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Image Analysis as a Tool for Estimation of Red Peppers' Color

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ABSTRACT

Red pepper is an excellent source of natural red color. The estimation of color is of great importance, as it is being used for grading red peppers to suit various applications in the food industry. A novel method for quick and easy determination of the color of red peppers by analyzing the color images obtained from a flatbed scanner was developed and validated. ImageJ software was used for measuring the RGB values of the images and the RGB values thus obtained were converted into the industrially accepted American Spice Trade Association (ASTA) color values in red peppers by using an empirical formula. The results were compared with color values obtained through ASTA chemical analytical method. The developed image analysis method was used for the analysis of red peppers with color values ranging from 15 to 154 ASTA units. The image analysis method showed good agreement with the chemical method for color values ranging from 40 to 140 ASTA units. The new method is also fast and easy to adopt and is deemed useful in the field, of processing and storage facilities, where access to sophisticated instrumentation for color estimation and color stability studies is limited.

Keywords: Image Analysis: Flatbed Scanner; Red peppers; ASTA Colour Value; RGB Colour Model

INTRODUCTION

Red pepper is being used both as a flavoring and coloring agent to culinary preparations. The red pepper extracts containing carotenoids, after the removal of pungent compounds like capsaicin, are used in the food industry for imparting shades of red color to products. In the spice industry, the accurate estimation of color is of great importance, as it is used for grading red pepper to suit various applications. A standardized arbitrary unit developed by American Spice Trade Association (ASTA), namely ASTA color units is being used to express the color of red pepper in trade and industry. The ASTA method involves the solvent extraction of coloring compounds from the red pepper followed by the measurement of absorbance using a uv-visible spectrophotometer (ASTA 2004, AOAC 1980).

Non-destructive and chemical-free analytical methods such as image-based estimations are being increasingly preferred to traditional chemical methods for the quality analysis of food products by the industry. Among the image analysis softwares, ImageJ holds a unique position as its source code is openly available to perform necessary modifications or insert add-ons to make it suitable for the required image analysis. ImageJ is a java-based, readily available, open-source, platform-independent, and public domain software developed by the National Institute of Health (NIH), Bethesda, Maryland USA (Rasband 2013). Studies have been reported on the application of ImageJ for color analysis in food products: e.g. apples (Garrido-Novella et al. 2012) and tomatoes (Lana et al. 2006). However, studies establishing a direct correlation between image analysis and standard chemical methods are rather limited. Availability of a non-chemical, image-based estimation technique for color evaluation in red pepper suitable to be used in food processing areas would be useful to monitor and manage the quality and consistency of chilly-containing food products. This study was aimed at the development of a flatbed scanner-based estimation of the color value of red pepper and to validate against the available chemical method of color value estimation.

MATERIALS AND METHODS

Tools and Materials

Red pepper: Samples (40 Nos.) of red pepper (*Capsicum annum*) powder were collected from the local markets of Maharashtra and Gujarat in India. The samples were powdered and sieved through a US No. 40 standard sieve (4760 microns), sealed, and stored at room temperature till analysis. Certified reference red pepper powders for color value were obtained from ASTA (category number 5500012).

Chemicals and Reagents: Acetone (spectroscopic grade), sulphuric acid, NIST-traceable potassium dichromate, and cobalt (II) chloride hexahydrate were purchased from Merck India.

Chemical estimation of color

About 0.0125 g potassium dichromate and 1.35 g of cobalt (II) chloride hexahydrate were dissolved in 50 mL 5% (v/v) sulphuric acid. The absorbance (A_{std}) of the solution was measured at 420nm, and the Instrument factor (I_f) was calculated (ISO 7541, 2010) using the equation $I_f = 0.315 / A_{std}$

The extractable color of red pepper powder was analyzed by ASTA Method No. 20.1 (ASTA 2004, AOAC 1980). About 0.1g (W_s) of red pepper powder was added into a 100 mL amber-colored volumetric flask and made up to the volume with acetone. This was kept for 16 h in dark for extraction. A reagent blank was also run simultaneously. The extracts were filtered through Whatman No. 1 filter paper and the absorbance of the extract at 460 nm for the sample (A_s) and the blank (A_b) were determined using a UV-visible spectrophotometer (Shimadzu Model UV 1700, Kyoto, Japan). The color value was calculated as:

$$Colour\ Values\ (ASTA) = \frac{[(A_s - A_b) \times 16.4 \times I_f]}{W_s}$$

Where 16.4 is the constant to convert the absorbance into ASTA color units.

