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Aroma enrichment and the change during storage of non-aromatic milled rice coated with extracted natural flavor

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Abstract

Three non-aromatic rice varieties (RD23, SPR1 and SPR90) were coated in a modified spouted bed with 30% sorbitol-plasticized rice-starch film containing 25% pandan (*Pandanus amaryllifolius* Roxb.) leaf extract produced by the supercritical fluid with carbon dioxide extraction method. The coated and uncoated samples and uncoated aromatic rice (KDML and PTT1 varieties) were packed in plastic bags (nylon15/PE20/LLDPE75) and stored at 25 °C for 6 months. Gas chromatography–mass spectrometry analysis revealed that the coating treatment resulted to similar flavor volatile profile as that of aromatic varieties, particularly the presence of 2-acetyl-1-pyrroline (ACPY) which is the main volatile compound responsible for the jasmine aroma. ACPY was absent in uncoated non-aromatic rice and ACPY content of non-aromatic rice coated with natural pandan extract was lower than that of uncoated aromatic rice. During storage, ACPY levels decreased. Coated non-aromatic rice retained higher ACPY levels than the aromatic PTT1 variety. The coating treatment also reduced the *n*-hexanal content of stored grains. Thus, the coating technique is a promising approach for rice aroma improvement and at the same time, for reducing the potential for oxidative rancidity during grain storage.

Keywords: *Oryza sativa* L.; Surface coating; Pandan; Aroma enrichment; Flavor volatiles; ACPY; Lipid oxidation; Grain storage

1. Introduction

Rice (*Oryza sativa* L.) is a major component of the diet of people in many countries. Thailand is the world's second largest rice exporting country. The milling product of greatest commercial importance is scented or aromatic rice. Khao Dawk Mali 105 (KDML) or Jasmine rice is a good example of the native variety which has the specific dominant trait of good jasmine fragrance. The steady increase in aromatic rice consumption and the growing export demand have brought keen interest on scented rice production and flavor improvement of non-aromatic rice. In the last two decades, attempts were made to better understand rice flavor chemistry. Buttery, Ling, and Juliano (1982) found that 2-acetyl-1-pyrroline (ACPY) is the organic volatile compound in cooked aromatic rice. This volatile compound could be a good indicator for identifying fragrance rice from ordinary or non-aromatic rice. ACPY was not found in non-aromatic rice and was present in low concentration in aged KDML rice. Some new aromatic lines of rice were found to produce ACPY ranging from 0.53 to 2.75 ppm (Bocchi, Sparacino, & Tava, 1995).

ACPY is the major volatile compound in pandan (*Pandanus amaryllifolius* Roxb.) leaves (Buttery, Juliano, & Ling, 1983; Laksanalamai & Ilangantileke, 1993). ACPY could be synthesized by using rhodium on alumina, reducing 2-acetyl pyrrole for 15 h, and hydrolyzing 2-(1-alkyloxyethenyl)-1-pyrroline compound with an acid. However, the yield is lower than 10%. Artificial pandan flavor is commercially available but it is ideal to use the natural source. Although pandan leaves are the best natural source of ACPY, the existing extraction procedure by steam distillation gives off-flavor artifacts. A new method, the supercritical fluid with carbon dioxide extraction (SC–CO_2), has been developed to extract the natural pan-

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dan flavor, with the highest yield of 716.5 ng/g obtained using 200 bar pressure, 50 °C temperature and 20 min contact time (Laohakunjit & Noomhorm, 2004a). Aside from solving off-flavor production, the method is advantageous as it uses CO_2 which is an inexpensive gas, non-harmful to human, and non-toxic to the environment.

Flavor enrichment of non-aromatic rice has been attempted earlier but a successful product has not been obtained (Donnarumma et al., 1973). The flavoring agent was either removed or not released or sensed during ingestion of the cooked grain. The use of sticking agents such as glucose, zein, edible oil, shellac or gluten, was tried but again, the flavoring material separated or sifted down in the package during handling, shipping and storage (Houston & Kohler, 1970; Kester & Fennema, 1986; Misuki & Yasumatsu, 1985; Nibler & Roseman, 1970). Some of the sticking agents were also not desirable food additives and produced off-flavor more rapidly than the rice or the flavoring material. It was concluded that it would not be possible to prepare and market a flavored dried rice product in a package.

