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Validation of the dye colour chart method for pH determination of rice grains

R.J.-C. Hsu and S. Lu

China Grain Products R&D Institute, 12-6, Hsiakutze, Bali Dist., New Taipei, 24937, Taiwan; Rachel.Hsu@CGPRDI.org.tw

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Abstract

During storage of rice, acidity is gradually generated in the rice grains. This increase in acidity results from an accumulation of compounds including tricarboxylic acid cycle metabolites, amino acids, and free fatty acids, and is used as an index for the length of storage or the freshness of rice grains. The acidity can be conventionally determined and expressed using a pH meter. A dye colour-chart method has been developed and is currently used to determine the pH of rice grains by using a bromothymol blue-methyl red dye solution and a pH colour chart. The method is low cost and convenient. To validate the repeatability and reproducibility of the dye colour-based method in measuring the pH of rice grains, an international ring test including 14 laboratories was performed. The high consistency and low standard deviation of repeatability and reproducibility showed that this method was acceptable and worthy to promote. The results show that the dye colour chart method is conditionally acceptable and highly comparable to the conventional pH meter method. In addition, training in this method is suggested for reducing variation.

Keywords: dye-colour chart method, freshness of rice kernels, rice kernels, rice pH, validation of pH

1. Introduction

During storage, lipid, carbohydrate, and protein components in rice grains gradually degrade into organic acids such as amino acids, free fatty acids, and phytic acid (Dhaliwal *et al.*, 1991). As acidity increases, rice quality decreases and the colour changes (Lii *et al.*, 1999).

Generally, the pH value of freshly harvested rice is at or above 7.2 and decreases gradually with a prolonged storage period. After years of storage, the pH value may decline to less than 6.0. If the rice pH value is less than 6.2, an apparent yellowish colour and rancid odour is detected, indicating unacceptable quality (Chang *et al.*, 2000). A rice pH of 6.3 has been recommended as the cut-off value for rice quality control by warehouses and manufacturers. Kumagai *et al.* (1978) first used an acidity dye indicator to rapidly detect rice freshness. This method has also been adopted by the Food Agency of Japan. However, this dye indicator method determines rice freshness only by visual examination and is only used for qualitative detection. Lii *et al.* (1999) in Taiwan developed a novel means of analysing the soluble acidity of rice grains based on the high correlation between the pH value (pH 5-8) and methyl red dye indicator absorbance at 630 nm. The dye colour chart method for determining rice pH, using a board with standard colour panels as shown in Figure 1, was first developed by the Agriculture and Food Agency, Council of Agriculture in Taiwan for rapid quality control of rice stock storage for years (Lii *et al.*, 1999). The standard colours on the chart were strictly selected from the international DIC Pantone Standard Colour Chart (DIC Corporation, Tokyo, Japan), according to the dye colours that appeared under different pH values. For example, the DIC Pantone Standard Colour Chart colour density is K = 1.85±0.05, C = 1.85±0.05, M = 1.70±0.05, and Y = 1.80±0.05.

The dye colour chart method for rice pH determination is a rapid way to check rice pH values for brown or milled rice kernels, and for both japonica and indica varieties. The principle of this method is the use of a pH-sensitive dye indicator mixture for the water-soluble organic acids that are released from rice kernels, by using bromothymol blue and methyl red indicators. Then, whether the rice is old or fresh can be rapidly determined by comparison with

