Relationship Between Color and Tannin Content in Sorghum Grain: Application of Image Analysis and Artificial Neural Network

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ABSTRACT

The relationship between sorghum grain color and tannin content was reported in several references. In this study, 33 phenotypes of sorghum grain differing in seed characteristics were collected and analyzed by Folin–Ciocalteu method. A computer image analysis method was used to determine the color characteristics of all 33 sorghum phenotypes. Two methods of multiple linear regression and artificial neural network (ANN) models were developed to describe tannin content in sorghum grain from three input parameters of color characteristics. The goodness of fit of the models was tested using R^2, MS error, and bias. The computer image analysis technique was a suitable method to estimate tannin through sorghum grain color strength. Therefore, the color quality of the samples was described according three color parameters: L* (lightness), a* (redness – from green to red) and b* (blueness – from blue to yellow. The developed regression and ANN models showed a strong relationship between color and tannin content of samples. The goodness of fit (in terms of R^2), which corresponds to training the ANN model, showed higher accuracy of prediction of ANN compared with the equation established by the regression method (0.96 *vs*. 0.88). The ANN models in term of MS error showed lower residuals distribution than that of regression model (0.002 *vs*. 0.006). The platform of computer image analysis technique and ANN-based model may be used to estimate the tannin content of sorghum.

INTRODUCTION

Sorghum grain (*Sorghum bicolor*) is an important ingredient in poultry diets with approximately 95% of the nutritional value of corn (Hulan & Proudfoot, 1982; Dowling *et al*., 2002; Black *et al*., 2005). Certain agronomic characteristics, such as drought tolerance, allow sorghum to be grown in diverse geographical regions. Tannin content in the pericarp is one of the most important factors affecting the feeding value of sorghum grain (Nyachoti, 1997; Selle *et al*., 2010) and adversely affects its metabolizable energy and protein utilization in poultry (Boren & Waniska, 1992; Black *et al*., 2005; Selle *et al*., 2010). An initial study on Iranian sorghum grain indicated that its true metabolizable energy corrected for nitrogen (TMEn) content ranged between 3003 and 3899 kcal/kg (Sedghi *et al*., 2011), and protein content between 10.9 and 13.8% (Ebadi *et al*., 2011) in high- and low-tannin sorghum, respectively. Tannin can reduce feed intake (Oduhu & Baker, 2005; Bryden *et al*., 2009b), metabolizable energy (Perez-Maldonado & Rodrigues, 2009; Bryden *et al*., 2009b; Sannamani *et al*., 2010; Sedghi *et al*., 2011) and amino acid digestibility (Selle *et al*., 2010; Ebadi *et al*., 2011) in broilers when present in sorghum. Therefore, performance can be reduced when broiler are fed diets containing sorghum (Donald *et al*., 2008; Oduhu & Baker, 2005).

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The color of sorghum grain varies greatly due to pericarp color and thickness, presence of testa, and endosperm texture and color. The relationship between sorghum color and tannin content was previously reported (Hahn & Rooney, 1985). Phenolic compounds, particularly especially tannin, may change the pigmentation of the pericarp and testa in sorghum grain (Rooney & Miller, 1982). Asquith *et al.* (1983) reported that sorghum cultivars may be divided into three categories, depending on their genotypes and tannin contents: type I sorghums do not have a pigmented testa and are tannin free; type II sorghums have a pigmented testa layer that contains condensed tannins; and Type III sorghums contain tannin both in the testa and the pericarp. Dykes & Rooney (2006) recorded tannin concentrations of 0.28, 4.48 and 11.95 g/kg in Type I, II and III sorghum categories, respectively.

The determination of color can be carried out by visual (human) inspection or by using a color-measuring instrument. Determination of color by human inspection is extremely variable from observer to observer. Therefore, standard colors are often used as reference for color determination. Food color is measured by L*a*b* units, using either a colorimeter or specific data acquisition and image processing systems. L*a*b* is an international standard for color measurements and widely used in agriculture (Fathi *et al.*, 2009; Wan *et al.*, 2011; Afshari-Jouybari & Farahnaky, 2011).