Image Analysis

Generating and Analyzing Images: A sample loading system with multiple sample wells was fabricated using a transparent plastic sheet of 7 mil (0.18 mm) thickness and circular rings of about 5 cm in diameter and 3 mm height, thus enabling multiple samples (up to 8) to be loaded at the same time. About 2 g each of the samples were loaded into the well and gently tapped down to form a

uniform surface against the plastic sheet. The setup was then scanned by placing it in a flatbed scanner (HP Scanjet model G2410) at a scanning resolution of 300 pixels per inch (ppi) and stored as images in JPEG format. The individual images were then loaded into ImageJ software and cropped into rectangular sections of uniform color (Fig. 1). The 'Measure RGB' plugin of ImageJ (Rasband, 2018) was used to analyze the images to obtain the R, G, and B values corresponding to the image. The performance of the scanner was verified by scanning a white paper of 80 gsm (g/m^2). The resultant image was analyzed in ImageJ and the R, G, B values were found to be within 250± 0.01.

Calculating Color Values: A general equation for the calculation of the color of red pepper from the RGB values obtained from a digital photograph of the powder can be stated as follows:

$$Color\ Value\ (Image\ Analysis) = rR + bB + gG$$

where R, G, and B are the red, green, and blue components obtained from the image analysis of the sample, and r, g, and b are coefficients that determine the contribution of red, green, and blue components to the final color of the red pepper powder. The values of these coefficients can be chosen so as to give the best correlation with the values obtained from standard methods. The coefficients were calculated by resolving 3 equations generated from the image analysis and chemical analysis of 3 samples with varying color values get the best fit with the empirical values as determined by the ASTA chemical method.

$$C_i = r_i R + b_i B + g_i G$$

where C is the color value obtained through ASTA chemical method, i = 1, 2, or 3

A plugin was written for ImageJ by incorporating the coefficients r, g, and b obtained through 5 sets of experiments (r = 1, g = -1.5, b = 0.4) which calculates the color of red pepper from the digital image (Fig. 1).

Method Validation

The precision of the method was validated in terms of repeatability and intermediate precision. Repeatability was studied by analyzing 5 replicates of each sample using both the chemical and image analysis methods. Intermediate precision of chemical method included the analysis of 3 replicates of each sample, analyzed by three analysts at three different times. Intermediate precision of the image analysis method was established by analyzing the same sample on two different scanners (HP Scan jet model G2410 and Canon LiDe 110) on different days. The ruggedness of the image analysis method was established by analysis of the same sample at scan resolutions of 150, 200, 300, 600, 1200, and 4800 ppi. The efficacy of the method was confirmed by analyzing 40 red pepper powder samples with varying color intensities simultaneously using both image analysis and chemical methods.

Statistical Analysis

Results were reported as mean ± standard deviation of 5 trials. The image analysis and ASTA methods were compared by regression analysis (Linnet 1993) and Bland-Altman plot (Altman and Bland 1983; Dewitte et al. 2002). Statistical analysis was performed using Microsoft Excel 2007

RESULTS AND DISCUSSION

Method Development

The RGB color model was chosen for image analysis. This is an additive color model in which three primary colors, viz. red (R), blue (B), and green (G), are added together to form a broad array of colors (Burger and Burge 2008). In this model, the (R, G, B) values of black color are (0, 0, 0) and those of white color are (255, 255, 255). Other colors are represented as combinations R, G, and B, varying from 0 – 255. It was established initially that varying the scan resolution from 150 – 4800 ppi did not

significantly affect the results; hence an optimum scan resolution of 300 ppi was chosen based on acceptable visual image quality and scan time. Ten red pepper powders of widely varying color values (30 – 100 ASTA units), including 2 certified reference materials supplied by ASTA, were selected for optimization of the method. Images of these samples were generated and the 'Measure RGB' plugin of ImageJ (Rasband 2004) was used to obtain the R, G, and B values from the images. The values of the coefficients r, g, and b were fixed so as to obtain the best correlation with the reference values obtained from the chemical analysis of these samples.