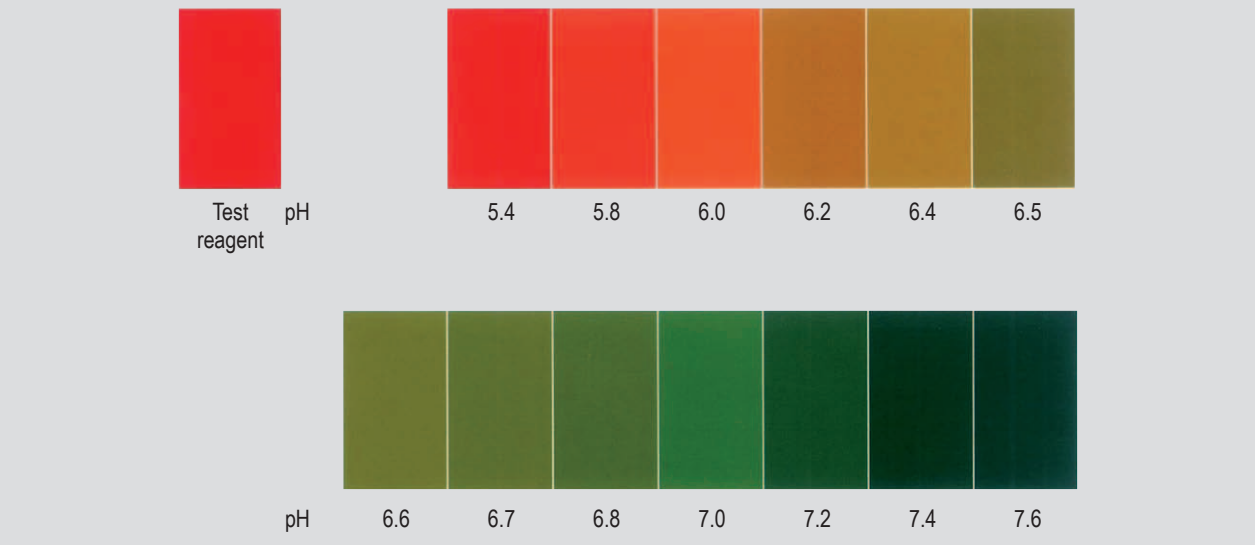

Figure 1. Dye colour chart board. Each tested sample was compared by colour to determine its pH value.

the colour chart. In this study, a ring test was performed to understand the reproducibility and repeatability of this rapid method.

2. Materials and methods

Stocked japonica paddy rice which had been stored for 0-4 years was purchased from a local warehouse (Tainan, Taiwan). All the stock rice had been stored in a local warehouse at room temperature. Because there are two harvest seasons for rice in Taiwan, 8 samples (level 1-8) were harvested and collected in different years and seasons, coded by 06-1, 06-2, 07-1, 07-2, 08-1, 08-2, 09-1 and 09-2. Paddy rice was dehulled and milled under the same conditions with 10% refining. After milling, rice kernel samples were quickly vacuumed in 80 plastic bags (15 g/bag) and randomly numbered. All vacuumed samples were stored at room temperature, as shown in Figure 2.

The samples were randomly picked and delivered at room temperature to collaborative laboratories within 2 weeks of bagging. Each laboratory received 8 samples (level 1-8) and was asked to run five repeats for analyses within 1 week of sample delivery. Fourteen laboratories participated in this validation test, including one from Spain, two from Italy and 11 from Taiwan, as shown in Table 1.

Test reagents from bromothymol blue and methyl red were freshly prepared. Three grams of rice kernels were mixed well with the test reagents in tubes and the tubes were inverted 5 times. Five min later, the tubes were inverted again. For pH determination, the colour of the test solution was compared to the colours on the dye colour chart. The representative pH value of the specific colour was recorded. The results were determined within 30 min, since the apparent colour slowly changes with time. All the data were statistically analysed according to ISO 5725-2:1994, 'Best method for determination of repeatability of a standard measurement method'.

3. Results and discussion

The averages and standard deviations of rice pH at 8 age levels from all 14 laboratories are shown in Table 2. There is an increasing trend in pH from level 1 to 8, i.e. from longer to shorter storage times.

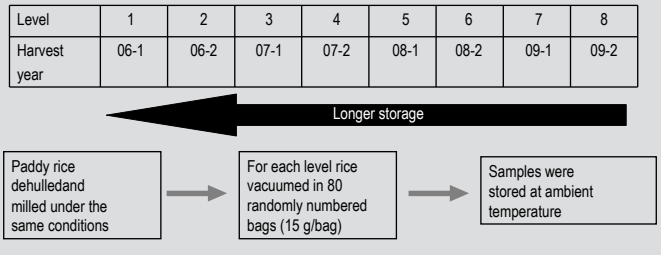

Figure 2. Preparation of paddy japonica rice samples that had been stored for 0-4 years in a local warehouse.

