MIND TECH

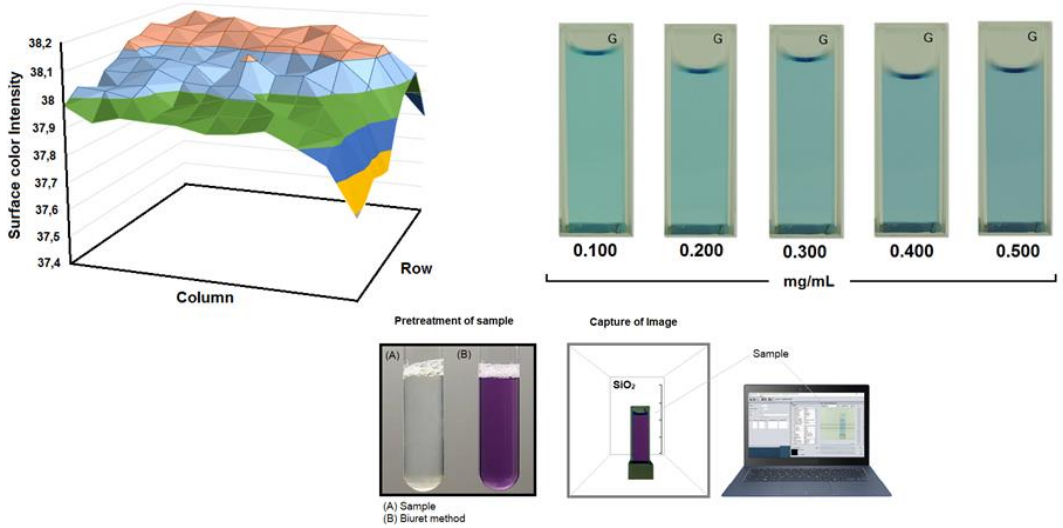
**Journal of Science with Technological Applications**
Research Article

**Tulio A. Lerma**[1,2*], **Jina M. Martínez**[2], **Enrique M. Combatt** [3]

[1] *Department of Chemistry, Faculty of Natural and Exact Sciences, Universidad del Valle, Cali – Colombia.*
[2] *Mindtech Research Group (Mindtech-RG), Mindtech S.A.S., Cali – Colombia.*
[3] *Department of Agricultural Engineering and Rural Development, Faculty of Agricultural Sciences, Universidad de Córdoba, Monteria – Colombia*

*correspondence author: tulio.lerma@correounivalle.edu.co

**Graphical abstract**




# Determination of total protein content by digital image analysis: An approach for food quality remote analysis

**Abstract**

The objective of this work was to evaluate the potential of digital image analysis based on color (DIAC), using surface color index defined for RGB space, for the determination of total protein content (TPC) by Biuret method. Approximation of surface color index was used to describe quantitatively the color intensity in the digital image of samples and standards. It was concluded that DIAC is a fast and simple analytical alternative for the determination of TPC. Detection and quantification limits were 0.02 mg/mL and 0.08 mg/mL, respectively. In addition, measurements of absorbance can be satisfactorily correlated with measurements of surface color intensity ($R^2$ = 0.9957). In addition, the importance of control of internal brightness in the measuring device was evidenced from distribution of values of color intensity into the images. It was concluded that DIAC is a

**Keywords**
RGB model
Color intensity
Digital image
Total protein content

useful tool for fast and simple remote analysis with potential applications in quality control during tests on site of raw materials.

# Determinación del contenido total de proteínas mediante análisis de imagen digital: un enfoque para el análisis remoto de la calidad de los alimentos

**Resumen**

El objetivo de este trabajo fue evaluar el potencial del análisis de imagen digital basado en el color (DIAC), utilizando el índice de color de superficie definido para el espacio RGB, para la determinación del contenido de proteína total (TPC) por el método de Biuret. La aproximación del índice de color de la superficie se utilizó para describir cuantitativamente la intensidad del color en la imagen digital de muestras y estándares. Se concluyó que DIAC es una alternativa analítica rápida y simple para la determinación de TPC. Los límites de detección y cuantificación fueron 0,02 mg/mL y 0,08 mg/mL, respectivamente. Además, las mediciones de absorbancia pueden correlacionarse satisfactoriamente con mediciones de la intensidad del color de la superficie ($R^2$= 0.9957). Además, la importancia del control del brillo interno en el dispositivo de medición se evidenció a partir de la distribución de valores de intensidad de color en las imágenes. Se concluyó que DIAC es una herramienta útil para el análisis remoto rápido y simple con aplicaciones potenciales en el control de calidad durante las pruebas en el sitio de las materias primas.