Color values of red peppers

The ASTA method, as an internationally accepted method being used in trade and industry for the evaluation of color in red pepper, was taken as the gold standard to develop the image analysis method. The efficacy of the method was verified with 40 samples ranging from 15.7 to 153.7 ASTA units. The results for each sample obtained by image analysis and chemical methods were compared. It was observed that the results of the samples studied could be divided into three ranges based on the level of correlation of the image analysis results with ASTA results - Range 1: results < 40 ASTA units, Range 2: results from 40 – 140 ASTA units and Range 3: results > 140 ASTA units. It was observed that in Range 1, the results from image analysis deviated widely from the results from chemical analysis. Range 2 showed a good correlation between the image analysis and chemical analysis results, to the extent of ≤ 10%. In Range 3, the degree of correlation between methods decreased but was still seen to be within ≤ 25%. Hence it was concluded that the method has a linear range within 40-140 ASTA units. The repeatability and intermediate precision obtained for the two methods in the three ranges are shown in Table 1.

To evaluate the correlation between the two methods in Range 2, two statistical methods were used. The regression analysis using a scatter plot of image analysis results versus chemical analysis results is given in Fig. 2. A Bland-Altman plot was constructed by plotting the difference vs. mean of the results obtained from the two methods, as given in Fig. 3. The regression analysis showed that the data obtained from the two methods showed a good correlation between the methods, with a correlation coefficient of 0.9895 ($P < 0.01$), slope 0.9405 ($P < 0.01$), and intercept 5.1895 ($P = 0.06$). From the Bland-Altman plot, the limits of agreement were calculated at 95% confidence limits as $d + 2s = 7.95$ and $d - 2s = -8.86$, where d is the mean difference between the methods and s is the standard deviation of the differences. Since this difference is not significantly important in measuring the color values of red peppers, it is inferred that the two methods can be used interchangeably within the range of 40 – 140 ASTA units. In the case of repeatability, the average %CV was found to be the lowest in Range 2 in both methods. In this range, the %CV for the chemical method was 0.6 and for the image analysis method was 2.0. In other ranges, the values were slightly higher. Intermediate precision (%CV) for Range 2 for chemical method and image analysis method were 1.3 and 2.0 respectively.

CONCLUSION

A novel method was developed by which the color of red peppers can be quantitatively estimated using a flatbed scanner and image analysis software. The image analysis method showed good agreement with the standard method for color values ranging from 40 to 140 ASTA units and was shown to be statistically comparable to the accepted method. Optimization of the

coefficients for different scanning instruments can be easily done by comparing the results with the ASTA color values obtained from the chemical method. The process can be adopted inexpensively by small-scale spice processers lacking instrumentation for chemical estimation of color, for a quick estimate of the color of sample lots and to ensure color consistency of sample lots. It can also be used for studies on the stability of color on storage, where the absolute correlation with the chemical method is not necessary. The image analysis method is also much faster and easier to perform than the corresponding chemical methods.

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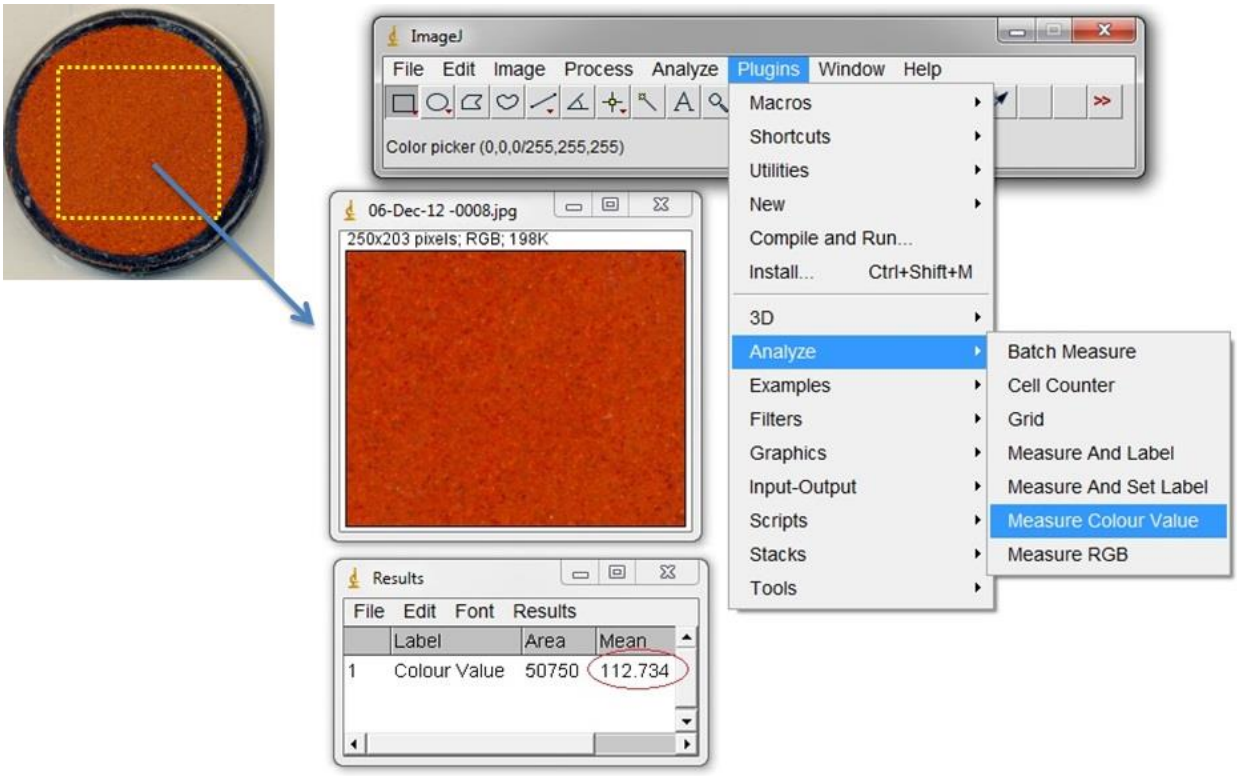