Table 1. Participating laboratories.

Code	Name
Z	China Grain Products Research & Development Institute (CGPRDI), Taiwan
A	Taiwan Forestry Research Institute (TFRI), Taiwan
B	Food Industry Research and Development Institute(FIRDI), Taiwan
C	Agriculture and Food Agency-Southern Region Branch (AFA-S), Taiwan
D	Super Laboratory Co. (Slaboratory), Ltd., Taiwan
E	Agriculture and Food Agency-Northern Region Branch, (AFA-N) Taiwan
F	SGS Taiwan Limited (SGST), Taiwan
G	Agriculture and Food Agency (AFA), Taiwan
H	Taiwan Agricultural Research Institute (TARI), Taiwan
I	Agriculture and Food Agency-Eastern Region Branch (AFA-E), Taiwan
J	Agriculture and Food Agency-Central Region Branch (AFA-C), Taiwan
K	Unità di Ricerca per la Selezione dei Cereali e la Valorizzazione delle Varietà Vegetali (CRA-SCV), Italy
L	Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Spain
M	Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (INRAN), Italy

Table 2. Means and standard deviations of rice pH for 8 age levels.

Laboratory	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
Z	6.0±0.0	6.2±0.0	6.1±0.1	6.2±0.0	6.5±0.0	6.7±0.0	7.2±0.0	7.2±0.0
A	6.2±0.1	6.2±0.0	6.2±0.0	6.2±0.0	6.5±0.1	6.6±0.0	7.0±0.0	7.2±0.0
B	6.2±0.0	6.3±0.0	6.3±0.0	6.3±0.0	6.4±0.0	6.5±0.0	6.8±0.0	7.0±0.0
C	6.0±0.0	6.1±0.1	6.1±0.1	6.1±0.1	6.4±0.1	6.4±0.1	6.9±0.1	6.9±0.1
D	6.1±0.1	6.1±0.1	6.2±0.1	6.2±0.1	6.4±0.0	6.6±0.0	7.0±0.1	7.2±0.0
E	6.1±0.1	6.1±0.0	6.2±0.1	6.2±0.1	6.5±0.0	6.7±0.1	7.2±0.1	7.2±0.1
F	5.8±0.0	5.8±0.0	5.8±0.0	6.0±0.0	6.2±0.0	6.4±0.0	7.0±0.0	7.2±0.0
G	6.2±0.0	6.2±0.0	6.2±0.0	6.2±0.1	6.5±0.0	6.7±0.0	7.0±0.0	7.2±0.0
H	6.2±0.0	6.4±0.0	6.2±0.0	6.4±0.0	6.5±0.0	6.7±0.0	7.2±0.0	7.4±0.0
I	6.2±0.0	6.4±0.0	6.4±0.0	6.4±0.0	6.5±0.0	6.6±0.0	7.0±0.0	7.0±0.0
J	6.0±0.0	6.2±0.0	6.2±0.0	6.2±0.0	6.4±0.0	6.5±0.0	7.0±0.0	7.2±0.0
K	6.0±0.1	6.0±0.1	6.0±0.1	6.1±0.1	6.2±0.1	6.3±0.1	6.7±0.1	6.7±0.1
L	6.2±0.0	6.4±0.0	6.4±0.0	6.5±0.0	6.7±0.0	6.9±0.3	7.2±0.0	7.4±0.0
M	6.0±0.0	6.1±0.0	6.1±0.0	6.2±0.0	6.4±0.0	6.5±0.0	6.7±0.0	7.0±0.0

The between-laboratory consistency was calculated through Mandel's 'h' statistic. Specifically, the deviation of each laboratory from the overall average was divided by the standard deviation, as shown in Figure 3. There were no outliers in between-laboratory consistency, which implies a high consistency for all 8 levels.

Mandel's 'k' statistic was used to calculate the within-laboratory consistency, defined as the proportion of the standard deviation to the pooled standard deviation, for each laboratory, as shown in Table 3. There was an apparent within-laboratory variance in some laboratories, as can be seen in Figure 4.