**Palabras clave**

Modelo RGB
Intensidad de color
Imagen digital
Contenido total de proteínas

**Recived:** 01-02-2019
**Accepted:** 20-02-2019
**Publishing date:** 15 – May - 2019
**Revision Code:** 20190201-MFMC [Pag. 53 - 64]
**Corresponding author:**
tulio.lerma@correounivalle.edu.co

MIND TECH

Journal of Science with Technological Applications
Research Article

# Determination of total protein content by digital image analysis: An approach for food quality remote analysis

**Tulio Lerma**[1,2*], **Jina M. Martínez**[2], **Enrique Combatt**[3]

[1] *Department of Chemistry, Faculty of Natural and Exact Sciences, Universidad del Valle, Cali – Colombia.*
[2] *Mindtech Research Group (Mindtech-RG), Mindtech S.A.S., Cali – Colombia.*
[3] *Department of Agricultural Engineering and Rural Development, Faculty of Agricultural Sciences, Universidad de Córdoba, Monteria – Colombia*

*correspondence author: tulio.lerma@correounivalle.edu.co

**Abstract**
The objective of this work was to evaluate the potential of digital image analysis based on color (DIAC), using surface color index defined for RGB space, for the determination of total protein content (TPC) by Biuret method. Approximation of surface color index was used to describe quantitatively the color intensity in the digital image of samples and standards. It was concluded that DIAC is a fast and simple analytical alternative for the determination of TPC. Detection and quantification limits were 0.02 mg/mL and 0.08 mg/mL, respectively. In addition, measurements of absorbance can be satisfactorily correlated with measurements of surface color intensity ($R^2$ = 0.9957). In addition, the importance of control of internal brightness in the measuring device was evidenced from distribution of values of color intensity into the images. It was concluded that DIAC is a useful tool for fast and simple remote analysis with potential applications in quality control during tests on site of raw materials.

**Keywords**
RGB model
Color intensity
Digital image
Total protein content

## 1. Introduction

Image analysis is a promising tool for fast, remote, nondestructive and simple analysis of objects. The growing advances in the data analyses and in the development of devices for digital image acquisition (computer vision), personal computers, web services and multifunctional electronic devices have contributed to the development of new applications based on digital images; for example, measures of size in astronomy (Bartholdi et al 1984), changes of color in agricultural and food industries as, for example, visual perception of foods such as the degree of fruit ripening, chlorophyll content in crops, caramelizing of breads, biscuits and visual analysis of different manufactured products, among others (Rorie et al, 2011; Mogol et al, 2014; Baresel et al, 2017), recognition of patters in medicine and biomedical sciences (Shu et al, 2016; Litjens et al, 2017), and for other analytical applications in different sectors (Bezerra et al, 2015; Neeley et al, 1981). Though it is widely recognized that the main advantage of image analysis is its potential for nondestructive and objective analysis, at the present, with the advances in telecommunication technologies and internet, other important advantages must be indicated: (i) the possibility of remote analysis, suggesting that "the expert" and "the sample" have not to be in the same place in the same time; (ii) analysis can be performed in real-time by a fast and easy system for acquisition of data enabling that cellphone become an analytical measurements instrument and (iii) results can be easily computerized and contrasted with data bases and algorithms of analysis, which eases the routine

analysis after its adequate standardization (Mogol et al, 2014; Baresel et al, 2017; Roda et al, 2016; Richards and Jia, 2006).

On the other hand, color can be defined as a subjective quality of substances and objects. Different models for its description have been developed from color additive theory (Wandell and Silverstein, 2003). RGB model is the most common model used in electronic and computing devices for the storing of color information in digital formats mimicking human vision (Wandell and Silverstein, 2003; Rajinikanth and Couceiro, 2015). Other color spaces are CMYK and L*a*b* (Lab or some time referred as CIELAB), the latter has been suggested as more appropriate for food applications since an important attribute of the Lab model is device independence. However, a bitmap image represented as Lab requires more data per pixel to obtain the same precision as an RGB or CMYK bitmap and its use less generalized in the most devices of easy acquisition.

In the RGB model, any color is a vector resulting of the sum of three primary vectors $\hat{R} = (255,0,0)$, $\hat{G} = (0,255,0)$ and $\hat{B} = (0,0,255)$ where elements of all vectors are integer numbers between 0 and 255 (or 0 and 1) (Wandell and Silverstein, 2003). In the electronic devices, color information is captured and stored in digital format by bits using binary systems and, later, is shown in a 2D-image constructed from thousands of bits. Thus, for a single bit, any color is defined in RGB space by $\hat{x} = (R, G, B)$ and its magnitude $I_0$ can be described by

$$I_0 = \sqrt{R^2 + G^2 + B^2} \tag{1}$$

Where *R*, *G*, *B* are the magnitudes of vectors $\hat{R}, \hat{G}$ and $\hat{B}$, respectively.

Some researchers have proposed the use of RGB space to define an "amount" capable to describe the intensity of color; for that, mean values of vectors *R*, *G* and *B* are usually used (Nobrega et al, 2006; Foca et al, 2011; Lopez-Molinero et al, 2013). Also, mathematical transformations of color space have been used for the making of the calibration curve for the performing of measurements (Vedad et al, 2015). Recently, average surface color index ($I_{sc}$) have been suggested to describe the changes of color intensity on surface, mainly, for the description of membrane fouling and to measure the turbidity resulting from bacterial growth (Cajiao et al, 2016; Palencia et al, 2016). The $I_{sc}$ is a concept based in the following assumptions: (i) the sample can be described as a surface with a constant tone or a surface formed by different phases with different tones, (ii) color intensity is directly related with some characteristic associated with the tone (e.g., concentration), (iii) for a same phase, values of color intensity are randomly distributed and, in consequence, Gaussian statistics can be applied and (iv) color intensity can be quantified by vector sum of vectors *R*, *G* and *B* (Cajiao et al, 2016; Palencia et al, 2016). In addition, because a digital image is a two-dimensional collection of data, three values associated with vectors *R*, *G* and *B* for each pixel, mean values can be calculated for a same phase and, in consequence, a total vector can be calculated to describe the complete surface.