Recent developments in surface coating have caused renewed interest in using the coating material as flavor-complexing agent. The edible coatings are desired for obvious reasons and among these, starch is the most commonly used since it is cheap and highly available (Nisperos-Carriedo, 1994). Gelatinized starch is usually used in various studies on interaction with other molecules since the ungelatinized granules are relatively inert and can easily be penetrated by water (Reineccius, 1991). In an earlier study, the 30% sorbitol-plasticized rice starch film was found to be the best coating agent for rice (Laohakunjit & Noomhorm, 2004b). The present study determined the effects of coating with rice starch-natural pandan extract complex on the volatile profile, ACPY content and *n*-hexanal production of non-aromatic rice and compared the changes in volatile compounds to that of uncoated aromatic rice.

2. Materials and methods

2.1. Rice samples

Three non-aromatic rice varieties, RD23, Supanburi 1 (SPR1) and Supanburi 90 (SPR90), and three aromatic varieties, KDML and Pathumthani 1 (PTT1), were obtained from the Pathumthani Rice Research Center, Thailand. All paddy samples (rough rice) were harvested in October 2001 and contained 20% moisture. After 24 h from harvesting, the samples were sun-dried to about 12–13% moisture content (wet basis). Following drying, the samples were stored in closed containers for 30 days at ambient temperature (30 ± 2 °C). After equilibration, 500 kg samples from each cultivar were dehulled in a hulling machine and immediately milled and polished using a standard laboratory mill. The polished rice grains were packed in plastic containers and kept at ambient prior to experimental set-up. Some samples were stored for 6 months at ambient.

2.2. Pandan flavor extraction

Pandan leaf samples were prepared and subjected to SC–CO_2 extraction (Laohakunjit & Noomhorm, 2004a). Five grams of the prepared sample were extracted at 200 bar and 50 °C for 20 min.

2.3. Rice starch-pandan flavor complex

The best rice starch film (30% sorbitol-plasticized film) as established in earlier experiments (Laohakunjit & Noomhorm, 2004b) was used. The rice starch was a local product ('Saohai flour') and contain 30% amylose. The rice starch-pandan flavor complex as coating material consisted of 5% rice starch (w/w), 30% sorbitol (w/w), 25% natural pandan extract, and distilled water.

2.4. Coating and storage

One kilogram non-aromatic rice samples were coated with 40 g rice starch-flavor complex using a modified spouted bed (Fig. 1). The bed had a rectangular section 250 mm in diameter and 250 mm in height, and included an angle of 60° with an inlet orifice diameter of 21 mm. All parts were made of stainless steel. A peristaltic pump was used to control the rate of the mass flow of the coating material to the equipment. An atomizer was placed in front of the bed and the coating suspension was fed with the spouting air. The coating operation started with the loading of the rice samples into the bed. The spouting of this load was promoted by air injected at the base of the bed. As soon as spouting was established, the air was heated to the desired temperature. The inlet temperature of spouting gas was maintained at 45 °C in all experimental runs. After reaching thermal equilibrium, the coating suspension was fed together with the atomizing air at a pre-set flow

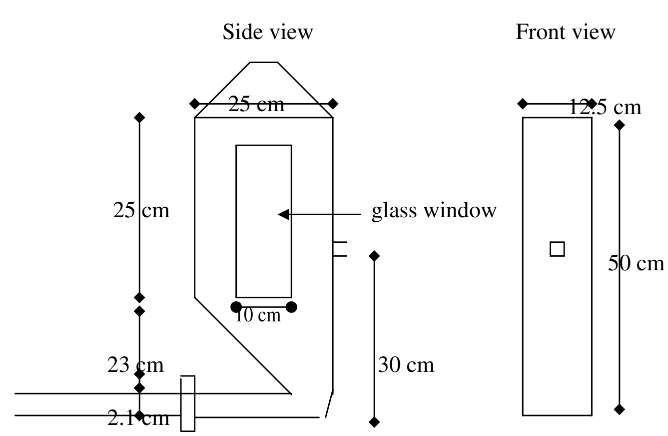

Fig. 1. Modified spouted bed used in the coating of rice with rice starch-pandan flavor complex.

