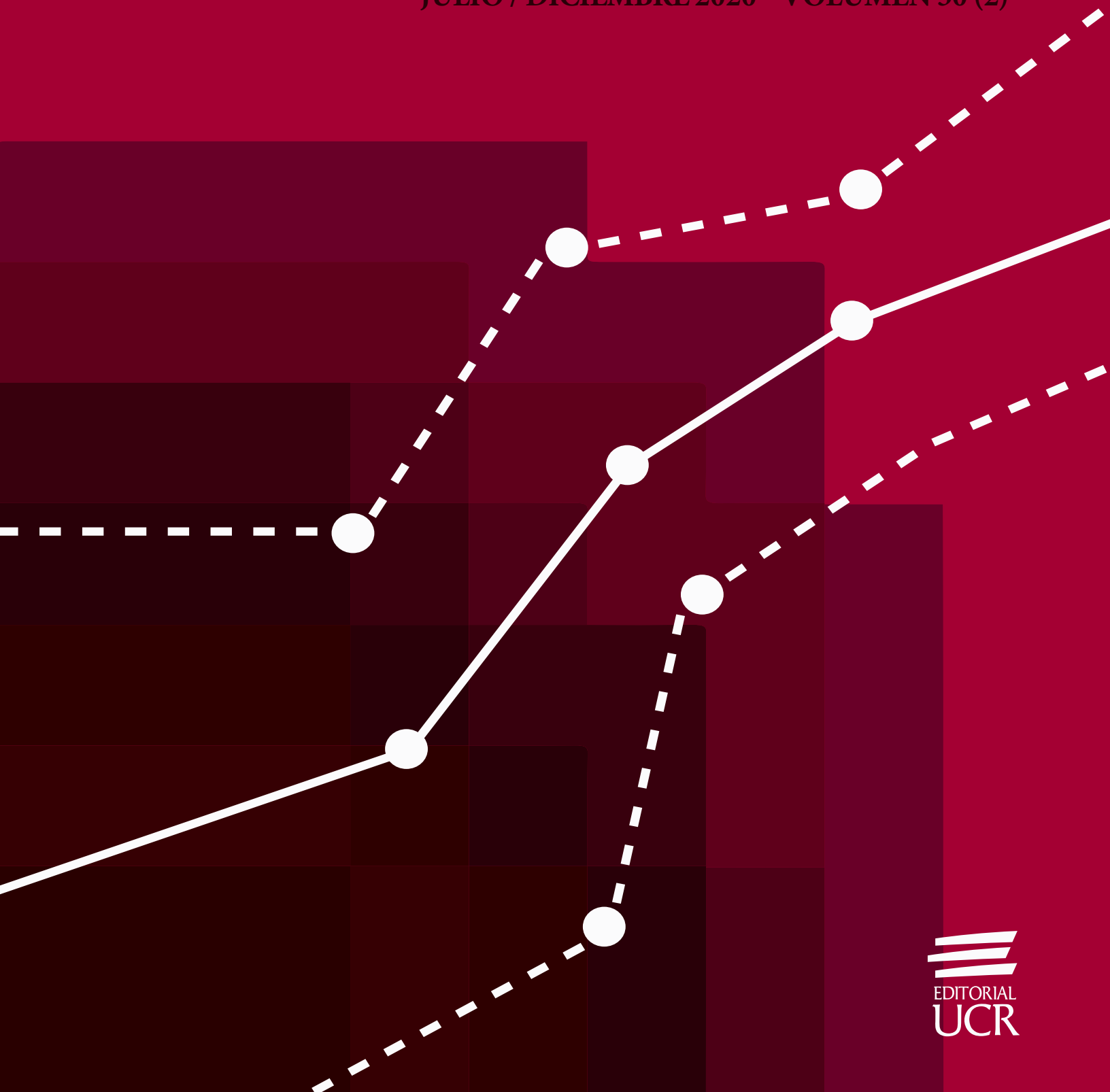


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Modelling Matambú bean (*Phaseolus vulgaris*) hydration kinetics using an automated digital image analysis

Modelado de la cinética de hidratación del frijol Matambú (*Phaseolus vulgaris*) utilizando un análisis automatizado de imágenes digitales