For a surface equal to one pixel, $I_{sc}$ is given by

$$I_{sc} = \frac{k}{I_0} \tag{2}$$

where *k* is a magnification factor used in order to increase numerical value of $I_c$ (here, we use $k = 1x10^5$ in order to obtain values in the 0-100 scale; but any value larger than zero is possible). Therefore, for a pixel set, $I_{sc}$ can be easily calculated by

$$I_{sc} = \frac{F_m}{\sqrt{r^2 + g^2 + b^2}} \tag{3}$$

with

$$r = \frac{1}{n_i}\sum_{i=1}^{n} R_i \tag{3a}$$

$$g = \frac{1}{g_i}\sum_{i=1}^{n} G_i \tag{3b}$$

$$b = \frac{1}{n_i}\sum_{i=1}^{n} B_i \tag{3c}$$

where *r*, *g* and *b* are the average values of *R*, *G* and *B*, respectively; and *n* is number of pixels in the analyzed surface.

It is important to indicate that minimal color intensity point in RGB model is the color black which is given by $\hat{x} = (0,0,0)$. This approach based on $I_{sc}$ uses the common strategy in chemical analysis which consists in the elimination of variability associated with the surrounding of the

experiment; for this case, the above is possible by the imposition of luminosity-controlled conditions and standardization of parameters for image capture. Similar strategies have been used in the food quality control (by using direct, ring and diffuse illuminators) (Wu and Sun, 2013). Thus, for example, different methods have been proposed for the protein determination. Jung (2017) evidenced that the use of histogram analysis method to detect the analyte concentration and calibration results shows good correlation between perceived color change and analyte concentration when RGB data are used. Sun et al (2008) developed a new method to determine the protein content of rice by combining digital image analysis technology and a traditional chemical approach. They concluded that digital chroma measurement is an applicable method to determine the protein content in rice. In comparison with Kjeldahl method and several traditional rapid protein determination methods, analysis condition of the digital chroma method is seen to have high precision, easy operation and high efficiency for applications in quality evaluation and supervising practice in the field of rice circulation. Kucheryavskiy et al (2014) developed an algorithm for extracting features from digital images of milk samples and evaluated its applicability of conventional digital imaging to quantitative determination of fat and total protein in cow's milk. Method was compared with previously published Vis/SW-NIR spectroscopic study of similar samples, but results were significantly worse. Other examples based on hyperspectral images also have proposed (Caporaso et al, 2018). Dutta et al (2017), recently demonstrated the utilization of smartphone as a detection platform for colorimetric quantification of biological macromolecules by the use of alternative representations of the RGB color model (HSV color space).

On the other hand, the biuret method is a chemical test commonly used for determining of peptide bonds by the complexation of cupper ions to functional groups in the peptide bonds of proteins. The biuret reaction can be used to measure the protein concentration because peptide bonds occur with the same frequency per amino acid in the peptide. Thus, according to the Beer-Lambert law, the intensity of the color, at 540 nm, is directly proportional to the protein concentration (Lubran 1978, Braun et al, 2001).

The objective of this work was to evaluate the potential of digital image analysis based on color (DIAC), using surface color index defined for RGB space, for the determination of total protein content by Biuret method. In particular, the determination of total protein content was selected because to that is an important parameter for food industry, by example, in the protein determination of food for humans and animals, in raw materials or for quality control in factory or in the market.

## 2. Experimental section

### 2.1 Reagents

Bovine serum albumin standard (BSA, Aldrich, USA) was used for the preparation of solutions, for calibration curve and as protein samples. Sodium hydroxide (Aldrich, USA), potassium sodium tartrate (Merck, USA) and $CuSO_4$ (Merck, USA) were used for preparation of Biuret Reagents A and B. Potassium phosphate monobasic (Merck, USA) and distilled water were used for the preparation of buffer solution at pH 6. As protein sources egg white from egg available in the local market was supplied by Incubadora Santander S.A. (GCI-FT-02, Reference Jumbo); for food industry requiring quality control analysis of egg protein these can be assumed to be models to raw material in poultry sector.

### 2.2. Preparation of standard samples for calibration curve

BSA (1.0015 g) was placed in a 100.0 mL volumetric flask and volume was completed with a buffer solution at pH 6.2 (acetic acid-ammonium hydroxide, with 0.1 mol/L with respect to acetate and ionic strength 0.025 mol/L); after, 1.0, 2.0, 3.0, 4.0 and 5.0 mL aliquots were placed in 10.0 mL volumetric flasks completing the volume, in all cases, with buffer solution to obtain concentrations with 0.10, 0.20, 0.30, 0.40 and 0.50 mg/mL, respectively. By this procedure, average correlation coefficient was 0.9964 ± 0.0023 at our experimental conditions.

### 2.3 Preparation of protein samples

Two protein sources were used: Egg white (1.0385 g in 25 mL of buffer solution) and BSA (1.0020 g in 100 mL of buffer solution).