rate and ambient temperature. The total coating time was kept at 30 min. The coated samples were withdrawn from the bed in the spout zone after drying for 15 min. Samples of the three non-aromatic varieties were coated with natural pandan extract while only SPR90 was coated with artificial pandan flavor. Some samples of coated and uncoated non-aromatic varieties and uncoated aromatic varieties were packed in plastic bags (nylon15/PE20/LLDPE75) and stored at 25 °C for 6 months. After each month of storage, samples were taken for analysis.

2.5. Analysis of volatile compounds

The solvent extraction method of Sarreal, Mann, Stroike, and Andrews (1997) and Bergman et al. (2000) was followed. A 0.3 g ground milled rice (100 mesh) was placed in a 12 × 32 mm vials with TFE septa and crimp top, and mixed with 0.5 mL of 459 ng/μL (0.459 ng/mL) solution of TMP in methylenechloride ($MeCl_2$). The TMP was employed as an internal standard. The sample vials were heated at 85 °C for 2.5 h and cooled in an autosampler tray at room temperature.

The different volatile compounds were analyzed with a Hewlett–Packard Model 5890 II gas chromatograph using Innowax capillary column (25 m length × 0.2 mm i.d. × 0.4 μm film thickness). A 2 μL solution of the cooled liquid extract was injected for analysis. Oven temperature was initially set at 50 °C for 2 min and programmed to increase from 50 to 170 °C at 7 °C/min and 170 °C, holding time was set for 5 min. Other operating conditions were as follows: injector, 170 °C; helium carrier gas flow rate, 0.6 mL/min; ion source temperature, 230 °C; electron multiplier voltage, 2600 V. The samples were injected in the splitless mode.

Mass spectra for ACPY and *n*-hexanal were determined using a Hewlett–Packard Model 5890 II gas chromatograph mass spectrometer (GC–MS). The GC conditions were the same as previously described. The MS was operated in a scan mode from m/z 50 to 400.

The identity of ACPY in sample extracts was confirmed by both chromatography with internal standards of TMP and mass spectral characteristic ions of ACPY. The concentration of ACPY was calculated as follows:

$$\text{ACPY concn.(ng/g)} = \frac{\text{area of ACPY} \times \text{conc. of TMP(ng/}\mu\text{L)} \times \text{vol. of injection(}\mu\text{L)}}{\text{area of TMP} \times \text{wt. of sample(g)}}.$$

The *n*-hexanal was identified by comparing GC–MS data and GC retention time of the sample with that of the authentic compound. Amounts of *n*-hexanal were determined by peak areas of a standard curve obtained by adding aliquots of standard solution ranging from 0.1 to 0.7 μg *n*-hexanal.

2.6. Statistical analysis

All data were analyzed by using SAS General Linear Model (GLM). Mean separation was performed by protected Least Significant Difference (LSD) method at $P \leqslant 0.05$ (SAS, 1991). The experimental design was CRD with three replications.

Table 1
ACPY content of uncoated aromatic and non-aromatic rice and pandan flavor-coated non-aromatic rice during storage for 6 months

Variety	Coating[a]	ACPY content (ng/g)						
		Duration of storage (month)						
		0	1	2	3	4	5	6
Non-aromatic rice								
RD23	NC	0f	0f	0f	0f	0f	0f	0f
	C	216.12c	176.77c	170.26c	158.55c	145.34b	118.70c	112.89c
SPR1	NC	0f	0f	0f	0f	0f	0f	0f
	C	210.56d	199.43b	178.74b	162.32b	123.33d	112.42d	104.38d
SPR90	NC	0f	0f	0f	0f	0f	0f	0f
	C	201.58e	163.53d	141.44d	138.41d	137.98c	136.45b	122.54b
Aromatic rice								
KDML	NC	324.45a	317.75a	280.99a	238.73a	192.82a	173.14a	153.09a
PTT1	NC	220.99b	142.29e	129.67e	114.41e	107.67e	90.108e	76.36e

Means within column not followed by a common letter differ at 5%, LSD.
[a] Coating treatment: NC – not coated, C – coated with natural pandan extract.