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Abstract

Common bean is one of the most consumed crops worldwide and of great importance for food security in Central America. In Costa Rica, there is not much information regarding the physical properties of the relatively new variety of Matambú bean. The aim of this study was to determine the speed in which Matambú bean (*Phaseolus vulgaris*) expands after grain soaking. An automated digital image processing pipeline for measuring individual grain volume and simultaneously retaining its integrity was validated and used. The previously described pipeline uses a mirror at 45 degrees so that a superior and lateral image could be taken and the 3 perpendicular diameters that compose a triaxial ellipsoid could be measured from grains. The geometric distances were calibrated with known geometries with high correlations ($R^2=0.999$) and the approximated volume was validated with a pycnometer with an average difference of 16 %. Volumetric expansion was evaluated with Peleg and sigmoid models for water temperatures of 20-70 °C. The equations kinetic parameters were described as functions of temperature using the Arrhenius equation with an activation energy of 22.410 kJ mol⁻¹. The obtained results are potentially useful for future studies on food properties, grain quality, process, and packaging design.

Keywords:

Digital image processing; Food properties; volumetric expansion; activation energy



Resumen

El frijol común es uno de los cultivos más consumidos en todo el mundo y es de gran importancia para la seguridad alimentaria en América Central. En Costa Rica no hay mucha información sobre las propiedades físicas de la variedad relativamente nueva de frijol Matambú. El objetivo de este estudio fue determinar la velocidad a la cual el frijol Matambú (*Phaseolus vulgaris*) se expande después del remojo. Se validó y utilizó un sistema de procesamiento de imágenes digitales automatizado para medir el volumen de granos individuales reteniendo su integridad. El sistema descrito anteriormente utiliza un espejo a 45 grados para que se pueda tomar una imagen superior y lateral para medir los 3 diámetros perpendiculares que componen un elipsoide triaxial a partir de granos. Las distancias geométricas se calibraron con geometrías conocidas con altas correlaciones ($R^2 = 0.999$) y el volumen aproximado se validó con un picnómetro con una diferencia promedio de 16 %. La expansión volumétrica se evaluó con modelos Peleg y sigmoides para temperaturas del agua de 20-70 oC. Los parámetros cinéticos de las ecuaciones se describieron como funciones de temperatura utilizando la ecuación de Arrhenius con una energía de activación de 22.410 kJ mol⁻¹. Los resultados obtenidos son potencialmente útiles para futuros estudios sobre las propiedades de los alimentos, la calidad del grano, el proceso y el diseño del empaque.

Palabras clave:

Procesamiento de imágenes digitales; propiedades de los alimentos; expansión volumétrica; energía de activación



1. INTRODUCTION

The common bean (*Phaseolus vulgaris*) is one of the crops with the longest history in the Americas and of the highest consumption worldwide especially in Latin America, Africa and Asia (Arias, Valverde, Fonseca & Melara, 2010). The plant has undergone a process of domestication where genotypes have been selected to grow in stressful environments and meet necessary nutritional demands (Rao, 2014). In Costa Rica, bean varieties have been bred with tolerance to disease and droughts from which the Matambú species is one of the most recent in 2013 (Hernández, Chaves, Araya & Beebe, 2018). Because of how recent and localized these developments are, information regarding its physical properties for post-harvest processing is scarce. Soaking is widely used as a pretreatment of cereals and legumes before other processing systems. In grains, soaking is used to hydrate a grain evenly in order to generate the necessary water for starch gelatinization and protein denaturation during cooking (Zanella-Díaz, Mújica-Paz, Soto-Caballero, Welti-Chanes & Valdez-Fragoso, 2014). It is also a very important step before processes such as fermentation (Egounlety & Aworh, 2003). A way to optimize the previously described processes is to describe physical changes in a biological material as a time dependent function.

Several models have been developed to describe the rate of grain soaking. Peleg's equation (eq. 1) corresponds to the most used model to describe hydration kinetics for food products. This is an empirical, two parameters equation for moisture absorption curves for materials exposed to the atmosphere for short times before the moisture level approaches equilibrium levels with medium (Peleg, 1988):

$$M(t) = M_0 + \frac{t}{(k_1 + k_2 t)} \quad (1)$$

This model has been used to describe products such as sorghum (Kashiri, Kashaninejad & Aghajani, 2010), soy (Quicazán, Caicedo & Cuenca, 2012), rice, and barley (Cardoso, Ascheri & Carvalho, 2014). The Peleg model despite being the most used model cannot describe an initial lag phase, observed during hydration of some dry grains, which has been corrected by the formulation of a sigmoid equation (eq. 2) (Kaptso et al., 2008). This is a three-parameter model where the delay time which describes a slow absorption phase and the moisture at equilibrium are considered.

