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Efficiencies of Chemical Techniques for Rice Grain Freshness Analysis

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Abstract: Two chemical methods which are commonly used for rice grain freshness determination were investigated for their efficiencies. Method 1 is made of bromothymol blue indicator, and the principle is based on indicator's color which is changed in according to pH of the stored rice grains. Method 2 is based on peroxidase activity which deteriorates during storage of rice grains. Both methods were used for determination of fresh-aged rice index of six Thai-rice cultivars, four from non-waxy rice cultivars (KDML 105, Chai Nat 1, Chai Nat 2 and Phitsanulok 2) and two from waxy rice cultivars (San-pah-tawng and RD6). Rice samples were kept in the forms of paddy and polished rice. Fresh-aged rice indices were determined using both methods every two weeks over the storage period of 24 weeks (six months). It was found that both methods were capable of detecting fresh-aged rice indices. The color of chemical solutions changed with regards to the age of rice grains and it could be detected spectrophotometrically. Rice grains which have been kept as paddy provided more consistent results. Method 1 is recommended for industrial application as it is simple, efficient and inexpensive.
Key words: fresh-aged rice index; peroxidase activity; bromothymol blue indicator; paddy; polished rice; chemical technique

After prolonged storage of paddy, hulled or milled rice, changes in the sensory attributes of the cooked rice can be perceived, which include aroma alteration and textural changes. Cooked rice prepared from aged rice grains becomes harder and the ratios of stickiness to hardness are lowered in comparison to those of fresh rice grains (Ohno et al, 2005, 2007). An increase in hardness and a decrease in stickiness of the cooked rice textures prepared from aged rice are desirable by consumers in some countries such as Thailand and India whilst others such as Japan and China flavor fresh rice (Sowbhagya and Bhattacharya, 2001; Zhou et al, 2002).

During ageing of stored rice grains, a number of physicochemical and physiological changes occur and they affect rice grain qualities (Chrastil, 1990; Noomhorm et al, 1997; Perdon et al, 1999; Suzuki et al, 1999; Sowbhagya and Bhattacharya, 2001; Zhou et al, 2002; Sodhi et al, 2003; Patindol et al, 2005; Singh et al, 2006; Sirisoontaralak and Noomhorm, 2007; Park et al, 2012). The ageing process can be monitored by measuring cooked rice textures or evaluating other physico-chemical properties, including thermal and pasting properties related to gelatinization behavior of the rice flour (Chrastil, 1992; Perdon et al, 1999; Sodhi et al, 2003; Zhou et al, 2003; Tananuwong and Malila, 2011). The obtained properties are then related to the freshness of rice grains. Freshness is considered so highly in Japanese rice markets and tests are devised for its measurement. Chemical properties can also be used for rice grain freshness determination. Several techniques were employed such as indicator's color changes due to pH and free fatty acids (Takashi et al, 2006) or some enzyme activities (Jin et al, 2007).

Rice grains include fats such as neutral lipid, bran lipid and phospholipid. These fats which are mostly neutral lipid, start deteriorating after harvest, undergo oxidation and decompose to produce free fatty acids. These fatty acids further decompose into hexanal. The produced fatty acids and hexanal increase in process of time, and the freshness of the grains can be determined by measuring the amount of the fatty acids. This can be conventionally measured by an indicator solution such as bromothymol blue (Takashi et al, 2006).

Peroxidase (EC 1.11.1.7) is present in rice grains. It is one of the key enzymes involved in reactive oxygen

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species scavenging of seeds. During storage, the loss of peroxidase activity may result in the deterioration of rice grains. Thus, measuring the activity of peroxidase makes it possible to discriminate rice grains from different harvests (Nandi et al, 1997). Therefore, this enzyme activity can be used for rice grain freshness indicator (Matsukura et al, 2000; Chen and Chen, 2003; Noda et al, 2005).

It has been known that extreme storage conditions especially high temperature and humidity affect the changes of both free fatty acids and enzyme activity (Chrastil, 1990, 1992). In practice, the colorimetric observations of free fatty acids and enzyme activity are usually detected using a spectrophotometer. Grain samples should be clean to avoid dust contamination of the solution.

