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REVIEW

Fluorescence Imaging with UV-Excitation for Evaluating Freshness of Rice

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Abstract

To measure the freshness of rice quickly and conveniently, we used a fluorescence imaging method with UV-excitation. The system consists of two UV-A fluorescent lamps and a blue LED with a band-pass filter, a cooled CCD camera with an IR cutoff filter to capture the fluorescence image on a PC, and image processing software for measuring the fluorescence intensity of the image. In a darkened box, sample materials are placed on a stage painted with non-fluorescent, flat black paint to minimize background scattering. Testing stored samples of unhulled rice and brown rice, we found a high correlation between the fluorescence intensity and conventional indices such as free fat acidity and guaiacol reaction. The fluorescence intensity increased as the storage temperature rose or the storage period lengthened. Eating quality decreased significantly as the fluorescence intensity rose. These results show that this system can estimate the loss of eating quality that occurs during storage of rice. Thus, fluorescence intensity could provide an effective and convenient way to evaluate rice quality.

Discipline: Postharvest technology
Additional key words: degradation, nondestructive inspection, palatability, quality evaluation method

Introduction

After harvesting, rice is dried and stored before consumption. The quality of the rice must be evaluated throughout this process and reported to producers, distributors, and consumers. Deterioration of rice after harvest, manifested as a reduction of freshness and flavor, occurs mainly as a result of conditions conducive to germination. Because rice is a perishable food, it must be kept fresh in storage for as long as possible. Deterioration of quality parallels a decrease in viability, changes in chemical components, and changes in physical characteristics. Conventionally, the freshness of rice is determined by the guaiacol reaction[14]. Rice grains are placed in a test tube filled with guaiacol solution and hydrogen peroxide (H_2O_2). The oxygen generated from the H_2O_2 by rice enzymes turns the colorless guaiacol into red tetraguaiacol. Fresh grains have a high level of enzyme activity, which will turn the grains and the solution reddish-brown, but stale grains will not change color. The fats and carbohydrates in stored grains break down over time to produce fatty acids and other organic acids. As fat breaks down most rapidly, the free fat acidity, a measure of fatty acids

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as byproducts of fat dissolution, is considered to be an important indicator of the deterioration of rice[7]. The free fat acidity is determined by the rapid method of the American Association of Cereal Chemists (AACC method 02-02), in which free fatty acids are extracted in benzene solution and titrated against KOH solution[8]. The problem with this method is that it relies on visual observations, and it is difficult to determine when to stop titrating. In the guaiacol reaction, the change of color is judged by eye as well, so the results are likely to fluctuate with the chemist and when the test is conducted.

Recently, two companies in Japan have put apparatus on the market that measure the freshness of rice more easily than these conventional methods[4,5]. Although the methods are different, both use specially developed reagents and a CCD line sensor, enabling a shorter analysis time and objective and constant results. However, these methods are not convenient for locations such as rice mills, which receive brown rice straight from growers. Efficient quality evaluation requires a quick and simple method without any reagents[3].

The goals of this study were to develop analytical techniques for locations such as rice mills and drying and processing facilities, and to establish a quick, simple, non-destructive method for evaluating the freshness of rice without the need for reagents.

Materials and methods

1. Evaluation system

(1) Concept of the system

We adopted a fluorescence imaging method based on UV-excitation[1,9,16] (Fig. 1, left). Fluorescence occurs when a molecule, atom, or nanostructure (the fluorophore) relaxes to its ground state after absorbing a photon. Usually the absorbed photon is in the UV range, and the emitted light is in the visible range, but this depends on the absorbance curve and Stokes shift of the particular fluorophore[1]. Once a molecule has absorbed photon energy, it can return to its ground state (the statistically most common energy state for room-temperature chemical reactions) via a number of routes. The diagram on the left of Figure 1, termed a Jablonski diagram, shows a few of these processes. If the photon emission occurs between states of the same spin state (e.g. $S_1 \rightarrow S_0$), this is termed fluorescence (line F). If it occurs between different spin states (e.g. $T_1 \rightarrow S_0$), this is termed phosphorescence (P). Since fluorescence is statistically much more likely than phosphorescence, the lifetimes of fluorescent states are very short (1×10^{-8} to 1×10^{-5} s), and those of phosphorescence are somewhat longer (1×10^{-4} s to minutes or even hours, such as seen in glow-in-the-dark toys)[1,16]. This method has been used to detect aflatoxins in hens' eggs and nuts[6]. Our system relies on the emission of light energy as fluorescence or phosphorescence. Rice quality is evaluated by taking an image of the fluorescence emitted from the rice through a CCD camera and measuring the brightness of that image on a PC.