$$M(t) = M_0 + \frac{M_{eq}}{1 + \exp[-k \cdot (t - \tau)]} \quad (2)$$

In his work for imbibed adzuki bean (*Vigna angularis*), Oliveira et al. (2013) showed that parameters k and τ have a temperature dependence considering the activation energy for which the Arrhenius equation was used. This equation represents the dependence of the rate of a chemical reaction with respect to temperature for a first order chemical reaction. The variation of a parameter P is presented in equation 3, which is the modified Arrhenius equation.

$$P(T) = P \cdot \exp\left(\frac{E_a}{R \cdot T}\right) \quad (3)$$

The previously described equation has been used for products such as red bean (*Phaseolus vulgaris* L.), chickpea (*Cicerarietinum* L.) (Shafaei, Masoumi & Roshan, 2016), cowpea (*Vigna unguiculata*), and Bambara groundnuts (*Voandzeia subterranea*) (Kaptso et al., 2008).

All the presented equations have been used to describe changes in moisture or mass, but studies that have modeled volumetric expansion as a function of time are limited in grains as it would generally require a time consuming individual analysis involved in traditional measuring methods. Most of the methods listed in the references are based in bulk analysis, disregarding individual grain variation. Because of this, the aim of this work is to describe bean volumetric expansion with a practical and accessible methodology through automated image processing. The method presented in this paper offers great reliability on the resulting data as it sheds light on many variables that are normally ignored.

2. MATERIALS AND METHODS

2.1 Location and plant material

This work was performed at the facilities of the Research Center of Grains and Seeds (CIGRAS) at the University of Costa Rica. Matambú bean samples (*Phaseolus vulgaris*) with 12.35 ± 0.78 % moisture (wet basis) were harvested in January 2016 at the experimental station Baudrit Fabio, Alajuela, Costa Rica. Prior to use, the product was classified using circular screens with perforations sizes of 4.37 and 4.76 mm. This created a homogenous sample that was manually cleaned, sealed in plastic bags, and stored in a dry place before using.

2.2 Image acquisition and analysis

The image acquisition was performed using a D7100 Nikon digital camera with a focal aperture of f/8, 5EV exposure, resolution of 6000 · 4000 pixels, and mounted on a rail, which maintains a constant distance from the observer to the object. All photographic equipment is confined in a chamber to avoid incidence of light from the outside with only white lights that are perpendicular to the line of sight. The action of the camera is controlled from a computer Intel Core i5 with a processor power of 3.00 GHz and 5.00 GB of RAM, using Camera Control Pro. Each grain was placed on a plate covered by blue germination paper (Hoffman Manufacturing) to create a contrasting background. A mirror at 45 degrees was used so that a superior and lateral image could be taken and the 3 perpendicular diameters (a, b, c) that compose a triaxial ellipsoid could be measured. A scalimeter was used to associate pixel value to SI units. The volume could then be calculated indirectly (eq. 4).

$$V = \frac{4\pi}{3} \cdot \frac{abc}{8} \quad (4)$$

In this experiment, c corresponds to the minor diameter observed from the lateral view given by the mirror while a and b describe the mayor and minor diameters from the superior image. Digital pictures were processed by a set of macros produced in ImageJ 1.50b (Schneider, Rasband & Eliceiri, 2012). The photographs were contrasted and then transformed to a black and white image where the beans were analyzed, using a relative L^*a^*b color scale to automatically separate the grains from their background. Consequently, each grain is studied as a particle that can be computationally approximated to an ellipse (Figure 1).

