Evaluation of Color Characteristics of Cross-Sectioned Wheat Kernels

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The color of wheat flour is an important commercial factor and improvement of the color characteristics of kernels is one of the breeding objectives in Japan. Methods were developed to measure color characteristics of both wheat kernels and wheat flour. Wheat kernels were fixed on a newly developed holder with light-curing resin, and a smooth cut-surface was prepared by a programmed cutting of a diamond wheel. Binder-free wheat flour was formed into cylindrical pellets to remove trapped air by repeated compression with a hydraulic press machine. Cut kernels and flour pellets were moistened and analyzed by a two-dimensional colorimetric method. Color changes of both specimens were stabilized after 3 h of moistening. And a comparison of their colorimetric results revealed the possibility of evaluating wheat flour color without milling the kernels. This is useful as an early generation selection method of breeding of wheat because the dissected embryo side retains the germination ability.

Keywords: wheat, color, flour, sectioning, colorimeter

A considerable part of Japanese domestic wheat varieties is milled into flour to produce noodles, and consumers prefer noodles, which have a pleasing brightness and whiteness. Noodles are prepared from the simple ingredients of flour, salt and water. Therefore, the color of the wheat flour is one of the most important factors in assessing commercial value (Miskelly, 1984; Moss, 1971). Many papers have reported the evaluation of wheat flour color (Kent-Jones & Martin, 1950; Patton & Dishaw, 1968; Ram et al., 2002; Shuey, 1975; Voss, 1992; Yasunaga & Uemura, 1962). All millers require white and creamy flour without contamination of particles from bran, because bran contains a reddish-brown pigment of xanthophylls (Markley & Bailey, 1935). The granularity of flour may also have an effect on the color, as the large granules cast shadows giving a gray shade. When flour is dipped into water, the color darkens as the result of the oxidase present. The color of flour has been evaluated by the Pékár color test in the milling trade. The quality of wheat endosperm is the major factor for the color development of noodles. At present, millers and processors measure the quality of endosperms with the Kent-Jones & Martin Flour Color Grader (Kent-Jones & Martin, 1950a), and some other methods (Dowell, 1997, 1998; Gillis, 1963; Ollivar et al., 1992; Wang et al., 1999) have been developed for evaluating the color of kernels or flour. However, no effective preparative methods have yet been developed to give satisfactory results for the two-dimensional analysis of color characteristics of wheat grains. Since differences in wheat endosperm quality play an important role in flour color (Barnes, 1986; Kowata et al., 1999), detailed studies of local variation of color in the kernel are required.

This paper presents, first, a method to study the color characteristics of wheat endosperm using kernel holders and a cutting device made for this purpose. Secondly, analytical methods of color characteristics of both wheat kernels and flour are presented.

Materials and Methods

Wheat samples Wheat samples were harvested in 2001 from a field in the National Agricultural Research Center in Tsukuba, Ibaraki Prefecture. After harvest, the kernels were dried in the air and stored at 4 ℃. Dissected wheat endosperms and flour were stored at 4 ℃.

Preparation of kernel holder A CNC vision measuring system (Quick Vision Pro, Mitsutoyo, Tokyo) was used to calculate the kernel size. This data was used in designing a kernel holder for 2-dimensional colorimetric measurement. A piece of acrylic resin (50×6×10 mm) was fixed on the cutting stage of a machining center (Lemoine, Kern Microtechnik, Murnau-Westried) with a vise, and a kernel holder was manufactured with cutting tools according to the machine programming.

The holder has 10 cavities each in front and back to accommodate 20 kernels (Fig. 1).

Fixation of kernels on the holder Light-cured reinforced glass ionomer cement (GC Fuji II LC, GC, Tokyo) was applied on the surface of the holder cavities. After placing each wheat kernel on the cement, it was cured for 20 s by the irradiation of light-curing apparatus (Candelux, J. Morita, Tokyo).

