Effect of Storage Conditions on 2-Acetyl-1-pyrroline Content in Aromatic Rice Variety, Khao Dawk Mali 105

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ABSTRACT: The effect of package and temperature on 2-acetyl-1-pyrroline content in milled aromatic rice during storage was investigated. 2-Acetyl-1-pyrroline content was decreased faster at higher storage temperature. However, fat acidity of rice was increased during storage and inversely correlated with 2-acetyl-1-pyrroline content at an early stage of storage. The difference in 2-acetyl-1-pyrroline recovery from the samples, which were extracted with ethanol at 40 °C and 75 °C, revealed that the starch bound and free forms of 2-acetyl-1-pyrroline may occur in aromatic rice. These results suggested that the biosynthesis of 2-acetyl-1-pyrroline before starch structure formation in rice kernel could play a key role in the aroma quality of aromatic rice.

Keywords: aromatic rice, 2-acetyl-1-pyrroline, gc-ms-sim, storage, fat acidity

Introduction

Consumers have become more quality-conscious toward the rice that they consume. Therefore, there is a need to focus on the quality of rice, which is the key to linking farmers to consumers. When farmers become conscious of the quality of their rice, they are driven to produce better rice. Aromatic rice varieties are very popular in South and Southeast Asia (Hori and others 1992, 1994) and have recently gained wider acceptance in the United States, Europe, and East Asia, especially in China. Because of their characteristic aroma and flavor, they are highly favored and command high prices in markets.

2-Acetyl-1-pyrroline, a “popcorn”-like flavor compound, was reported as a main active flavor component of aromatic rice (Butterly and Ling 1982). Also, this compound contributed to the “roasted aroma” of cooked beef and crusts of wheat and rye breads (Schieberle and Grosch 1985). The “aroma” characteristic of aromatic rice in sensory evaluation showed the strong correlation with its content (Ishitani and Fushimi 1994). We previously reported (Yoshihashi 2002) that 2-acetyl-1-pyrroline is not formed either during cooking or the postharvest processes of aromatic rice varieties. Instead, it is formed in the aerial parts of rice plants while they are growing in the fields. Because this compound is present at a relatively low concentration, it can be easily lost via diffusion out from the rice. Thus, it is quite important to preserve the content to maintain its flavor characteristics during distribution of aromatic rice.

Previously, 2-acetyl-1-pyrroline was analyzed using gas chromatography - flame ionization detector (GC-FID) (Buttrey and others 1986) or gas chromatography-mass spectrometry (GC-MS) (Tan-chotikul and Hsieh 1991). We also used GC-MS with a stable isotope dilution technique for its quantification (Yoshihashi 2002). However, these methods for farmers because of the availability and price of the equipment. Quantification of 2-acetyl-1-pyrroline in aromatic rice without this equipment is rather difficult compared with that of other components in rice. The relatively low level of 2-acetyl-1-pyrroline (approximately 300 ppb), high volatility, and the interaction effect of starch and proteins have complicated its determination. Therefore, it is necessary to find alternative factors that correlate with 2-acetyl-1-pyrroline content.

Fat acidity of rice was the most commonly used factor to determine rice deterioration during storage. Lipid oxidation is the major deterioration process during rice storage and releases free fatty acids and carbonyl compounds (Ohtsubo and others 1987). These compounds were known to contribute to the development of the rancid flavor of rice. Lipase and lipoxygenase play important roles in determining rice quality because of the influence of their reaction products in lipid oxidation. However, the factors controlling oxidation such as genetic traits and storage conditions are still not clearly understood. Fat acidity is expected to increase during the storage of aromatic rice, whereas 2-acetyl-1-pyrroline content is expected to decrease. Thus, it seems that fat acidity could be an alternative indicator for the prediction of 2-acetyl-1-pyrroline change during storage. Consequently, a quite simple and quick method for fat acidity, which is a commonly used index for rice inspection or quality evaluation, can be easily adopted to estimate probable “aroma” quality of aromatic rice under laboratory condition with a moderate level of equipment.

2-Acetyl-1-pyrroline is a highly volatile and lipophilic compound. Rice lipids are known to occur in both free and starch-bound forms. Free lipids adhered on the surface of starch granules are either-extractable, even at room temperature. However, higher extraction temperatures using ethanol are required for extraction of bound lipids located inside of starch granules. Because of the lipophilic characteristic of 2-acetyl-1-pyrroline, free and bound 2-acetyl-1-pyrroline are assumed to occur. Understanding the oc-
currence form of 2-acetyl-1-pyrroline is believed to be essential to help evaluate aromatic rice quality.