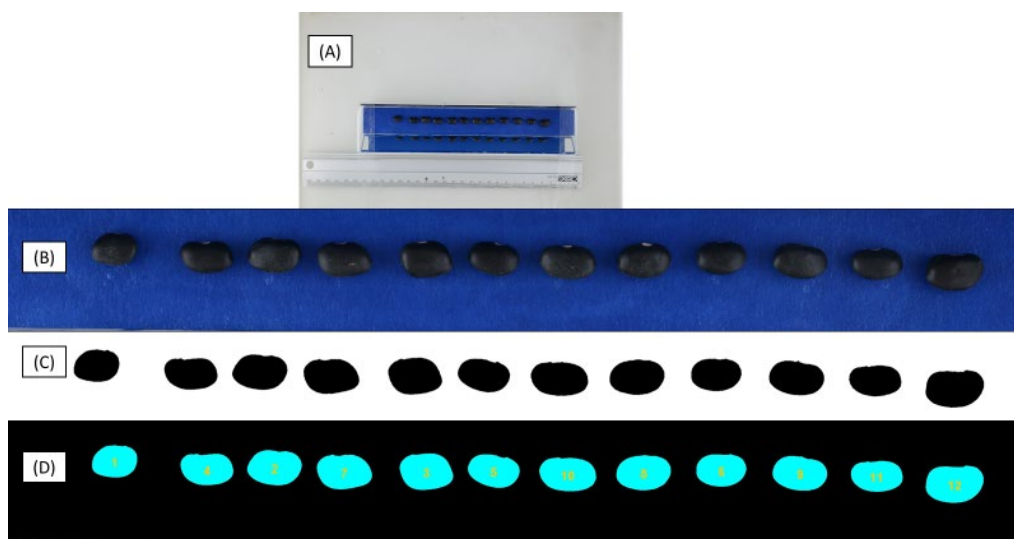


Figure 1. Digital image processing workflow: (A) camera photograph, (B) Cropped area of study, (C) Image binary conversion, (D) Image analysis

2.3 Calibration

The equipment was calibrated using an image that was generated in AutoCAD 2013 printed in 1: 1 scale of 9 ellipses and a sphere with different diameter values and centroid tilt angles. The printed imaged was analyzed using the digital image processing method and the ellipses parameters that were produced from ImageJ were compared with the real values from the original picture. As the macros try to compare individual shapes to virtual ellipses, the correlation between the theoretical and virtual ellipse parameters would display the precision and exactitude of the digital image processing method. A validation was done for 100 grains in which a digital Vernier caliper, image processing and pycnometer measurements where compared. An individual analysis was performed with consecutive use of a Vernier caliper, image processing, and pycnometer for each grain. The

results were compared using tests for the significance of the Pearson product-moment correlation coefficient and one-way ANOVA test with post-hoc Tukey test.

2.4 Determination of volumetric expansion during soaking

Once the computer digital image processing system was calibrated, this was used to calculate the initial and final volumes of beans during expansion. Separate grains were hydrated in divided petri dishes and were superficially dried, measured and then removed. Individual measurements of grain with 10 repetitions were made before and after pre-defined time intervals. Increasing time lapses were used to calculate the volumetric expansion ratio for each sample, up to a total soaking time which ranged from 1100 to 45 min. This was done for water temperatures of 20, 30, 40, 50, 60 and 70 °C that were thermostatically controlled. The volumetric expansion ratio was expressed as the coefficient between the final and initial volumes per individual grain.

Matambú bean volumetric expansion kinetics were evaluated using Peleg's equation and the sigmoidal model. For the models that appropriately described the experimental data, their parameters were modelled as a function of the process temperature. The criteria of model selection were the use of the Pearson Product Moment correlation coefficient and consistency regarding the process boundary conditions.

Additionally, the maximum volume expansion ratio was compared across different temperatures, based on samples of 20 grains that were exposed extended time periods and covering up to 24 hours.

3. RESULTS AND DISCUSSION

3.1 Validation of Digital Image Processing (DIP) equipment

The error caused by direct measurement of distance within the equipment used to determine the diameters of the approximate ellipse to beans was studied and found to be low (Figure 2). For this, high correlation coefficients were found (Table 1) where the Pearson coefficient showed a significant correlation ($p < 0.01$). No differences were found when comparing scalimeter values for the real image and the reflected by the mirror ($p < 0.05$) for 10 measurements of 1 mm, as such it can be assumed that that the virtual and real image are the same for the 45-degree position.

A significant correlation ($p < 0.05$) was also found when comparing the diameters a, b, and c for the Vernier caliper and the DIP methods (Figure 3). Despite having a significant correlation ($p < 0.05$) (Figure 4) and no significant differences between the average values ($p < 0.05$), the volume measurements displayed an error when using the triaxial ellipsoid as an approximation for the shape of Matambú beans. The average error for both the Vernier caliper and digital image processing remains as a 16 % lower than the reference value provided by the pycnometer (Table 2). An explanation can be that the flattened ellipsoid shape does not consider the size generated by the curvature of the grain as was observed for barley (Walker & Panozzo, 2012).