Several methods of chemical analysis were frequently used to determine phenolic compounds and tannin content (Price *et al.*, 1978; Hahn & Rooney, 1985; Makkar *et al.*, 2000). Tannin determination through chemical analysis is expensive and time consuming, thus, it is necessary to find an alternative method. Image analysis and artificial neural network (ANN) have widely been applied to determine quality parameters of food and agricultural products (Majumdar & Jayas, 2000; Lu *et al.*, 2000; Shafafi Zenoozian *et al.*, 2008; Fathi *et al.*, 2009).

The objective of this study was to use a computer image analysis method and ANN model to describe the relationship between sorghum tannin (as output) and color variation (as inputs). The predicted values of the developed ANN model were compared with those obtained with regression model.

MATERIAL AND METHODS

Sorghum samples and analyses

Thirty three phenotypes of sorghum grain differing in seed characteristics were collected. All samples were chemically analyzed in triplicate for total tannin content by Folin–Ciocalteu method (Makkar *et al.*, 2000). The picture of sorghum grain was taken using a scanner (model Hp 3800) and saved in JPEG format. Since the computer vision system perceived color as RGB (red, green and blue) signals, which is device-dependent (Fernandez *et al.*, 2005), a 200*200 pixel areas was cut from each image and converted into L*a*b* units to ensure color reproducibility. In the L*a*b* color space, L* is lightness component, ranging from 0-100, parameter a* is the redness component (from green to red) and parameter b* is the blueness component (from blue to yellow). L* and b* are two chromatic components, which range from −120 to 120. In the L*a*b* space, color perception is uniform, and therefore, the Euclidean distance between two colors corresponds approximately to the color difference perceived by the human eye (Pedreschi *et al.*, 2007). Transformation of RGB into L*a*b space was performed according to a direct model, similar to the procedure described by Leon *et al.* (2006). Image analysis was performed using ImageJ tool-box software version 1.40g (Edit/Selection/Specify/Plugins/Color space converter).

Model development and evaluation

The regression analysis was employed using L*, a* and b* as independent variables and sorghum tannin as the dependent variable. The model was fitted to data using PROC REG of the SAS (SAS Institute Inc., 2003).

An algorithm of feed-forward multi-layer perceptron with three inputs, one output (with linear activation function), and five hidden neurons (with hyperbolic tangent activation function) was considered to build the ANN models (Haykin, 1999). A Quasi-Newton training algorithm was used to train the network. The three input variables were L, a, and b. The 33 data lines were randomly divided into two sets of learning and testing, with 24 and 9 data lines, respectively. The data set was imported into the Statistica Neural Networks software version 8.0 (StatSoft, 2009). The goodness of fit of ANN models was determined using R2, MS error, and bias (Roush *et al.*, 2006).

RESULTS

The tannin content and color parameter values of sorghum phenotypes obtained from chemical and image analyses are listed in Table 1. It seems

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Table 1 – Values of tannin content and color parameters insorghum phenotypes obtained by chemical and image analyses.

Sorghum phenotypes	Color parameter[1]			Chemical analysis Tannin (%)[1]
	Blueness	Redness	Lightness	
1	26	18	43	0.587
2	27	5	70	0.049
3	13	16	36	0.589
4	24	16	44	0.357
5	25	18	46	0.515
6	28	18	49	0.246
7	22	7	58	0.058
8	24	17	48	0.476
9	26	20	45	0.420
10		6	71	0.059
11	24	19	47	0.399
12	18	17	40	0.607
13	17	15	62	0.313
14	21	13	45	0.483
15	23	18	49	0.422
16	18	13	39	0.567
17	26	18	51	0.282
18	29	8	63	0.059
19	28	6	68	0.021
20	21	22	41	0.484
21	26	5	73	0.020
22	23	16	44	0.443
23	36	9	63	0.129
24	31	7	69	0.063
25	17	13	36	0.681
26	29	7	64	0.046
27	31	8	65	0.072
28	10	6	42	0.409
29	28	17	45	0.379
30	27	13	45	0.556
31	26	19	50	0.236
32	23	17	42	0.525
33	18	15	37	0.662

