Measurement of the Time-Dependent Appearance of Discolored Spots in Alkaline Noodles by Image Analysis[1]

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Cereal Chem. 76(2):189–194

ABSTRACT

Fresh alkaline noodles prepared from patent flours of the Canadian wheat class Canadian Prairie Spring White (CPSW) AC Karma and AC Vista were characterized by image analysis to numerically evaluate the time-dependent formation of dark areas (spots) on the noodle surface. The system was able to rapidly detect, measure, and characterize such undesirable discolorations, which commonly form on the surface of raw noodles with time. Variations in minimum spot detection size (0.050–0.250 mm^2), in combination with a darkness threshold setting (Δ grey scale units of 2, 5, and 10) were investigated. A linear increase in the number of spots was observed over time for both cultivars with nine combinations of size and darkness. The total number of discolored spots measured was dependent on both detection size and sensitivity (threshold setting). Significant differences ($P < 0.05$) in the number of darkened spots were detected between the two cultivars only after 24 hr. Similarly, significant differences ($P < 0.05$) in size of the spots between the two cultivars was observed at 24 hr. Darkening was most rapid within the first hour for both cultivars, followed by a period of stability before a significant ($P < 0.05$) further increase by 24 hr. Characterization of the darkness distribution indicated significantly different distribution profiles for the two cultivars examined, which was consistent with their noodle brightness (L*) values.

Color and appearance are prime quality requirements for high-quality noodle products. As such, there is normally a preference for using patent flours because they produce noodles that are brighter and display fewer visible bran specks. Wet noodles under alkaline conditions are susceptible to time-dependent discoloration, giving the noodle a darkened appearance. The darkening occurs not only on the overall matrix of a noodle surface but is quite commonly accelerated in localized areas throughout the noodle, resulting in spots that yield a mottled appearance. The reason for the appearance of discolored spots is not totally understood but is believed to originate from loci of contaminating bran particles in the flour. It is a complex phenomenon that involves the enzyme polyphenol oxidase (PPO), phenolic components, alkaline oxidation, and their subsequent auto-oxidation products (Pierpoint 1969; Miskelly 1984; Miskelly and Moss 1985; Moss et al 1986; Singleton 1987; Taylor and Clydesdale 1987; Hatcher and Kruger 1993, 1997; Kruger et al 1994, 1995; Baik et al 1995).

Changes in the localized areas (defined by the discolored spots) on noodle surface with time are difficult to quantify. Indirectly, enzyme levels and reaction products associated with discoloration can be measured chemically, and this relates somewhat to the extent of overall discoloration (Hatcher and Kruger 1997). Additionally, a colorimeter can provide information on overall time-dependent discoloration and the level of colored pigments that are present. Neither method, however, can separate overall darkening from localized areas of accelerated darkening.

Imaging systems capable of detecting bran specks in flour (Evers 1993, Whitworth 1994) and of detecting specks in durum wheat semolina (Harrigan 1995, Symons et al 1996) have been reported. In our studies, we plan to apply image analysis to all aspects of color and appearance in noodles. The objective of the present study was to evaluate this technique for the specific measurement of spots in raw noodles and the changes associated with these spots, which occur independently of the noodle's background matrix color change over time.

METHODS

Flours

Two cultivars, AC Karma and AC Vista, from the Canadian Prairie Spring White (CPSW) wheat class were milled on the Grain Research Laboratory pilot mill using a commercial flow (Fajardo et al 1995). Individual streams were composited on the basis of increasing ash to yield a 60% patent flour. Characteristics of the flours used in this study are listed in Table I.

Analytical Methods

Protein content (%N × 5.7) was determined with a combustion nitrogen analyzer (CNA) (model FP-248, Leco Corp., St. Joseph, MI) calibrated with ethylenediaminetetraacetic acid (EDTA). Ash content, farinograph, moisture, and starch damage were determined by Approved Methods 08-01, 54-21, 44-15A, and 76-30A, respectively (AACC 1995). The PPO levels were determined by the method of Hatcher and Kruger (1993). Flour color was determined using a color grader (Series IV, Satake) that measures relative reflectance of a flour-water slurry. Results are standardized to the Satake international scale where the lower the number, the brighter the flour.