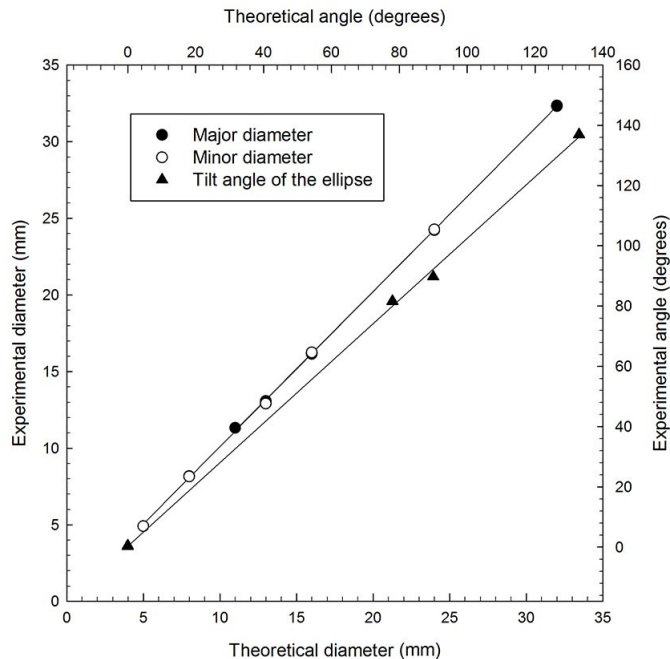


Figure 2. Comparison of the centroid tilt angle, minor and major diameters of the theoretical ellipses versus experimental data

Table 1. Theoretical versus experimental linear regression constants for digital image analysis of drawn ellipses with known parameters (Figure 2)

Variable	Equation f(x)	R ²
Major Diameter	0.069+ 1.008x	0.999
Minor diameter	-0.006+ 1.011x	0.999
Centroid Tilt Angle	4.467+ 0.982x	0.999

Table 2. Analysis of Vernier and Digital Image Processing (DIP) indirect volume measurement accuracy using pycnometer as reference

Instrument	Volume (mL)	Error (decimal)
Vernier	0.131 A ± 0,090	15.9 A ± 12,9
DIP	0.123 A ± 0,032	15.8 A ± 10,9
Pycnometer	0.140 A ±0,032	N/A

* Means with a common letter are not significantly different for different measurement methods (p> 0.05)

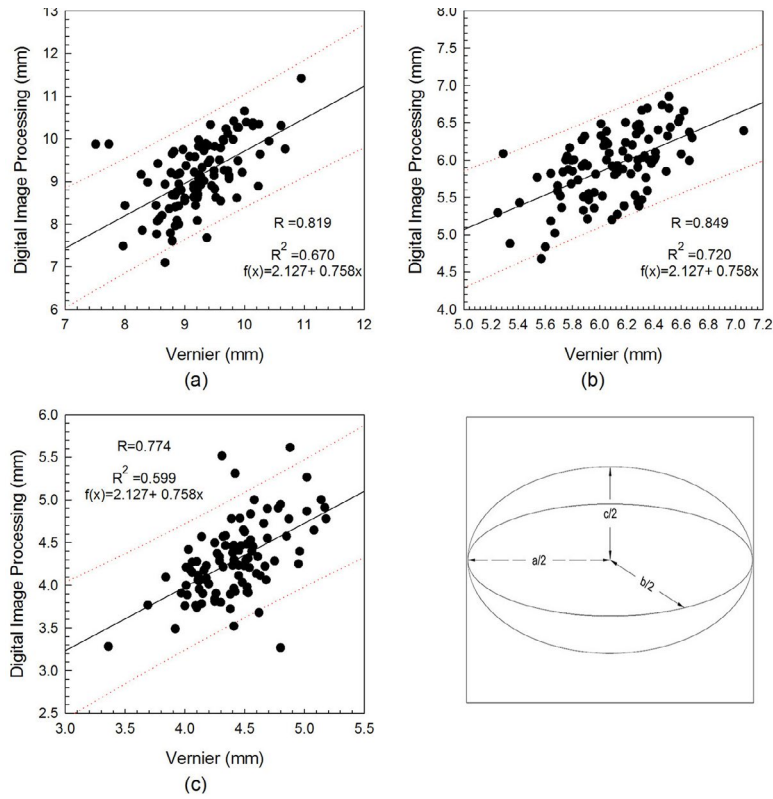


Figure 3. Diameter measurements a, b and c of Vernier caliper versus Digital Image Processing method