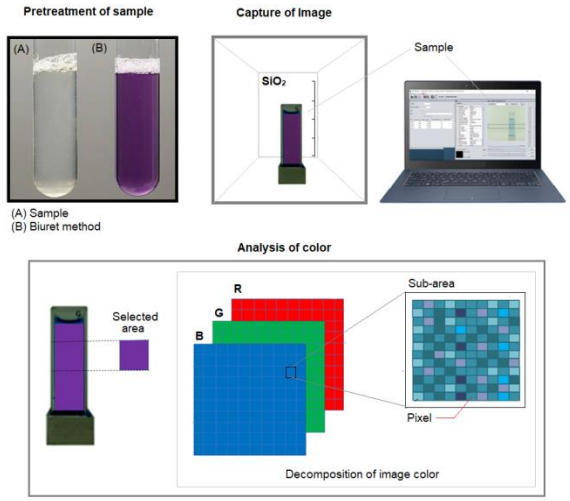



**Figure 1.** Stage of analysis: (a) pretreatnment of sample (application of Biuret method to sample and standards), (b) calibration of the device (analysis of standard, selection of area for analysis, fixing of measurements parameters) and (c) analysis of color (decomposition of image color, division of image in sub-areas and calculation of Isc for each sub-area and total area from vector components in each pixel).

Later, 3.5 mL of protein samples were diluted for respective analysis (3.5 mL of sample in 10 mL of buffer solution). Technical data per unity of GCI-FT-02: total fat (0.1 per unit), cholesterol (0.1 per unit), sodium (0.00426 per unit), total carbohydrate (0.02 per unit) and total protein (0.12 g per unity per unit).

### 2.4 Equipment and measurement procedure

A digital photometer was designed and evaluated by Research Group in Science with Technological Application of Universidad del Valle (Cali-Colombia) and Mindtech Research Group (Cali-Colombia) (Cajiao et al, 2016; Palencia et al, 2016). The main components of digital photometer are: (i) An image capture device (Nikon Coolpix P530 digital camera model with a 42x optical zoom), image sensor CMOS (1/2.3 in), operation temperature between 0 to 40 °C (32 to 104 °F), NIKKOR glass lens with Lens-shift VR and electronically-controlled 6-blade iris diaphragm, (ii) a cell sample holder (glass cell for spectrophotometry 45x12.5x12.5 mm, 10 mm pitch), in addition, distance between holder and camera lens was constant for all measurements (10 cm) (iii) aluminum foil coated $SiO_2$ was use as background surface in order to achieve greater visual uniformity when samples with a certain degree of transparency are analyzed, (iv) a cabin for analysis with an internal lighting system (light-emitting diode, LED, were placed around the sample holder in order to avoid shades, specifically, LED 4 watts, 260 lm and 200 ° defined by the ANSI), (v) a computer for processing information and (vi) a reading software of values of Isc from the captured image (Spectrum 2.1®, Mindtech s.a.s., Colombia); this software is specific for the determination of $I_{sc}$ from digital image and its algorithm was developed by Mindtech-RG researchers.

In general terms, Spectrum® 2.1 reads the RGB vector components into selected area which can be modified depending on interest of analysis and calculates the mean values of $I_{sc}$ with their respective standard (Cajiao et al, 2016; Palencia et al, 2016). Sequence of steps are given below, in addition, a scheme of procedure used for analysis is shown in the Figure 1:

- Image is capture and upload to the software.
- Selection of image section to be analyzed (e.g., 1 cm x 1 cm) is performed by the user. When the area is selected, automatically the relative position is defined and fixed for all samples. Thus, for images captured at the same conditions, the analysis area and its relative position respect to sample holder are constant. For that, it is used a reference scale on the posterior surface of the cabin.
- In the selected area, software drawing a grid with 10 rows and 10 columns. Thus, for each sub-area, RGB vector components which are defined to be R, G and B are determined for each pixel, and the average of them is determined and transformed to $I_{sc}$ by equations 2 and 3. Later, mean $I_{sc}$ values obtained for each sub-area are averaged through the respective rows and columns, in that order.

At the laboratory, the measurement procedure was similar to that commonly used in spectroscopy, the samples are individually placed in a glass cell and an image of the cell-sample assembly is taken under controlled light conditions, without activation of the flash, by manual capture operation and storage of the image at .jpg format (4608x3456 image size at pixels). Note that, analysis of images is relative to analysis conditions defined for the analysis of standard samples and, in consequence,

all measuring parameters must be initially fixed and maintained both for the standard as for the samples.

Samples and standards were analyzed by ultraviolet-visible spectroscopy (UV-vis) at 540 nm using a Spectronic 20 Genesys (Thermo Scientific). Significant differences between correlation coefficients, concentration and replicates were evaluated by Vassarstats® and Medcalc®.

### 2.5 Light homogeneity test

Determination of $I_{sc}$ assumes that surface of image which is being analyzed is homogeneous, i.e., ideally all pixel has exactly the same color and the same vector components into RGB space. Clearly, this is not true in many cases, therefore the user must do a correct selection of target area when solid surfaces are analyzed; however, in the case of solutions, this is an assumption more compatible because these are homogeneous systems by definition. On the other hand, when an image is captured in digital format, color depends on screen, but information captured by photodiodes in the camera are fixed as a result of interaction of photodiode with the light. Here, it is assumed that variation in the values of RGB vector magnitude in a homogeneous surface is resulted of random distribution of data due to the error inherent in the measure, in consequence, Gauss distribution and parametric statistical can be used to evaluate the homogeneity of surface color. In order to ensure that the conditions of internal lighting do not alter the perception of homogeneity of the solution and color (by shadows or reflections flashes), a verification of the homogeneous distribution of color was made by the following procedure: (a) one standard solution was prepared and corresponding images was captured, (b) by the use of software, several areas were selected (4 areas in total) and $I_{sc}$ was determined for each of them, (c) variance homogeneity test (Fisher's F-test) and mean tests were performed.