Noodle Preparation

Noodles were prepared on three separate days using the method previously described by Kruger et al (1994). A 1% *kansui* solution (9:1 potassium and sodium carbonate) was added to 200 g of flour in a mixer (N50, Hobart Canada, North York, ON) over a 30-sec interval at the slow speed setting to achieve a final water absorption of 32%. The material was mixed for an additional 4.5 min (Kruger et al 1994). The crumbs were sheeted on a laboratory noodle machine (Ohtake, Tokyo, Japan) with an initial gap setting of 3.00 mm. Two passes were made at this setting, with the noodle sheet being folded between passes to ensure homogeneity. A representative section of the noodle sheet (25 cm long) with reproducible weight was used in the subsequent sheeting passes. Seven sheetings with gap settings of 3.00, 2.55, 2.15, 1.85, 1.57, 1.33, and 1.10 mm were made. Total force of individual sheetings was measured using an analog-digital board (Labmaster DMA, Scientific Solutions, Solon, OH) interfaced to a personal computer using software (Labtech Notebook, Laboratories Technologies, Wilmington, MA) to ensure reproducibility between the triplicate preparations.

The noodle sheet was cut into two pieces with one piece being used for spectrocolorimetric color measurements and the second piece being used for image analysis measurements.

Noodle Color Measurement

Noodle color was measured with a spectrocolorimeter (Labscan II, HunterLab, Reston, VA) equipped with a D65 illuminant using the (CIE 1976) L*, a*, and b* color scale. Measurements were made in triplicate at two random locations on the surface for each sample. For fresh alkaline noodles, high values of L* (brightness)

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Publication no. C-1999-0215-03R.

are preferred, with consumers showing a preference for high to moderate *b** (yellow) values. Extremes on either side of the *a** scale (negative showing increasing green levels; positive showing elevated red levels) are considered deleterious.

Image Analysis

Noodle images were captured using a color camera (CD-950, Sony of Canada, Willowdale, ON) attached to a macroscope (model M-8, Wild Leitz Canada, Willowdale, ON). The red-green-blue signal from the camera was interfaced to the computer through a framegrabber and software (Matrox Meteor and Intellicam v. 2.0, Matrox, Montreal, Canada). An 8-Mb video card (Matrox Millenium) was used to allow 32-bit color display of the noodle surface. Computer control was provided by a Pentium-based (200 mHz) computer with 128 Mb RAM.

A ring lamp located around the macroscope lens supplied lighting, through fiber optics from a 3,200 K lamp (model CLS 150, Leica AG, Heerbrugg, Switzerland). Illumination levels were controlled by adjusting the camera gain to a constant value from a Kodak #3 grey scale (Eastman-Kodak, Rochester, NY). This target was also imaged and stored daily for use during image processing to correct for minor daily variation in the image capture system. Reproducible spatial scaling was ensured by the daily capture of a metal washer (7.15 mm) image, previously measured with a micrometer to yield a grey scale factor of 0.0250 mm/pixel.

Images were captured from six different areas on each noodle sheet using a grid-positioning system developed for this method. This positioning system ensured that the noodle was relocated exactly the same for each series of measurements and that the same six regions were measured at each time period. The camera-macroscope complex was located within a self-enclosed cabinet to prevent interference from overhead lighting. The noodle sheet was stored in a plastic box at 25°C and ≈95% rh, except during photography. Each image represented a 1.5- × 1.1-cm section of the noodle surface for a total of 9.9 cm^2.

