Diversity of Physicochemical Properties of Different Rice Varieties Produced in Regions of Hokkaido, Japan through Eight Years

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A combination of physical and chemical properties defines the rice quality. During grain filling, kernel location in the panicle and ambient air temperature influence its physicochemical properties thereby affecting the quality of rice. Here, we implemented principal component analysis (PCA) to reduce the dimensionality of 10 characteristics of 719 harvested rice samples, comprised of 10 varieties produced from 2010 to 2017 in regions of Hokkaido, Japan, into latent variables PC-1 and PC-2, which explained 69.7% of the total variance. The model increased the interpretability of the relationship among physicochemical properties of brown rice and temperatures during grain filling in a visually intuitive manner. Results showed that high temperatures in August lowered the quality of samples produced in some regions in 2013 and 2016 by increasing the percentage of immature kernels and protein. Also, high temperatures in September increased the quality of samples produced in some regions in 2011 and 2012 by increasing the percentage of mature kernels and decreasing amylose and protein levels. Moreover, in 2015 and 2017, low temperatures in August and September increased amylose levels, which negatively affected rice quality attributes. This information could contribute to the production of high-quality and palatable rice demanded by Japanese consumers.

Keywords: grain filling temperatures, physicochemical properties, principal components analysis, rice quality

INTRODUCTION

Although rice (Oryza sativa L.) production in Japan has remained constant in recent years, per-capita consumption has decreased (Fujibayashi, 2017). Moreover, approximately 66% of Japanese prioritize palatability over price in the rice they consume. Therefore, since Japan has become a more wealthy society, consumers have been demanding higher quality and more palatable rice (Hori et al., 2016; Ohtsubo and Nakamura, 2017).

Rice quality is defined by a combination of physical and chemical properties. Physical properties comprise the grain’s external and structural characteristics (Bhattacharyya, 2011a); meanwhile, chemical properties determine its sensory characteristics after cooking (Siebenmorgen et al., 2013). Therefore, because rice is handled, processed, and cooked before consumption, its physicochemical properties play a major role in influencing its quality (Bhattacharyya, 2011b; Siebenmorgen et al., 2013).

In Japan, physicochemical measurements and sensory testing are used to evaluate rice quality. Physicochemical measurements involve evaluating moisture, protein, and amylose content using near-infrared (NIR) spectroscopy and measuring the percentage of sound whole kernels using a visible light (VIS) grain segregator (Kondo and Kawamura, 2013). Sensory testing involves evaluating the taste. Trained panel members taste cooked rice samples and give scores for appearance, flavor, taste, hardness, stickiness, and overall sensory properties (Ohtsubo and Nakamura, 2017).

Rice varieties with lower protein and amylose content, which appear as soft and sticky grains after cooking, are considered very palatable among consumers in Japan and in Northeast Asian countries. Residents in Hokkaido, Japan found milled rice of the Yumepirika variety with an amylose content of < 19% and protein content of < 7.5% and/or amylose content of ≥ 19% and protein content of ≤ 6.8% to be highly palatable (Kawamura et al., 2013). Similarly, brown rice with an average moisture content of 15% and sound whole kernel rate of more than 80% was considered to be of high-quality (Mizuho National Foundation, 2011).

During grain filling, both kernel location in the panicle and ambient air temperature have an enormous impact on rice quality through their influence on the physicochemical properties of rice (Patindol et al., 2015).

Rice grain filling depends on the flowering order of spikelets and is a long process that happens from the top of the panicle downward (Myers McClang, 2004). Therefore, it significantly affects protein and amylose content by influencing kernel thickness, weight, and maturity within the bulk of harvested rice. In general, kernels growing in the upper part of the rice panicle experience a longer growing period. They are therefore more mature, with lower levels of amylose and lower levels of protein, and are consequentlly of higher quality and more palatable than kernels growing in the lower part (Matsue et al., 1994a; 1994b,

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physical properties of each sample were determined in an rice scanned by an ES-1000 VIS grain segregator were obtained from approximately 1,000 kernels of brown mature kernels (Mature) and immature kernels (Immature) the rice kernel surface.