The Cochran's test was used to check the outliers by comparing the standard deviation of a laboratory with the highest standard deviation for each level. If the proportion was higher than 0.304, the laboratory was defined as an outlier. Under the calculation, there were outliers for all 8 levels, as shown in Table 4.

Using the Cochran's test, most of the laboratories standard deviations for each level were zero, leading the remaining non-zero standard deviations to be very sensitive when performing a Cochran's test. In other words, the non-zero standard deviations tended to be declared as statistical outliers. Therefore, we used the control chart for standard

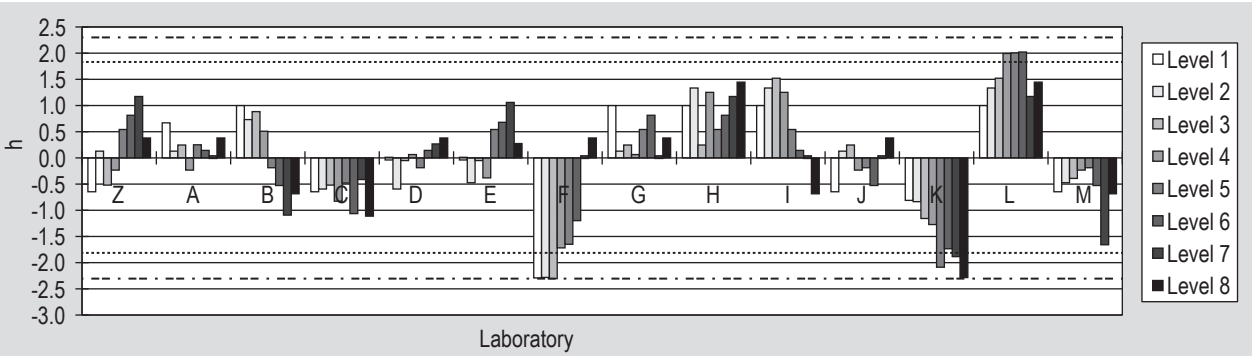

Figure 3. Mandel's between-laboratory consistency h-statistic of 14 laboratories and 8 age levels, calculated according to ISO 5725-2:1994. The 1% and 5% significance levels for p=14 are shown: h=2.30 (outliers) and h=1.86 (stragglers), respectively.

Table 3. Mandel's 'k' statistic of all laboratories for all 8 age levels.

Laboratory	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
Sp[1]	0.041	0.044	0.051	0.049	0.031	0.077	0.050	0.046
Z	0.000	0.000	2.131	0.000	0.000	0.000	0.000	0.000
A	2.160	0.000	0.000	0.000	1.733	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	2.495	2.131	2.254	2.830	0.581	2.191	2.408
D	2.646	2.495	1.740	1.840	0.000	0.000	1.789	0.000
E	1.080	0.000	1.066	0.920	0.000	0.581	0.894	0.983
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	1.840	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
K	1.080	1.248	0.870	1.127	1.733	1.087	2.280	2.692
L	0.000	0.000	0.000	0.000	0.000	3.485	0.000	0.000
M	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

For p=14 and n=5: k=1.75 at the 1% significance level (outliers) and k=1.52 at the 5% significance level (stragglers), respectively.
[1] Sp = standard deviation pooled for all laboratories.