Individual images were analyzed by in-house software developed in KS400 software (Carl Zeiss Vision, Eching Germany). Before

TABLE I
Proximate Analysis of Flour Samples (14% mb)

	AC Vista	AC Karma
Protein, %	10.9	10.4
Ash, %	0.38	0.43
Color	–3.8	–4.4
Starch damage	8.9	8.0
Moisture, %	14.0	14.0
Farinograph measurements		
Absorption, %	61.8	60.0
Development time (min)	6.25	2.25
Mixing time (BU)	25	90
Stability (min)	22.5	2.5

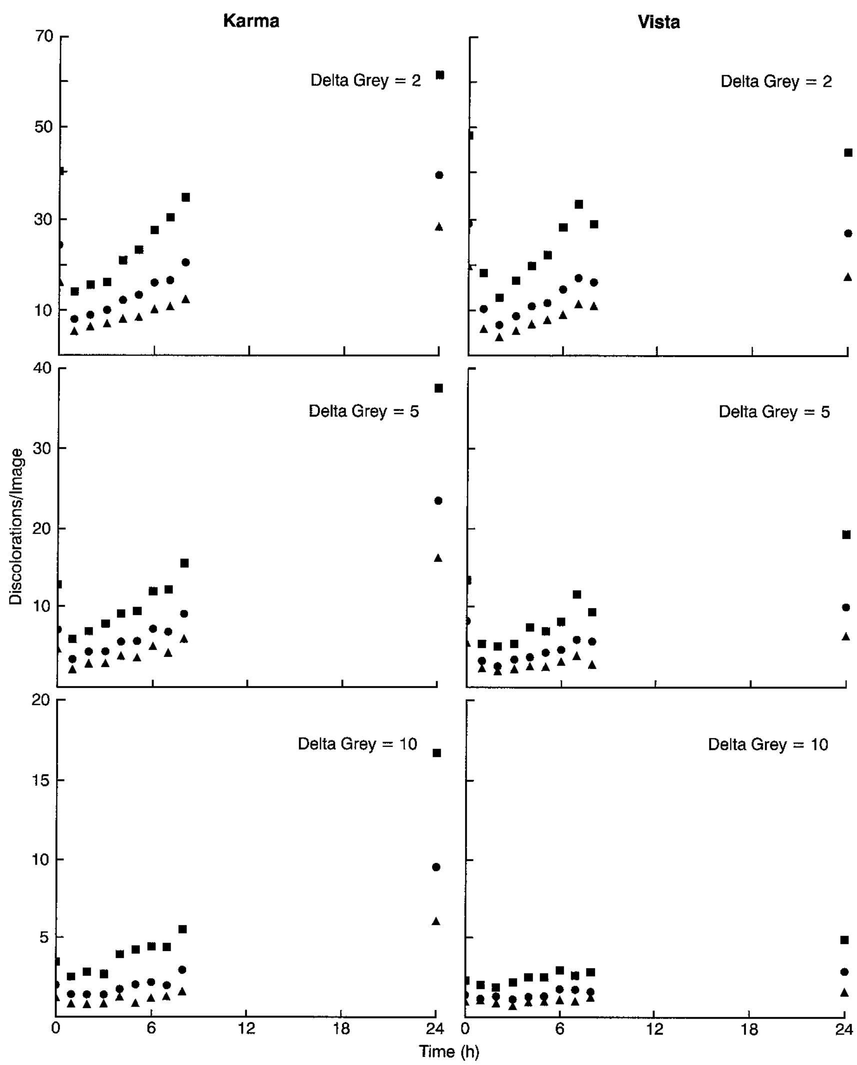

Fig. 1. Comparison between cultivars AC Karma and AC Vista of the average number of detectable spots within a 1.65 cm^2 image of alkaline noodles over time. Minimum size threshold: 5 (■), 10 (●), 15 (▲) pixels at Δ grey levels of 2, 5, and 10.

analysis, each image was corrected for minor variations in the image capture system using the white background image captured daily. Preliminary experimentation had shown that a white background setting of 140 on the 0–255 grey scale was optimum. The difference between the daily white background image and grey scale value 140 (target level) was determined. To identify areas that were darker than the noodle surface, the noodle image was first corrected for any variation from the target grey value of 140. The surface was then corrected for any variation in brightness within the image itself. The grey values within the image were remapped so that only pixels associated with discolored regions had grey value <255 (pure white). Image segmentation to form a binary mask inverted the grey scale so that background pixels of 255 were translated into grey value 0 (black) and those pixels <255 in value were translated to 255 (white).