rpm to obtain a high hulling rate and to avoid scratches on hulled using FC2K impeller huller (Otake, Aichi, Japan) to Fukuroi, Shizuoka, Japan). Next, the dried samples were laboratory grain test dryer (Shizuoka Seiki Co., Ltd., approximately 15 of the initial moisture content using a

Thus, it is evident that kernel location in the panicle and ambient air temperature during grain filling affect rice quality. However, no detailed information is available on the variations in the physicochemical properties of harvested rice produced from 2010 to 2017 in various regions of Hokkaido, Japan, depending on the variety, production region and year, as well as ambient temperature during grain filling. Such information would contribute to the production of the high quality and palatable rice demanded by Japanese consumers.

MATERIALS AND METHODS

Rice samples
A total of 719 rough rice samples were collected from different rice-producing regions of Hokkaido, Japan, between 2010 and 2017. They included Hidaka, Hiyama, Ikuri, Ishikari, Kamikawa, Oshima, Shiribeshi, and Sorachi regions. The sample set comprised ten non-waxy Japonica rice varieties, namely Daichinohoshi, Fukkinoko, Hoshimaru, Hoshinoyme, Kiraran-397, Kitakurin, Nanatsuboshi, Oborozuki, Sorayuki, and Yumepirika.

Sample preparation
The rough rice samples collected were dried to approximately 15% of the initial moisture content using a laboratory grain test dryer (Shizuoka Seiki Co., Ltd., Fukuroi, Shizuoka, Japan). Next, the dried samples were hulled using FC2K impeller huller (Otuke, Aichi, Japan) to obtain brown rice. The impeller huller was set to 3,500 rpm to obtain a high hulling rate and to avoid scratches on the rice kernel surface.

Methods of measurement and devices
Collection of physical property data
Physical properties, such as estimated percentages of mature kernels (Mature) and immature kernels (Immature) were obtained from approximately 1,000 kernels of brown rice scanned by an ES-1000 VIS grain segregator (Shizuoka Seiki Co., Ltd., Fukuroi, Shizuoka, Japan). The physical properties of each sample were determined in an average of five repeats.

Collection of chemical property data
Amylose content (Amylose) of milled rice, was determined by iodine colorimetry using a Solid Prep III auto-analyzer (Bru-Link, Norderstedt, Germany) following the protocol of Williams et al. (1958) with modifications by Inatsu (1988) Absorption of the amylose-iodine complex was measured at 620 nm wavelength with a spectrophotometer comprising an auto-analyzer. The amylose content was quantified against a calibration curve. The Hoshinoyume variety (moisture content 13.1%, amylose content 21.1%) and Hakuchoumochi glutinous rice (amylose content 0%) grown in Hokkaido were used as standards.

Amylose content was calculated for an average of 3 measurements per sample and expressed as a percentage of the total starch in milled rice using an auto-analyzer (%, MR, Auto-analyzer).

Protein content (Protein) of milled rice was determined by NIR spectroscopy using an Omega Analyzer G BR-5000 NIR transmittance spectrometer (Shizuoka Seiki Co., Ltd., Fukuroi, Shizuoka, Japan).

The NIR calibration model was developed by the chemometric technique partial least squares regression (PLS) based on Kjeldahl reference chemical method.

Temperatures during grain filling period
The months of August and September represent the rice grain filling period in Hokkaido, Japan. Accordingly, average temperature and average minimum and maximum temperatures within a day (24 hours) in August (Aug_ave, Aug_min, and Aug_max) and September (Sep_ave, Sep_min, and Sep_max), respectively, were used in this study. Average temperatures for each region and year of production were collected from the Japan Meteorological Agency homepage: http://www.jma.go.jp/jma/index.html.

Data analysis
The explorative chemometric technique principal component analysis (PCA) within the statistical software Unscrambler version 10.3 (CAMO software, Oslo, Norway) was used to process the data.

PCA was performed to reduce dimensionality and increase the interpretability of the relationship among physicochemical properties of brown rice and temperatures during grain filling considering varieties and both production region and year in a visually intuitive manner. It involved projecting the information from the original multivariate data (10 characteristics of 719 rice samples) onto a smaller set of latent variables called principal components (PCs) (Bro and Smilde, 2014; Jolliffe and Cadima, 2016).