Recently, chemical techniques for rice grain freshness determination that are more convenient than textural or sensorial techniques have gained interest. However, the research in this area is very limited. No scientifically proven evidences on the use of chemical methods are available. Thus, this study investigated the efficiencies of two chemical methods used for the determination of rice grain freshness in order to provide better understanding and application recommendations.

MATERIALS AND METHODS

Rice materials

Six rice cultivars, four from non-waxy rice cultivars (KDML 105, Chai Nat 1, Chai Nat 2 and Phitsanulok 2) and two from waxy rice cultivars (San-pah-tawng and RD6) were obtained from several provincial rice research centers in Thailand. Rice samples were freshly harvested during November to December, 2010.

Methods

Fresh-aged rice index

Rice samples were kept in the forms of paddy and polished rice at tropical ambient environments, with 28–30 °C average temperature, following general commercial storage. Chemical solutions were prepared. Method 1 is made of pH-adjusted bromothymol blue indicator (Takashi et al, 2006), and the principle is based on indicator's color which is changed according to pH induced by the changes of lipids in rice grains during storage. Method 2 is based on peroxidase activity which deteriorates during storage of rice grains. Peroxidase color reactions were detected using the mixed solutions containing dianisidine, guaiacol, H_2O_2 and phosphate buffer (Chen and Chen, 2003). Both methods were used to detect fresh-aged rice indices of the samples, every two weeks over the period of six months. Rice samples which have been kept as paddy were dehulled and polished using a laboratory rice mill (Satake Lab Mill, Satake (Thailand) Co., Ltd.) prior to analysis. The analysis was conducted using the test tube technique (10 g of sample in a test tube containing sufficient chemical solution) and the single grain technique (one single grain per well in a micro-plate containing sufficient chemical solution). The absorbance (A) of chemical solutions after reactions (10 min) was determined using a spectrophotometer for test tube technique or a micro-plate reader for single grain technique. For test tube technique, at least 25 test tubes were conducted at each time. For single grain technique, 96 grains were tested (a 96-well plate) at each time. Average A values of were used for calculation of fresh-aged rice index, as follows:

$$\text{Fresh-aged rice index (Method 1)} = A_{615} - A_{690}; \quad (1)$$

$$\text{Fresh-aged rice index (Method 2)} = 1 / A_{385}; \quad (2)$$

Fresh-aged rice indices obtained from both methods under all different test conditions were plotted against storage times. Average and standard deviation values were used to plot the graphs. Linear regression analysis was conducted using Microsoft Excel version 2007. Linear equations and r^2 values were used to judge the difference between methods.