Two imaging variables were used during noodle analysis. The first of these, Δ grey, represents a threshold value of darkness that must be exceeded for a discolored region to be identified. This factor was applied during the formation of the binary mask when the image grey values were remapped. Δ Grey values of 2, 5, and 10 were used. The second imaging parameter was a value for selecting discolored spots based on size. This parameter was applied to the binary mask to remove those regions smaller than the minimum size parameter. Size values of 5, 10, or 15 pixels were used corresponding to an actual size of 0.003, 0.0063, and 0.0094 mm^2. The software subsequently analyzed the original noodle image using the binary mask to identify areas of localized darkening on the basis of size and darkness.

Statistical Analysis

All statistical analyses (regression, analysis of variance [ANOVA], multivariate analysis of variance [MANOVA], and frequency distributions) were performed with SAS statistical software (version 6.11, SAS Institute, Cary, NC). The general linear model (GLM) procedure with the repeated and polynomial options were utilized for the MANOVA analyses. These options were necessary to effectively interpret the influence of time on the variables.

RESULTS

Δ Grey Parameters and Time of Noodle Aging

The minimum difference in darkness between the spots and the noodle surface was set as a fixed constant referred to as Δ grey. This required that the noodle surface be of a constant brightness. A common problem with standard colorimetric methods that affects the measurement of initial noodle color is the uneven hydration of flour particles at the low 32% water absorption level. This results in a mottled surface that disappears with time as uniform hydration occurs. The imaging software was developed to adjust for this unevenness in color on the surface of the noodle so that noodle spots were identified relative to the grey level brightness of the noodle

