

คุณลักษณะของข้าวหอมมะลิ (*Oryza sativa* L.) หลังการขัดสี และเปลี่ยนแปลงสภาพธรรมชาติของเอนไซม์น้ำมันรำ

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อประเมินผลของเทคนิคหลังการเก็บเกี่ยวในการป้องกันการเสื่อมสภาพของน้ำมันรำที่ติดอยู่บนพื้นผิวของเมล็ดข้าวสารหลังการขัดสี ดำเนินการทดลองโดยให้ความร้อนแก่ข้าวสารภายใต้สภาวะสุญญากาศด้วยเตาไมโครเวฟ ให้ความร้อนด้วยไอร้อนชื้น และไอร้อนชื้นร่วมกับการใช้เอทานอลปริมาณต่างกัน หลังการให้ความร้อนและเก็บรักษาตัวอย่างข้าวสาร 3 เดือน ทำการประเมินลักษณะทางเคมีกายภาพที่เกี่ยวข้องกับคุณภาพของข้าว ผลการทดลองพบว่า การให้ความร้อนด้วยไอร้อนชื้นและไอร้อนชื้นร่วมกับการใช้เอทานอลสามารถลดปริมาณกรดไขมันอิสระบนพื้นผิวของเมล็ดข้าวสารลง เมื่อเปรียบเทียบกับตัวอย่างข้าวที่ให้ความร้อนด้วยคลื่นไมโครเวฟและข้าวปกติ การให้ความร้อนแก่ข้าวสารทุกวิธีการไม่มีผลต่อการเปลี่ยนแปลงสีของข้าวเมื่อประเมินด้วยค่า L^* a^* และ b^* การวิเคราะห์ด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดแสดงให้เห็นว่า สภาพพื้นผิวของเมล็ดข้าวสารและลักษณะของเม็ดแป้งข้าวไม่ได้รับผลกระทบที่เกิดจากการควบแน่นของเอทานอลหรือจากความร้อนที่เกิดขึ้นในระหว่างการทดลอง ซึ่งสอดคล้องกับผลการวิเคราะห์สมบัติความชื้นหนืดของข้าวทุกตัวอย่างที่พบว่าไม่แตกต่างกัน การศึกษาผลของการพ่นเอทานอลโดยตรงต่อข้าวในระหว่างขั้นตอนการขัดเงาพบว่า ข้าวสารที่ได้มีค่า b^* และปริมาณเอ็กซานเนลต่ำกว่าการพ่นด้วยละอองน้ำ นอกจากนี้พบว่า ข้าวสารที่ได้จากการขัดเงาทั้งสองวิธีการมีปริมาณสารหอม 2-อะเซทิล-1-พิริโรลีน ไม่แตกต่างกัน ผลการทดลองแสดงถึงศักยภาพของการใช้เอทานอลในระหว่างขั้นตอนการขัดเงาของระบบการสีข้าวแบบปกติในการเพิ่มคุณภาพทั้งด้านสีและกลิ่นของข้าวหอมมะลิ

คำสำคัญ : คุณภาพของข้าว / กรดไขมันอิสระ / สมบัติความชื้นหนืด / 2-อะเซทิล-1-พิริโรลีน / เอ็กซานเนล

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Characteristics of Jasmine Rice (*Oryza sativa* L.) after Milling and Denaturing of Bran Oil Enzyme

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Abstract

To produce a premium grade of milled jasmine rice, postharvest techniques employing heat and ethanol were evaluated. Milled rice samples were heated using a modified vacuum-microwave oven or exposed to heat-moisture treatment and heat-moisture treatment in combination with different amounts of ethanol vapors. These treatments were done in order to prevent degradation of the residual bran oil on the surface of the milled rice. After treating and storage for 3 months, the physico-chemical characteristics related to quality of milled rice samples were determined. The results showed that heat-moisture treatment and heat-moisture treatment in combination with ethanol vapor reduced free fatty acids on the milled rice surface comparing with microwave and untreated rice. All heat and heat in combination with ethanol treatments did not affect color of the milled rice as verified by L^* a^* b^* values. Scanning electron microscope (SEM) analysis indicated that the surface of milled rice and the starch granule morphology were not affected by condensation of the ethanol vapor or heat generated during treatments and confirmed by the similar pasting curves in all samples analyzed. In the present study, the effect of direct spraying of ethanol into milled rice during polishing step was investigated. It was found that the ethanol assist-polished rice had low b^* and n -hexanal values comparing with ordinary water assist-polished rice. Both polishing techniques produced milled rice having the same amount of 2-acetyl-1-pyrroline. Application of ethanol therefore, shows the potential of this practice in improving color and aroma quality of the jasmine rice.