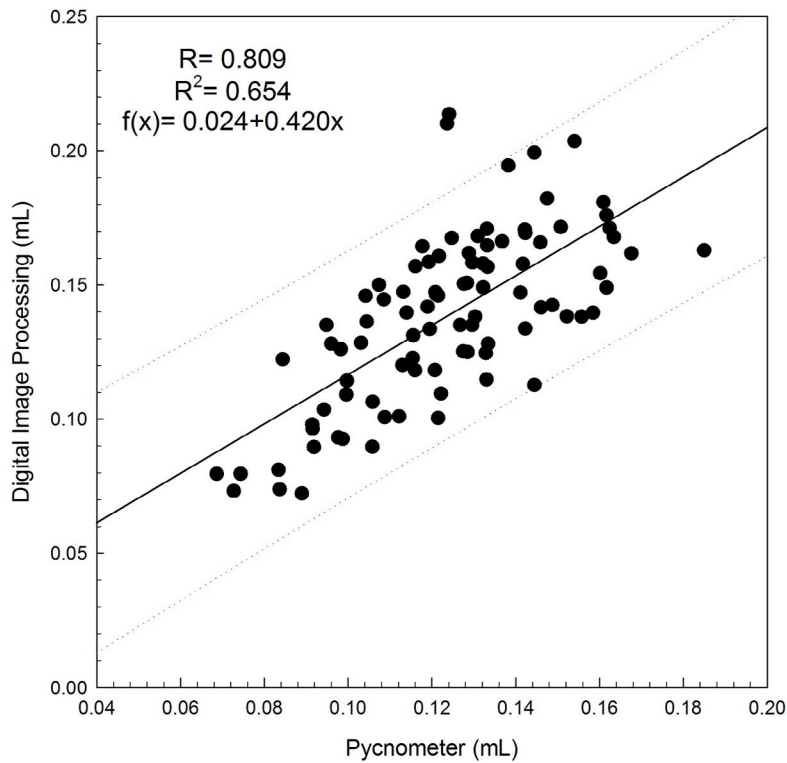


Figure 4. Measurement of volumes of pycnometer versus Digital Image Processing method

3.2 Models for volumetric expansion ratio

It was found that, after the grains gain their maximum mass and reach equilibrium, there are no significant differences for the average values in the volumetric expansion ratio ($p > 0.05$) and the temperature range studied (Table 3). It is determined that for the studied bean variety, there is an equilibrium point of 2.427, which is a constant calculated from the average of the maximum volumetric expansion ratios of the analyzed temperatures. With this consideration, Peleg's equation for volume ratio did not show acceptable results due to the impossibility of forming an equilibrium point (Figure 5.A, $R^2 > 0.9$). This effect is corrected on the sigmoidal model (Figure 5.B, $R^2 > 0.9$). Table 4 and 5 show the regression constants for the described equations.