(2) Hardware components of the system

Figure 1 (right) shows a schematic diagram of the system. The sensor module consists of a CCD camera and a control unit. The CCD images 1360 × 1024 pixels and is thermoelectrically cooled by a one-stage Peltier device. The dynamic range of the camera is 16 bits, and

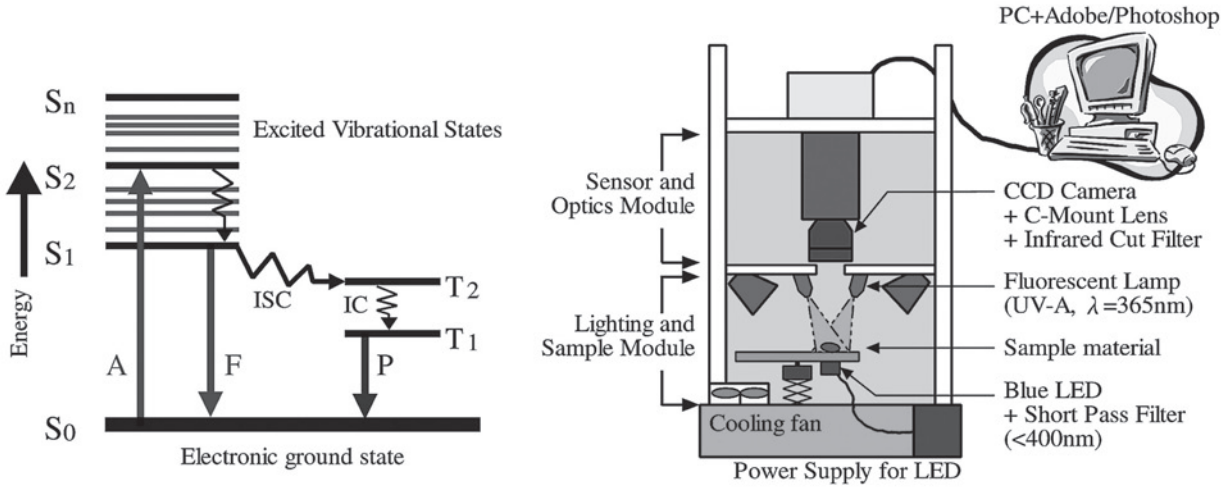

Fig. 1. Photochemical basic process and schematic diagram of the freshness evaluation system of rice
A: Photon absorption, F: Fluorescence (emission), P: Phosphorescence,
S: Singlet state, T: Triplet state, IC: Internal conversion, ISC: Intersystem crossing

the raw data are saved to a PC. The optics module consists of a spectrograph coupled with a standard *f* 1.4 C-mount lens attached to the CCD camera head.

The IR cut-off filter in front of the lens cuts radiation longer than the approximate IR range. A preliminary analysis using excitation–emission matrix spectroscopy showed great differences in fluorescence intensity in the range of 440–500 nm, but almost no differences in the range >600 nm. Therefore, we designed our system to cut out near-IR light and detect fluorescence at shorter wavelengths. We installed two kinds of excitation light sources: a pair of UV-A fluorescent lamps and a blue LED. The former have a central wavelength of 365 nm and are mounted above the sample stage to supply near-uniform excitation energy. The latter incorporates a short-pass filter (<400 nm) and is mounted beneath the sample stage. The system is mounted in a darkened box fitted with a fan to prevent heating of the samples by the light sources. Samples are placed on a stage painted with non-fluorescent, flat black paint to minimize background scattering.