Keywords : Rice Quality / Free Fatty Acid / Pasting Property / 2-acetyl-1-pyrroline / n -hexanal

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1. Introduction

Jasmine rice is a very popular aromatic rice variety in Thailand. It is called Khao Hom Mali in the domestic market and the name implies the pleasant aroma and color of the jasmine flower. Because of its superior flavor and texture characteristics, the demand for jasmine rice in both the domestic and international markets increased over the years. Research conducted in the United States revealed that the most important acceptance factors for Asian consumers living in the United States were cooked rice appearance and aroma, and imported Thai jasmine rice was preferred over any other rice in that market [1]. Recently, competition in producing and exporting aromatic rice has increased dramatically and aroma quality of rice becomes a major determinant for consumer. To sustain Thai export competitiveness, pre- and postharvest technologies that improve rice quality should be sought continuously.

In the rice industry and its supply chain system, rough rice must be processed to milled rice by removing husk and bran layer and then stored for periods of time prior to consumption. During processing, lipid bodies of the rice bran layer are disrupted and bran particles are removed from rice kernel. However, some residues of bran oil are still remained on the surface of the milled rice kernel. This non-removing bran oil is contact with the enzyme lipase and rapidly hydrolyzed to free fatty acid (FFA). A research demonstrated that 0.1% of FFA was reached after 8 days when fully milled rice was stored under 37°C and 70% relative humidity [2]. Moreover, Lam and Proctor [3] reported that FFA on the surface of milled rice was increased exponentially during early phase of storage periods and this occurrence was due to bran lipase activity. The increasing of FFA in later storage periods

was resulted from microbial lipase activity. The generated FFA was then oxidized to carbonyl compounds and associated with high rancid odor of the stored milled rice. Of these compounds, n-hexanal produced from linoleic acid breakdown increase significantly with storage time and had the highest influence on odor change of milled rice [4-6].

To preserve the high quality of rice and rice products, attempts were made to stabilize brown rice from lypolytic hydrolysis utilizing aqueous ethanol [7] and ethanol vapor [8-9] and by using microwave heat treatment for rice bran product [10-12]. These postharvest techniques were successful in stabilization of the rice produces by mean of denaturation of the lipase enzyme. Since milled rice contains a significant amount (0.8%) of lipids [13] and some of them are located at surface of the milled rice. These surface lipids are therefore susceptible to hydrolytic deterioration via the action of both bran and bacterial lipases. If the stabilization practices mentioned above are applied to rice during or immediately after whitening stage of the milling process, the bran oil enzyme may be possibly inactivated and subsequently contributed to better flavor in stored milled rice. However, studies on these postharvest techniques on milled rice, especially jasmine or other aromatic rice cultivars are limited. To fill the gap, the study was therefore undertaken to evaluate the effects of microwave, heat-moisture treatment and heat-moisture treatment in combination with ethanol vapor on the denaturation of bran oil enzyme retarding FFA generated on the surface of milled jasmine rice kernels. The effect of direct spraying of ethanol mist to rice during polishing process was also determined. Changes due to these postharvest treatments were monitored using some physico-chemical properties that are related to the quality characteristics of the aromatic rice.

2. Materials and Methods

2.1 Rice samples

Paddy cv. Khao Dawk Mali 105 (jasmine rice) was de-hulled by a McGill sample sheller and the resulting brown rice was milled for 30 seconds in a friction-type miller. The head rice was then separated from the broken kernel by a cylinder grader and used for subsequent treatments. A head rice sample gave the following composition: amylose content of 17.59 % (w/w) determined by the method of Juliano et al. [14], and protein (Nx5.95) of 7.64% and lipid contents of 0.88 %, both determined by AOAC [15] standard methods.