The data set was standardized before being processed. The standardization was carried out by mean centering and scaling the data. Scaling was carried out by multiplying each X-variable with the inverse of the standard deviation of the corresponding variable (USD). As a result, each standardized X-variable got the same variance and could not dominate over another and thus influence the model (Bro and Smilde, 2014). The model was validated by cross-validation with 8 segments, where a segment represented one production year.
Physicochemical properties of rice and temperature data

Average values of physicochemical properties of rice varied somewhat by year of production and among the years of production (Table 1). The difference in soil type and fertility, in rice cultivar and panicle characteristic, and mostly in ambient air temperature and humidity during the kernel development period affected the physicochemical properties values among the production regions and the production years (Kinoshita et al., 2017).

Reduction of the dimensionality of the multivariate data of rice produced in Hokkaido

The first four PCs accounted for 87.7% of the total data variation. However, the first two PCs (PC-1 and PC-2) explained 69.7% of the total variance in the original multivariate data (10 characteristics of 719 rice samples). Consequently, PC-1 and PC-2 enabled us to easily explore the data.

PC-1 explained 50.4% of the total variance which represented the maximum variance in the data. PC-1 correlated positively with protein content (Protein) and the percentage of mature kernels (Mature) as well as with average temperatures in August (Aug_ave, Aug_min, and Aug_max) and September (Sep_ave, Sep_min, and Sep_max), but correlated negatively with amylose content (Amylose) and the percentage of immature kernels (Immature) (Table 2). Moreover, PC-1 correlated highly significantly (P < 0.05) with the percentage of mature kernels and average temperatures in August and September. It also correlated significantly (P < 0.05) with Amylose and the percentage of immature kernels but did not correlate significantly with Protein (Table 2). Accordingly, PC-1 was primarily a measure of temperature, specifically the daily average (Sep_ave) and daily average minimum temperatures in September (Sep_min) (Table 2).

PC-2, which accounted for 19.3% of the data variation, correlated positively with Protein, percentage of immature kernels, and average temperatures in August (Aug_ave, Aug_min, and Aug_max), but correlated negatively with Amylose, the percentage of mature kernels, and average temperatures in August and September (Sep_ave, Sep_min, and Sep_max) (Table 2). Also, PC-2 correlated highly significantly (P < 0.01) with Protein and the percentage of mature and immature kernels but did not correlate significantly with average temperatures in August and September (Table 2). Accordingly, PC-2 was primarily a measure of maturity (Mature and Immature) (Table 2).

Based on the observed variance in the data and the significant correlation values discussed above, we concluded that the model is primarily a measure of PC-1. Consequently, the daily average (Sep_ave) and daily average minimum temperatures in September (Sep_min) indicated the highest effect on the model. Accordingly, daily average temperature in September had a higher effect on physicochemical properties of brown rice.

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**Table 1** Summary of physicochemical properties of rice and temperature information per year of production.

| Production year | n | Amylose, % | Protein, % | Mature, % | Immature, % | Aug_ave, °C | Aug_min, °C | Aug_max, °C | Sep_ave, °C | Sep_min, °C | Sep_max, °C |
|-----------------|---|------------|------------|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 2010            | 88| 16.5       | 8.1        | 60.4     | 30.1        | 23.0        | 19.1        | 27.9        | 17.8        | 12.9        | 23.3        |
| 2011            | 107| 18.0       | 7.9        | 67.5     | 26.0        | 21.9        | 17.6        | 27.2        | 17.6        | 13.4        | 22.4        |
| 2012            | 41 | 15.6       | 7.3        | 74.7     | 23.1        | 22.1        | 18.3        | 26.8        | 20.7        | 16.7        | 25.7        |
| 2013            | 95 | 18.2       | 8.1        | 59.5     | 27.4        | 21.9        | 18.2        | 26.7        | 17.0        | 12.2        | 22.0        |
| 2014            | 92 | 19.4       | 7.6        | 52.9     | 31.7        | 21.0        | 16.8        | 25.9        | 15.7        | 10.6        | 21.8        |
| 2015            | 103| 19.8       | 7.7        | 63.8     | 26.4        | 21.1        | 17.2        | 26.0        | 16.6        | 12.2        | 21.8        |
| 2016            | 100| 18.2       | 8.0        | 61.6     | 28.9        | 22.5        | 18.1        | 27.6        | 17.8        | 13.5        | 22.9        |
| 2017            | 92 | 21.0       | 7.2        | 36.5     | 31.9        | 20.0        | 16.4        | 24.5        | 15.9        | 10.6        | 21.7        |
| Mean            | 18.3| 7.7       | 62.1       | 28.2     | 21.7        | 17.7        | 26.6        | 17.4        | 12.7        | 22.7        |
| Range           | 15.6–21.0| 7.2–8.1 | 52.9–74.7 | 23.1–31.9| 20.0–23.0 | 16.4–19.1 | 24.5–27.9 | 15.7–20.7 | 10.6–16.7 | 21.7–25.7 |