Figure 1. Image of red pepper powder used for analysis (300 ppi, HP Scanjet model G2410) and the ImageJ plugin used for estimation of color value

Table 1. Repeatability and Intermediate Precision data for Chemical and Image Analysis methods for estimation of color in red peppers

		Chemical Analysis		Image Analysis	
	ASTA Values	**Repeatability (%CV)**	**Intermediate Precision (%CV)**	**Repeatability (%CV)**	**Intermediate Precision (%CV)**
Range 1	< 40	1.5	3.4	3.3	5.7
Range 2	40 – 140	0.6	2.0	1.3	2.0
Range 3	>140	1.1	2.5	1.4	1.7

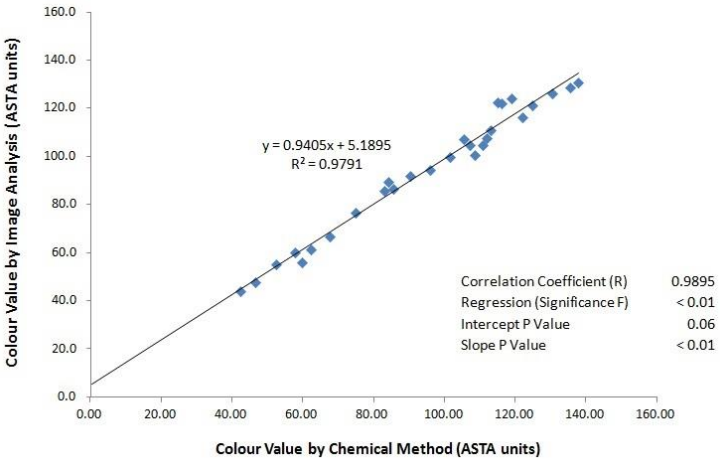

Figure 2. Regression analysis of results of colour values of red peppers from image analysis and chemical analysis methods in the range 40 – 140 ASTA units (mean of five trials)

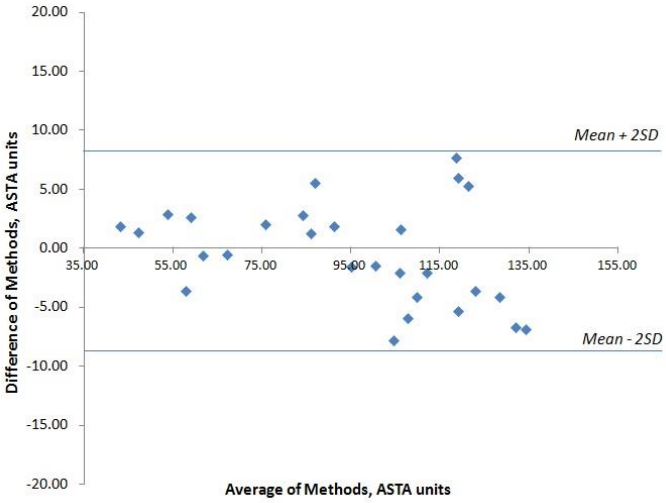

Figure 3. Bland-Altman plot for results of colour values of red peppers from image analysis and chemical analysis methods in the range 40 – 140 ASTA units (X-axis = $[C_{image}+C_{chem}] / 2$, Y-axis = $[C_{image} - C_{chem}]$, where C_{image} is the colour value obtained by image analysis and C_{chem} is the colour value obtained by chemical analysis.