2.2 Postharvest treatments

In this study, two experiments were conducted. Fig. 1 shows a schematic diagram indicating the process of rice milling and stages of the two experiments undertaken. The first experiment was done to estimate the effects of microwave heat treatment (MHR), heat-moisture treatment (AHR) and heat-moisture treatment in combination with ethanol vapor (EVR) on some physico-chemical properties that related to milled rice quality. Milled rice samples, 400 g each, were vacuum-packed in 20x30 cm nylon laminated bags.

The bags were then heated, one at a time, at 100% power for 1 min in a microwave oven (Model XT-20MS/W, Whirlpool (Thailand) LTD., Thailand) at 800 W and 2,450 MHz. Unheated bags served as control. For AHR and EVR, an aluminum container (11 cm height x 8.5 cm diameter) equipped inside with a wire mesh screen at the bottom position was used. Ethanol (95%, v/v) 0, 2 or 4 mL was added and a rice sample (400 g) was placed on the screen inside the container. The container was sealed and heated for 15 min at 100°C to allow the ethanol to boil using an automatic autoclave (SS-320, Tomy Seico Co. Ltd., Wako, Saitama, Japan). After heating process, the rice samples were left in the sealed container for 2 h. Samples were then packed in nylon laminated bag and vacuum-sealed. All samples were stored for 3 months at room temperature (~25 °C) before their properties were determined.

The second experiment was done to evaluate the effect of ethanol when applied during polishing of the rice kernel. Either ethanol (70%, v/v) or water (conventional polishing method) mists were sprayed into the polishing machine and the polished rice samples were analyzed for color and aroma quality.

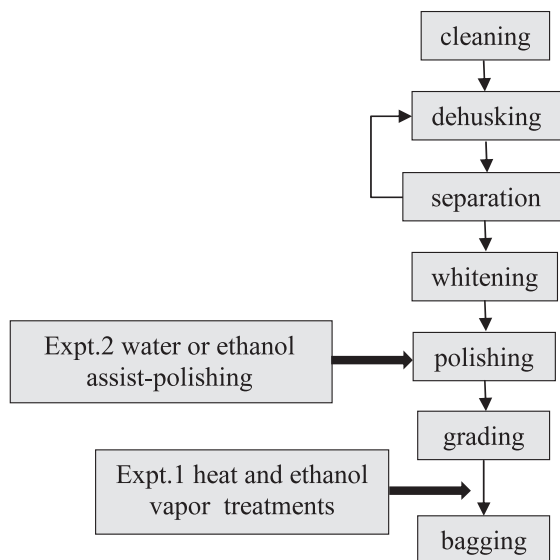


Fig. 1 Schematic diagram of rice milling process and the stages of experimental treatments undertaken.

2.3 Determination of free fatty acid (FFA)

The lipid on the milled rice surface was extracted using the method of Lam and Proctor [16]. Lipid was removed from the surface of milled rice by vortexing 10 g of sample for 2 min with 4 ml of isopropanol (IPA). An additional 4 ml of IPA was then added and the sample was vortexed again for 2 min. The extract was centrifuged at 2,500 rpm for 10 min to remove bran particles. FFA in the extract was determined by the colorimetric method of Walde and Nastruzzi [17]. An assay solution containing 0.375 mL of solution A (0.1M tris/HCL, pH 9.0), 0.125 mL of solution B (2 mM phenol red in 0.1M tris/HCl, pH 9.0) and 50 mL of solution C (50 mM bis (2-ethylhexyl) sodium sulfosuccinate in isooctane) was prepared. The assay solution (1 mL) was placed in a 1-cm cuvette with 30 μ L of the IPA extract and shaken for 1 min before measuring the absorbance at 560 nm. The FFA content of each extract was read from a calibration curve of oleic acid and was expressed as the percentage of the original rice weight.