1 - Blueness, redness, lightness and tannin values of 33 sorghum samples. Each value is the mean of a triplicate.

that computer image analysis technique is a suitable method for predicting tannin in sorghum grain both by regression and ANN models. A linear regression model was applied to all data (Table 1), and the following relationship was established:

Tannin = 1.167 (SE= 0.141) -0.015 (SE= 0.003) × L* + 0.004 (SE=0.004) × a* -0.004 (SE=0.004) × b*
R^2= 0.88

Where L*, a*, and b* are lightness, redness, and blueness, respectively. Redness and blueness were not significant ($p > 0.05$).

The regression and ANN model plots of predicted vs. actual values of tannin content are shown in Figures 1 and 2, respectively. The comparison between actual and predicted output values may describe the behavior of the prediction model from the investigating inputs. The calculated coefficients of determination indicated good agreement between the observed and the predicted tannin values both in the regression and ANN models (training and testing sets). The goodness of fit statistic values derived from the regression and ANN models to predict the sorghum tannin content are shown in Table 2. The regression model (equation 1) adequately (R^2= 0.88) explained the variation among tannin contents of different sorghum samples (Figure 1). The goodness of fit in terms of R^2 corresponding to training of the ANN model showed a higher accuracy of prediction than the equation established by the regression method (0.96 vs. 0.88). In terms of MS error, the ANN model showed lower residuals distribution than that of regression model (0.002 vs. 0.006).

DISCUSSION

The objective of this study was to explore the possibility of using digital image analysis to estimate the tannin content of sorghum grain from its color. Nowadays, digital camera systems and image analysis techniques are well established, robust, and relatively cheap and can form the basis of a real-time observation
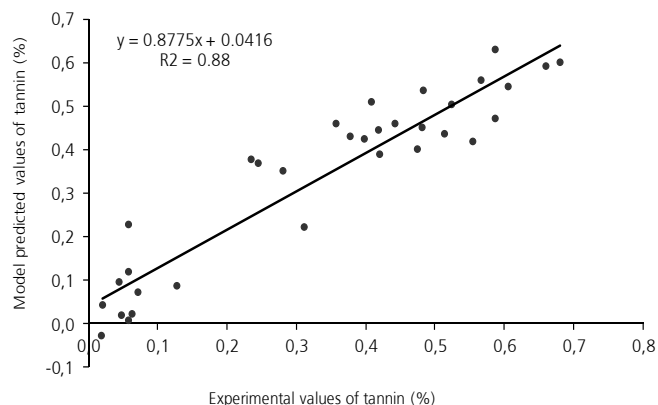
Figure 1 - The scatter plot of experimental vs. predicted values of sorghum tannin content obtained by regression analysis (black dots) using entire data set (n=33). Solid line indicates the fitted simple regression line on scattered points.

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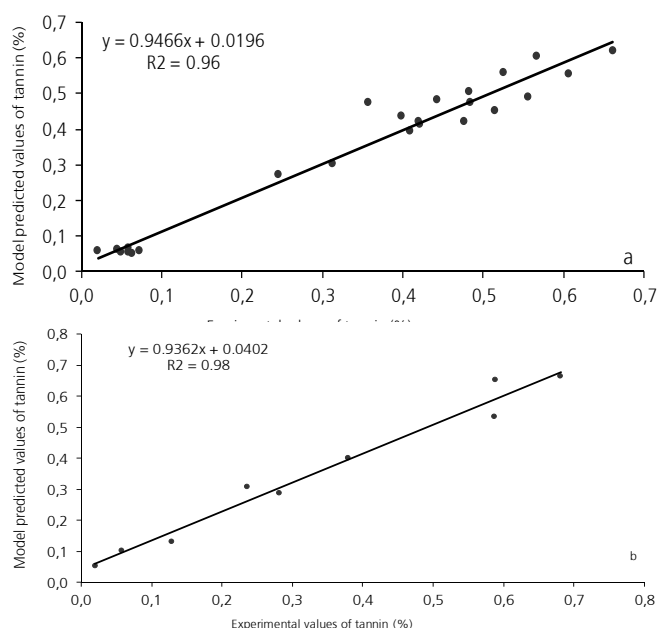
Figure 2 - The scatter plot of experimental vs. predicted values of sorghum tannin content obtained by artificial neural network model (black dots). a: Training set (n=24); b: Testing set (n=9). Solid line indicates the fitted simple regression line on scattered points.