2. Rice samples

(1) Sample storage and treatment

We tested grain of the cultivar 'Haenuki' from Yamagata Prefecture. Unhulled rice and brown rice were stored for 180 days in paper bags typically used for rice at eight different storage temperatures: –20, 0, 5, 10, 15, 20, and 30°C, and room temperature (RT, 4.5–33.5°C). Samples were withdrawn twice a month. The unhulled rice was then hulled in a commercial impeller husker (Model FC2K, Otake Seisakusho Co., Ltd.) to obtain brown rice. The brown rice was milled in a commercial abrasive mill (Model VP-32T, Yamamoto Co., Ltd.). The yield of each milled sample was 90.0% ± 0.5% of the brown rice weight.

(2) Evaluation of relationship between degree of milling and fluorescence intensity

To identify the site of the fluorescent component, we tested brown rice and rice milled to 90.0% ± 0.5%, 80.0% ± 0.5%, and 70.0% ± 0.5%. The same rice as above was stored at RT for 60 days.

3. Methods

We measured fluorescence intensity, free fat acidity, gelatinization properties, guaiacol reaction, and rice palatability (by a sensory test in which panelists taste cooked rice).

Free fat acidity was determined by titration[8]. Gelatinization properties were measured with a Rapid Visco Analyser (RVA; Newport Scientific, Warriewood, NSW, Australia). Palatability was based on the subjective judgment of a panel of 26 to 35 tasters. The sample stored at –20°C was used as a reference. Panelists graded the samples by comparison with the reference sample on a scale of +3 to –3.

To collect the fluorescence image data, we place sound whole rice grains in a laboratory dish 30 mm in diameter, then set it on the sample stage. A 30-s image is taken by the CCD camera and saved as a computer file. In Adobe Photoshop CS, the file is opened and the pixel intensity of the fluorescence image is calculated.

Results and Discussion

1. Evaluation of aging of stored rice by conventional freshness indices

Figure 2 shows the change in guaiacol reaction during storage at each temperature. The guaiacol reaction indicates the freshness of the rice: the higher the enzyme activity of the rice, the quicker and deeper the change in color. The enzyme activity decreased with both increasing length of storage and increasing storage temperature. The difference during storage at low temperatures (–20, 0, 5°C) was minor, but quality decreased rapidly from 30 days at higher temperatures (20°C, 30°C, RT).

Figures 3 and 4 show the changes in free fat acidity and maximum viscosity (gelatinization property) of brown rice during storage. Both measures increased as the storage temperature rose and as the length of storage increased. They started to increase immediately after the beginning of storage at high temperatures (20°C, 30°C, RT), but increased only slightly during 6 months' storage at low temperatures (–20, 0, 5, and 10°C). Differences

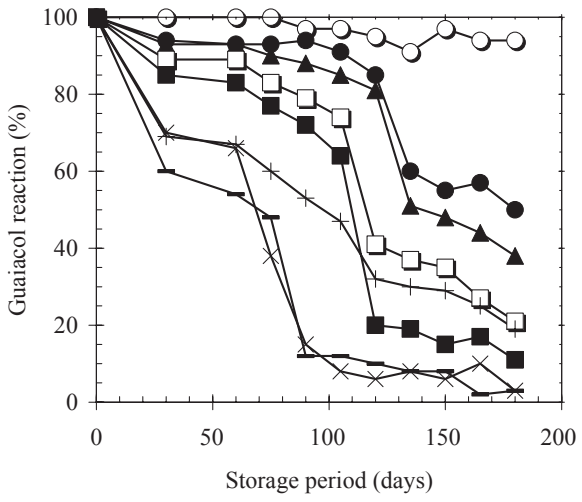

Fig.2. Change in guaiacol reaction
○: –20°C, ●: 0°C, ▲: 5°C, □: 10°C, ■: 15°C, +: 20°C, –: 30°C, ×: RT

among temperatures became clearer after a month.