The objectives of this study were to examine the effects of storage conditions, especially package and storage temperature, on 2-acetyl-1-pyrroline content in milled aromatic rice, which are commonly distributed in markets and to investigate the relationship between 2-acetyl-1-pyrroline content and fat acidity. Moreover, extractable 2-acetyl-1-pyrroline was determined with use of the high and low extraction temperature to elucidate the presence of the free and bound forms of 2-acetyl-1-pyrroline.

Materials and Methods

Rice samples and preparations

Rough rice samples of the Khao Dawk Mali 105 variety grown in 2000 and 2001 at Tungkularonghai experimental paddy field of Land Development Dept., Roiet, Thailand, were obtained from the Soil Science Div., Dept. of Agriculture, Bangkok, Thailand, and stored at 5 °C in sealed polypropylene bags before the experiment. They were hulled using a rice huller (TR-200; Kett, Tokyo, Japan) and milled for 30 s using a friction-type grain testing mill (Pearltest; Kett). Broken kernels were removed by an automated rice analyzer (RN-500; Kett). Rice samples used in the milling degree test were milled using an abrasive-type grain testing mill (TM-05C; Satake, Hiroshima, Japan) until a desired milling degree.

Milled rice samples were ground with a 0.5-mm mesh screen (ZM-100; Retsch, Haan, Germany). Moisture content of the samples was measured using oven drying to constant weight at 105 °C for 3 h.

Chemicals

Iodomethane-13C was purchased from CDN isotopes (Quebec, Canada). Ethanol (residual pesticide analysis grade) was bought from Wako Chemicals (Osaka, Japan). Other reagents used were of analytical grades.

Synthesis of 2-acetyl-1-pyrroline and 2-acetyl-(methyl-13C)-1-pyrroline

2-Acetyl-1-pyrroline was synthesized as described by De Kimpe and others (1993). 2-Acetyl-(methyl-13C)-1-pyrroline was also synthesized following the same procedure using methyl-13C-magnesium iodide from iodomethane-13C. Purity of each compound was confirmed by GC-MS and 1H and 13C-NMR (nuclear magnetic resonance); yields were 35% and 34%, respectively. The yield of last reaction for labeling acetyl group was 99%. Actual concentrations were confirmed by 1H-NMR using ethanol as an internal standard. Stock solutions (2%) were prepared with dichloromethane and stored at −80 °C until use.

Storage test

Milled rice samples were stored at 5 °C, 20 °C, 25 °C, and 30 °C and 75% relative humidity (RH) under atmospheric pressure in sealed low-density polyethylene (LDPE) bags or in nylon mesh bags, which allow gas transfer easily between the products and the storage atmosphere. 2-Acetyl-1-pyrroline solution put on filter bags, which allow gas transfer easily between the products and the sealed low-density polyethylene (LDPE) bags or in nylon mesh bags. An appropriate amount of the samples was removed every wk for quantification of 2-acetyl-1-pyrroline and determination of fat acidity.

Effect of milling degree on storage

Brown rice samples produced in 2001 were milled until 85% to 95% weight of a brown rice basis and stored at 30 °C and 75% RH in nylon mesh bags. An appropriate amount of the samples was removed every wk for quantification of 2-acetyl-1-pyrroline.

Effect of extraction temperature on 2-acetyl-1-pyrroline liberation

The effects of 2 extraction temperatures (40 °C and 75 °C) on 2-acetyl-1-pyrroline recovery from rice samples were investigated to elucidate the presence of 2-acetyl-1-pyrroline in milled rice using an aluminum heating block.

2-Acetyl-1-pyrroline extraction from rice samples

The extraction vials used were 12 × 32 mm and closed with a PTFE septa and screw caps. 2-Acetyl-1-pyrroline was extracted from 200 mg samples using 0.75 mL ethanol containing 200 ppb 2-acetyl-(methyl-13C)-1-pyrroline as an internal standard for 2 h. After centrifugation, the supernatant was subjected to gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM) analysis.