Preparation of cross-section of endosperm Each holder with 20 kernels was fixed on the stage of computer programmed
cutting instrument (Plant Cutter-NT02, Takachiho Seiki, Tokyo) equipped with a diamond wheel (A5232, Horico, Berlin). The wheel (22 mm in diameter, 0.3 mm thickness, electrodeposited 200 mesh of diamond) rotated at 30,000 rpm. Wheat kernels were cut crosswise with reciprocal movement (100 mm/min) of the wheel. The endosperms were finally ground to a depth of 0.05 mm to make a smooth cut-surface for colorimetric measurement (Fig. 2a). The complementary embryo-side of the kernels fixed on a supporting plate with both sides taped (Fig. 2b) was recovered for the germination test.

**Analysis of color characteristics of wheat endosperm** A two-dimensional colorimeter (CI-1040i, Minolta, Tokyo) equipped with integrating sphere (d254 mm) was used to analyze color characteristics of the wheat endosperm. Color measurement was performed using the CIELAB color space (International Commission on Illumination, 1976). The CIELAB color value was determined using a D_65 illuminating light source and an 8 degree eyesight mode. Calibration of L* was made by the reflection standard white ceramics plate (Ever-Colors, Evers Co., Osaka). The color value was obtained as rectangle pixel unit (0.098×0.114 mm) for two-dimensional analysis and L* values were calculated statistically with the analytical software for two-dimensional analysis (Pixel Analyzer-02, Chihoda Seiko, Tsukuba). Five holders (Fig. 3, b, c, d, e, f) each with 20 cross-sectioned wheat kernels were placed in a protective plastic case (Fig. 3a). L* values of the cut-surfaces of the kernels were measured on the glass plate (40×40 mm) on the integrating sphere of the
two-dimensional colorimeter. The $L^*$ value was affected by the difference of light transmitted between mealy and glassy kernels in the dry state. Therefore, the cut-surface was moistened with distilled water soaked in a paper filter (No. 2, Advantec, Tokyo). The protective plastic case accommodating the holders was placed on a wet filter paper for time-dependent measurement of endosperm color. The color change of the moistened endosperm was measured on the glass plate of the measuring window of the colorimeter.

Preparation of wheat flour and pellets  Wheat was milled to 60% flour extraction from conditioned kernels with a Buhler MLU-202 experimental mill fitted with an automatic feeder at 20°C and 60% humidity. The milling method followed the procedure of the Japan Wheat Flour Institute. It was reported by Kent-

Fig. 6.  Two-dimensional color analysis of 100 wheat endosperms. a, c, e, cross-sectioned endosperm of Bandowase, Toyohokomugi, Norin 61, before moistening. b, d, Bandowase and Toyohokomugi moistened for 24 h. Four images of endosperms are enlarged, and the distributions of lightness chosen in the lines (black, green and red) are shown.
Jones and Martin (1950b) that trapped air interferes with the colorimetric measurement of the wheat flour suspension. Binder-free wheat flour (300 mg) was formed into cylindrical pellets (10 mm in diameter, 3 mm in height) by applying repeated compression pressure (35 MPa) with a hydraulic press to remove the trapped air, thus improving reproducibility in the analyses.

Analysis of color characteristics of wheat flour Nine compressed wheat flour pellets were placed in a measurement holder (Fig. 4). The holder was placed on a wet filter paper to prevent drying of the pellets and time-dependent change of the color was analyzed by the two-dimensional colorimetric method.