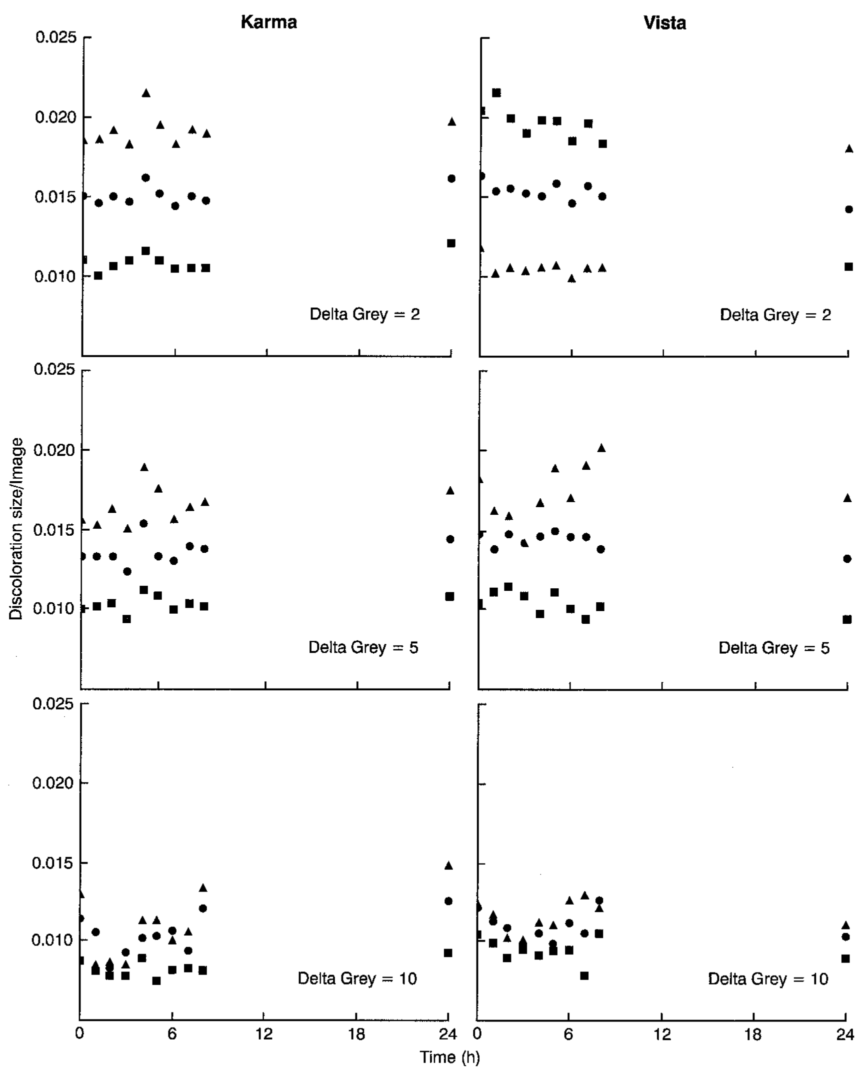

Fig. 2. Comparison between cultivars AC Karma and AC Vista of the average size of a discolored spot within a 1.65 cm^2 image of alkaline noodles over time. Minimum size threshold: 5 (■), 10 (●), 15 (▲) pixels at Δ grey levels of 2, 5, and 10.

surface immediately surrounding them. The influence of the unevenness in noodle surface darkness could not be totally eliminated when the noodle was imaged immediately following preparation. At a Δ grey value of 2 (which detected spots just visibly different from the noodle background), it allowed small surface variations to be detected and counted as spots. For this reason, statistical analyses do not include the initial readings which contained a disproportionate amount of variance.

For both cultivars tested, the number of dark spots counted by the imaging method decreased with increasing Δ grey values (Fig. 1). As the Δ grey value increased, the differences between the darkness of the noodle surface matrix and the spots increased. The number of spots counted increased continuously with time for all samples at each of the three Δ grey levels. For the lowest Δ grey value (Δ grey 2), as many as 62 ± 3.45 darkened spots per noodle image, corresponding to 37.6/cm^2, were detected within the noodle after 24 hr for Karma (Fig. 1A) and 45 ± 2.51 darkened spots for Vista (Fig. 1D). The high number of spots counted by the imaging system at low Δ grey values shows the ability of the imaging system to detect and measure spots that differ only slightly in darkness from the noodle surface in an objective and reproducible manner. Maximum variation in quantitation occurred at Δ grey 2 (minimum size 5) with an average standard deviation of 3.45. Minimum variation occurred at Δ grey 10 (minimum size 15) with an average standard deviation of 0.28.

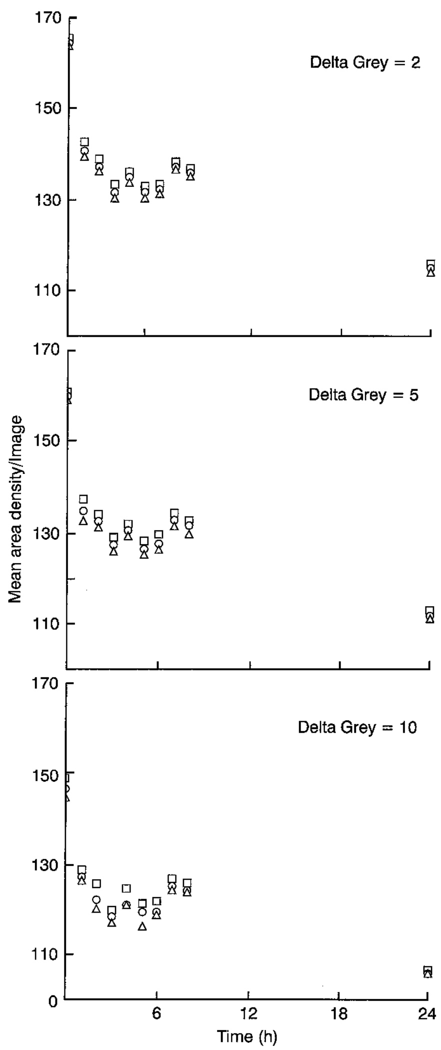

Fig. 3. Changes in mean densities for areas of accelerated darkening over time for the cultivar AC Karma. Minimum size threshold: 5 (□), 10 (○), 15 (Δ) pixels at Δ grey levels of 2, 5, and 10.