3. Results and discussion

3.1. ACPY levels

ACPY content of aromatic rice varied from 221 to 324 ng/g in fresh or unstored grains (Table 1). KDML contained the highest ACPY level while PTT1, the lowest. ACPY was absent in fresh and stored grains of uncoated non-aromatic varieties and in SPR90 coated with artificial pandan flavor. Coating with natural pandan extract remarkably increased the ACPY content of non-aromatic rice to 201–216 ng/g, with RD 23 (Fig. 2) containing the highest level while SPR90, the lowest. With storage, ACPY content decreased regardless of variety and coating treatment. The rate of ACPY loss was highest in PTT1 and lowest in coated SPR90 (Fig. 3). At the end of the 6-months storage period, KDML had still the highest ACPY content (153 ng/g) but it represented more than 50% loss relative to the initial content. PTT1 which exhibited the highest rate of ACPY loss, had only 76 ng/g ACPY or about 65% lower than its initial content. It was even lower than the ACPY content of coated non-aromatic rice which ranged from 104 to 122 ng/g.

ACPY is an important compound, chiefly responsible for the characteristic pandan-like aroma of aromatic rice varieties. ACPY content differed with variety indicating that it is under genetic control. The results also demonstrate that natural ACPY enrichment of non-aromatic rice using pandan leaf extract in rice-starch coating is highly possible. However, this may depend on the rice variety. Varietal differences in ACPY content among coated non-aromatic rice were obtained, suggesting that the retentive