2.4 Determination of pasting property

Rice samples were ground and pass through a 0.5 mm screen (Cyclotec 1093 sample mill, Tecator, Hogenas, Sweden) and the pasting property of the flour samples were analyzed using a Rapid Visco Analyser (RVA) (Model 4D, Newport Scientific, Warriewood, NSW, Australia). The flour samples, each weighing 3.00 ± 0.01 g, were placed in test canisters to which distilled water was added to make the weight of each 28.00 ± 0.02 g. The samples were then analyzed using the profile outlined by Approved Method 61-02 [18]. The RVA pasting parameters recorded were pasting temperature, peak viscosity, viscosity at 95°C after holding (trough), viscosity at 50°C (final viscosity), breakdown based on peak viscosity minus trough, and setback based on final viscosity minus peak viscosity.

2.5 Determination of kernel color

Kernel color was measured using a color meter (ColorQuest® XE, Hunterlab, Reston, Virginia, USA), using the 1976 Commission Inter-

nationale de l'Éclairage L^* , a^* and b^* color system. Color parameters interpreted by L^* , a^* and b^* values described the brightness, redness and yellowness of samples, respectively.

2.6 Scanning electron microscopy investigation

Change in morphology of starch granules of rice kernel was investigated. Cross section of middle part of the treated and untreated rice kernels including their corresponding flour samples were mounted on metal stubs using double-sided adhesive tape and coated with gold palladium (~6 nm thickness) using a SPI-MODULE™ Sputter Coater (SPI Supplies® Division of Structure Probe, Inc., Japan). The samples were then observed under a scanning electron microscope (JEOL JSM-5910 LV, JEOL Technics LTD., Japan) at an accelerating voltage of 15 kV. The SEM images were captured by automatic image capturing software (SEM Control User Interface Version 5.08, JEOL Technics LTD., Japan) and the magnification was 5,000 times.

2.7 Analysis of *n*-hexanal and 2-acetyl-1-pyrroline

The relative amounts of *n*-hexanal and 2-acetyl-1-pyrroline (2AP) concentrations, representing the key off-odor and aroma compounds of the rice samples, were analyzed using the headspace-gas chromatography (HS-GC) method developed by Sriseadka et al. [19]. Milled rice samples were ground to pass through a 0.5 mm screen. The resulting flour, weighing exactly 1.000 g was placed into a 20-ml headspace vial. An internal standard of 1 μ L of 0.50 mg/ml 2,6-dimethylpyridine (DMP) in benzyl alcohol was added to the vial, which was then immediately sealed with a PTFE/silicone septum (Restek Corp., Bellefonte, Pennsylvania, USA)

and an aluminum cap. An Agilent Technologies (Wilmington, Delaware, USA) gas chromatograph (GC), model 6890N was used. The GC was equipped with a headspace autosampler (Agilent Technologies model G1888) and a fused silica capillary column, HP-5, with a 5% phenyl-95% dimethylpolysiloxane 1.5 μ m film thickness chemical coat and dimensions of 30 m \times 0.53 mm i.d. (J&W Scientific, Folsom, California, USA). The sample headspace vial was equilibrated at 120°C for 9 min in the autosampler before the rice headspace was transferred to the injection port of the GC. The GC condition was set as follows: the column temperature program started at 50°C and increased at a rate of 1°C/min to 70°C, the injector and flame ionization detector temperatures were 230°C and 250°C, respectively. Purified helium was used as carrier gas at a flow rate of 7 mL/min. The relative amounts of *n*-hexanal were derived from the ratio of the peak areas of *n*-hexanal and DMP. Concentrations of 2-acetyl-1-pyrroline in the rice samples were determined using a standard calibration curve.

2.8 Statistical analysis

Experimental data of heat and ethanol vapor treatments were subjected to an analysis of variance (ANOVA) and a least significant difference (LSD) test was done to separate the means ($P < 0.05$). For data on the application of ethanol in polishing step of milling process, a T-test was used to determine the difference between treatments ($P < 0.05$).