Results and Discussion
The shapes and sizes of wheat kernels differ depending on the cultivation conditions and the difference in varieties. Applications of the size-measuring machine and the machining center facilitated designing and producing of the holder for a variety of kernel samples. The holder designed here accommodated 20 kernels, which were fixed securely by light-curing resin. Movement of the cutting wheel was programmed so as to repeat a reciprocal motion, thus preventing deformation of the thin diamond wheel by hard kernels. Cutting speed was restricted to 100 mm/min to reduce the excessive force being applied to the kernels. Cutting scars on the measuring surface lowered the accuracy of reflectance colorimetric measurement. However, the problem was solved effectively by a final polishing (0.05 mm in depth) of the cut-surface of the endosperms. The embryo in the complementary half of the cut kernels retained germination ability (Fig. 5). Furthermore, this study showed the promising possibility of selecting kernels among a large number of hybrids in early generations, because the surface of endosperm can be stained with a dyeing reagent. Figure 6 shows two-dimensional color analysis of 100 wheat endosperms. Four images of endosperms are enlarged, and the distribution of lightness chosen in the lines (black, green and red) are shown. As shown in the color bar, the intensity of $L^*$ was replaced by colors from blue ($L^*$ is less than 45.0) to red ($L^*$ is more than 70). In the study, a clear mapping image of two-dimensional colorimeter was obtained from a smoothly cut-surface of endosperms of the kernels. In the image, a dry mealy kernel (a: Bandowase) showed almost equal $L^*$, whereas dry glassy kernels (c: Toyohokomugi) showed wide distribution of $L^*$. Norin 61 (e) showed a medium distribution. After 24 h of moistening, the uniformity of $L^*$ was increased in mealy kernels (b: Bandowase), while local difference was still observed in glassy kernels (d: Toyohokomugi).

Changes of $L^*$ of wheat endosperm are shown in Fig. 7. The inclination of $L^*$ value gradually became gentle after 2 h moistening and the data stabilized within 3 h. The $L^*$ was shown as an average of the measurement data of each pixel. The numbers of kernels were 40 (31,992 pixels), 40 (33,016 pixels) and 60 (55,002 pixels) in Bandowase, Norin 61 and Toyohokomugi, respectively. The average value and standard deviation of $L^*$ were 66.43±5.06, 64.10±4.78 and 60.81±4.27 in Bandowase, Norin 61 and Toyohokomugi, respectively. This tendency continued until 6 h after moistening. However, after 12 h, the difference of $L^*$ among the specimens decreased with time.

An important purpose for measuring the color of wheat endosperm is to predict the color of wheat flour. The change in the time-dependent brightness of a compressed cylindrical pellet of flour is shown in Fig. 8. Brightness of the pellet decreased rapidly during 1 h and stabilized within 3 h after moistening. The moistened surface of flour pellets became sticky with time, while the order of the brightness was maintained for 12 h. The $L^*$ was shown as the average of 3 cylindrical pellets of Bandowase, Norin 61 and Toyohokomugi. The numbers of pixels were 14,004, 14,787, 19,864 and the average value and standard deviation of $L^*$ were 81.54±1.09, 82.28±1.19 and 83.70±1.43 in Bandowase, Norin 61 and Toyohokomugi, respectively. This tendency continued for 6 h after moistening, whereas endosperms were swollen after 12 h and the difference of color was decreased. In the study, wheat flour was compressed to discharge remaining air of the water in the cylindrical pellets. The optimum analysis time after moistening for the evaluation of wheat flour and endosperm seems to be 3 h, judging from the results of Fig. 7 and Fig. 8.

Recently, it was reported that the $L^*$ value of cross-cut en-
Conclusions

A simplified procedure was developed to analyze the color characteristics of cross-sectioned wheat kernels. A smooth-cut surface of kernels was easily prepared with a newly developed kernel holder and cutting device. Compressed cylindrical flour pellets with a flat plane improved the reproducibility of the color analysis and facilitated the handling of the flour sample. Optimal moistening time of wheat kernels and flour was 3 h at 4°C for measurement of the L* using a two-dimensional colorimetric method. The measurement method with the cross-cut surface of a kernel is simple and accurate enough to document differences in the distribution of color characteristics including wheat color properties. This method is also useful as an early generation selection method of breeding because the dissected embryo-side retains the germination ability.

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