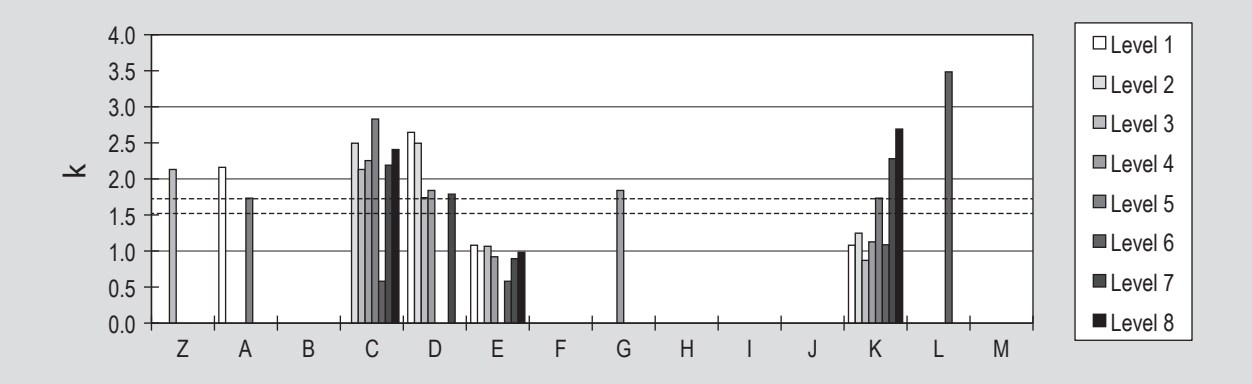

Figure 4. Mandel's within-laboratory consistency k-statistic of 14 laboratories and 8 age levels, calculated according to ISO 5725-2:1994. The 1% and 5% significance levels for p=14 and n=5 are shown: k=1.76 (outliers) and k=1.52 (stragglers), respectively.

Table 4. Cochran's test for outliers.

Laboratory	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
Z	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000
A	0.008	0.000	0.000	0.000	0.003	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.012	0.012	0.012	0.008	0.002	0.012	0.012
D	0.012	0.012	0.008	0.008	0.000	0.000	0.008	0.000
E	0.002	0.000	0.003	0.002	0.000	0.002	0.002	0.002
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
K	0.002	0.003	0.002	0.003	0.003	0.007	0.013	0.015
L	0.000	0.000	0.000	0.000	0.000	0.072	0.000	0.000
M	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Σs^2	0.024	0.027	0.037	0.033	0.014	0.083	0.035	0.029
C value[1]	0.500	0.444	0.324	0.364	0.571	0.867	0.371	0.517

For p=14 and n=5: C=0.304 at the 1% significance level (outliers) and C=0.255 at the 5% significance level (stragglers), respectively.
[1] If C value >0.304 than outliners were found.

deviations (the σ chart) instead. A general formula for the control limits for the σ chart is:

$\sigma=\overline{\sigma}\pm3\sigma_{\sigma}$,

where $\overline{\sigma}=\sum_{i=1}^{p} s_i$,

$\sigma_{\sigma}=\sqrt{\sum_{i=1}^{p}(s_i-\overline{\sigma})^2/(p-1)}$,

and s_i is the observed standard deviation of laboratory *i*.

However, as shown in Table 5, all of the resulting lower limits turned out to be less than zero. Because σ cannot be a negative value, we set the lower limit equal to zero in these cases. Almost all of the standard deviations in each level fell within the control limit. We then concluded that none of them were outliers (Grant and Leavenworth, 1980).

Grubb's test was used to identify the laboratory outlier observations. First, the maximum and minimum pH for each level were determined. Their deviation from the average pH was divided by the standard deviation, resulting in Gp and G1, respectively. At the 1% significance level, a Gp or G1 higher than 2.75 would indicate an outlier. As can be seen in Table 6, there were no outliers when using this test, which was consistent with the results of the Mandel's 'h' statistic.

All of the averages from the 14 laboratories are listed in Table 7. The younger rice samples had higher pH values. The standard deviations of repeatability for each age level ranged between 0.032 and 0.077, with an average of 0.048. The standard deviations for reproducibility for each age level ranged between 0.127 and 0.192, with an average of 0.160. This indicates that the test is reliable when run in different laboratories.

When the rice pH values were combined with storage time as shown in Figure 5, an obvious linear correlation was revealed between pH 6.2 and pH 6.8. In the standard colour chart, the colours changed sensitively with 0.1 changes in pH between pH 6.4 and 6.8. When the pH of rice was less than 6.2, the rice was rancid and beyond quality specification. However, the method is very sensitive for rice pH higher than 6.2, which means that this rapid method is good for rice quality control.