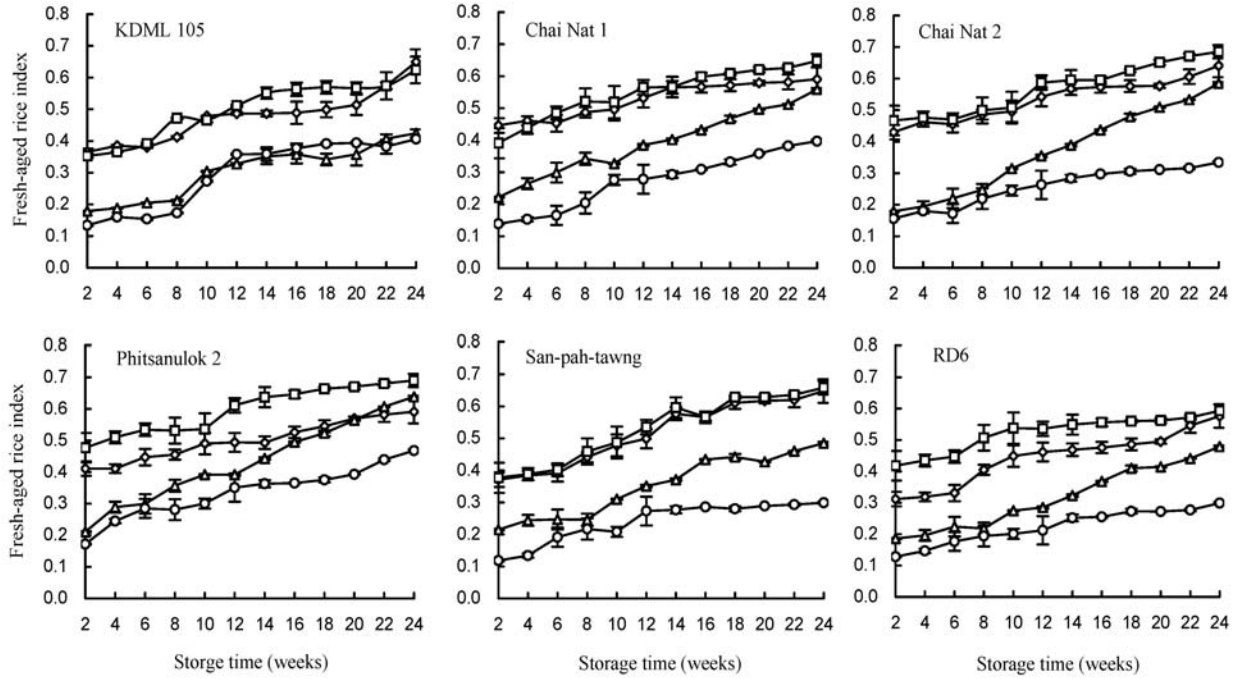
Color band for fresh-aged rice determination

In order to develop the color band for field uses, the solution's color obtained from the test tube technique was captured using a digital camera (Canon EOS 600D, Japan). The captured pictures were assessed for their color dimension in Lab system using the ImageJ image analysis software (National Institutes of Health, USA) (Sheffield, 2007).