Free fat acidity is considered to be an important indicator of rice quality[10]. When free fatty acids (age-related breakdown products) bind with starch, they prevent the gelatinization of the starch during cooking and harden aged rice[11]. It has been reported that there is a high correlation between the maximum viscosity and breakdown as measured using the amylogram and palatability of cooked rice. Breakdown is the difference between the maximum and minimum viscosities, and shows the degree of viscosity drop due to heating, which is believed to be caused by the disintegration of starch granules. Rice with a higher maximum viscosity and/or a larger breakdown is considered to have better palatability in Japan[10,15], but each measure increases with age also, and these increases do not accompany an increase in palatability. Two theories have been proposed to explain why maximum viscosity increases with the aging of rice: (1) amylase activity decreases during storage, and (2) the substances that make up the cell wall are denatured[12,13]. It has been suggested that an increase in maximum viscosity or in breakdown during storage should be interpreted as deterioration of palatability, although each is usually supposed to mean an increase in palatability of fresh rice[15].

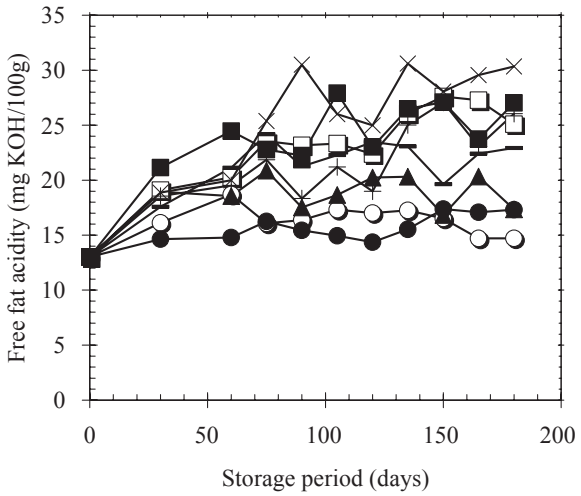

Fig.3. Change in free fat acidity
○: –20°C, ●: 0°C, ▲: 5°C, □: 10°C, ■: 15°C, +: 20°C, –: 30°C, ×: RT

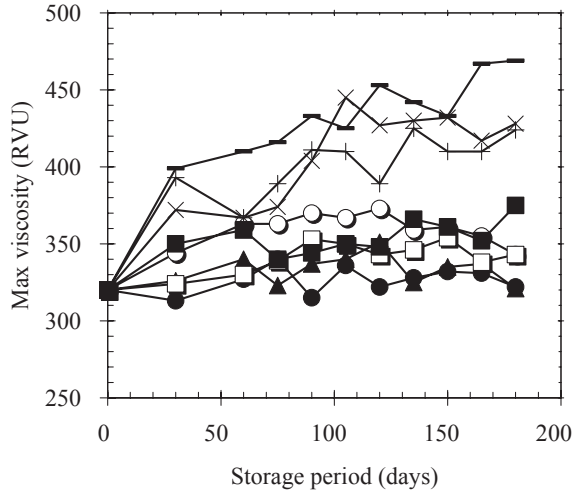

Fig.4. Change in maximum viscosity
○: –20°C, ●: 0°C, ▲: 5°C, □: 10°C, ■: 15°C, +: 20°C, –: 30°C, ×: RT

The results of the palatability test are shown in Table 1. The samples stored at 30°C for 60 days or longer were extremely deteriorated and had the typical aged-rice smell, and thus were excluded from the test. Taste deteriorated as the temperature rose and as storage increased. No significant difference was found between 0°C and 5°C; those samples maintained high palatability. The palatability of the rice stored at 10–15°C was slightly reduced. The palatability of the rice stored at higher temperatures was further reduced.