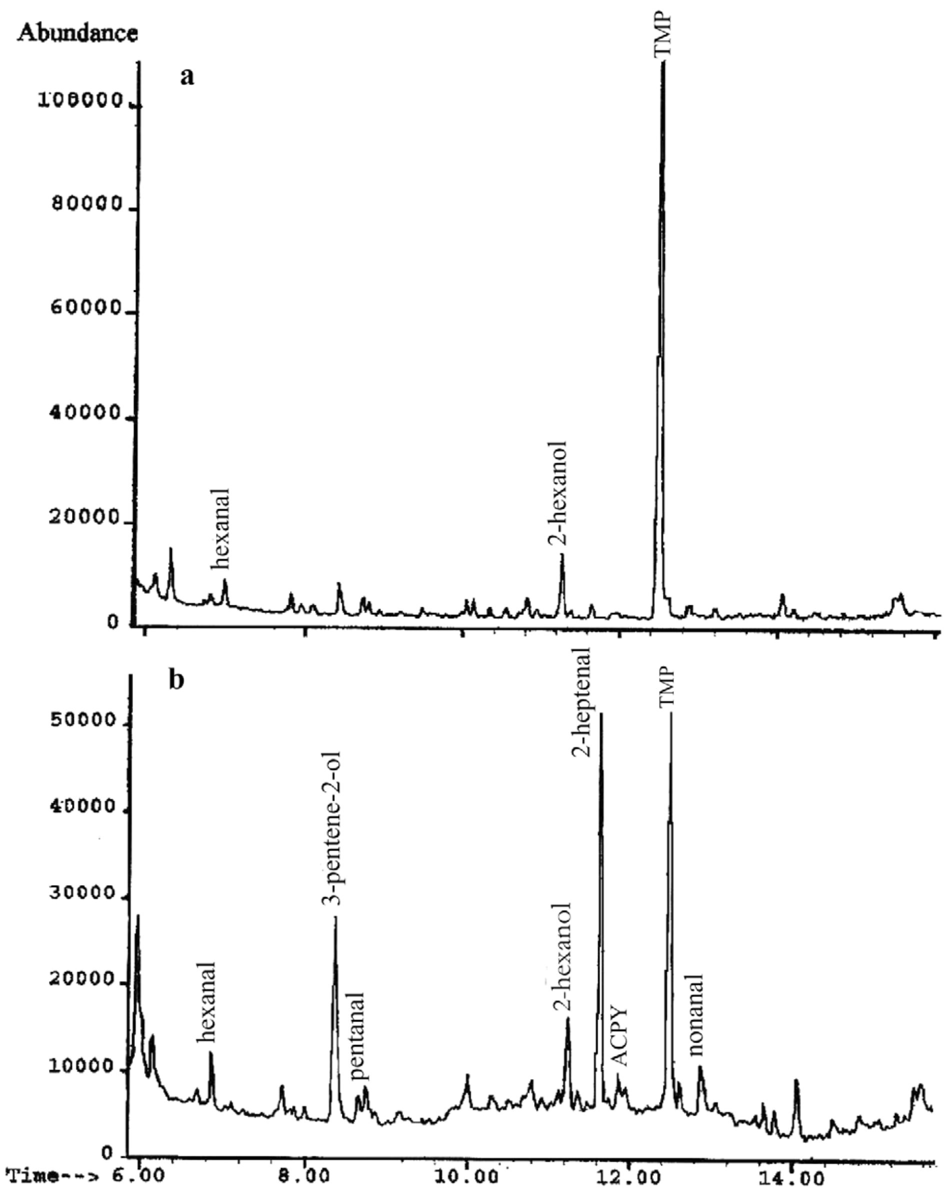

Fig. 2. GC chromatogram of volatile compound of RD 23 (a) before coating (b) pandan extract-coated RD 23.

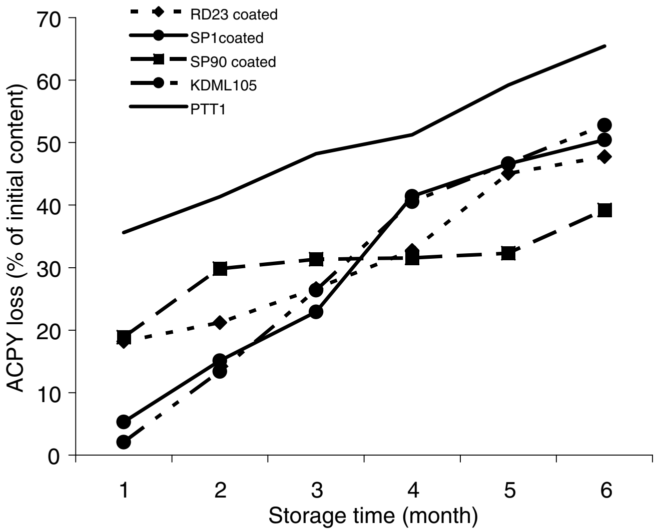

Fig. 3. ACPY loss during 6-months storage of coated non-aromatic rice and uncoated aromatic rice.

capacity for the coating material differed with variety. Furthermore, it was noted that the rice-starch film did not completely prevent the loss of ACPY during storage. This was probably because the film had only intermediate property for binding with pandan extract. In an earlier study, it was found that only 30% ACPY was sorbed with rice-starch film (Laohakunjit & Noomhorm, 2004b). Apparently, the applied thin film of coat was not a good barrier to prevent ACPY from diffusing through the rice starch and retarding loss of ACPY. The results compared well with that of Debeaufort and Voiley (1997) in which aroma transfer through hydrophilic film (methyl cellulose based film) strongly increased with increasing WVTR due to the plasticization of the polymer network by water. However, it can be seen from Table 1 and Fig. 3 that the rate of ACPY loss was relatively lower in the coated non-aromatic rice than the loss of native ACPY of aromatic rice. This can be attributed to the lower amylose content of aromatic rice and hence lower binding capacity with the aroma compound compared to non-aromatic rice.