**Table 2** Correlation loadings of physicochemical properties of rice and temperature during grain filling period for principal components PC-1 and PC-2.

| Physicochemical properties and temperature | PC-1 (50.4% of total variance) | PC-2 (19.5% of total variance) |
|-------------------------------------------|---------------------------------|---------------------------------|
| Amylose                                   | −0.53 *                         | −0.30                           |
| Protein                                   | 0.17                            | 0.66 **                         |
| Mature                                    | 0.47 **                         | −0.76 **                        |
| Immature                                  | −0.33 *                         | 0.80 **                         |
| Aug_ave                                   | 0.59 **                         | 0.25                            |
| Aug_min                                   | 0.85 **                         | 0.19                            |
| Aug_max                                   | 0.72 **                         | 0.26                            |
| Sep_ave                                   | 0.92 **                         | −0.08                           |
| Sep_min                                   | 0.93 **                         | −0.11                           |
| Sep_max                                   | 0.84 **                         | −0.06                           |

Significance level: *P < 0.05 and **P < 0.01
We developed a two-dimensional plot of loadings for PC-1 and PC-2 to analyze the relationship among physicochemical properties and temperatures during grain filling in a visually intuitive manner (Fig. 1). Within the plot of loadings, we considered properties and/or temperatures close to each other, and those located in the same quadrant, to have high positive correlation, and we considered properties and/or temperatures in diagonally opposed quadrants to be negatively correlated (CAMO Software As, 2014).

Average temperatures in August (Aug_ave, Aug_min, and Aug_max), much like average temperatures in September (Sep_ave, Sep_min, and Sep_max), correlated positively amongst themselves. In addition, average temperatures in August and September correlated positively with each other (Fig. 1).

Protein correlated positively with average temperatures in August, albeit mostly to the daily average maximum in August (Aug_max) (Fig. 1). Protein content also correlated positively with the percentage of immature kernels (Immature) and negatively with the percentage of mature kernels (Mature) (Fig. 1).

Amylose content (Amylose) correlated negatively with average temperatures in August and September (Fig. 1).

We created the bi-plot for scores of 719 rice samples and loadings of physicochemical properties of brown rice and temperature during grain filling for PC-1 and PC-2 considering rice varieties produced in Hokkaido. In the plot, each marker represents a sample within a variety and each arrow represents a property and/or temperature (Fig. 2).

Some Oborozuki samples correlated positively mostly with average temperatures in August and some Yumepirika samples mostly with average temperatures in September. Both groups of samples correlated negatively with Amylose (Fig. 2). This is an expected result considering that Oborozuki and Yumepirika are the low amylose content varieties of Hokkaido. However, some Oborozuki and Yumepirika samples, as well as those of Nanatsuboshi, Sorayuki, Kirara-397, and Fukkurinko, correlated positively with Amylose (Fig. 2).

Some Daichinohoshi, Kitakurin, Hoshimaru, Fukkurinko, Oborozuki, and Yumepirika samples, which also correlated positively with average temperatures in August, correlated positively with the protein and the percentage of immature kernels. Consequently, they correlated negatively with Amylose (Fig. 2).

Some Nanatsuboshi, Fukkurinko, Yumepirika, and Hoshinoyume samples, which also correlated positively with average temperatures in September, correlated positively with the percentage of mature kernels and negatively with Amylose (Fig. 2). These samples had low levels of
Amylose, Protein, and immature kernels as well as high percentages of mature kernels, and we therefore considered them to be of high quality.