2. Evaluation of aging of stored rice by fluorescence intensity

Figures 5, 6, and 7 show the change in fluorescence intensities of brown rice, milled rice, and unhulled rice

Table 1. Results of palatability evaluation test

Storage Temp.	Storage days				
(°C)	30	60	90	120	150
0	–0.30	–0.04	–0.33	–0.15	–0.07
5	–0.26	–0.31	–0.33	–0.15	–0.14
10	–0.11	–0.04	–0.00	–0.11	–0.00
15	–0.04	–0.15	–0.04	–0.04	–0.55
20	–1.33**	–0.73*	–1.07**	–1.44***	–0.69**
30	–2.15**	–	–	–	–
RT	–0.67*	–0.27	–0.52	–0.56*	–1.14***

Note 1: This test was a comparative evaluation of rice samples stored at various temperatures were compared with standard rice. Panelists taste the reference sample first and grade it as 0. Then, each samples are tasted and graded on the basis of comparison with the reference sample. Evaluation is graded and recorded on a +3 to –
Note 2: Standard rice was unhulled rice stored at –20ºC.
Note 3: 26–35 panelists assessed the cooked rice samples.
*,**,***: Significant at the 5%, 1% , and 0.1% levels respectively.

during storage at each temperature. The fluorescence intensity increased with temperature and storage period regardless of storage form, and remained almost constant over time at low temperatures (–20, 0, 5, 10°C; not all data shown). The fluorescence intensity of milled rice increased more slowly than that of brown rice. This suggests that the main fluorophore is concentrated in the bran layer, and that its quality alters over time. The fluorescence intensity of brown rice stored as brown rice changed more than that of brown rice stored as unhulled rice.

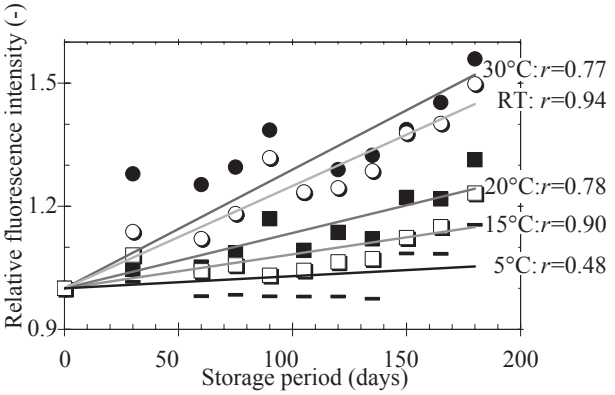

Fig.5. Transition of fluorescence intensity of brown rice in storage form "brown rice"
–: 5°C, □: 15°C, ■: 20°C, ●–: 30°C, ○: RT

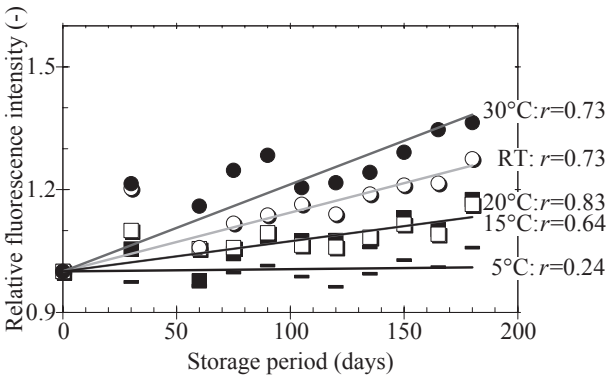

Fig.6. Transition of fluorescence intensity of milled rice in storage form "brown rice"
–: 5°C, □: 15°C, ■: 20°C, ●–: 30°C, ○: RT

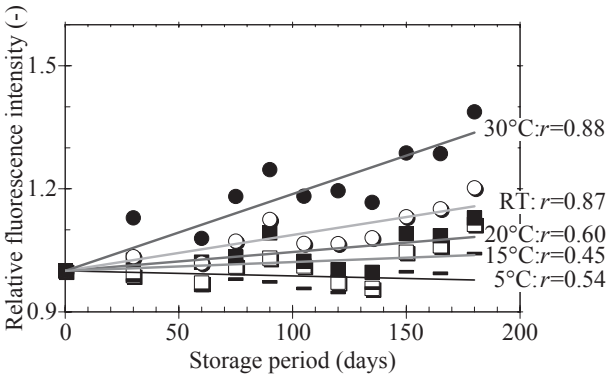

Fig.7. Transition of fluorescence intensity of brown rice in storage form "rough rice"
–: 5°C, □: 15°C, ■: 20°C, ●–: 30°C, ○: RT