Gas chromatography-mass spectrometry-selected ion monitoring

The extract (0.2 μL) was injected into a fused silica capillary column (DB-WAX; 60 m × 0.25-mm inner dia × 0.25-μm film thickness; J&W Scientific, Folsom, Calif., J&W Scientific, Folsom, Calif.) installed in Hewlett-Packard (HP) 5980 series 2 gas chromatograph (Palo Alto, Calif., U.S.A.). Helium gas (purity 99.9999%, passed through a molecular sieve and an oxygen trap) with a head pressure set at 40 p.s.i. was used as the GC carrier gas. The injector and the GC-MS interface temperatures were set at 150 °C and 250 °C, respectively. The column temperature was held initially at 40 °C for 2 min, and then temperature was increased at a rate of 10 °C/min to 100 °C and was further increased to 140 °C at a rate of 5 °C/min. Finally, the column temperature was maintained isothermally at 250 °C for 10 min. An HP 5989A mass spectrometer (Palo Alto, Calif., U.S.A.) was used in the electron ionization mode with the ion source temperature set at 250 °C, the analyzer temperature set at 100 °C, and the ionization energy set at 70 eV. SIM was set up to monitor m/z 111 for 2-acetyl-1-pyrroline and m/z 112 for 2-acetyl-(methyl-13C)-1-pyrroline. MS detection dwell time was set at 100 ms for each ion. Under these conditions, the retention times of these compounds were found to be 12.47 and 12.46 min, respectively. Quantification was performed by measuring the area ratios between ions at m/z 111 and 112, corresponding to 2-acetyl-1-pyrroline and 2-acetyl-(methyl-13C)-1-pyrroline, respectively. Each extract was analyzed in triplicate. The content in the samples was calculated from a calibration curve constructed by plotting area ratio of various concentration for synthetic 2-acetyl-1-pyrroline to that of the internal standard against a known amount of synthetic 2-acetyl-1-pyrroline. Data were expressed as the mean of triplicate measurements ± standard deviation.

Fat acidity

Fat acidity of rice samples was determined following a colorimetric method of Onitsubo and others (1987). Rice flour samples were used within 2 h after grinding. Two grams of the sample were weighted exactly and the fat was extracted by 6 mL toluene for 1 h at 30 °C. Extract was transferred into a tube containing 4 mL chloroform and 2.5 mL copper reagent, which was prepared by mixing of 45% (v/v) 1 M triethanolamine, 5% (v/v) 1 M acetic acid, and 50% (v/v) 6.45% copper nitrate solution, followed by vigorous shaking. Finally, 0.1% diethylthiocarbamate isobutanol solution (0.5 mL) was added into 3 mL of the chloroform layer, and an absorbance at 440 nm was measured. The fat acidity was expressed as mg/100 mL of potassium hydroxide needed to neutralize fatty acid released from 100 g of rice flour.

Differential scanning calorimetry

Gelatinization temperature of the ground rice sample was ana-
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Effect of storage on 2-acetyl-1-pyrroline

The content of 2-acetyl-1-pyrroline in rice for both production years was decreased during storage (Figure 1). This result agrees with results reported by Widjaja and others (1996), but there is no information available relating to the mechanism for changes in 2-acetyl-1-pyrroline content during postharvest processes. Authentic 2-acetyl-1-pyrroline on filter paper decreased rapidly at an early stage of storage test. 2-Acetyl-1-pyrroline was reported as a highly volatile compound. Evaporation of authentic standard 2-acetyl-1-pyrroline in the same storage condition showed it to be relatively faster than that in rice. Moreover, during storage at 25 °C to 30 °C and 75% RH, the concentration of 2-acetyl-1-pyrroline did not change significantly after 7 wk of storage. Therefore, it is suspected that there are other factors that hold 2-acetyl-1-pyrroline and slow its later release from rice. Package materials of rice affected the preservation of 2-acetyl-1-pyrroline; however, the effect was only moderate during storage. LDPE did not inhibit penetration of 2-acetyl-1-pyrroline. Therefore, it is needed to test other package materials for better storage of aromatic rice.

Effect of storage temperature

With increased storage temperature, the content of 2-acetyl-1-pyrroline decreased even faster (Figure 1). Cold storage at 5 °C in sealed LDPE bags preserved the contents most effectively. Generally, rice storage at low temperature retarded flavor quality deterioration caused by intermolecular reactions operating in parallel. Especially, enzyme catalyzed reactions were drastically inhibited at low storage temperature (Yasumatsu and others 1964; Chikubu 1970). Because 2-acetyl-1-pyrroline is quite volatile, it is recommended that aromatic rice variety be stored and handled at a low temperature.