4. Conclusion

In conclusion, the results show that the dye colour chart method is acceptable and highly comparable to the conventional pH meter method. Training could improve method performance and reduce variation.

Acknowledgements

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Table 5. The σ chart test for outliers.

Laboratory	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
Z	0.000	0.000	0.110	0.000	0.000	0.000	0.000	0.000
A	0.089	0.000	0.000	0.000	0.055	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.110	0.110	0.110	0.089	0.045	0.110	0.110
D	0.110	0.110	0.089	0.089	0.000	0.000	0.089	0.000
E	0.045	0.000	0.055	0.045	0.000	0.045	0.045	0.045
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.089	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
K	0.045	0.055	0.045	0.055	0.055	0.084	0.114	0.122
L	0.000	0.000	0.000	0.000	0.000	0.268	0.000	0.000
M	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
upper limit	0.133	0.143	0.161	0.152	0.102	0.250	0.159	0.147
lower limit	0	0	0	0	0	0	0	0

Table 6. Grubb's test for outliers.

	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
X_{max}	6.20	6.40	6.40	6.50	6.70	6.88	7.20	7.40
X_{min}	5.80	5.80	5.80	6.00	6.14	6.32	6.66	6.70
avg	6.08	6.18	6.16	6.23	6.43	6.58	6.99	7.13
std	1.32	1.09	0.83	0.59	0.39	0.21	0.17	0.29
single high, Gp	0.09	0.20	0.29	0.45	0.70	1.45	1.22	0.94
single low, G1	0.21	0.35	0.44	0.39	0.73	1.25	1.96	1.48

For p=14: G=2.755 at the 1% significance level (outliers) and G=2.507 at the 5% significance level (stragglers).

Table 7. Means, repeatability and reproducibility standard deviations for all age levels.

	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
n	5	5	5	5	5	5	5	5
p	14	14	14	14	14	14	14	14
Mean pH	6.079	6.179	6.161	6.231	6.426	6.579	6.993	7.129
$S^2(rj)$	0.0017	0.0019	0.0026	0.0024	0.0010	0.0059	0.0025	0.0021
$S^2(dj)$	0.074	0.138	0.123	0.091	0.094	0.111	0.156	0.176
$S^2(Lj)$	0.014	0.027	0.024	0.018	0.019	0.021	0.031	0.035
$S^2(Rj)$	0.016	0.029	0.027	0.020	0.020	0.027	0.033	0.037
S_r	0.041	0.044	0.051	0.049	0.032	0.077	0.050	0.046
S_R	0.127	0.171	0.163	0.141	0.140	0.164	0.182	0.192

Abbreviations used: $S^2(rj)$ = repeatability variance; $S^2(dj)$ = between-laboratory standard deviation; $S^2(Lj)$ = between-laboratory variance ($S^2(Lj)=(S^2(dj)-S^2(rj))/n$); $S^2(Rj)$ = reproducibility variance; S_r = repeatability standard deviation; S_R = reproducibility standard deviation.

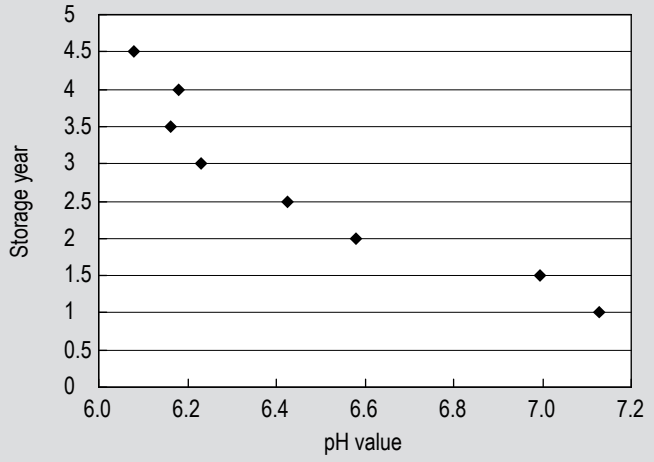

Figure 5. The correlation between storage time and pH values of rice kernels.

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