## 3. Results and discussion

### 3.1 Analytical determinations

Values of $I_{sc}$ for standard dissolution from 0.1 to 0.5 mg/mL are shown in the Table 1. The $I_{sc}$ changed from 38.31 ± 0.11 to 50.45 ± 0.15 CIU for the lowest and the highest protein concentration, respectively. It can be seen that values of vector components decrease for all cases as concentration is increased; however, *G* and *B* vectors linearly decreased whereas the *R* vector exhibits a behavior relatively constant with increasing of concentration. Clearly, color of samples resulting of Biuret method is localized in the visible spectrum to wavelengths closer to blue color than red color, in consequence, it is expected a major contribution by blue and green components, i.e., for Biuret method, changes in the dissolution color by changes of protein concentration are defined in the image by changes in the *B* and *G* components (Lubran, 1978).

Where as magnitude of vector RGB decreases as concentration is increased, $I_{sc}$ is increased with the concentration. Since relation between vector RGB and $I_{sc}$ is a constant (equation 2), it is clear that the effect resulting of Isc definition is a better coherence (i.e., an increase of protein concentration is described by an increase of $I_{sc}$ instead of a decrease of magnitude of vector RGB). Thus, the linear correlation equation using vector RGB as a function of protein concentration (C) is RGB = -35.13C + 265.14 ($R^2$ = 0.9896 ± 0.0010) whereas the same equation using $I_{sc}$ is: $I_{sc}$ = 5.44C + 37.66 ($R^2$ = 0.9869 ± 0.0009).

The magnitude order obtained for vector components was *G* > *B* > *R*. It can be seen that, in consequence, it can be concluded that *R*, *G* and *B* vectors are not adequate for the quantitative determination of protein by Biuret Method.

However, values of Ics increased from 38.31 ± 0.11 to 50.45 ± 0.15 as protein concentration is increased. In addition, it was seen that coefficients of variation (CV) of surface color intensity values of standards were similar to those corresponding to protein concentrations between 0.100 and 0.500 mg/mL. Mean CV was 0.35 ± 0.04 %.

The above can be explained considering the nature of data. Thus, *R*, *G* and *B* vectors are the combination of values required to defined the magnitude of resulting vector in the space RGB, in consequence, different combinations can define a same tone since function domain for individual vectors is the same: integer numbers from 0 to 256. Thus, defining 2 values, e.g., *R* and *G*, the third value corresponding to *B* is assigned (i.e., values of B is the needed to complete the tone detected by camera) (Wandell and Silverstein, 2003).

**Table 1.** Surface color intensity for protein standards by digital photometry (analyzed image surface: 246016 pixel$^2$). CIU: Color intensity unit

| [Protein] (mg/mL) | Average components of RGB vector | | | $I_{sc}$ |
|---|---|---|---|---|
| | *R* | *G* | *B* | (CIU) |
| 0.100 | 118 ± 1.8 | 171 ± 0.11 | 158 ± 0.11 | 38.31 ± 0.11 |
| 0.200 | 118 ± 1.1 | 168 ± 1.9 | 157 ± 2.1 | 38.69 ± 0.14 |
| 0.300 | 117 ± 2.1 | 164 ± 0.5 | 157 ± 2.1 | 39.15 ± 0.13 |
| 0.400 | 114 ± 1.1 | 160 ± 1.0 | 156 ± 1.4 | 39.86 ± 0.15 |
| 0.500 | 113 ± 2.6 | 156 ± 1.2 | 155 ± 1.5 | 50.45 ± 0.15 |

**Table 2.** Protein concentration determined by digital photometry and UV-vis spectroscopy for samples with known concentration ($\varepsilon_{rel}$: relative error. Absolute difference between methods is shown in the right column).

| Protein Concentration | Digital photometry | | UV-vis spectroscopy | | Difference |
|---|---|---|---|---|---|
| | Concentration | $\varepsilon_{rel}$ | Concentration | $\varepsilon_{rel}$ | |
| (mg/mL) | (mg/mL) | (%) | (mg/mL) | (%) | (mg/mL) |
| 0.10 | 0.12 | 20 | 0.11 | 10 | 0.01 |
| 0.20 | 0.19 | 5 | 0.20 | 2 | 0.01 |
| 0.30 | 0.27 | 10 | 0.28 | 7 | 0.01 |
| 0.40 | 0.40 | 0.0 | 0.39 | 2 | 0.01 |
| 0.50 | 0.51 | 2 | 0.52 | 3 | 0.01 |

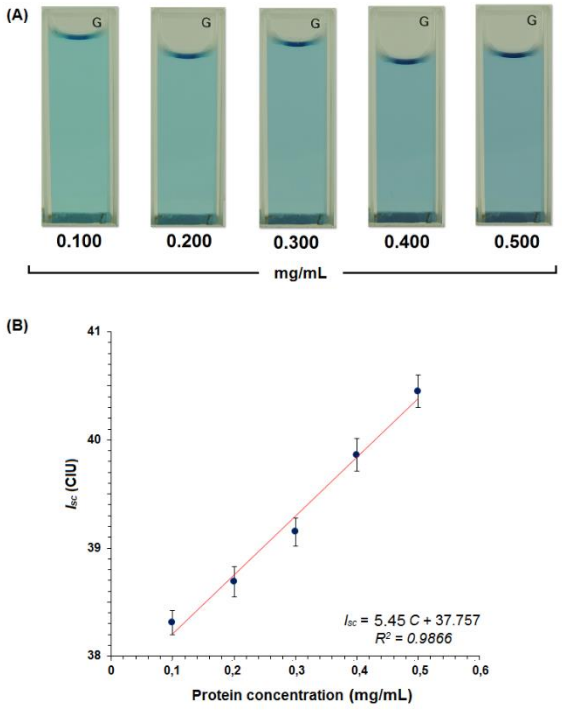



**Figure 2.** (A) Photos of digital images of protein standards and (B) calibration curve obtained by digital photometry.