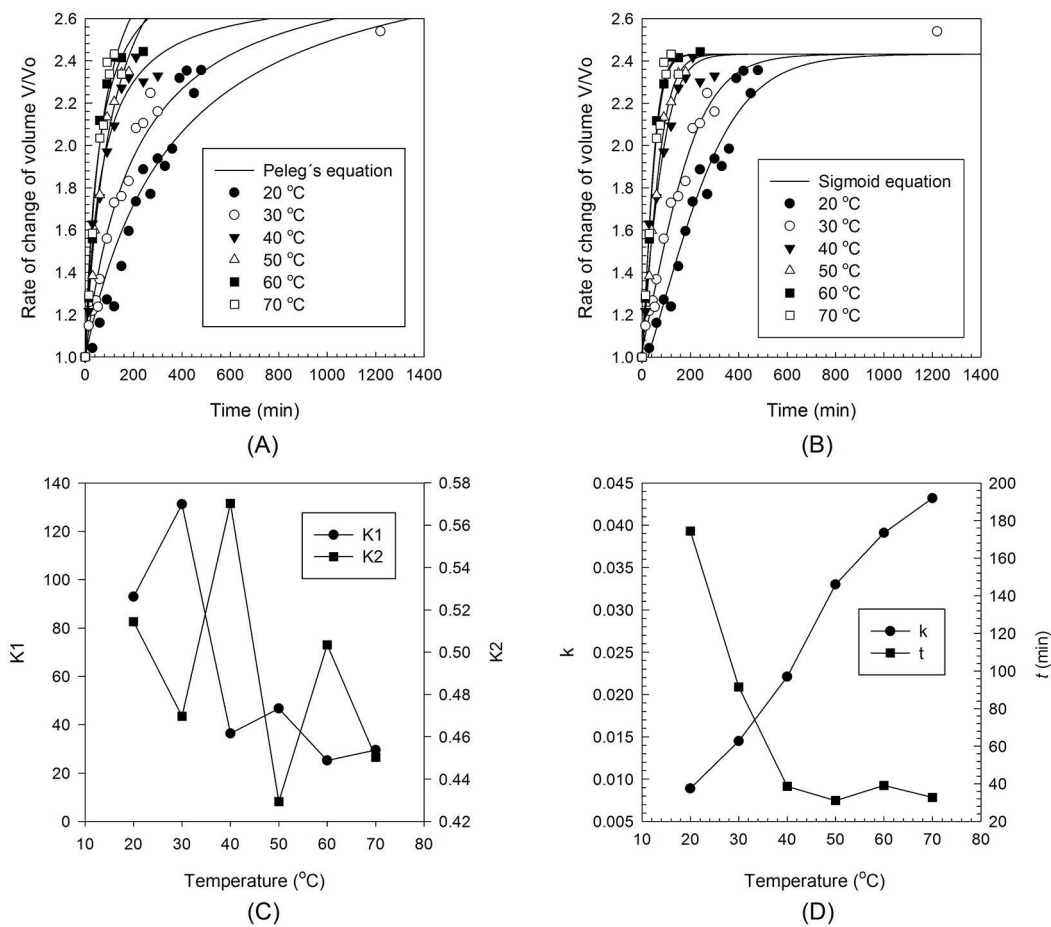


Figure 5. Volumetric expansion for (A) Peleg and (B) sigmoid equations, as well as parameter variations with temperature for (C) Peleg and (D) sigmoid models

Table 3. *Volumetric expansion equilibrium values for the temperature range studied*

T(°C)	V _{eq}
20	2,339 ± 0,247
30	2,539 ± 0,447
40	2,558 ± 0,295
50	2,346 ± 0,387
60	2,443 ± 0,151
70	2,337 ± 0,44
Total Average	2,427

Table 4. *Regression constants for Peleg's equation*

Temperature (°C)	Parameter	Coefficient	Standard Error	t-value	P-value	R ²	Residuals
20	k ₁	92,947	20,192	4,603	<0,0001	0,900	0,380
	k ₂	0,514	0,042	12,235	<0,0001		
30	k ₁	131,173	26,868	4,882	<0,0001	0,972	0,087
	k ₂	0,470	0,038	12,39	<0,0001		
40	k ₁	36,374	11,481	3,168	0,003	0,977	0,053
	k ₂	0,570	0,051	11,171	<0,0001		
50	k ₁	46,716	14,713	3,175	0,003	0,984	0,028
	k ₂	0,429	0,08	5,378	<0,0001		
60	k ₁	25,208	7,378	3,417	0,001	0,969	0,073
	k ₂	0,504	0,047	10,694	<0,0001		
70	k ₁	29,549	9,180	3,219	0,002	0,969	0,068
	k ₂	0,450	0,066	6,879	<0,0001		

Table 5. Regression constants for sigmoid equation

Temperature (°C)	Parameter	Coefficient	Standard Error	t-value	P-value	R ²	Residuals
20	M _{eq}	2.432	0.024	99.906	<0,0001	0.959	0.156
	τ (min)	80	8.047	9.942	<0,0001		
	K	0.007	0	16.406	<0,0001		
30	M _{eq}	2.432	0.024	99.906	<0,0001	0.984	0.051
	τ (min)	34.226	5.763	5.94	<0,0001		
	k	0.009	0.001	14.05	<0,0001		
40	M _{eq}	2.432	0.024	99.906	<0,0001	0.968	0.074
	τ (min)	10.235	4.023	2.544	0.014		
	k	0.019	0.002	10.355	<0,0001		
50	M _{eq}	2.432	0.024	99.906	<0,0001	0.994	0.011
	τ (min)	15.641	3.453	4.529	<0,0001		
	k	0.024	0.002	10.144	<0,0001		
60	M _{eq}	2.432	0.024	99.906	<0,0001	0.997	0.007
	τ (min)	11.547	2.226	5.188	<0,0001		
	k	0.036	0.004	9.19	<0,0001		
70	M _{eq}	2.432	0.024	99.906	<0,0001	0.986	0.031
	τ (min)	11.259	2.348	4.795	<0,0001		
	k	0.034	0.003	10.638	<0,0001		