A strong linear relationship ($P < 0.05$) was also observed between aging time (to 24 hr) and the increasing number of spot discolorations using Δ grey levels of 5 and 10 for both Karma (Fig. 1B and C) and Vista (Fig. 1E and F). Examination of an 0–8 hr period for Karma showed a strong relationship (r^2 = 0.75–0.99) for all combinations of Δ grey value and size. For Vista, these relationships were more varied (r^2 = 0.26–0.89). Over 8 hr, the lowest correlations were found at the extremes of Δ grey 10 (minimum size 15) in both cultivars. Increasing Δ grey values resulted in a corresponding significant ($P < 0.05$) decreases in the number of spots detected. Furthermore, there was a highly significant (P = 0.0001), interaction effect between time and Δ grey value, indicating that both factors contribute to the number of discolored spots detected.

Effect of Size Limits on Spot Numbers

For each Δ grey value, the size threshold for detecting discolored spots varied from 5 to 10 or 15 pixels. As the size threshold increased, the number of spots detected decreased (Fig. 1). This change in size threshold, however, did not change the linear relationship between number of darkened spots and time, although there was a significant difference ($P < 0.05$) in the number of darkened spots detected between size thresholds. At Δ grey 2 for example, increasing the size threshold to the 10 or 15 pixel limit reduced the number of spots detected at 24 hr to 40 and 28 spots per area imaged for Karma and 24 and 17 spots per area for Vista, respectively (Fig. 1A and D). The number of spots were also significantly influenced (P = 0.0001) at the different threshold levels by both time and Δ grey value, confirming that both factors influence the number of spots detected.

Δ Grey Values and Detection Size Limit Variations Influence on Comparative Noodle Speckiness

When Karma is compared to Vista, a significant difference in the number of detectable spots was found only at 24 hr (P = 0.001) (Fig. 1) for all combinations of Δ grey values and size thresholds. This indicates that the time at which assessment is made is particularly important when comparing noodles prepared from different wheat flours. Although the general increase in spots detected over time for Karma was also present in the Vista series, the increase in numbers of spots was not as large as that found for Karma. Regression analysis of the mean from triplicate replicates for each cultivar under the respective combinations of Δ grey and size threshold showed a minimum value of r^2 = 0.92 for Karma, while for Vista the range was r^2 = 0.81–0.96.

Noodle Aging Time and Size of Darkened Spots

Although increasing in number with time, the actual areas of the individual darkened spots, determined under varying Δ grey and size threshold combinations, remained relatively constant over a 24-hr time period for both Karma and Vista (Fig. 2). As anticipated, the size-rejection threshold values significantly (P = 0.0001) influenced the mean area of the observed discolorations in both Karma and Vista. As the size threshold increased, darkened spots with an area smaller than the threshold limit were simply rejected, causing an increase in mean area of the darkened spots. For a Δ grey 2 in Karma (Fig. 2A), the average area of a darkened spot was 0.019 mm^2 at a 15 pixel threshold value compared to 0.015 and 0.011 mm^2 for the 10 and 5 pixel levels, respectively. In each case, the coefficient of variation was <5.5%. Furthermore, increasing the Δ grey value (2, 5, and 10) significantly increased (P = 0.0001) the average area for each size-rejection threshold studied. No significant difference was detected in the size of discoloration between the two cultivars as this parameter was imposed by the imaging system.