3. Results and Discussion

The concentration of FFA on the surface of milled jasmine rice was decreased after the rice samples were exposed to heat-moisture treatment and heat-moisture treatment in combination with ethanol vapor (Fig. 2). Although, heat-moisture

treatment (AHR) by autoclaving could reduce the FFA content on the rice surface, rice heated using an autoclave in combination with 2 mL of ethanol (EVR1) got a better result. The content of FFA in EVR1 was only 0.045 % of the rice weight, while for untreated sample (ordinary rice, OR) and AHR, the FFA were 0.24 and 0.08 %, respectively. Ethanol vapor was reported to be successful in stabilizing lipid in brown rice [9]. However, an unexpected higher FFA content was found in the sample heated in the autoclave in combination with 4 mL of ethanol (EVR2). This result would

be attributed to starch lipids from the inner layer of EVR2 milled rice samples be extracted and diffused to the surface of rice kernel. This diffusion may occur after the ethanol vapor treatment possibly facilitated by the excess amount of ethanol in EVR2. Rice starch lipids were found to contain a significant portion of FFA [20] and could be best extracted with hot aqueous ethanol [21]. In the current study, higher FFA content on the surface of EVR2 rice, compared to AHR and EVR1, could be attributed to higher amount of ethanol given to this heat-moisture treatment.

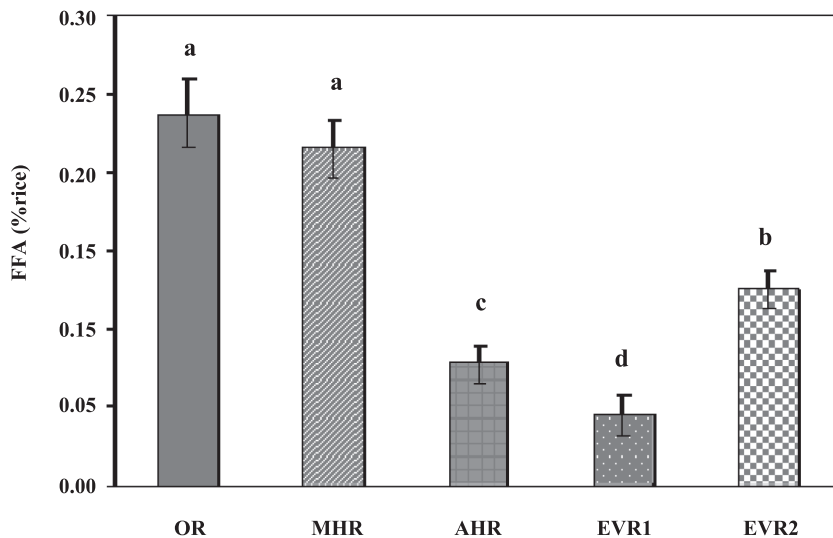


Fig. 2 Changes in FFA content of milled jasmine rice after treating with different postharvest treatments. OR=milled rice vacuum-packed in nylon laminated bag; MHR=milled rice vacuum-packed in nylon laminated bag and heated 1 min with microwave; AHR, EVR1, 2=milled rice packed in containers with 0, 2 or 4 mL of ethanol added and heated at 100°C for 15 min in autoclave. Vertical bars (\pm SD) with different letters are significantly different at $P < 0.05$, LSD.

A small decrease in FFA of MHR in this study indicated that the heat amount or the heating time were not enough for deactivating the bran oil enzyme. Ramezanzadeh et al. [11] conducted a study on rice bran and concluded that microwave heat can be used for inactivation of lipase. In their study, a microwave oven (850 W and 2450 MHz)

was used as a heat source to stabilize 150 g (21% moisture content) of bran for 3 min at 100% power. The heat generated in their samples was thus higher than in our samples in which 400 g of dry milled rice (13.4% moisture content) was heated for 1 min in a microwave oven at 800 W.

Table 1 Color (L^* , a^* and b^*) parameters of milled jasmine rice after treating with different postharvest treatments. OR=milled rice vacuum-packed in nylon laminated bag; MHR=milled rice vacuum-packed in nylon laminated bag and heated 1 min with microwave; AHR, EVR1, 2=milled rice packed in containers with 0, 2 or 4 mL of ethanol added and heated at 100°C for 15 min in autoclave.