RESULTS

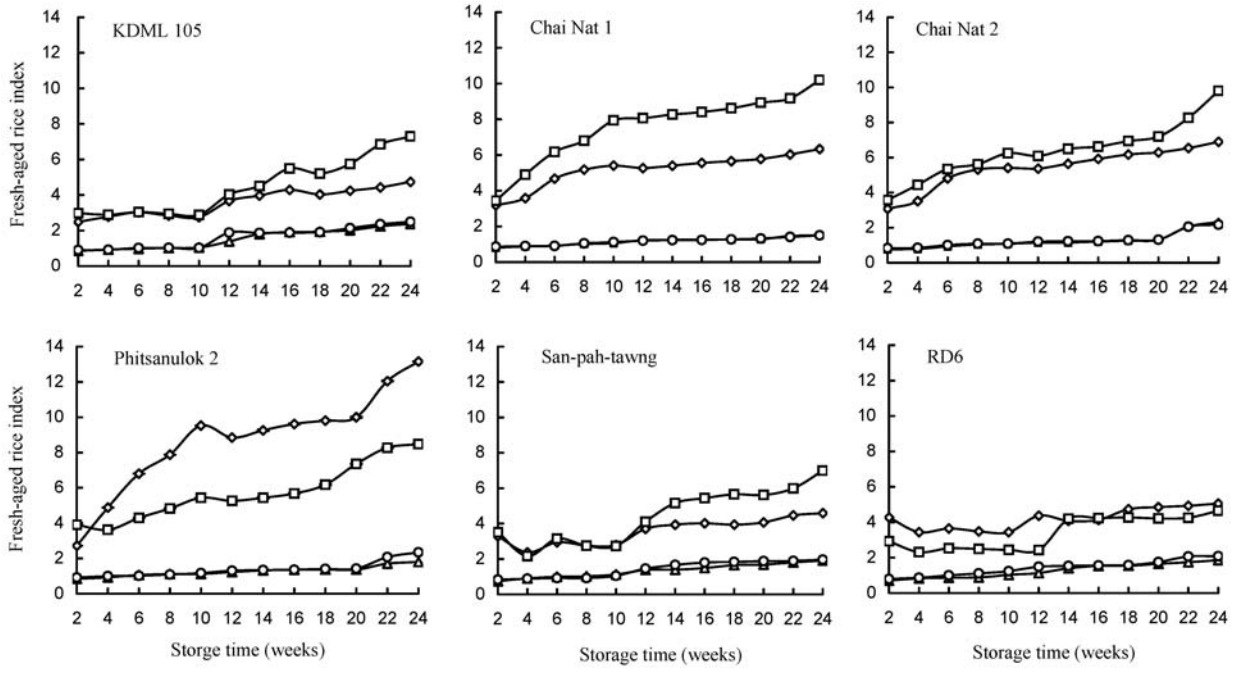
Fresh-aged rice index

Based on the results, it could be clearly seen that both methods were capable of detecting fresh-aged rice indices of all rice cultivars. The indices increased as the storage time increased (Figs. 1 and 2). Rice grains which have been kept as paddy provided higher fresh-aged indices than those obtained from polished rice samples. It implied that both methods were more suitable for use in paddy samples. Moreover, both methods could also be used for rice grains stored as milled



□, Stored as paddy and tested using single grain technique ◊, Stored as paddy and tested using test tube technique
○, Stored as polished rice and tested using single grain technique Δ, Stored as polished rice and tested using test tube technique

Fig. 1. Fresh-aged rice indices obtained from Method 1 (bromothymol blue indicator).
Error bars denote standard deviation.



□, Stored as paddy and tested using single grain technique ◊, Stored as paddy and tested using test tube technique
○, Stored as polished rice and tested using single grain technique Δ, Stored as polished rice and tested using test tube technique

Fig. 2. Fresh-aged rice indices obtained from Method 2 (peroxidase activity).
Error bars denote standard deviation.

Table 1. Linear regression equations and r^2 values of fresh-aged rice indices obtained from Method 1 (bromothymol blue indicator).

Material	Paddy-test tube	Paddy-single grain	Polished rice-test tube	Polished rice-single grain
KDML 105	$y = 0.02x + 0.33$ ($r^2 = 0.89$)	$y = 0.02x + 0.16$ ($r^2 = 0.92$)	$y = 0.02x + 0.34$ ($r^2 = 0.92$)	$y = 0.03x + 0.11$ ($r^2 = 0.86$)
Chai Nat 1	$y = 0.01x + 0.43$ ($r^2 = 0.93$)	$y = 0.03x + 0.20$ ($r^2 = 0.99$)	$y = 0.02x + 0.41$ ($r^2 = 0.94$)	$y = 0.02x + 0.11$ ($r^2 = 0.97$)
Chai Nat 2	$y = 0.02x + 0.42$ ($r^2 = 0.96$)	$y = 0.04x + 0.12$ ($r^2 = 0.99$)	$y = 0.02x + 0.43$ ($r^2 = 0.96$)	$y = 0.02x + 0.15$ ($r^2 = 0.95$)
Phitsanulok 2	$y = 0.02x + 0.39$ ($r^2 = 0.98$)	$y = 0.04x + 0.19$ ($r^2 = 0.99$)	$y = 0.02x + 0.47$ ($r^2 = 0.94$)	$y = 0.02x + 0.19$ ($r^2 = 0.94$)
San-pa-twang	$y = 0.03x + 0.34$ ($r^2 = 0.96$)	$y = 0.03x + 0.18$ ($r^2 = 0.95$)	$y = 0.03x + 0.35$ ($r^2 = 0.95$)	$y = 0.02x + 0.13$ ($r^2 = 0.85$)
RD6	$y = 0.02x + 0.29$ ($r^2 = 0.93$)	$y = 0.03x + 0.14$ ($r^2 = 0.98$)	$y = 0.02x + 0.43$ ($r^2 = 0.87$)	$y = 0.02x + 0.13$ ($r^2 = 0.96$)

Table 2. Linear regression equations and r^2 values of fresh-aged rice indices obtained from Method 2 (peroxidase activity).