Changes in Darkness of Spots with Noodle Aging

The image analysis system provides the capability to characterize the degree of darkness, computed as a mean grey value of the pixels for each of the individual discolored spots on the noodle surface as it aged with time. Lower measured values indicate darker spots. Over the 24-hr time period that the noodles were examined, significant changes in mean darkness of the areas were found (Fig. 3). The largest change in darkening was noted at 1 hr, probably as a result of continued dispersion of bound water within the noodle. Following this initial rapid change there was a relatively stable 8-hr period, culminating in significantly ($P < 0.05$) darker spots on the noodle surface by 24 hr.

Differences in mean intensities of darkening were found between Karma and Vista by 3 hr ($P < 0.05$) of noodle storage, with specks of Karma appearing darker for each Δ grey and size threshold combination (results not shown). Such darkness was not significantly influenced by changing the minimum size threshold limit. With increasing values for Δ grey, the mean intensity of spot darkening significantly increased ($P = 0.0001$) for both cultivars. This indicates a significant range of darkness within individual discolored spots because with higher values for Δ grey, those spots appearing lighter are not detected and measured (Fig. 3).

Intensity Differences of Dark Spots Between Cultivars

Very profound differences were found in the relative distribution in the darkness intensities of the areas of discoloration in the noodles made from the two wheat cultivars examined in this study (Fig. 4). Comparisons made between Karma and Vista (Fig. 4A) on noodles analyzed after 2 hr of aging at Δ grey 2 show that over half of the specks (AC Karma [58.8%], AC Vista [65.1%]) have a darkness value of ≥140 (larger values indicate lighter colored speck) for both cultivars. For AC Karma, this percentage declined to 39.5% at Δ grey 5 and further decreased to 7.8% at Δ grey 10. For AC Vista, the corresponding distributions had values of 43.8 and 21.2%, respectively.

Comparing the two time periods, distributional differences in relative intensities of the spots changed with time for both cultivars and become more definitive (Fig. 4D–F) at 24 hr. Thus, the spots for AC Karma were significantly darker ($P < 0.05$) than those for AC Vista. By 24 hr (Fig. 4D), 36.8% of the spots in AC Vista were located in the lighter region at ≥130 at Δ grey 2. AC Karma had no spots in this lighter region. Even at Δ grey 10, AC Vista maintained 28.7% of its spots in the light range. At 24 hr, AC Karma had no portion of its specks in the lighter categories at any Δ grey value. Furthermore, AC Karma displayed a much narrower distribution pattern than Vista at all Δ grey values.

These findings were consistent with the observed differences in noodle brightness (L^*) over time (Table II). Although both Karma and Vista decreased in brightness by the same extent (9 units) over the 24-hr period, the Vista samples were brighter at each time period examined. At 24 hr, the significantly darker Karma regions and narrower density distributions were also manifested in the observed shift in redness (a^*) displayed by Karma (+0.62) versus Vista (+0.22) (Table II).

Time-dependent changes in mean darkness of the spots as well as changes in distributions of darkening between the two cultivars are believed to be the direct result of chemical changes that occur in the noodle as a result of hydration and, in particular, the level of activity of the enzyme PPO. Differences observed between these two cultivars, however, can not be attributed to enzyme levels. Analysis of the AC Karma patent flour for PPO detected only 4 nmol of O_2/g/min of activity while the corresponding Vista flour with particles with lighter spots, yielded a higher, but not detrimental level, of 48 nmol of O_2/g/min of activity. The role of the enzyme PPO and phenolic compounds are factors strongly believed to be responsible for the formation of discoloration in noodles (Hatcher and Kruger 1997). PPO has also been associated with bran com-