The results further show that coating with natural pandan flavor of non-aromatic rice resulted to lower levels of ACPY compared to that of aromatic rice. This could be due to losses of some ACPY during the coating process probably initiated by the high air velocity and high temperature during spouted bed operation. The temperature and mechanical techniques of spouted bed could be further studied and optimized to prevent or minimize loss of valuable volatiles such as ACPY during coating.

3.2. *n*-Hexanal production

n-Hexanal content differed with variety and was highest in fresh and stored grains of uncoated aromatic KDML variety with more than 20 μg/g (Table 2). Fresh grains of the other varieties had less than 15 μg/g *n*-hexanal. Coating significantly decreased *n*-hexanal levels relative to their uncoated counterpart. The same trend was obtained during the 6-month storage period. In general, *n*-hexanal contents increased with increasing period of storage. At the end of the 6-month storage period, all aromatic varieties had more than 20 μg/g *n*-hexanal while that of coated and uncoated non-aromatic rice, below 20 μg/g. Coating did not arrest the increase in hexanal content with storage. In SPR90, it even resulted to a higher rate of increase (from 7.93 to 12.55 μg/g) than without coating (from 13.57 to 14.65 μg/g). However, the coated non-aromatic rice had consistently lower hexanal content than the uncoated ones, except for SPR90 coated with artificial pandan flavor which showed more than 100% increase (from 8.01 to 16.44 μg/g) in hexanal content at the end of storage.

n-Hexanal, a carbonyl compound, is a volatile compound associated with a specific aspect of rice aroma. Of the carbonyls, hexanal increases the most during storage of rice and consequently has been used as an indicator

Table 2
n-Hexanal content of pandan extract-coated and uncoated non-aromatic rice and uncoated aromatic rice during storage for 6 months

Variety	Coating[a]	*n*-Hexanal content (μg/g)						
		Duration of storage (month)						
		0	1	2	3	4	5	6
Non-aromatic rice								
RD23	NC	13.08b	13.94b	15.05b	15.25b	15.33c	17.17c	18.65c
	C	8.69e	10.15e	10.82d	10.99e	11.09e	11.70e	12.24f
SPR1	NC	12.13c	12.95cd	13.10c	15.80b	16.71b	18.53b	19.39c
	C	10.79d	12.23d	13.62c	14.19c	14.19d	14.66d	14.77d
SPR90	NC	13.57b	13.60bc	13.66c	13.84d	14.05d	14.12d	14.65d
	C	7.93e	9.59e	10.02d	10.05e	10.45e	11.44e	12.55e
Aromatic rice								
KDML	NC	20.37a	21.40a	22.02a	21.92a	22.90a	23.28a	24.76a
PTT1	NC	13.93b	14.34b	14.58b	15.00bc	16.95b	18.45b	20.60b

Means within column not followed by a common letter differ at 5%, LSD.
[a] Coating treatment: NC – not coated, C – coated with natural pandan extract.

of rancidity (Champagne & Hron, 1993; Yasumatsu, Moritaka, & Wada, 1966a). It is associated with lipid deterioration and formed by the auto-oxidation of linoleic acid resulting in the development of off-flavors and stale flavors (Champagne & Hron, 1993; Shibuya, Iwasaki, Yanase, & Chikubu, 1974; Shin, Yoon, Rhee, & Kwon, 1986; Tsugita, Ohta, & Kato, 1983; Yasumatsu, Moritaka, & Wada, 1966b). The results of the present study show that the potential for rancidity development during rice storage was higher in aromatic varieties than in non-aromatic ones. Coating further lowered such potential for rancidity development as it markedly reduced the *n*-hexanal content of the stored grains. The film coating could have served as a gas barrier limiting oxygen availability and lipid oxidation. Thus, the coating treatment not only improve the flavor but also the storage potential of the grains.

4. Conclusions

Rice-starch coating containing natural pandan extract produced non-aromatic rice with aroma compounds similar to that of aromatic rice. It was effective in adding ACPY which is mainly responsible for the jasmine aroma of aromatic rice. It also reduced the potential for rancidity development during grain storage by lowering *n*-hexanal levels. *n*-Hexanal production and oxidative rancidity were potentially higher in aromatic rice than in non-aromatic rice.

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