Diversity of physicochemical properties by region of production

We created another bi-plot for scores of 719 rice samples and loadings of physicochemical properties of rice and temperature during grain filling for PC-1 and PC-2 according to the variety of rice. Each marker represents a sample within a variety and each arrow represents a property and/or temperature.

We identified that those Oborozuki and Yumepirika samples that correlated positively with the average temperatures in August and September and negatively with Amylose (described in the previous section), were mostly produced in Oshima and Sorachi as well as in some areas of the Ishikari, Kamikawa, and Shiribeshi regions. Also, those Oborozuki, Yumepirika, Nanatsuboshi, Sorayuki, Kirara-397, and Fukkurinko samples that showed higher level of Amylose (described in the previous section), were...
mostly produced in Kamikawa, Oshima, and Sorachi and some areas of the Iburi region (Fig. 3).

We also identified that those Daichinohoshi, Kitakurin, Hoshimaru, Fukkurinko, Oborozuki, and Yumepirika samples that correlated positively with average temperatures in August and were of low quality based on high protein level and high percentage of immature kernels (described in the previous section), were mostly produced in Sorachi and some areas of the Ishikari, Oshima, and Shribeshi regions (Fig. 3).

Moreover, we identified that those Nanatsuboshi, Fukkurinko, Yumepirika, and Hoshinoyme samples that correlated positively with average temperatures in September and were of high quality based on low levels of Amylose, Protein, and immature kernels and high percentage of mature kernels (described in the previous section), were produced in areas of the Kamikawa, Ishikari, Oshima, Shribeshi, and Sorachi regions (Fig. 3).

**Diversity of physicochemical properties by production year**

We also created a third bi-plot for scores of 719 rice samples and loadings of physicochemical properties of rice and temperature during grain filling for PC-1 and PC-2; in this case, considering the year of production. In the plot, each marker represents a sample within a year of production and each arrow represents a property and/or temperature (Fig. 4). Accordingly, we were able to identify the year of production of each sample within a variety produced in regions described in the previous section as well as its relationship with physicochemical properties and temperature.

We identified that those Oborozuki and Yumepirika samples that correlated positively with average temperature in August and September and negatively with Amylose, and were mostly produced in Oshima and Sorachi and in some areas of the Ishikari, Kamikawa, and Shribeshi regions (described in previous sections), were collected in 2010, 2012, and 2016. However, Oborozuki samples that correlated positively with average temperatures in August were mostly produced in 2010 and a few of them in 2016. Meanwhile, Yumepirika samples that correlated positively with average temperatures in September were mostly produced in 2012 and a few of them in 2016 (Fig. 4).

Moreover, we identified that Daichinohoshi, Kitakurin, Hoshimaru, Oborozuki, and Yumepirika samples that correlated positively with average temperatures in August, were of low quality based on high Protein level and high percentage of immature kernels, and were produced in Sorachi and some areas of the Ishikari, Oshima, and Shribeshi regions (described in previous sections), were mostly collected in 2010 and a few of them in 2011, 2013, and 2016 (Fig. 4).

In addition, we identified that Nanatsuboshi, Fukkurinko, Yumepirika, and Hoshinoyme samples that correlated positively with average temperatures in September, were of high quality based on low levels of Amylose, Protein, and immature kernels as well as high percentages of mature kernels, and were produced in areas of the Ishikari, Oshima, Shribeshi, and Sorachi regions (described in previous sections), were mostly collected in 2011 and 2012 and a few of them in 2015 and 2016 (Fig. 4).
PHYSICOCHEMICAL PROPERTY OF RICE

DISCUSSION

Because the island of Hokkaido is located at the northern limit for rice cultivation, cultivars must be able to adapt to low temperatures and long natural day length during the growing period. Consequently, Hokkaido rice cultivars have been bred specifically to have extremely low photoperiod sensitivity for production on the island (Fujino and Sekiguchi, 2005). They are therefore genetically similar (Kinoshita et al., 2017). Accordingly, physicochemical properties of brown rice and temperature values during the filling period varied somewhat by year of production and among the years of production (Table 1).