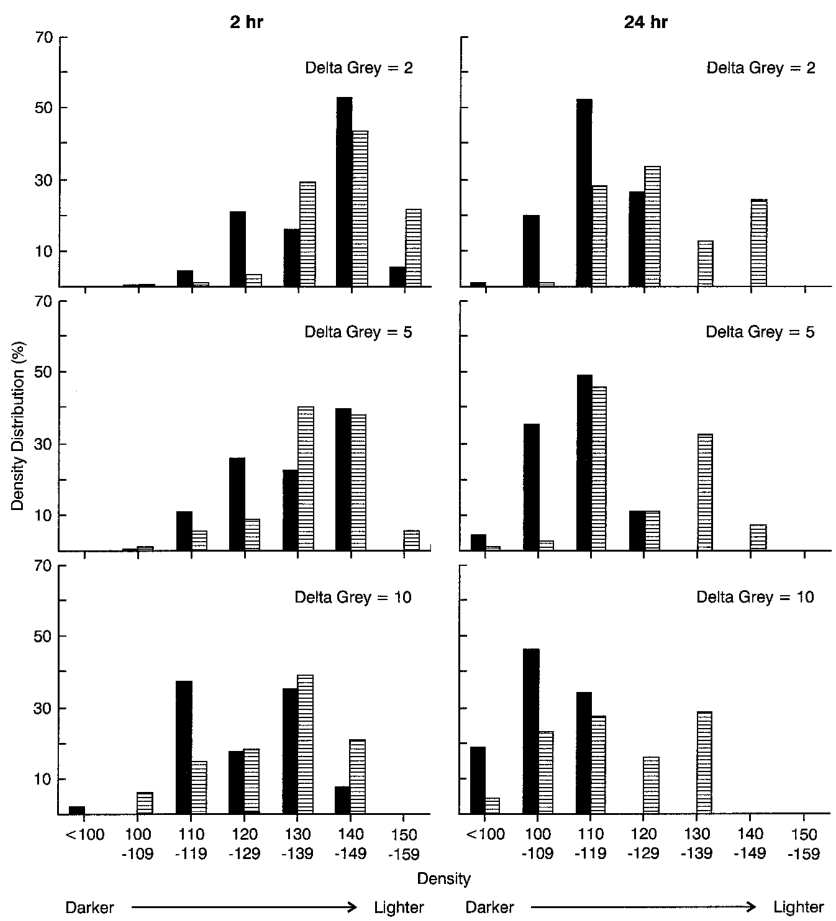

Fig. 4. Profiles of darkness distribution of specks for cultivars AC Karma (solid) and AC Vista (striped) at 2 and 24 hr at Δ grey levels of 2, 5, and 10.

TABLE II
Color Analyses of Alkaline Noodles Over Time

Color	AC Vista				AC Karma			
	0 hr	2 hr	24 hr	Δ 24 hr	0 hr	2 hr	24 hr	Δ 24 hr
L^*	87.69	83.80	78.64	–9.05	86.12	82.69	77.09	–9.03
a^*	–0.80	–0.82	–0.58	0.22	–0.43	–0.46	0.19	0.62
b^*	21.01	25.51	27.43	6.42	22.99	27.10	28.71	5.72

[a] L^* = degree of lightness, a^* = red-green, and b^* = yellow-blue.

ponents (Marsh and Galliard 1986) and highly correlated with millstream ash content (Hatcher and Kruger 1993). The implication from this study is that the observed increase in areas of attenuated noodle darkening with time likely originate from bran contamination in the flour but are not solely enzyme-dependent as previously believed.

CONCLUSIONS

The image analysis method presented is capable of detecting areas of accelerated darkening that form spots on a wet noodle's surface with time. Furthermore, it is able to objectively characterize these darkened spots based on size, darkness intensity, and density distributions. The current image analysis system is both sufficiently sensitive and robust enough to be capable of detecting differences in noodle quality appearance between cultivars within the same wheat class. The method is extremely adaptable, using combinations of Δ grey values and minimum size thresholds for establishing quality control criteria that are exempt from individual bias. The benefits of this system allow for consistent measurement of noodle quality between laboratories and would be extremely applicable to multisite commercial operations concerned with noodle quality control.

ACKNOWLEDGMENTS

We wish to gratefully acknowledge the assistance of R. Desjardin and J. Burrows for milling the wheat, H. Facto and M. J. Anderson for the preparation of the noodles, and E. Basilly, research associate at GRL, who guided us through the statistical analyses.

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[Received June 26, 1998. Accepted August 26, 1998.]