Material	Paddy-test tube	Paddy-single grain	Polished rice-test tube	Polished rice-single grain
KDML 105	$y = 0.21x + 2.30$ ($r^2 = 0.90$)	$y = 0.15x + 0.56$ ($r^2 = 0.95$)	$y = 0.43x + 1.70$ ($r^2 = 0.90$)	$y = 0.16x + 0.59$ ($r^2 = 0.91$)
Chai Nat 1	$y = 0.24x + 3.70$ ($r^2 = 0.82$)	$y = 0.06x + 0.76$ ($r^2 = 0.95$)	$y = 0.50x + 4.30$ ($r^2 = 0.88$)	$y = 0.06x + 0.80$ ($r^2 = 0.97$)
Chai Nat 2	$y = 0.30x + 3.50$ ($r^2 = 0.88$)	$y = 0.11x + 0.51$ ($r^2 = 0.76$)	$y = 0.43x + 3.60$ ($r^2 = 0.91$)	$y = 0.10x + 0.61$ ($r^2 = 0.77$)
Phitsanulok 2	$y = 0.74x + 3.90$ ($r^2 = 0.87$)	$y = 0.08x + 0.77$ ($r^2 = 0.93$)	$y = 0.42x + 3.00$ ($r^2 = 0.92$)	$y = 0.11x + 0.68$ ($r^2 = 0.79$)
San-pa-twang	$y = 0.17x + 2.40$ ($r^2 = 0.78$)	$y = 0.11x + 0.66$ ($r^2 = 0.99$)	$y = 0.40x + 1.80$ ($r^2 = 0.84$)	$y = 0.12x + 0.62$ ($r^2 = 0.91$)
RD6	$y = 0.13x + 3.30$ ($r^2 = 0.65$)	$y = 0.11x + 0.55$ ($r^2 = 0.97$)	$y = 0.23x + 1.90$ ($r^2 = 0.73$)	$y = 0.12x + 0.66$ ($r^2 = 0.97$)

or polished rice. From Tables 1 and 2, Method 1 provided more consistent results than Method 2, giving higher r^2 values. Low r^2 values were observed for some samples such as Chai Nat 2 (both paddy and polished rice), Phitsanulok 2 (polished rice), San-pa-twang (paddy) and RD6 (both paddy and polished rice). Published literatures on this issue are very limited. This study demonstrates that chemical techniques can be used to differentiate the fresh and aged rice grains and they can detect the changes within the first few weeks (Figs. 1 and 2). It also highlights here that common indicator solution such as bromothymol blue indicator can be efficiently used to detect the fresh or aged rice grains. Moreover, the use of indicator is very simple and the chemical solution can be easily prepared. Therefore, we recommend Method 1 for application in industry.

In terms of cultivars, Method 1 was found to be capable of detecting fresh-aged rice indices in both waxy and non-waxy rice cultivars (Figs. 1 and 2). Changes in rice lipids during storage or ageing are the key principle for Method 1. The changes occurred in waxy and non-waxy rice cultivars could be different but they did not affect the efficiency of Method 1. However, some inconsistent results especially in non-waxy rice samples (Figs. 1 and 2) were observed using Method 2 and thus they represent poor efficiency of Method 2.

Color band for fresh-aged rice determination

Based on the results from previous section, Method 1 was found to be capable and thus it was used for development of color band. It was found that the color pictures obtained from the solutions of Method 1 in all rice cultivars studied were similar. Therefore, the results here only showed the color band obtained from the Phitsanulok 2 cultivar (Fig. 3).

From Fig. 3, it was clear that the colors of solution change with regard to the ageing of rice and they could be visually distinguished. Using the image analysis software, the color space in Lab could be determined for reproduction of the color band. The

1-month ageing	2-month ageing	3-month ageing	4-month ageing	5-month ageing	6-month ageing
L : 59–63	*L* : 54–57	*L* : 49–51	*L* : 46–49	*L* : 28–30	*L* : 25–27
a : (-26)–(-28)	*a* : (-24)–(-27)	*a* : (-21)–(-26)	*a* : (-18)–(-20)	*a* : (-13)–(-15)	*a* : (-10)–(-14)
b : 17–22	*b* : 15–18	*b* : 12–16	*b* : 10–12	*b* : 6–8	*b* : 1–3

Fig. 3. Color band and Lab color space for fresh-aged rice analysis based on bromothymol blue indicator (Method 1).