system. There is a clearly considerable potential benefit in using image-based estimation of tannin content in sorghum grain instead of chemical analysis.

The ANN-model seems to be a more accurate ($R^2 >$ 0.96) tool for predicting tannin content both in training and testing data sets (testing data were not used during the training processes). Moreover, the trained ANN model of tannin was statistically balanced in two subsets of training and testing data (Table 2). This may suggest that there was no overlearning during the training process, and that developed model has good generalization ability (Figures 2b) when faced with completely novel datasets (Lou & Nakai, 2001; Ahmadi & Golian, 2010).

Multiple linear regression (MLR) and artificial neural network (AAN) models were previously used to describe the correlation between chemical compositions and TMEn value of sorghum grain in poultry (Sedghi *et al.*, 2011; Ebadi *et al.*, 2011). The results of these studies showed that the ANN model may more accurately estimate TMEn of feed ingredients than those using the MLR model. Furthermore, the results of those studies indicated that TMEn and amino acid digestibility were negatively correlated with polyphenol content in sorghum grain.

Sorghum grain characteristics have been documented by Rooney & Miller (1982). Sorghum appearance and quality are significantly affected by genetically-controlled characters. Several studies reported that there is a relationship between sorghum grain color and tannin content (Hahn & Rooney, 1985; Leeson & Summers, 2005). In contrast, Boren & Waniska (1992) suggest that seed color is not a good parameter to predict tannin content. Rooney & Miller (1982) reported that phenolic compounds, like tannin, change the pigmentation of the pericarp and testa of sorghum grain. Sorghum phenols, pigmentation and color of the pericarp and testa are controlled by the R, Y, B1, B2 and S genes. When both R and Y are dominant, anthocyanidin pigments increase and the pericarp becomes red. Dominant B1 and B2 genes control the presence of pigmented testa layer and tannin in sorghum. When S is dominant, in the presence of B1 and B2, the level of total phenols and tannin in the whole grain, pericarp and testa layers increased (Hahn & Rooney, 1985; Rooney & Miller, 1982). Since the precision of tannin estimation from color may be different in other sorghum varieties, further research on this field is required in order to ensure tannin estimation in sorghum grain based on grain color.

Table 2 - Statistical values derived from regression and artificial neural network models to describe tannin content in sorghum grain based on grain color parameters.

Statistics	Artificial neural network model		**	Regression model
	Training set	Testing set		Entire data
R^2	0.96	0.98		0.88
Mean square error	0.002	0.002		0.006
Bias	-0.004	-0.019		0.000
Type of network	Three layers perceptron			
Training algorithm	Quasi-Newton			
Number of hidden neurons	4			
Type of activation function in hidden neurons	Hyperbolic tangent			

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CONCLUSION

The objective of this study was to explore the possibility of using digital image analysis and ANN to predict tannin content in sorghum grain based on its color. Although the precision of tannin estimation depends on many factors, based on our procedures and results, the development of a practical imaging system for tannin estimation in sorghum grain is feasible. However, more sorghum varieties must be analyzed to find a more accurate relationship between tannin and its color. The image analysis results may be used in ANN models instead of regression models to increase accuracy of tannin prediction based on color of sorghum grain.

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