Effect of milling degree on 2-acetyl-1-pyrroline preservation

As we reported previously, the content of 2-acetyl-1-pyrroline in rice bran was higher than that in milled rice. Lower milling degree increased 2-acetyl-1-pyrroline content because of its higher recovery rate from rice bran (Figure 2). However, 2-acetyl-1-pyrroline content was relatively unchanged after 6 wk of storage. These results suggested that 2-acetyl-1-pyrroline in rice bran evaporated faster than that in rice kernel, where bound 2-acetyl-1-pyrroline was released with difficulty, even after a long storage period.

Correlation between fat acidity and 2-acetyl-1-pyrroline content during storage

Fat acidity increased during storage, except storage at 5 °C (Figure 3). Higher storage temperature increased the fat acidity faster. The increase in fat acidity was believed to be the result of lipid oxidation as the major degradation processes, which release free fatty acids and carbonyl compounds. Correlation between fat acidity and 2-acetyl-1-pyrroline content was shown to be inverse at an early stage of storage. Hence, fat acidity could be a useful index in estimating the decrease in 2-acetyl-1-pyrroline content in rice during an early stage of storage.

Results and Discussion

Effect of storage time and package

Effect of extraction temperature on 2-acetyl-1-pyrroline recovery and its relationship with gelatinization

We have reported that the optimal extraction condition for 2-acetyl-1-pyrroline recovery from milled rice sample was 2 h at 75 °C, whereas the optimal extraction condition for seedlings or callus was at room temperature. This phenomenon suggested that the rice component, of which the structural conformation is changed at around 70 °C, may keep or interact with 2-acetyl-1-pyrroline and be associate with its liberation from rice. Sood and Siddiq (1978) reported the technique for rice aroma evaluation using alkaline solution or boiling, which induced starch gelatinization in milled rice samples. Furthermore, the gelatinization temperature of rice starch measured by using DSC was 63.7 °C. In fact, extraction at 40 °C was expected to liberate free 2-acetyl-1-pyrroline, which did not form a complex with starch. On the other hand, at a
higher extraction temperature, the majority of 2-acetyl-1-pyrroline was believed to be extracted from milled rice (Tanaka and others 1978; Juliano and Goddard 1986). Figure 4 shows 2-acetyl-1-pyrroline recovery at different tested extraction temperatures during storage. Extraction at 40 °C provided a lower recovery of 2-acetyl-1-pyrroline than that at 75 °C, and its content decreased rapidly at an early stage of storage. A relatively slow release of 2-acetyl-1-pyrroline from rice between 40 °C and 70 °C indicated a complex form of starch and 2-acetyl-1-pyrroline. The gelatinization process has resulted in collapses of starch structure and its complex with 2-acetyl-1-pyrroline. Therefore, it can be expected that starch-bound 2-acetyl-1-pyrroline in milled rice is liberated during starch gelatinization processes, such as during alkaline extraction or boiling.

Conclusions

2-Acetyl-1-pyrroline content in milled rice was decreased during storage. Hence, postharvest processes like drying, milling, and storage are particularly important for aroma and flavor preservation of aromatic rice. Therefore, it was assumed that storage and handling at low temperature would inhibit not only the generation of off-flavors but would also minimize volatilization of 2-acetyl-1-pyrroline from rice. 2-Acetyl-1-pyrroline on filter paper decreased faster than that present in rice. Its liberation from rice samples depended on the extraction temperature. All of these clearly indicated the possibility of the presence of 2-acetyl-1-pyrroline and starch granule complex, which may slow its evaporation. This finding was supported by the fact that the techniques widely used for aroma evaluation applied alkaline extraction or boiling, which caused rice starch gelatinization accompanied by collapses of its complex with 2-acetyl-1-pyrroline, as well as with the assumption that this compound was released during cooking of aromatic rice, where similar processes take place. Our recent study reported that 2-acetyl-1-pyrroline in aromatic rice was formed in the paddy field (Yoshihashi and others 2002). Taken together, these results suggested that the biosynthesis of 2-acetyl-1-pyrroline before formation of starch structure in rice kernel might play a key role in the aroma quality of aromatic rice.

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References