Coefficients of correlation between conventional indices and fluorescence intensity ranged from 0.65 to –0.82, and were significant for all comparisons (Table 2). The correlation between palatability and fluorescence intensity was –0.45 ($P < 0.05$, Fig. 8), but there was no high correlation with physico-chemical properties such as guaiacol reaction. To accurately estimate rice palatability from fluorescence intensity, we need to collect more data.

3. Relationship between milling degree and fluorescence intensity

Figure 9 indicates that the fluorophores are concen-

Table 2. The correlation coefficients between each freshness index (n=88)

	Guaiacol reaction	Max viscosity	Free fat acidity
Max viscosity	–0.720**	–	–
Free fat acidity	–0.765**	0.570**	–
Fluorescence intensity	–0.819**	0.765**	0.650**

**: Significant at the 1% level.

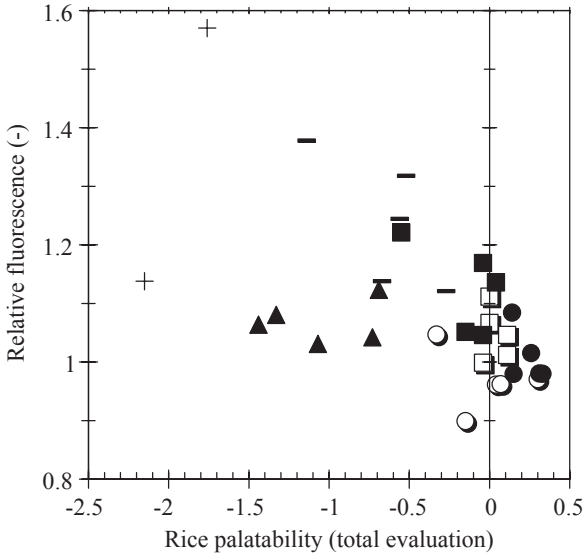

Fig.8. The relationship between rice palatability and fluorescence intensity
○: 0°C, ●: 5°C, □: 10°C, ■: 15°C, ▲: 20°C, +: 30°C, –: RT

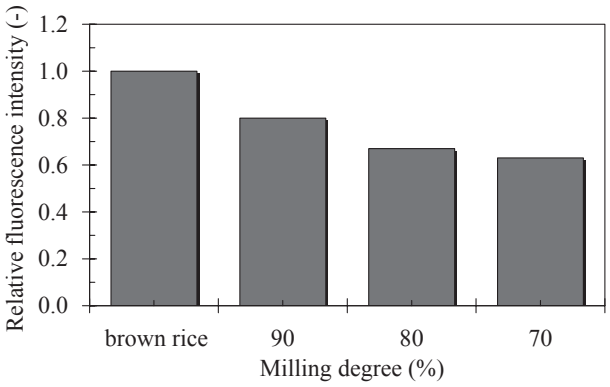

Fig.9. The relationship between milling degree and fluorescence intensity

trated in the bran layer, but some occur in the endosperm as well. It is possible that several different fluorophores are dispersed throughout the rice kernel. Further biochemical research to identify the main components related to deterioration and to clarify the mechanisms responsible for changes in fluorescence is needed.

Conclusions

We have designed and developed a system for evaluating the freshness of brown rice from fluorescent images. The use of fluorescence techniques appears to be a promising method for evaluating the deterioration of rice. Evaluation of the method showed a good relationship between fluorescence intensity and conventional indices of freshness, indicating that it can be used for monitoring aging of rice. Our results were obtained with only a few samples in one year. To evaluate the accuracy of the system more precisely, we need to test it with a greater number of cultivars over several harvest years.

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