L, *a* and *b* values are Hunter Lab color values. A Lab color space is a color-opponent space with dimension *L* for lightness and *a* and *b* for the color-opponent dimensions, based on nonlinearly compressed CIE XYZ color space coordinates.

decrease of *L* values (darker color) was observed as the ageing progressed. The changes of *L* values were accompanied with the changes of *a* and *b* values (Fig. 3). The use of color band may be helpful for small enterprises and on field applications.

DISCUSSION

The changes of fresh-aged rice indices over the storage period were observed by both methods (1 and 2). However, obvious changes, from both methods, were detected in rice samples which have been kept as paddy. These changes could be the influences from pH which was induced by lipid oxidation, free fatty acids and enzyme activity. It has been reported that the outer layers of rice kernels (that is in the bran including the germ) have larger amounts of lipid than the inner parts (that is in the core or inner endosperm) (Juliano, 1992; Ogawa et al, 2002). Though, rice lipids are usually stable in the intact spherosomes in the cells. When the lipid membrane is destroyed by phospholipase, physical injury or high temperature, lipid hydrolysis is initiated by the action of lipases (Takano, 1989). Of the various lipid fractions, the greatest proportional change was observed in free fatty acids (Zhou et al, 2002). The changes in fatty acid profiles and increases of free fatty acids during storage have been substantiated in numerous studies (Dhaliwal et al, 1991; Deka et al, 2000; Nishiba et al, 2000). In this study, high temperature (28–30 °C) storage in tropical environment played an important role in the changes of lipids in paddy and consequently led to the changes of indicator's color. It has been reported that lipid content in brown rice was stable during storage for 12 months at 5 °C but decreased significantly during storage at 35 °C (Shin et al, 1986).

In this study, smaller changes of the indices during storage were observed in milled or polished rice samples as compared to paddy samples. For polished rice, the outer layers which are rich in lipids are removed during milling and polishing (Watson et al, 1975; Ito et al, 1983; Hemavathy and Prabhakar, 1987). Therefore, active substances e.g. free fatty acids that can induce the reactions with the test chemicals are minimal. We recommend that other techniques such as texture or sensory tests may be used in combination with chemical techniques when analyzing the fresh-aged rice indices of polished rice grains. Notably that texture measurements should be done on rice aged at least three months after harvest to be able to observe major changes (Perez and Juliano, 1981).

This study suggested that Method 1 could be used for both waxy and non-waxy rice cultivars. Changes in lipids occur differently in waxy and non-waxy rice. It has been reported that non-waxy milled rice had proportionally more starch lipids (bound lipids) and less non-starch lipids (free lipids) than waxy milled rice (Choudhury and Juliano, 1980). Free fatty acids were the highest in stored, waxy, milled rice (Perez and Juliano, 1981). However, in this study, it is suggested that bromothymol blue indicator (Method 1) was capable of detecting changes and consequently could differentiate fresh and aged rice by means of fresh-aged rice indices.

There is still a need for research in this area. Most of current reports focused on the changes of enzyme activities for detecting the freshness of rice grains (Ushio et al, 2000; Chen and Chen, 2003; Noda et al, 2005). Effects of storage conditions and rice cultivars can be the factors for further studies.

CONCLUSIONS

Chemical techniques can be used to differentiate the fresh or aged rice grains. This technique can provide more consistent results than the sensory technique. This study confirms the use of two chemical methods to detect fresh-aged rice indices of six rice cultivars, both non-waxy and waxy cultivars. We recommend the use of Method 1 which is based on bromothymol blue indicator for industrial use as it is inexpensive, simple and fast. For small food enterprises or field applications, the color band which based on the chemical solution's color can be used for convenience.

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