The explorative technique PCA allowed us to reduce the dimensionality of 10 characteristics of 719 rice samples onto latent variables PC-1 and PC-2 which explained 69.7% of the total variance and to increase the interpretability of the relationship among physicochemical properties of rice and temperatures during grain filling considering varieties and both production region and year in a visually intuitive manner.

The model revealed that the Nanatsuboshi, Fukkurinko, Yumepirika, and Hoshinoyume samples were of high quality (Fig. 2), produced in areas of the Ishikari, Oshima, Rumoi, Shiribeshi, and Sorachi regions (Fig. 3), mostly in 2011 and 2012 and a few in 2015 and 2016 (Fig. 4). These samples experienced higher average temperatures in September; as a result, they produced a higher percentage of mature kernels, a lower percentage of immature kernels as well as lower protein and amylose content. Matsue et al. (1994a; 1994b); Matsue et al. (2001); Nitta (2010) and Ma et al. (2017) reported similar findings and indicated that mature kernels, which grow in the upper part of the rice panicle and have a longer growing period, had lower levels of protein and amylose than that of kernels growing in the lower part of the rice panicle. Protein content decreases because the molecular weight of glutelin, which is the major storage protein of rice, decreases with grain-fill (Shih, 2004).

Moreover, the lower quality samples were comprised of the Daichinohoshi, Kitakurin, Hoshinmaru, Fukkurinko, Oborozuki, and Yumepirika (Fig. 2) produced in Sorachi and some areas of the Ishikari, Oshima, and Shiribeshi regions (Fig. 3), mostly in 2013 and 2016 and few of them in 2010 (Fig. 4). This was owing to higher average maximum temperatures in August, which caused samples to have a higher percentage of protein content and immature kernels. Accordingly, this indicated that those samples suffered high-temperature injury at the beginning of the grain filling period (Morita et al., 2016). This confirmed the findings of Siebenmorgen et al. (2013) and Patindol et al. (2015).

On the other hand, the model revealed that in recent years, specifically in 2014, 2015, and 2017 (Fig. 4), there was an increase in levels of amylose content in samples of varieties such as Oborozuki, Yumepirika, Nanatsuboshi, Sorayuki, Kirara-397, and Fukkurinko in principal Hokkaido rice producing regions such as Kamikawa, Oshima, and Sorachi and some areas of the Iburi region (Fig. 3). This was because of cool temperatures during the grain filling period (August and September) (Kinoshita et al., 2017). Researchers must consider this in further analysis because it strongly affects rice quality attributes in Japan.

CONCLUSIONS

This study involved an explorative and interpretative analysis of the physicochemical properties of rice produced in various regions of Hokkaido from 2010 to 2017 in a visually intuitive manner. Results indicated that temperature during grain development had an effect on physicochemical properties of rice, and also revealed a relationship among the properties. The model showed that as average temperatures (mostly the daily average maximum) in August increased, there was an increase in protein content and in the percentage of immature kernels as well as a decrease in amylose content. Also, as average temperatures in September increased, there was an increase in the percentage of mature kernels and a decrease in amylose content, protein content, and the percentage of immature kernels. We linked these characteristics to better quality samples.

Based on the higher average temperatures in September, Nanatsuboshi, Fukkurinko, Yumepirika, and Hoshinoymure samples produced in the Ishikari, Oshima, Rumoi, Shiribeshi, and Sorachi regions mostly in 2011 and 2012 and a few in 2015 and 2016, were found to be higher quality samples. Moreover, based on the higher average maximum temperatures in August, Daichinohoshi, Kitakurin, Hoshinmaru, Fukkurinko, Oborozuki, and Yumepirika samples produced in the Sorachi, Ishikari, Oshima, and Shiribeshi regions mostly in 2013 and 2016 and a few in 2010, were found to be of lower quality.

The model also revealed that cooler temperatures during the grain filling period (August and September) in recent years (2014, 2015, and 2017) resulted in an increase in levels of amylose content in samples of the principle varieties produced in the major rice producing regions in Hokkaido. Researchers must consider this phenomenon since it strongly affects rice quality attributes.

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