Digital images of protein standards are shown in the Figure 2A and respective calibration curve is shown in the Figure 2B ($I_{cs}$ = 0.545 C + 37.657 where C is the protein concentration; quadratic correlation coefficient ($R^2$) was 0.9866).

A point to highlights from Figure 2A is the high-resolution capacity of colors that can be obtained by digital images analysis. It is observed that for human eye, color differences between dissolutions are not perceptible in the images, and therefore, it can be stated that the measure using $I_{sc}$ is not based on the tone or intensity of color, but rather in, this is based on primary information capture by the device (i.e., emitted visible radiation by the substances).

Samples with known concentrations were analyzed by DIAC, analysis of relative error associated with the results are shown in the Table 2 and compared with results obtained by UV-vis spectroscopy. It can be seen that similar results are obtained by both methods, being the absolute difference in all cases 0.01 mg/mL.

Detection limit (i.e., lowest detectable value) and quantification limit (i.e., lowest quantifiable value), denoted to be DL and QL, respectively, were calculated from several measurements of Isc using the blank experiment (sample without protein which was analyzed by Biuret method and DIAC). DL and QL are defined to be 3 and 10 times the standard deviation of blank experiment. Thus, for DIAC the DL and QL were 0.02 mg/mL and 0.08 mg/mL, respectively.

On the other hand, DIAC and UV-vis spectroscopy were compared by correlation analysis using Pearson correlation coefficient ($R^2$) which is a

**Table 3.** Protein concentration determined by digital photometry and UV-vis spectroscopy for two problem samples: Egg white solutions at two concentrations and commercial albumin solutions.

| | DP-DIAC | | UV-vis spectroscopy | |
|---|---|---|---|---|
| **Sample** | $I_{sc}$ | **[Protein] (mg/mL)** | **Absorbance** | **[Protein] (mg/mL)** |
| *Egg solution 1 | 41.03 | 0.62 | 0.125 | 0.52 |
| *Egg solution 2 | 38.79 | 0.21 | 0.065 | 0.27 |
| Albumin | 39.42 | 0.32 | 0.085 | 0.35 |

*Egg solution corresponding to samples obtained from local market

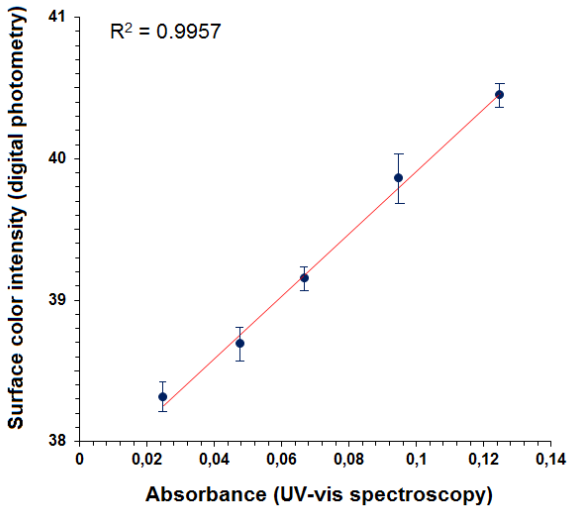



**Figure 3.** Comparison of digital photometry and UV-vis spectroscopy by correlation analysis.

bivariate analysis that measures the strengths of association between two variables and the direction of the relationship (see Figure 3). It can be seen that the value of $R^2$ obtained was 0.9957; this result suggests that methods produce similar results in the concentration range between 0.10 and 0.50 mg/L of protein.

The application of Biuret method in the determination of total protein content for DIAC and UV-vis spectroscopy of problem samples is shown in the Table 3.

Results evidence that digital photometry can be used in the determination of total protein content in samples with complex composition (water, proteins as ovalbumin, ovotransferrin, ovomucoid, ovoglobulin among other, trace minerals, fatty material, vitamins, and glucose (Sunwoo and Gujral, 2015)).

Results show that protein concentration can be correctly determined by DIAC for low protein concentrations using Biuret method. The above can be explained understanding the operating principle in the obtaining of a digital image. Thus, a digital camera is a device which uses a standard format for computers for the storing of information obtained by a CCD (Charge Coupled Device); the CCD is a light sensitive sensor, consisting of millions of small semiconductor silicon capable of capturing photons and generate an electrical response that subsequently is converted into digital data that constitutes a pixel, which in turn constitute each point into the image (Richards and Jia, 2006). In consequence, CCD is an artificial vision system or a visible light sensor. It is in the pixel where the color information that can be analytically useful is stored. These data can be used for analytical applications since these are not subjective in nature (although its projection on a monitor or photograph follows being subjective). Consequently, under analytical approaches, a device for the making of a digital image is a capture instrument of electromagnetic information emitted by the sample in the visible region of the electromagnetic spectrum. Thus, it is clear that the quality of such information and the ability of its quantitative interpretation are the determinants of appropriate quantitative application.