Treatments	brightness (L^* value)	redness (a^* value)	yellowness (b^* value)
OR	51.80±0.46 ^a	0.97±0.06 ^b	6.98±0.36 ^c
MHR	51.90±1.06 ^a	1.00±0.09 ^b	6.88±0.33 ^c
AHR	51.20±1.28 ^a	1.02±0.05 ^b	6.96±0.21 ^c
EVR1	50.80±1.76 ^a	0.99±0.09 ^b	6.95±0.29 ^c
EVR2	51.70±0.53 ^a	0.97±0.03 ^b	7.10±0.06 ^c
CV. (%)	2.19	-6.89	3.90

Means (\pm SD) followed by the same superscript in a column are not significantly different at $P < 0.05$, F-test.

CV = Coefficient of variation

Neither heat nor ethanol vapor altered the kernel color. Treated and untreated samples showed similar color as indicated by comparable L^* , a^* and b^* values (Table 1). Heating milled rice with high temperature could result in yellow color of the milled rice [22]. Yellow color of heated rice was attributed to Maillard browning reaction occurring mainly between reducing sugars and free amino acids. This browning reaction was enhanced by temperature level and heating periods. The comparable color parameters of rice samples in the present study indicated that the temperature or heating time given to milled rice samples were satisfactory. Visual observation and SEM investigation results revealed neither fissuring nor changes in kernel appearance and destruction of starch granular structure of the treated rice samples. Photographs and SEM micrographs of untreated and treated samples are shown in Fig. 3. These micrographs

show that surface of the starch granules of all samples retain their flatness and smoothness indicating no effect of the condensation of ethanol vapor or heat during treatments. Similar results were also found in RVA analysis wherein significant changes in pasting parameters were not found. Both treated and untreated rice samples performed similarly in their pasting behavior and therefore only the pasting curves of untreated, MHR and EVR1 treated samples are shown in Fig. 4. Similar RVA pasting characteristics of the untreated and treated rice moreover, implied that there was no alteration in cooking quality of the rice after treatments. Results from the present study suggested that the given heat-moisture treatment was suitable for inactivating bran oil enzyme. Moreover, when applied this technique to milled rice with an appropriate amount of ethanol (2mL of ethanol per 400 g of milled rice) a better result was obtained.