The variation of both Peleg (Figure 5.C) and sigmoidal equation's parameters (Figure 5.D) was studied for the temperature range of 20-70 °C. Only on the second model a clear tendency is found. The kinetic parameter increases proportionally with temperature while the delay time decays and remains as a constant due to the lack of any sigmoidal form on the expansion kinetics. As an indicator of the process, the kinetic parameter was described according to the Arrhenius modified equation (eq. 5, R²=0.963):

$$k(t) = 119.673 \cdot \exp\left(\frac{-22410.739}{R \cdot T}\right) \quad (5)$$

While the delay time was described with an exponential decay equation (eq. 6, $R^2=0,920$):

$$\tau(t) = 503.567 \cdot \exp(-T \cdot 0.055) \quad (6)$$

Which both combined produce the general equation (eq. 7):

$$V(t) = \frac{2.427}{1 + \exp\left[-119.673 \cdot \exp\left(\frac{-22410.739}{R \cdot T}\right) \cdot (t - 503.567 \cdot \exp\{-T \cdot 0.055\})\right]} \quad (7)$$

The obtained results serve as a tool to predict volumetric expansion in a way that can be used for food process design and grains technology. More importantly, it is a basis that will be used in future studies to describe mass expansion using more complex and sophisticated models that require knowledge of water concentration, volume, and superficial area. The presented results might not be as accurate as 3D reconstructions methods (Roussel, Geiger, Fischbach, Jahnke & Scharr, 2016) as it remains dependent of geometric approximations; however, these are necessary as they are requirements for an statistical shape generalization for grains.

3. CONCLUSIONES

A tool based on image analysis (DIP) to determine the dimensions of beans in the three orthogonal positions was developed. The results are statistically similar to measurements made with Vernier and with the advantage of eliminating the bias produced by the operator. This provides an innovative, low cost methodology that enables an analysis that otherwise would be impractical. The operational time of the described method for 10 grains requires seconds of computer camera control and data processing whereas a Vernier and a pycnometer would require, respectively, 5 minutes and half an hour to provide the same amount of results. Despite that the system presented in this paper is accurate, the geometric assumption that the Matambú bean has an ellipsoid shape has a difference or error of 16 %. However, the previously described error is not significantly different according to ANAVA tests. This occurs due to the variance that tends to exist in biological materials, but it does not invalidate the notion that the flattened ellipsoid shape does not consider the size generated by the curvature of the grain.

Peleg's equation is not a valid model to describe beans volumetric expansion while the sigmoid equation provides a good general description of the process, despite not having an initial low absorption phase. Temperature is not a factor on the volume kinetics equilibrium point, which allows for the sigmoid model to be used as a tool to predict volume change and support food process

design and quality control (Shafiur Rahman, 2005). The volumetric expansion curves produced in this paper can now be used as a starting point to understand more complex processes used for grain quality. Soaking can reduce cooking time as it is dependent on bean humidity and variety from which even color remains an important distinction (Kinyanjui et al., 2017). Determining the specific point in the volume kinetics, which can optimize any post-harvest process, is a task for future studies. As this paper focuses on studying a relatively recent variety of bean that is important for Costa Rican culture, the previous results are uniquely important.

NOMENCLATURE

a, b, c	major diameters (mm)
$w.b.$	wet basis
E_a	activation energy (kJ mol^{-1})
k	kinetic constant sigmoid (s^{-1} , min^{-1} , h^{-1})
k_1	absorption rate Peleg ($\text{w.b.}^{-1} \text{ h}$)
k_2	absorption capacity Peleg (w.b.^{-1})
$M(t)$	moisture at time t (w.b)
M_0	initial moisture content (w.b)
M_{eq}	equilibrium moisture content (w.b)
$M(t)$	moisture at time t
P	pre-exponential factor
R	universal gas constant ($8.314 \text{ kJ mol}^{-1} \text{ K}^{-1}$)
R^2	determination coefficient
t	time (s, min, h)
T	temperature ($^{\circ}\text{C}$, K)
τ	delay time sigmoid (s, min, h)
V	volume (m^3)
V_0	initial volumetric expansion ratio
V_{eq}	equilibrium volumetric expansion ratio
$V(t)$	volumetric expansion ratio at time t

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