### 3.2 Instrumental aspects: Illumination

At the present, image capture devices available in the market offer a wide and excellent image resolution in term of quality. However, the main factor, for analytical applications of this kind of devices, is an adequate sample illumination. Illumination can be affected by external radiation (e.g., flashes and lights from other sources) and by shadow effect (i.e., non-random darkening of the tones as a consequence of the obstruction of light by an adjacent object, which results in a change in the intensity of light emitted and recorded by the CCD). By the above, it is clear that a strategy for evaluation of measurement quality is required.

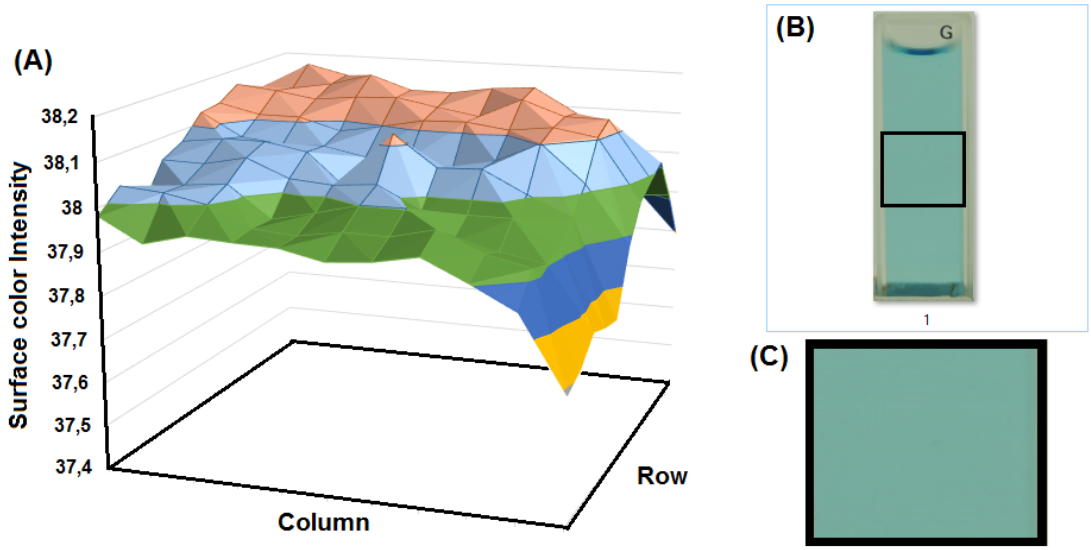



**Figure 4.** (A) Average surface color intensity corresponding to analyzed section in the image, (B) image of sample in the measure cell and (C) analyzed surface by a grid with 110 sampling subzones.

Here, it is proposed a light homogeneity test which was previously described in the experimental section.
From homogeneity test the distribution of data was analyzed in the image. For that, image is assumed to be a surface and is divided in 110 subzones (11 rows and 10 columns). Distribution of intensity values is exemplified in the Figure 4.
It can be seen that image is not homogeneous though apparently the coloration is completely homogeneous. Thus, for the example that is shown in the Figure 4, it can be seen that lowest valued of color intensity are sited on the right on the figure whereas the highest intensity values are sited in the upper area. In consequence, in order to minimize the data dispersion, analysis zone in the image must be selected to be more homogeneous zone. Thus, homogeneity test can be considered as a necessary tool for the evaluation of internal lighting.

## 4. Perspectives

With the advances in the image capture devices in relation with resolution capacity and small size, the portability and possibilities of designs for different applications, DIAC is a potential analytical strategy to enter in a generalized market.
Analytical strategy can be easily transferred to non-specialized populations in order to advance to new analytical applications. A generalized analytical configuration can be defined to be constituted by:

• Acquisition of data by any non-specialized person or basic training,
• Transformation of data and comparative analysis by a web application,
• Interpretation of information by a specialized person in another place and,
• The use of information by end users.

Thus, examples are: remote medical diagnosis, remote agricultural evaluation, integrated systems for monitoring of water quality in cities, etc.

## 5. Conclusions

It is concluded that digital photometry and digital image analysis based on color is an analytical alternative for determination of total protein content by Biuret method. Detection and quantification limits were 0.02 mg/mL and 0.08 mg/mL, respectively. In addition, measurements of absorbance can be satisfactorily correlated with measurements of surface color intensity. Distribution of values in the images showed the importance of control of internal brightness in the measuring device.

**Conflict of interest**

No conflict of interest

**Acknowledgments**

The authors thank to the Universidad del Valle and Mindtech S.A.S for financial support.