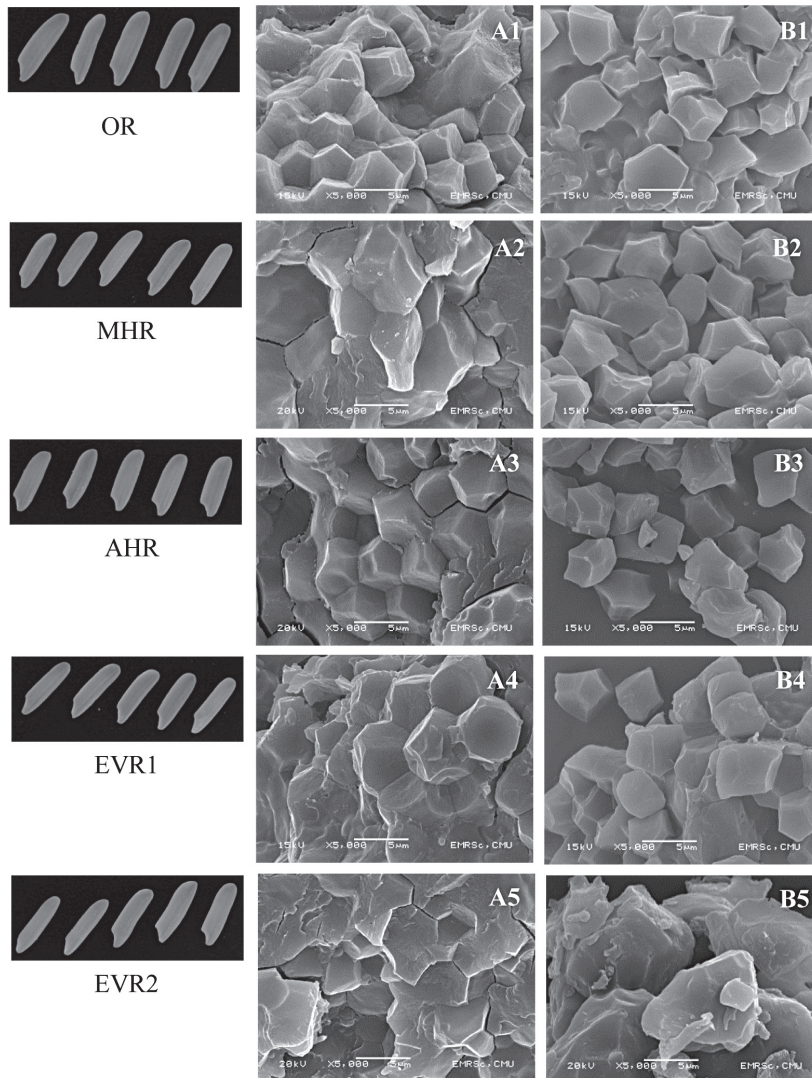


Fig. 3 Kernel appearance and starch granule morphology of milled jasmine rice after treating with different postharvest treatments. OR, MHR, AHR, EVR1 and EVR2 are the respective photographs of milled rice vacuum-packed in nylon laminated bag; milled rice vacuum-packed in nylon laminated bag and heated 1 min with microwave; milled rice packed in containers with 0, 2 or 4 mL of ethanol added and heated at 100°C for 15 min in autoclave. A1-A5 and B1-B5 are the respective SEM micrographs of starch granule of kernel cross section and flour of OR, MHR, AHR, EVR1 and EVR2.

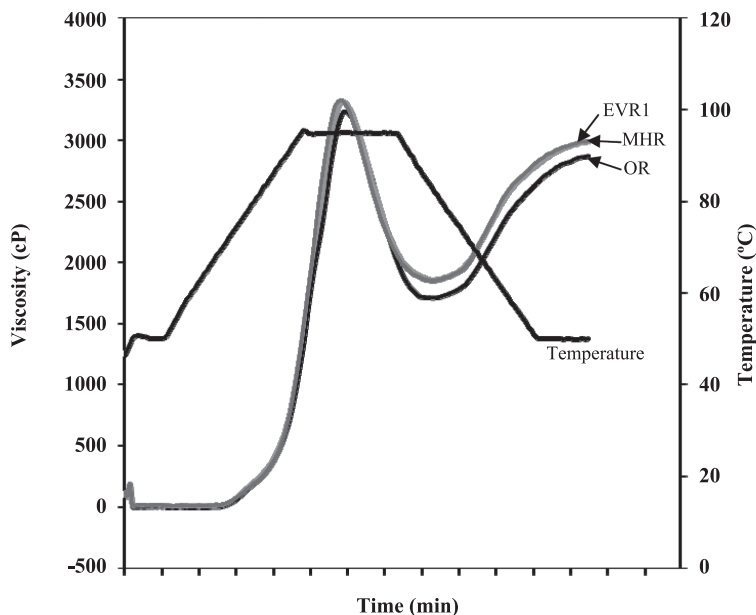


Fig. 4 RVA pasting curves of flour from ordinary (OR), microwave-heated (MHR), and ethanol vapor-treated (EVR1) milled rice.

For a more practical application, ethanol (70%, v/v) was directly sprayed into milled rice during polishing step. The results showed that more bran oil residue adhering to the surface of the rice kernel was wiped out. The ethanol assist-polished rice (EPR) appeared whiter compared to the ordinary water assist-polished rice (OPR) as shown in Table 2. Moreover, small amount of bran oil residue on the surface of EPR kernel led to lower *n*-hexanal, the prime rancidity compound of milled rice (Fig. 5A). The ethanol treatment did not decrease the quantity of key aromatic compounds evidenced by comparable contents of 2-acetyl-1-pyrroline in EPR

and OPR samples (Fig. 5B). The ethanol-polishing practice required small amounts of ethanol and short exposure time, reducing the chance of affecting the 2-acetyl-1-pyrroline content of EPR. Better quality in terms of whiter color and lower *n*-hexanal of the EPR sample was attributed to the capability of ethanol, a high-polarity organic solvent, in denaturing bran and microbial origin lipases. Ethanol assist-polishing could also help to remove residual bran oil from the surface of milled rice resulting to limited substrate for lipases. The EPR sample was consequently whiter and lower stale odor compared to OPR rice.

