air at 40 and 50 °C were slightly greater than that achieved from sun-drying. On average, the head rice percentage obtained from drying paddy with modified air (30 and 40 °C) and hot air (40 and 50 °C) was 56.48%, as compared with 53.64% obtained from the sun-drying method. These results also demonstrated that there was a slight variation in the percentage of head rice obtained from each drying treatment with storage time. Fig. 5 shows the effect of the drying method on the percentage of whiteness of milled rice. Similar to the percentage of head rice, the whiteness of milled rice was generally low when obtained from paddy dried by high temperature hot air (70 °C). Surprisingly, the percentage of whiteness of milled rice from paddy dried by modified air at 30 °C was also low. On average, the percentages of whiteness of milled rice was 44.30%, and 44.79%, as obtained from paddy dried by high temperature hot air (70

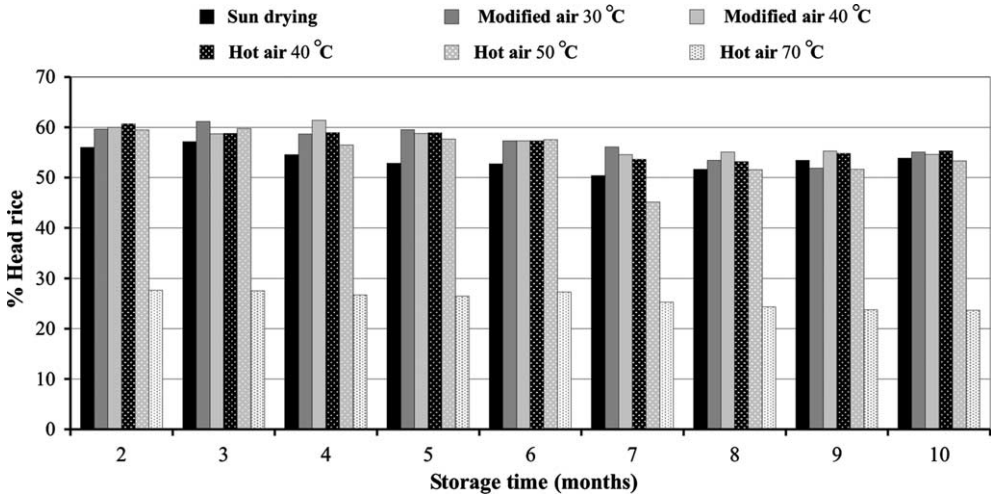

Fig. 4. Effect of drying method on percentage of head rice obtained for KDML 105 rice samples stored as whole grains for 10 months.

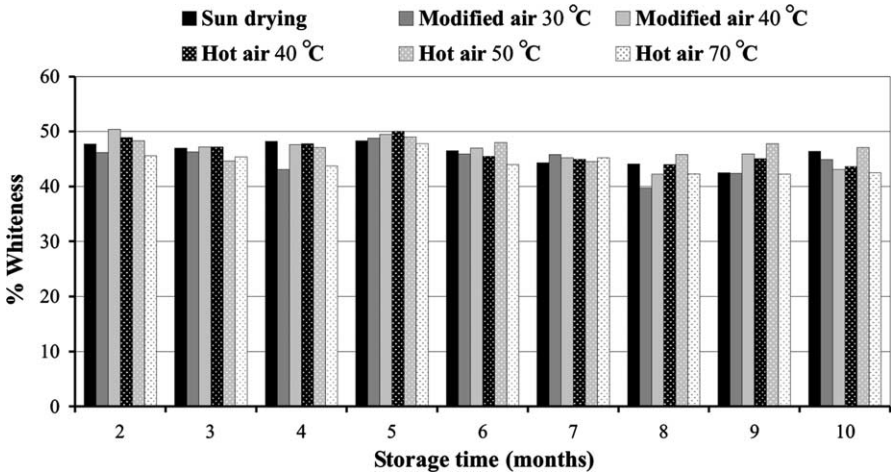

Fig. 5. Whiteness of KDML 105 rice samples subjected to the 6 methods of drying at monthly intervals up to 10 months of storage.

°C) and modified air at 30 °C, respectively. Fig. 5 also illustrates that there was slight variation in the percentage of whiteness obtained from each drying treatment with storage time. Generally speaking, sun-drying and the drying methods using hot air at 40 and 50 °C and modified air at 40 °C resulted in higher percentages of whiteness of the milled rice.

4. Conclusions

The drying method and storage time, as important post-harvest processing variables for rice grains, have significant effects on the aroma and milling quality of aromatic rice KDML 105 stored as whole grains. An appropriate post-harvest treatment for KDML 105 rice, according to this study, should be one which employs drying methods with low temperature, for example, modified or hot air at 30–40 °C, together with as short a storage time as possible to ensure better aroma quality. From the results of this work, drying by hot air at 70 °C should be discounted as it gives a percentage of head rice less than one half of that obtained by the other drying methods, regardless of the storage time.

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