**References**

[1]. Baresel J.P., Rischbeck, P., Hu, Y., Kipp, S., Hu, Y., Barmeier, G., Mistele, B. & Schmidhalter, U. (2017). Use of a digital camera as alternative method for non-destructive detection of the leaf chlorophyll content and the nitrogen nutrition status in wheat. Computers Electronic Agriculture, 140,25-33

[2]. Bartholdi, P., Burnet, M. & Rufener F., (1984). Three statistical tests for digital photometry. Astronomy Astrophysics, 134, 290-295

[3]. Bezerra, G., Sousa, D.D., Almeida, V.E., Policarpo, T.S., Melo, J.P., Gonçalves, P.H. & Véra, G. (2015). Digital image-based classification of biodiesel. Talanta, 139, 50–55

[4]. Braun, J.P., Guelfi, J.F. & Pages, J.P. (2001). Comparison of four methods for determination of total protein concentrations in pleural and peritoneal fluid from dogs. American Journal of Veterinary Resource, 62, 294-296

[5]. Cajiao, N., Mora, H. & Palencia, M. (2016). Determination of microorganism cellular concentration by digital image quantitative analysis. Journal of Science with Technological Applications, 1, 77-86

[6]. Caporaso, N., Whitworth, M. B., & Fisk, I. D. (2018). Protein content prediction in single wheat kernels using hyperspectral imaging. Food Chemistry, 240, 32-42

[7]. Dutta, S., Saikia, G. P., Sarma, D. J., Gupta, K., Das, P., & Nath, P. (2017). Protein, enzyme and carbohydrate quantification using smart-phone through colorimetric digitization technique. Journal of Biophotonics, 10(5), 623-633

[8]. Foca, G., Masino, F., Antonelli, A. & Ulrici, A. (2011). Prediction of compositional and sensory characteristics using RGB digital images and multivariate calibration techniques. Analytical Chemical Acta, 706, 238-245

[9]. Jian-Ping S., Cai-yun H., Jing F., Xu W. Determination of the protein content in rice by the digital chromatic method. Journal of Food Quality, 31, 2008, 250-263

[10]. Jung Y. Development of smartphone based optical device. Doctoral thesis. School of Mechanical Engineerig. Purdue University. 2017

[11]. Kucheryavskiy, S., Melenteva, A., & Bogomolov, A. (2014). Determination of fat and total protein content in milk using conventional digital imaging. Talanta, 121, 144-152

[12]. Litjens, G., Kooi, T., Bejnordi, B.E., Adiyoso, A.A., Ciompi, F., Ghafoorian, M., van der Laak, J., van Ginneken, B. & Sánchez, C.I. (2017) A survey on deep learning in medical image analysis. Medical Image Analysis, 42, 60-88

[13]. Lopez-Molinero, A., Tejedor, V., Domingo, R. & Sipiera, D. (2013). Feasibility of digital image colorimetry-Application for water calcium hardness determination. Talanta, 103, 236-244

[14]. Lubran, M.M. (1978). The Measurement of Total Serum Proteins by the Biuret Method. Annals Clinical Laboratory Science, 8, 106-110

[15]. Mogol, B.A. & Gökmen, V. (2014). Computer vision-based analysis of foods: A non-destructive colour measurement tool to monitor quality and safety. Journal Science and Food Agriculture, 94, 1259-1263

[16]. Neeley, W.E., Epstein, D. & Zettner, A. (1981). An instrument for digital matrix photometry. Clinical Chemistry, 27, 1665-1668

[17]. Nobrega, E., Lacerda, V., da Silva, W., de Almeida, L., da Silva, E. & Ugulino, M. (2006). Digital image-based titrations. Analytical Chemical Acta, 570, 283-290

[18]. Palencia, M., Lerma, T. & Palencia, V.J. (2016). Description of fouling, surface changes and heterogeneity of membranes by color-based digital image analysis. Journal of Membrane Science, 510, 229-237

[19]. Rajinikanth, V. & Couceiro, M.S. (2015). RGB Histogram Based Color Image Segmentation Using Firefly Algorithm. Procedia Computer Science, 46, 1449-1457

[20]. Richards, J.A. & Jia, X. (2006). Remote Sensing Digital Image Analysis. Springer-Verlag, Berlin Heidelberg, pp. 385

[21]. Roda, A., Michelini, E., Zangheri, M., Di Fusco, M., Calabria, D. & Simoni, P. (2016). Smartphone-based biosensors: A critical review and perspectives. Trends Analytical Chemistry, 79, 317-325

[22]. Rorie, RL., Purcell, L.C., Karcher, D.E. & King, C.A (2011). The Assessment of Leaf Nitrogen in Corn from Digital Images. Crop Science, 51, 2174-2180

[23]. Shu, J., Dolman, G.E., Duan, J., Qiu, G. & Ilyas M. (2016). Statistical colour models: an automated digital image analysis method for quantification of histological biomarkers. BioMedical Engineering, 15-46

[24]. Sun, J. P., Hou, C. Y., Feng, J., & Wang, X. (2008). Determination of the protein content in rice by the digital chromatic method. Journal of food quality, 31(2), 250-263

[25]. Sunwoo, H. & Gujral, N. (2015). Chemical Composition of Eggs and Egg Products. In: Hanbook of food chemistry. Berlin, Springer, 2015, pp 331-363

[26]. Vedad, J., Solivas, J.L., Santos, J.H. & Rosario, E.J. (2015). Determination of Chromium and Manganese in Steel Using Digital Photometry of Solutions. Philippine Journal of Science, 144 (2015) 117-127

[27]. Wandell, B.A. & Silverstein, L.D. (2003). Digital Color Reproduction. In: The Science of Color. Ed. Elsevier, pp. 281-316

[28]. Wu, D. Sun., D. (2013). Color measurements by computer vision for food quality control - A review. Trends in Food Science and Technology, 29, 5-20