Table 2 Color (L^* , a^* and b^*) parameters of milled jasmine rice after ordinary water assist-polishing (OPR) and ethanol assist-polishing (EPR).

Treatments	brightness (L^* value)	redness (a^* value)	yellowness (b^* value)
OPR	53.12±0.46 ^a	-0.46±0.03 ^a	10.44±0.23 ^a
EPR	53.21±0.91 ^a	-0.54±0.06 ^a	9.81±0.16 ^b

Means (±SD) followed by the same superscript in a column are not significantly different at $P < 0.05$, T-test.

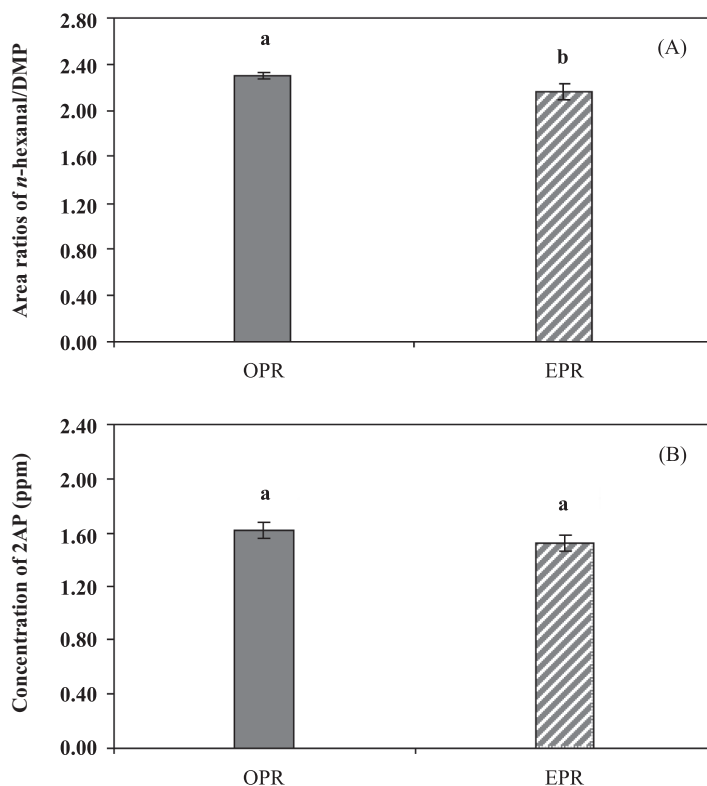


Fig. 5 The content of key odor compounds, *n*-hexanal (A) and 2-acetyl-1-pyrroline (B), of milled jasmine rice after ordinary water assist-polishing (OPR) and ethanol assist-polishing (EPR).

Vertical bars (±SD) with different letters are significantly different at $P < 0.05$, T-test.

4. Conclusions

Heat-moisture treatment and heat-moisture treatment in combination with ethanol vapor reduced the concentration of FFA on the surface of milled jasmine rice when compared to untreated and microwave heated rice. These postharvest techniques inactivated lipase on the milled rice surface without changing kernel appearance, morphological characteristic and pasting property. Application of ethanol in place of water during the polishing of the milling step demonstrated a potential of this practice in improving color and maintaining the aroma quality of the fragrant rice. The experimental results point to the advantages if this adopted postharvest practice is used in producing milled rice that is wash free and safe for long storage.

5. Acknowledgments

The authors would like to acknowledge the Center of Excellence for Innovation in Chemistry, Commission on Higher Education, Ministry of Education, for its support in lending the HS-GC instrument. Our special thanks are given to Mr. Tinakorn Sriseadka for his assistance. We also would like to acknowledge “Hands-on research and development project” of Rajamangala University of Technology Lanna.

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